XXII nd CONGRESS OF THE WVPA
World Veterinary Poultry Association

Verona (Italy)
September 4-8, 2023

CEVA & PARTNERS POSTERS

XXII[nd] CONGRESS WVPA, 4-8 SEPTEMBER 2023, VERONA (ITALY)

Preface

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Assessment of day-old-chick's vocalizations at the hatchery after maternal pheromone exposure during incubation

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Introduction

The first days of the chick's life are critical during the poultry rearing process. The use of objective indicators on the physical, sanitary and welfare status of the chick's are very relevant. Sounds emitted by day-old chicks, called vocalizations, can be used to determine their welfare status during the hatchery processing. Moreover, multiple vocalizations in hatchery baskets (Containing around one hundred chicks each) require the use of a signal processing model for differentiation. Embryonating chicken eggs received a single spray of a pheromone solution 48 hours before hatch. This study used signal processing for the generation of several numerical descriptions and statistical analyses to differentiate the day-old chick's vocalizations from the pheromone treated group (SecureChick®) vs. a negative control group the day of hatch.

Materials and Methods

Location: This field study was conducted in a commercial hatchery, collecting the fertile eggs from 5 breeder flocks and randomly distributing them in 10 hatchers (Chickmaster ®) for this evaluation.

Treatment: A total of 272,160 embryonated eggs from broiler breeders (Ross 308) of different ages were selected for a single spraying of the pheromone solution. A commercial batch of SecureChick® was sprayed (w/ Desvac Eleckit) on the eggshells surfaces and hatchers 48 hours before hatch using 20 ml per 1,000 eggs (A total of 546 ml of SecureChick® were used to sprayed a total of 27,300 eggs per hatcher).

Groups: The fertile eggs selected were randomly assigned to two treatment groups, the treated one (Group 1) was sprayed with the pheromone SecureChick® and compared with a negative control group (Group 0) without any treatment.

Audio-recordings: A microphone was placed in randomly selected trolleys carrying the day-old chicks' baskets from both groups to record the sounds per basket for 90 seconds each time at three different levels of the trolley (Top, middle and bottom). A total of 210 sound recordings in three rounds were analyzed with the algorithm developed for this purpose. The algorithm applied techniques to process the field audio recordings, such as frequency filtering, signal reconstruction and feature extraction using the Python programming language. Then, the sound files were classified according to sound engineered features to train a machine learning classifier by frequency filtering, signal reconstruction and feature extraction such as: Amplitude, Spectral centroid and bandwidth, and zero-crossing rate. This process allows to automatically identify differences in the vocalizations between the groups (Figures 1 & 2).

Algorithm Model Selection: A model selection process was conducted using several machine learning algorithms being the best performing ones the Random Forest (RF) and the Neural Network (NN) models (Table 1).

Table 1. Machine learning algorithms being the Random Forest model the best performing one.

Algorithm Selected Model	Random Forest	Neural Network
Accuracy Test Mean	73%	72%
Precision Test Mean	83%	80%
ROC AUC Test Mean*	75%	71%

** ROC AUC: Mean test area under Receiver Operating Characteristic Curve.*

Results & Discussion

The comfort vocalizations of the chicks may be characterized by an individual sound from low tone to high tone in a relative brief period in contrast with a middle tone sound to low tone for an extended period, which is characteristic of a vocalization of distress.

The algorithm applied techniques to process the field audio recordings of multiple vocalizations (100 day-old-chicks per basket) such as frequency filtering, signal reconstruction and feature extraction using the Python programming language, helped in the data analysis and visualization. A model selection process using several machine learning algorithms allowed to automatically identify differences in the vocalizations between the two treatment groups.

Figure 1. Schematic view of chicks' vocalization assessment through signal processing

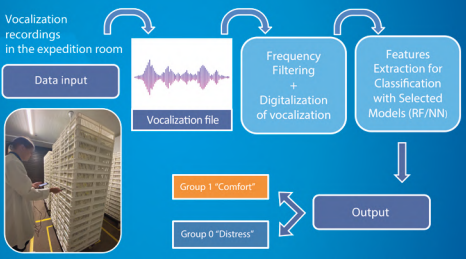
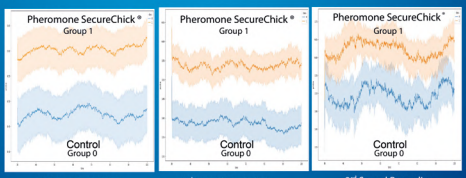


Figure 2. Time series curves showing the average probability of the classification for the 2 treatment groups: Pheromone SecureChick® treated vs. the negative control group.



The curves using the selected classifier shows the average probability of the classification with a 95% confidence interval around the mean for multiple sounds in the time, classified as Group 1 "comfort" for the Pheromone SecureChick ® and Group 0 "distress" for the negative control vocalizations .

Conclusions

- The results of the sound analyses allowed us to differentiate two distinct patterns of vocalizations between the chicks treated with the pheromone SecureChick® versus the negative control group.
- The differentiation patterns found among the audio recordings analyzed through the algorithm selected model (Random Forest) confirmed that there is a "product-take" after spraying the embryonating eggs in the hatcher which was detectable at hatch day.
- Bird vocalization is a helpful behavioral sign which associated with selected algorithms and machine learning techniques, might provide a valuable input for an automated assessment of broiler welfare in hatcheries.

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Displacement of field IBDV strains on layer pullets switching from a combination of HVT-ND/IBD + 2 x live intermediate IBD vaccines to a single immune-complex-IBD vaccination program.

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Introduction

A Belgian layer rearing organization observed inconsistent growth in their pullets during the rearing period. To address this issue, they implemented an IBDV monitoring program that includes serology and PCR testing.
Four locations were chosen: three with impaired pullet growth (A, B and C) and one without impaired growth (D). The study spanned six consecutive rearing cycles, from February 2021 to March 2023 (except location D for only one cycle).
Two different Gumboro vaccination schemes were applied as follow (table 1):

Cycle 1 + 2	Location A, B, C, D	HVT-ND/IBD + 2 x live intermediate IBD vaccines
Cycle 3 + 4	Location A, B, C	Novamune (immune-complex, Ceva Animal Health)
Cycle 5	Location A, B	HVT-ND/IBD + 2 x live intermediate IBD vaccines
	Location C	Novamune (immune-complex, Ceva Animal Health)
Cycle 6	Location A, B, C	HVT-ND/IBD + 2 x live intermediate IBD vaccines

Table 1: IBDV vaccination schemes according to cycle and location.

Materials and Methods

Both HVT-ND/IBD and Novamune vaccines are administered subcutaneously, in combination with Rispens CVI-988. Live intermediate IBD vaccines are given at 25 and 32 days of age through drinking water. Live vaccines are only added after HVT-ND/IBD hatchery vaccination.

Blood collection at 0, 3, 5, 7, 10, 13 and 16 weeks of age, for Elisa serology.
15 to 20 blood samples / sampling moment.
Bursa of *Fabricius* swabbing at 3, 5, 7, 10 and 13 weeks of age, for PCR IBDV incl. sequencing.
1 pool of 6 bursa swabs / sampling moment.

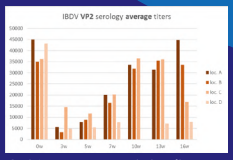
Due to Avian Influenza restrictions, some sampling moments could not be performed. Those results are missing. No sampling on cycles 2 and 4.

Serology and molecular analyses (RT-PCR) were performed at PoulPharm laboratories (Izegem, Belgium) and at the Laboratory of Microbiology of the MAPS Department of the University of Padova (Legnaro, Italy).

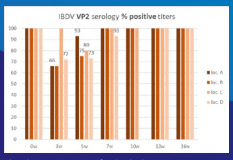
2 kits were used to perform serology: 'IBD Virus Antibody Test' kit from BioChek and 'ID Screen® IBD VP2 Elisa' kit from Innovative Diagnostics.
Molecular investigations were performed in 2 steps. Detection of positive / negative samples was performed at PoulPharm Belgium (Kylt® IBDV Screening Real Time RT-PCR, Anicon) and partial VP2 sequencing at the University of Padova. Sequencing was not always possible due to insufficient genetic material. Those results are labelled as "positive".

Results

1st cycle: Anti-VP2 antibodies showed an increase (seroconversion) in average titers over the rearing period (with location D being slightly lower) :



Graph 1: Average anti-VP2 antibody profile over time with HVT-ND/IBD + 2 x live intermediate IBD vaccination scheme. Locations A, B, C and D.
ID Screen® IBD VP2 Elisa kit.

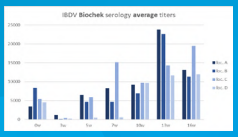


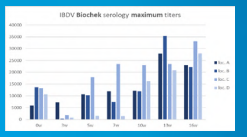
Graph 2: Percentage of individual positive anti-VP2 antibody profile over time with HVT-ND/IBD + 2 x live intermediate IBD vaccination scheme. Locations A, B, C and D.
ID Screen® IBD VP2 Elisa kit, cut-off value 1,325.

Amongst the sampled birds, some did not produce anti-VP2 antibodies until week 10 (graph 2, individual titers, cut-off value 1,325). At 3 and 5 weeks of age, up to 33% of birds sampled have no anti-VP2 antibodies and may not be protected (Gumboro immunity-gap).

In that same 1st cycle, regular IBD Elisa (BioChek kit) profile shows increase of active IBDV antibodies after the decline of passive Maternally Derived Antibodies. Average (graph 3) and individual maximum titers (graph 4) exceed titer 4,000 and 12,000, both reference maximum values for respectively vectorised HVT-ND/IBD vaccines and intermediate live IBDV vaccines (ref. BioChek). Possibly due to field IBDV infection, breaking through the immunity-gap.

All four locations have maximum titers that at one point during rearing exceed titer value 12,000.





Graph 3 + 4: Average and Maximum individual IBDV antibody profile over time with HVT-ND/IBD + 2 x live intermediate IBD vaccination scheme. IBD Virus Antibody Test kit Elisa (BioChek).

PCR results (table 2):
Cycle 1,
Location A positive for '99.8% identical to D78' IBDV strain before life vaccination at 25 and 32 days of age. The live vaccine strain, however, could not be isolated after vaccination.
Location B positive (no sequencing) prior to vaccination with live vaccines.
Location C positive prior live vaccine and at 5 weeks of age, a field strain genogroup A3 showing '98% homology with reassortant MN786768' is sequenced.
Location D: thought to be in a lower IBDV pressure area and no suspicion of sub-clinical Gumboro, positive prior life IBDV vaccine and later positive for a field strain genogroup A3 showing homology with MN786768 reassortant.

Several interpretations are possible:
- Negative IBDV PCR after live vaccination on loc. A may indicate a failure of drinking water vaccination.
- Positive vvIBDV PCR after HVT-ND/IBD vaccination suggest the inability of the vaccine to block field strains, despite correct application.
- The IBDV immunity-gap with HVT-ND/IBD exceeds 7 weeks of age (serology and PCR).

Cycle 3, first introduction of Novamune alone on locations A, B and C. No serology available. Focus was on bursa blocking capabilities. No Gumboro field strain have been identified. Instead, SYZA26 vaccine strain was detected on the bursa's. Novamune induces displacement of field strains from the first introduction on the different locations.

Cycle 5: locations A and B vaccinated with Novamune alone. Novamune was able to colonise the Bursa of Fabricius and block the field strains. Novamune protection as early as 3 weeks of age.
On location C, vaccinated with HVT-ND/IBD + 2 live int. IBD vaccines, a field strain genogroup A3 showing '98% homology with reassortant MN786768' reappeared on the birds. Meaning a constant pressure of field strains from the environment. Novamune alone was previously able to block this strain in the 3rd cycle.

Cycle 6: Locations A and B have positive IBDV PCR. Unfortunately, no sequencing was possible. Location C negative after 2 x live IBD vaccination.

Cycle 1	IBDV vaccination	3w	5w	7w	10w	13w
Loc. A		99,8% D78	negative	99,8% D78	positive	positive
Loc. B	HVT-ND/IBD + 2 x live	positive	99,8% D78	99,8% D78	positive	positive
Loc. C		positive	A3 vvIBDV	A3 vvIBDV	positive	positive
Loc. D		positive	positive	A3 vvIBDV	A3 vvIBDV	negative
Cycle 3			**5w**	**7w**	**10w**	
Loc. A				negative	positive	
Loc. B	Novamune 1st cycle		SYZA26			
Loc. C			SYZA26	SYZA26	SYZA26	
Cycle 5		**3w**	**5w**	**7w**		
Loc. A	Novamune 3rd cycle	SYZA26	SYZA26	positve		
Loc. B		SYZa26		positve		
Loc. C	HVT-ND/IBD + 2 x live	negative	A3 vvIBDV			
Cycle 6		**3w**	**5w**	**7w**		
Loc. A				positive		
Loc. B	HVT-ND/IBD + 2 x live		positive			
Loc. C			negative			

Table 2: Summary of PCR results.

Conclusion

When considering the potential harm of field IBDV strains on bird immunity, it appears that the new immune complex Novamune is more effective in blocking the bursa of *Fabricius* and reducing the Gumboro immunity-gap. The combination of HVT-ND/IBD + 2 x live intermediate IBD vaccination was unsuccessful in preventing the bursa of *Fabricius* to be colonized by field strains.

Novamune not only prevents Gumboro infection in birds but also displaces field IBDV strains found in various locations.

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Detection of Infectious Bronchitis Variant viruses in commercial broiler flocks in Pakistan

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Introduction

Infectious bronchitis (IB), caused by an Infectious bronchitis virus (IBV), is characterized by the highest frequency of new variants amidst all coronaviruses. The actual number of existing variants in the world is not fully known as some of these variants are restricted to some geographic areas. Valastro et al (2016) proposed a unified nomendature based on genotypes and lineages. In Pakistan, IB causes major economic losses in broilers, resulting in poor weight gain, poor feed conversion and poor litter quality. Additionally, this virus can be associated with other respiratory pathogens aggravating the situation that may lead to high mortality rates. There are multiple studies determining the prevalence or seropositivity rates of IBV in Pakistan with less focus on novel variants. Furthermore, at national level, the incidence of the disease is not well documented due to the lack of a consolidated surveillance plan. This study aims at determining which IBV strains are circulating in vaccinated commercial broiler chicken flocks in Pakistan, impacting the respiratory integrity and performance of the birds.

Materials and Methods

A total of 13 broiler farms with prior history of respiratory clinical signs, were surveyed from April 2021 to June 2022 in Punjab, Pakistan. Vaccination was performed in the hatchery by spray method (hand sprayer and local made hatch-sprayer). GI-1 (Mass) was used in two flocks, GI-13 (793B) in eight flocks, or a combination of both (three flocks). The samples were collected during the clinical outbreaks or at slaughter age and included cecal tonsils and tracheal swabs smeared on FTA cards for molecular detection (RT-PCR) and further sequencing of the positive results. IBV ELISA serology (IDVet) was carried out at slaughter age .

Results

All vaccinated flocks showed clinical signs compatible with IB. Total mortality in the monitored flocks ranged from 3.08% to 49%, with an average of 13.23%. A total of 52 cecal tonsils and tracheal swabs from 13 flocks (4 birds per flock) were smeared on FTA cards for RT-PCR analysis. All flocks (100%) tested positive to IBV RT-PCR. The genetic material in 4/13 flocks (31%) was not enough to perform sequencing. The remaining 8/13 (69%) flocks tested positive to GI-13 (793B) vaccine strains. One flock 1/13 (8%) showed high mortality (49%) with severe clinical signs and LPAI H9 coinfection was confirmed by molecular detection (RT-PCR) and high HI titres (10 $\log_2$) at slaughter age. In this flock, the tracheal plugs found yielded positive results to a GI-24 IBV strain with 99% similarity to MW525215.1. (*Fayyaz, A., et al.,* In Press). IBV ELISA tests (IDVet) at slaughter age showed an average GMT of 4,502 and a CV of 41%, confirming that the flocks were exposed to circulating IBV strains.

Flocks Numbers	IBV Vaccination Program	Age & Method of Vaccination Program	Equipments Type (All are Local Random Supplier)	Mortality (%age)	IBV Serology (IDVet ELISA)	CV %age	Slaughter age (Days)	RT-PCR Result
1	Mass + 793B	Spray Method in DOC's in hatcheries	Hand sprayer	11.03%	5918	33%	38	Weak IBV Positive
2	Mass + 793B		Hatchsprayer	8.43%	6528	47%	38	Weak IBV Positive
3	**Mass + 793B**		**Hatchsprayer**	**49.00%**	**4502**	**57%**	**29**	**IBV Variant**
4	Mass only		Hand sprayer	18%	8078	34%	35	Weak IBV Positive
5	Mass only		Hand sprayer	5%	1630	43%	29	Weak IBV Positive
6	793B		Hatchsprayer	5.60%		Not Done		793B group Positive
7	793B		Hatchsprayer	3.08%	6895	40%	36.5	793B group Positive
8	793B		Hatchsprayer	5%	4159	70%	36.5	793B group Positive
9	793B		Hatchsprayer	13.10%	6704	31%	39	793B group Positive
10	793B		Hatchsprayer	19.12%	7764	36%	38	793B group Positive
11	793B		Hatchsprayer	8.20%	5898	33%	36	793B group Positive
12	793B		Hatchsprayer	17.80%	5523	44%	39.5	793B group Positive
13	793B		Hatchsprayer	8.78%	5835	40%	43	793B group Positive

Table. 1. IBV serology (IDVet ELISA) & RT- PCR results in 13 flocks

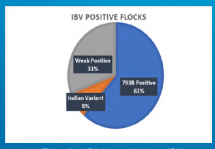

Fig. 1: IBV positivity (%)

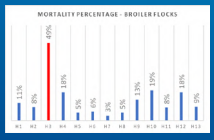

Fig. 2. Mortality (%)

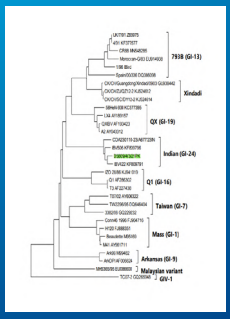

Fig. 3. GI-24 Phylogenetic tree

Conclusions:

The repeated detection of G1-24 (Indian Variant IBV genotype) in this study shows that this strain is widespread in Pakistan, confirming a previous work *(Saba, R., et al., 2018)*. These findings highlight the need for continuous epidemiological surveillance to identify the IBV strains circulating in Pakistan. These IBV variant strains enhance the pathogenicity of co-infective pathogens like LPAI H9 (*Samy et al. 2018*). As a preventative measure, it is recommended improving the quality of broiler spray vaccination in the hatchery by means of modern equipment and adapted vaccination protocols that ensure good vaccine uptake and to protect clinically against circulating GI-24 IBV variant strains.

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Reduce layer mortality at 5 weeks of age by using immune-complex vaccine (SYZA-26) in the hatchery in Indonesia

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Introduction

Infectious bursal disease virus (IBDV) is still a major infectious disease in Indonesia causing high morbidity and high mortality at 5 weeks of ages in commercial layer. Biosecurity and disinfectant cleaning were not sufficient to control the disease. When considering vaccination to control IBD, the main objective must include both protection against clinical sign and prevention. This study aims to assess the use of IBD immune- complex vaccine consisting of the SYZA-26 strain in the hatchery to reduce mortality in layer.

Materials and Methods

A total of 36 flocks with 838,961 pullet were vaccinated with IBD immune-complex (SYZA-26 strain) and rHVT Newcastle disease (ND) vaccine in the hatchery. These day-old-pullets were then placed in farms previously with IBD problem. In some flocks, at 12-26 days of age, a live IBD was administered by oral drop as per farmers' preferences.

Monitoring criteria:

- At 1, 21, 28, 35 and 42 day of age, 15 sera were collected for IBD antibodies monitoring using a commercial ELISA test kit (BioChek)
- Five (5) individual bursas were collected from flock at 35 days of age and sent for reverse transcriptase polymerase chain reaction (RT-PCR) technique follow by sequencing to identify SYZA-26 strain.

Results were compared between flock using conventional IBD vaccine and flocks using immune-complex (SYZA-26) in the hatchery

Results

Maternal derived antibody (MDA) serology at day-old-pullet were around 10,000 ELISA units and birds started to show active seroconversion at 21 days of age (fig 1). Mortality % at five (5) weeks of age improved drastically from 11.7% in previous flocks to 3.0% after vaccinated with IBD immune-complex (SYZA-26) vaccine with no IBD clinical signs reported (fig 2). Average IBD titre at 35 day of age was 11,222 ELISA units (Biochek). Molecular detection showed SYZA-26 strain in the bursa showing a proper vaccine-take of the vaccine giving protection to the pullets. (fig 3)

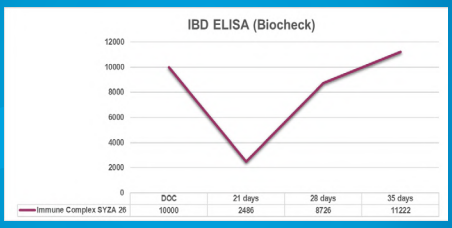

Fig. 1. MDA and seroconvertion started at 21 days

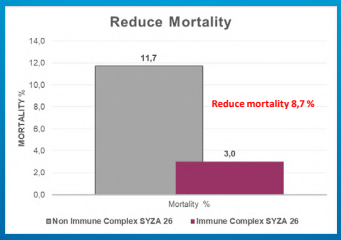

Fig. 2. Lower mortality using immune complex SYZA-26.

Ceva Phylaxia Code	Original Data	Organ Tested	IBDV PCR / Novamune qPCR	Sequence
D6442/1	Age 35 Days At Hatchery, DO : Novamune	bursa	IBDV Positive SYZA 26	-
D6442/2		bursa	IBDV Positive SYZA 26	-
D6442/3		bursa	IBDV Positive SYZA 26	-
D6442/4		bursa	IBDV Positive SYZA 26	-
D6442/5		bursa	IBDV Positive SYZA 26	-

Fig. 3. PCR results identifying SYZA-26 strain replication in the bursa.

Conclusion

The significance of SYZA-26 strain as an effective IBD immune-complex vaccine was demonstrated in this large-scale field trial in layer farms Indonesia. The mechanism of action of this IBD immune-complex vaccine includes the colonization of the bursa of Fabricious by the vaccine virus as evidenced at 35 days of age in majority of the samplings. This colonization of the bursa by the vaccine virus occurred in alignment with the ELISA antibody response that could be detected at 21 day of age. Adopting SYZA-26 strain as the IBD immune-complex vaccine for layer is safe, convenient and efficacious.

References:
Palya, V., Walkóné-Kovács, E., Homonnay, Z. G., Kiss, I., Matló, T., Gardin, Y. (2015) Development of differential real-time RT-PCR method for the assessment of field and vaccine IBD virus replication in vaccinated chickens. AAAP Symposium, 12-14 July, Boston, USA

Pathology of avian adenovirus infections

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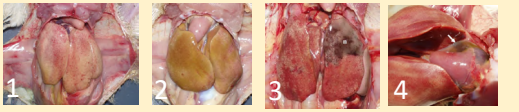
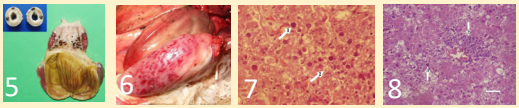
1. *Aviadenovirus* Infections

1.1. Inclusion body hepatitis

Results of our research: Adeno positive PCR for **FAdV2.**

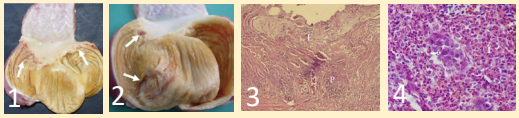
- A characteristic macroscopic lesion is the enlarged, dystrophic liver with yellowish colour and crumbly texture (1). Manifestations of total icterus (2) or large-scale necrosis are possible (3). Hydropericardium is an accompanying finding in 10-15% of cases (4). With a similar frequency of distribution are also found petechial and larger hemorrhages in the gastric mucosa around the papillary orifices of the glands (5). Often, ecchymoses and striated haemorrhages in skeletal muscles are observed (6).
- Microscopically, extensive dystrophic changes and necroses of liver parenchyma are detected. In the nuclei of hepatocytes, basophilic or eosinophilic inclusion bodies are detected (7). Although very rarely, at the same time with the intranuclear inclusions (arrow-a), perivascular mononuclear proliferates (arrow-b) are present (8).





1.2. Gizzard erosions (GE) in broilers.

- Grossly they are manifested by erosions affecting the koilin membrane and the underlying tissues of the gizzard (1 and 2).
- Microscopically, Deeper erosions are manifested through focal necrotic lesions, desquamation and lack of glandular epithelium (3). Basophilic intranuclear inclusions in the gland epithelial cells (arrow 1) and an inflammatory-cellular infiltrate consisting of mainly heterophilic granulocytes (arrow 2), (4).



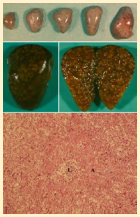
2. *Siadenovirus* Infections

2.1. Hemorrhagic enteritis in turkeys

- Grossly the small intestine, especially the duodenum, has a dark red colour and ramiform blood vessels prominating under the serous coat, and sometimes, haemorrhages are seen through the intestinal wall (1). The extent of lesions of the intestinal mucosa varies from hyperaemic to haemorrhagic, severe haemorrhagic or fibrinous necrotic inflammation (2). The liver is enlarged, crumbly and mottled with multiple haemorrhages, varying from petechiae to ecchymoses (3). The spleen of infected birds is typically enlarged, haemorrhagic, crumbly, mottled or marble-like (4). Later, the spleen reduces its size 2-3 times and acquires a specific silvery-grey colour (5).
- A characteristic diagnostic sign is the discovery of large acidophilic, rarely basophilic intranuclear inclusion bodies in the reticuloendothelial cells of the spleen (the green arrows). The displaced and condensed nuclear chromatin pattern around the inclusion bodies often looks like a crescent (the white arrows). Similar inclusion bodies could be sometimes found out in lamina propria of the interstitial mucosa (6).

2.2. Marble spleen disease in pheasants

Marble spleen disease (MSD) of pheasants. MSD affects captive-reared ring-necked pheasants (*Phasianus colchicus*) between 2 and 8 months of age.
At necropsy, the spleen and lungs are the only organs with gross lesions. The spleen is markedly enlarged and mottled (above). The lungs are edematous.
Microscopically, the spleen has marked reticuloendothelial cell hyperplasia and amyloid degeneration (below). Deposition of amyloid masses (A) from the periphery to the center of the lymphatic follicles (L) of the white pulp.



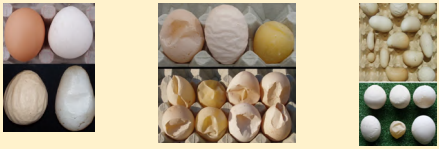
2.3. Splenomegaly in chickens

Avian adenovirus splenomegaly. Avian adenovirus splenomegaly is characterized by enlargement of the spleen, pulmonary oedema and congestion. The etiological agent belongs to the genus *Siadenovirus*. It is observed in broiler breeders at the age of 20–45 weeks. Its course is peracute or acute. The mortality could reach 8–9%. The most typical lesions are splenomegaly, mottled or marble-like appearance of the spleen, oedema or hyperaemia of lungs.

3. Atadenovirus Infections

3.1. Egg drop syndrome 1976

The egg drop syndrome 1976 (EDS 76) is an infectious disease in layer hens manifested by a quick drop in egg production, failure to reach peak production, irregularly shaped eggs, soft-shelled or shell-less eggs and depigmentation.
The first sign is the loss of egg pigmentation, rapidly followed by the appearance of soft-shelled, shell-less of deformed-shell eggs.
The drop could be sudden or prolonged. Usually, it lasts for 4-10 weeks and the egg production is reduced by about 40%. Apart the inactive ovaries and oviduct atrophy, other lesions are not discovered.



MUSCULOSKELETAL PATHOLOGY IN BROILER CHICKENS

CLINICAL-MORPHOLOGICAL STUDIES IN PROBLEMATIC CONDITIONS

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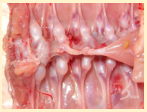
INTRODUCTION: This research presents data, analyses and conclusions from large-scale field surveys of many years' duration on problematic conditions related to musculoskeletal pathology in broiler chickens. The reviewed disorders constitute a major part of broiler pathology and their consequences can cause substantial economic losses.

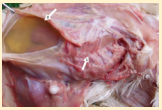
1. LEG SKELETON PATHOLOGY

1.1. RICKETS. Determination of the incidence of different rickets forms with regard to pathomorphological diagnosis on the basis of gross and microscopic lesions in field clinical rickets cases in broiler chickens.

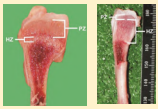
1.1.1. Incidence of forms of rickets and their association with other pathological states in broiler chickens. Determination of the forms of clinical rickets and their association with other disease states in broiler chickens.



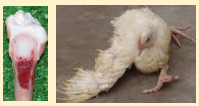
*Gross rickets lesions in the area of costochondral junctions – **rachitic rosary** in a 10-day-old chicken*



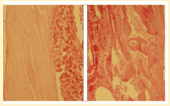
Chest deformity in the area of chondrocostal junctions (arrow 1) and ascites syndrome (arrow 2) in a 35-day-old chickens after recovery from hypophosphataemic rickets.



Comparison between the gross lesions of hypocalcaemic (left) and hypophosphatemic (right) rickets

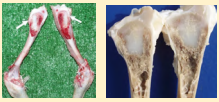


Growth of wedge-shaped cartilaginous masses in the proximal tibiotarsus in a chicken survived rickets. Spondylolisthesis-like signs in a 34-day-old chicken after recovery from hypocalcaemic rickets.



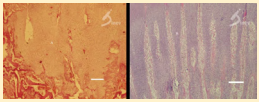
Thickness of normal bone wall. Strong thinning of the cortex, remnants of bone and growth of fibrous tissue in rickets.

1.2. TIBIAL DYSCHONDROPLASIA. Determination of the prevalence of TD lesions that lead to lameness in commercial broiler chickens**.**



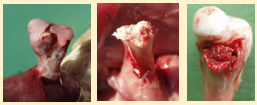
In general, TD lesions are bilateral and symmetric.

Bending (breaking) of the proximal part of the tibia, just below the abnormal cartilage mass, with a tendency for fracturing.



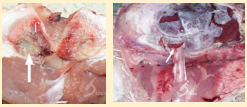
Longitudinal section of the proximal end of the tibia in a broiler chicken. Left – TD affected bone: overgrowth and accumulation of pre-hypertrophic cartilage with lack of a distinct boundary between the proliferating and hypertrophic cartilage. Right – normal bone: regularly formed cartilage columns in the metaphyseal region, H/E, Bar = 50 µm.

1.3. FEMORAL HEAD NECROSIS. Determination of the prevalence of femoral head necrosis (FHN) as a cause of lameness in broiler chickens.

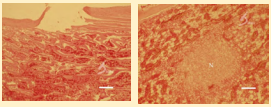


Extremely thinned cortex of the femoral neck and initial fracture.

Incomplete fracture of the femoral head (left). Complete femoral head fracture (right).



Necrosis (arrow) and sequestration of the metaphyseal part of the femur (f), concomitantly with osteomyelitis.

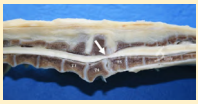


A significant cortical thinning, especially in the proximal femoral neck region. In the medullary bone zone, there were necroses (N).

In the cases of femoral bone fractures, with simultaneous osteomyelitic lesions, mainly *E. coli* – more than 90% were isolated from the bone marrow samples.

2. AXIAL SKELETON PATHOLOGY

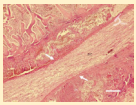
•Pathological conditions of the axial skeleton, prevalent among broiler chickens: spondylolisthesis; spondylosis; spondylitis; scoliosis.
•All they result mostly in posterior paralysis, immobilisation and death.



***Spondylolisthesis**. Ventral dislocation of T4, whose posterior end lifts T5*

***Spondylitis**. A dorsoventral spread of the inflammatory swelling with dorsal shift and compression of overlying spinal cord.*

***Osteochondrosis.** Articular cartilage at the junction of T4 and T5 was necrotic*



Epidural pseudocysts (PC) in cases of vertebral osteomyelitis, resulting in dorsoventral spinal cord compression

3. SKELETAL MUSCLES AND TENDON PATHOLOGY

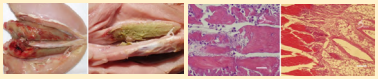
3.1. *Rupture of the gastrocnemius tendon (RGT).*
A significant problem associated with lameness and high culling rates in modern broiler breeder industry is the RGT.

The ruptured end of the gastrocnemius tendon (arrow). In old lesions, abnormal masses of various size could be detected subcutaneously. The ends of ruptured tendon are encircled by the newly grown fibrous tissue (arrows).

Haemorrhagies between the tendon and the tendon sheath. New connective tissue growth (b) embracing the end of ruptured tendon (a).

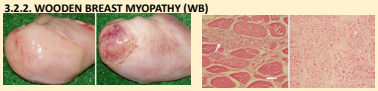
3.2. PECTORAL MYOPATHIES IN BROILER CHICKENS

3.2.1. Deep pectoral myopathy (DPM)



The affected muscles are intensive green and with multiple haemorrhages. Degenerative necrobiotic changes (Zenker's degeneration),. Muscle necrosis replaced by fibrous and adipose tissue.

***3.2.2.* WOODEN BREAST MYOPATHY (WB)**



WB is a myopathy primarily affecting the superficial breast muscle *pectoralis major* in high-breast-yield broiler chickens. Active phagocytosis of fragmented myofibril material. High-grade interstitial thickening of newly grown fibrous tissue.

3.3. CONTACT DERMATITIS IN BROILER CHICKENS

Moderate to strong swelling affecting the metatarsal region due to perifocal inflammatory oedema in plantar pododermatitis.

Microscopic lesions exhibiting defective keratinization in Str. intermedium, especially around the ulcer and heterothallic infiltration.

Conclusions:

I. Broiler chickens

A) The pathological conditions of the leg are of highest prevalence: rickets (mainly due to impaired Ca/P ratio); tibial dyschondroplasia; femoral head necrosis, osteomyelites and coxofemoral arthrites, associated with *E.coli*.
B) Next in prevalence come the conditions related to axial skeleton pathologies: spondylolisthesis, spondylosis, spodylitis, scoliosis.
C) Some forms of contact dermatiitis in broilers as plantar pododermatitis and contact dermatitis in the tarsometatarsal joint region are a serious cause for lameness in this category poultry.
D) A relatively rarely encountered pathology in broiler chickens, although of increasing prevalence lately, is deep pectoral myopathy.

II. Broiler breeders

A) In broiler breeders at productive age, the rupture of the gastrocnemius tendon is an important problem resulting in serious losses.

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World Veterinary Poultry Association
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Field comparative efficacy study of Newcastle disease vaccination program with r-HVT ND against inactivated ND vaccine in broiler with Real World Evidence (RWE) method in Indonesia

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Introduction

Newcastle disease (ND) is a highly contagious disease causing significant economic losses in global poultry industry. Current vaccination program using inactivated ND vaccine at one day old chicks in hatchery still encountered high mortality and economic losses in Indonesia. With the recent availability of new technology recombinant ND vaccine, this study aims to evaluate the efficacy of rHVT-ND vaccine from Ceva Animal Health through ND HI serology and flock performance in comparison with inactivated ND vaccine in Indonesia.

Materials and Methods

From August to December 2020, three million (3,000,000) broilers were vaccinated with inactivated ND and live ND IB (Massachusetts' strain) in the hatchery. Also in 2020 until 2022, 13,200,000 broilers were vaccinated with rHVT-ND vaccine and live ND IB (Massachusetts' strain) vaccine in the hatchery. Both groups of broilers received a live ND (La Sota) at 10-12 days of age in farm.

Monitoring criteria:

- production performances till the end of harvest (35 days),
- HI ND serology at day 35

Results were compared between flock using inactivated ND vaccine and flocks using rHVT-ND vaccine.

Results

Body weight, mortality, performance index and ND HI serology result were compared using Real World Evidence statistical method. The body weight, mortality, performance index and FCR of rHVT-ND vaccinated group was significantly different ($p<0.05$) (fig 1 & 2) compared to the inactivated ND group. The serology test using HI ND titer tests in rHVT-ND vaccine group showed significantly lower ($p<0.05$) (fig. 3) suggesting reducing of field ND exposure.

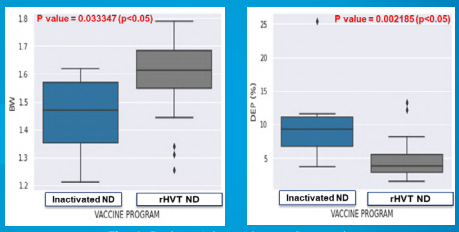

Fig. 1. Body weight and mortality results.

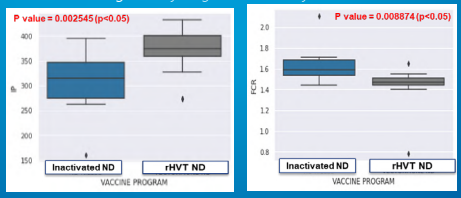

Fig. 2. Performance index and FCR results.

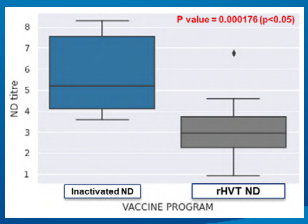

Fig. 3. Serology ND HI titer results.

Conclusion

The effectiveness of Ceva rHVT-ND vaccine in broiler was confirmed in this large scale field trial in Indonesia. The production performances reached the target, showed a much lower mortality rate and better zootechnical performance. The ability of rHVT-ND induce strong reduction of viral shedding explained the lower ND HI serology at 35 days of age with no ND clinical signs reported.

Therefore a better control of ND risk in Indonesia could be achieved with better performance.

References :
F. Rauw, Y. Gardin, V. Palya, T. van den Berg & B. Lambrecht, Avian Pathology, 2014, Vol. 43, No. 1, pp. 26–36 The combination of attenuated Newcastle disease (ND) vaccine with rHVT-ND vaccine at 1 day-old is more protective against ND virus challenge than when combined with inactivated ND vaccine.

Protection against a Brazilian, Variant 2-type (GI-23) infectious bronchitis virus with the combination of BR-I-type (GI-11) and Mass-type (GI-1) vaccines

T. Tatár-Kis[1], B. Felföldi[1], Z. Homonnay[1], E. Walkó-Kovács[1], J. Chacón[2], L. Sesti[3], V. Palya[1], T. Mató[1], C. Cazaban[4], I. Kiss[1]

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Introduction

Infectious bronchitis virus (IBV) is one of the most important poultry pathogens. Due the high variability among IBV strains and the simultaneous presence of different genotypes in the same geographical regions, the combined use of different heterologous vaccine strains - which can bring a broad cross-protection- became an important tool to control the disease. Most of the published cross-protection studies focused on the combination of Mass-type (GI-1) and 793B-type vaccines (GI-13). In 2021, Variant-2 type (GI-23) IBV strains appeared in the field in Brazil (Ikuta, 2023; Trevisol, 2023). Only Mass-type (GI-1) and Brazilian variant-I-type (GI-11) vaccines were available, therefore there was a need to test the ability of these vaccine strains to provide satisfactory protection against the recently introduced Variant-2 virus.

Materials and Methods

Efficacy of the combined use of a Mass-type, H120 vaccine strain (Cevac Bron 120 L vaccine) and BR-I vaccine strain (Cevac IBras vaccine) was tested in SPF chickens against challenge with a recent Var2-type isolate from Brazil. Protection was evaluated based on the severity of respiratory signs, ciliostasis, histological lesions in the trachea (tr.) and kidney (k.), and challenge virus load in the trachea and kidney.

Table 1. Study design

Group	Vaccination at day-old	Challenge at 22 days post-vacc.	No of birds	Post-challenge tests at 5 days post-chall.
V-Ch	**Mass-type and 793B-type vaccine in combination** - Eye drop - 3.5 $\log_{10}$ EID_{50}/dose each	**Var2-type Brazilian IBV isolate** (D6609/22 BR) - Oculo-nasal route - 4.0 $\log_{10}$ EID_{50}/dose	n=15	- **respiratory noise scoring**[A] - **ciliostasis test**[B] - **histopathology** (tr., k.)[C] - **challenge virus load** (tr., k.)[D]
NV-Ch	No		n=10	
NV-NCh		No	n=5	

A: Score from 0 to 3 (no detectable noise to severe respiratory signs)
B: Ten rings tested from each bird; each ring scored from 0 (no detectable ciliostasis) to 5 (full ciliostasis); scores 0-2 reflects at least 50% of cilia beating. Protected bird according to the European Pharmacopoeia: at least 90% of rings show at least 50% ciliary activity after the challenge.
C: H-E staining. All HP lesions were scored from 0 (no lesion) to 3 (severe lesion).
D: Selective one-step RT-real-time PCR for Var2-type viruses was used (Kylt® IBV-Variant 02 kit). Standard curve was prepared from dilution series of trachea homogenate pool from the NV-Ch group which was titrated in embryonated SPF hen's eggs.

Results

Mild-moderate rales were heard in the controls, and only mild or no rales in vast majority of vaccinates (Fig. 1A). There was almost complete ciliostatic effect of challenge in non-vaccinated controls, whereas ciliostasis was significantly prevented by vaccination (Fig. 1B). This corresponds to 100% protection in the vaccinated group and 0% protection in the controls according to the Eur. Ph. methodology.

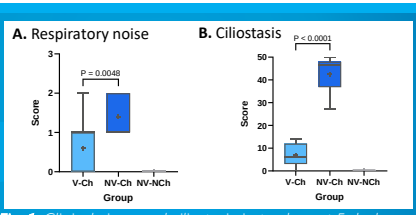

Fig. 1. Clinical signs and ciliostasis in trachea at 5 dpch.
Comparison of V-Ch and NV-Ch groups was done by Mann-Whitney test (p<0.05 indicates significant difference).

Severity of histological lesions was significantly reduced by vaccination both in the trachea and in the kidney (Fig. 2).

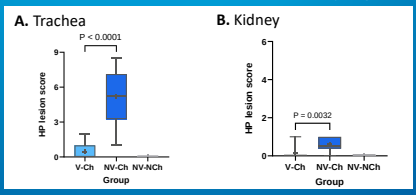

Fig. 2. Severity of histological lesions at 5 dpch.
Comparison of V-Ch and NV-Ch groups was done by Mann-Whitney test (p<0.05 indicates significant difference).
Maximum score was 9 for trachea (mean of two sections representing the upper and lower part) and 6 for kidney.

Challenge virus replication was significantly suppressed by vaccination in both organs (Fig. 3). The very low titers in vaccinated birds indicate, that RT-qPCR could detect and quantify so weak IB virus replication which would have not been detected by titration in embryonated eggs.

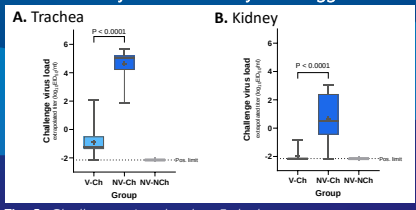

Fig. 3. Challenge virus load at 5 dpch.
Comparison of V-Ch and NV-Ch groups was done by Mann-Whitney test (p<0.05 indicates significant difference).
Abdominal surface of kidney was removed before sample collection for PCR to avoid possible contamination from air sacs.
There was a single bird with an extrapolated titer of trachea sample above 0.1 log_{10} EID_{50}/ml in the vaccinated group.

Conclusion

The BR-I vaccine strain in combination with the H120 vaccine strain provided significant protection against the Brazilian isolate of Var2-type heterologous IBV challenge as shown by the suppression of challenge virus replication and consequent reduced severity of lesions in the trachea and kidney.

References:
Ikuta, N.; Kipper, D.; Freitas, D.S.S.d.; Fonseca, A.S.K.; Lunge, V.R. Evolution and Epidemic Spread of the Avian Infectious Bronchitis Virus (IBV) GI-23 in Brazil. Viruses 2023, 15, 1229. https://doi.org/10.3390/v15061229
Trevisol, I.M.; Caron, L.; Mores, M.A.Z.; Voss-Rech, D.; da Silva Zani, G.; Back, A.; Marchesi, J.A.P.; Esteves, P.A. Pathogenicity of GI-23 Avian Infectious Bronchitis Virus Strain Isolated in Brazil. Viruses 2023, 15, 1200. https://doi.org/10.3390/v15051200

Efficacy of vector vaccines against Newcastle disease (Vectormune ND) and Infectious laryngotracheitis (Vectormune FP-LT+AE) in comparison with live vaccines in commercial layer pullets

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Introduction

Newcastle disease (ND) and Infectious laryngotracheitis (ILT) are two of the most important viral infections in poultry causing serious clinical disease. The emergence of new technology vector vaccines has set new standards in the prevention of ND and ILT.

The **aim of this study** was:

1. to assess antibody seroconversion conferred by the use of a recombinant herpesvirus of turkey (HVT+ND) and a Fowl Pox (FP-ILT+AE) vector vaccine in field conditions of layer production and
2. to evaluate the differences compared to the use of commercial live vaccines.

Materials and Methods

NDV testing

Sixteen (16) commercial layer pullet flocks vaccinated with Vectormune ND at day old (DO) vs 13 flocks vaccinated with a Hitchner B1 at DO and 3 La sota vaccines (Days 15, 50, 85)

Monitoring criteria:

- 12 serum samples collected from all flocks before transfer of pullets
- For 12 of the Vectormune ND flocks, a double sampling scheme was followed (1st at 6 weeks old and 2nd before transfer to the laying unit)

All samples were tested for NDV/NDVF serology (Biochek© ND/NDVF kit).

ILT testing

Twelve (12) commercial layer pullet flocks vaccinated with Vectormune FP-ILT+AE between 7-9 weeks vs 10 flocks vaccinated with a live attenuated chicken embryo originated (CEO) ILT vaccine between 7-9 weeks.

Monitoring criteria:

- 12 serum samples collected from all flocks 6 weeks after vaccination
- For 11 of the Vectormune flocks, a double sampling scheme was followed at 4 and at 6 weeks after vaccination

All samples were tested for ILT serology with both Biochek© ILT and IDVET© ILTgB kit.

All flocks were clinically inspected after vaccination to evaluate post vaccination reactions after ILT vaccination.

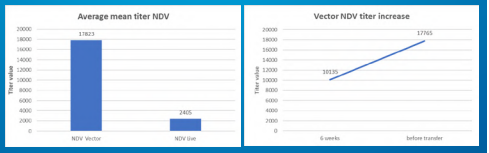
Results

NDV testing

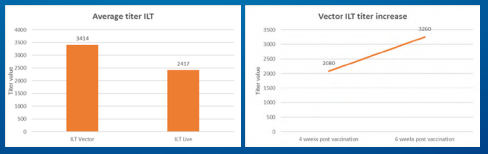
The mean antibody titer was higher for the fllocks vaccinated with Vectormune ND showing also a lower vaccination coefficient of variation (CV: 46 and higher antibody positivity: 94%) vs flocks vaccinated with live NDV vaccines (CV: 89, antibody positivity: 65%).

ILT testing

The mean antibody titer was higher for the fllocks vaccinated with Vectormune FP-ILT showing a higher vaccination CV (CV: 98 and antibody positivity: 89%)vs flocks vaccinated with a live CEO ILT vaccine (CV: 60, positivity: 86%)



Figures 1a & 1b - ND serological results



Figures 2a & 2b - ILT serological results

	Vector ILT	Live CEO ILT
Total number of vaccinated flocks	12	10
Number of flocks with post vaccine reactions	0	7

Table 1 - Monitoring of ILT post vaccine reactions

Conclusion

This study suggests that the use of recombinant rHVT-ND and rFP-ILT+AE vaccines activates a high level of antibody seroconversion in comparison to an alternative vaccination schedule, including live vaccines, while no post-vaccination reactions were recorded after the use of rFP-ILT+AE vaccine in comparison to a live CEO ILT vaccination.

Abstract ID:2300277

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Transmission capacity and dissemination of two avian reovirus strains belonging to the same genetic cluster and isolated from the same geographic region

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Introduction

There has been an increasing focus on avian reovirus (ARV) in the recent years due to its impact on the broiler industry. ARV is a ubiquitous virus having segmented genome consisting of 10 double stranded RNA molecules, which leads to high diversity. ARVs show a high genetic variability and large differences of pathogenic potential among the strains. This latter aspect is particularly challenging due to the lack of established methodology to evaluate the pathogenicity in a standard way which mimic the natural infection. The most used method for pathogenicity assessment is the foot pad test with direct inoculation of the ARV into the foot pad followed by evaluation based on macroscopic lesions in the legs. This methodology eliminates the potential impact of natural barriers that can have a significant impact on the ability of ARV to infect the birds and efficiently disseminate in their body.

Materials and Methods

The present study aimed at the comparison of the pathogenicity of two ARV strains including assessment of dissemination in the body and transmission in parallel to the macroscopic foot-pad evaluation. The two strains selected were from the same genetic cluster (cluster 1), isolated from joint samples from clinical cases in the same year (2021) from the same country (Table 1).

Table 1. Study design

	Strain 1	Strain 2
Origin of the strain	Breeder (grandparent)- 56 days of age / Joint sample	Pedigree- 19 days of age/ Joint sample
Cluster	1	1
Plaque purification	yes	yes
Test animals	Day-old SPF chickens (layer)	
Challenge dose	4.3 $\log_{10}TCID_{50}$/bird (4.0 both routes)	
Challenge method	Footpad route and per os (in parallel)	
Sampling date	5th and 7th day post-infection	

Direct-infected chickens (n=20) were co-mingled with the negative hatchmates (‚contacts', n=10) from two hours after infection. The chickens were reared in isolators for 7 days after infection. Ten infected and five contact chickens were sampled both at 5- and 7-days post-infection to determine the virus load in the different organs or swab samples and investigate the transmission capacity of these ARV strains, as measured with a published M1 gene-specific RT-qPCR.

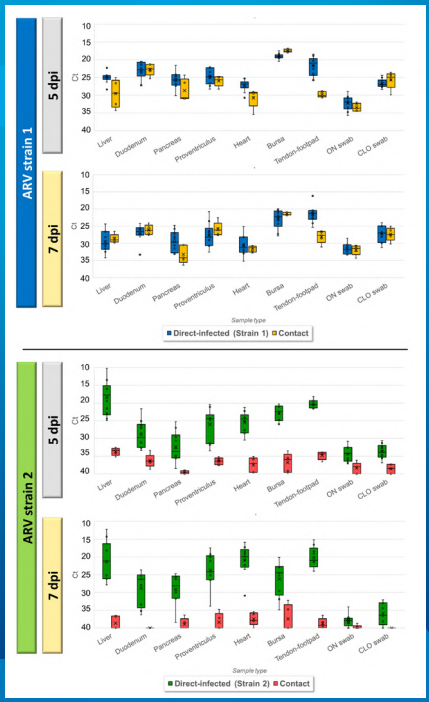

Fig. 1.: Virus load in the different organ and swab samples in the direct-infected and contact animals at 5 and 7 days post-infection

Results

Both ARV strains caused mild-moderate swelling of the inoculated leg at 5 and 7 days post-infection (dpi), no difference was observed according to the most used foot pad test evaluation between them. Contacts did not show any macroscopic change of the foot pad or shank during the seven days long observation period.

Spread of the virus and dissemination in the contact chickens was fast after infection with ARV strain 1. This indicated an efficient transmission capacity and ability to initiate a productive replication after natural infection, reaching the tendon-footpad already by 5 dpi .

In case of ARV strain 2, although all the contact birds became infected, they replicated the virus in much lower amount compared to the infected birds.

Conclusion

These results clearly demonstrate that the current molecular classification scheme(s) does not allow direct prediction of the pathogenicity of ARV strains. To find a correlation between pathogenicity and genetic characteristics, a broader scale molecular analysis of isolates would be certainly needed accompanied by a standardized pathogenicity test covering more aspects than the regular foot pad test (e.g. dissemination in the body, shedding).

Reference:

Yi Tang, Huaguang Lu (2016). Whole genome alignment based one-step real-time RT-PCR for universal detection of avian orthoreoviruses of chicken, pheasant and turkey origins. - Infection, Genetics and Evolution 39, 120-126.

Prevention of transmission of DMV1639 infectious bronchitis virus in broilers using a vGA08/Mass heterologous live-attenuated vaccine

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Introduction

Infectious bronchitis (IB) is a highly contagious gammacoronavirus that causes clinical outcome in chickens varying from suboptimal performances to acute clinical disease. DMV1639 variant (GI-17) is currently the most widespread circulating IBV in the United States causing respiratory signs, kidney damage or reproductive tract lesions. This study tested if heterologous vaccination with a live vGA08 (GI-27) and Mass (GI-1) would control DMV1639 transmission in broiler chickens challenged directly or indirectly.

Materials and Methods

At 27 dpv, 20 vaccinated and 20 non-vaccinated birds were challenged with a virulent DMV1639 intra-ocularly. Twenty-four hours later 10 challenged vaccinated birds were introduced into the 10 contact vaccinated non-challenged birds, exposing them to the DMV1639 challenge (Rep 2). As a control, 10 DMV1639 challenged nonvaccinated birds were mingled with 10 non-vaccinated contact birds (Rep 2). RT-qPCR of choanal-cleft swabs were taken from individual birds to ascertain viral shedding (DMV1639) at 1-14 dpc.

Results

Fig. 1. *Phylogenetic tree showing relatedness of S1 region of vaccine and challenge strains.*

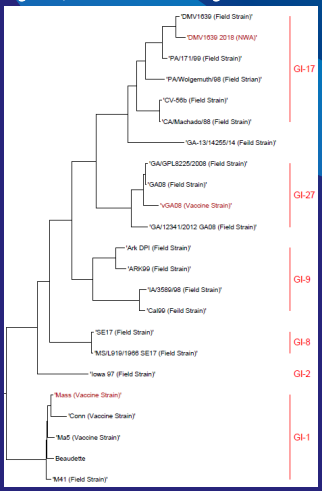


Fig. 2. *Titers of IBV at one doa and 27 dpv measured with Biocheck IBV ELISA kit.*

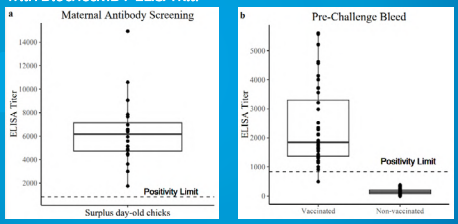
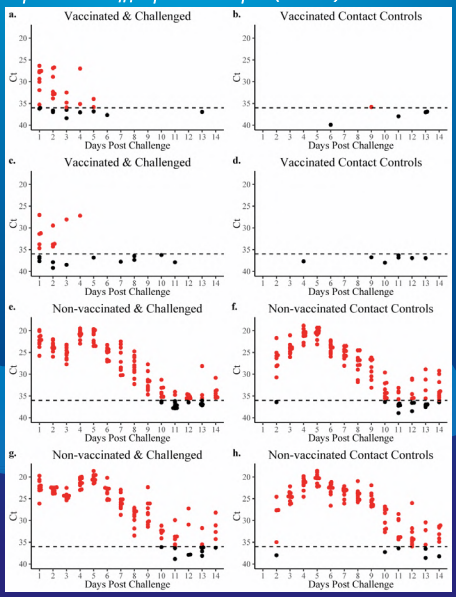


Fig. 3. *RT-qPCR detection of DMV1639. Dotted line represents cutofffor positive samples (≤ to 36).*



Conclusion

Vaccination with vGA08 and Mass not only shortened the time for clearance of IBV in challenged birds, but eliminated transmission of DMV1639 to vaccinated contact birds. This data confirms the reduction of DMV1639 RNA detection and DMV1639 transmission observed in field settings when heterologous vaccines are utilized.

XXII nd CONGRESS OF THE WVPA World Veterinary Poultry Association

Verona (Italy)
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Usage of Vectormune FP ILT+AE in Broiler Breeders in Germany: Insights from the Field about Health and Economical Parameters

Franziska Kloska[1], Jaime Sarabia Fragoso[2], Francesca Tonellato[3], Matthias Todte[4], Sandra Gunter[4]
[1] Ceva Tiergesundheit GmbH, [2] Ceva Animal Health, [3] Ceva Salute Animale, [4] Vet Practice MMT Germany

Introduction

In challenging times it becomes more and more important to consider sustainable poultry production both under health and economical aspects. Infectious Laryngotracheitis (ILT) of poultry leads to a severe inflammation of the trachea correlated with increased mortality and decreased production parameters, e.g. a decrease of egg production in layer-type and breeder birds. Layer-type and breeder birds are routinely vaccinated against Avian Encephalomyelitis (AE) to protect them against a decrease in egg production and hatchability and to protect their offspring via maternally derived antibodies from the classical manifestation of AE related to apathy, ataxia, tremor and increased mortality. Fowl Pox can either manifest on the skin or the mucosa of poultry with the expression of crustaceous vesides leading to pain, decreased production and increased mortality rates.
Vectormune FP ILT+AE is a pox vectored vaccine for poultry expressing a glyco- and nonstructural protein of ILTV (gB and UL-32) which is mixed with a live attenuated AE Calnek strain. It is applied via wingweb between eight to 13 weeks of age and protects poultry from Fowl Pox, ILT and AE.
In this report we describe the usage of Vectormune FP ILT+AE in broiler breeders, both under health and economical aspects.

Results

At 13 dpv 99% of birds showed the characteristic occurrence of the vaccine pox at the wingweb injection site. Seroconversion against ILT and AE was demonstrated at the age of 18 weeks. AE AMT titers are not significantly different between groups (Fig. 1). In the Vectormune vaccinated group birds are showing homogenous seroconversion in the ILT gB ELISA (Fig. 1) and nearly no seroconversion in the BioChek ILT ELISA (data not shown) as it does not specifically detect gB antibodies, but all antibodies directed against ILTV. Production parameters and mortality rates were better in the Vectormune FP ILT+AE group, especially at the beginning of production. There was no significant difference in both groups with regard to cumulative mortality at 55 weeks of age. Return of investment was 1:80 at 55 weeks of age in the Vectormune FP ILT+AE group compared to the standard group taking vaccine prices, egg and meat price as well as slaughter weight into account (Fig. 2).

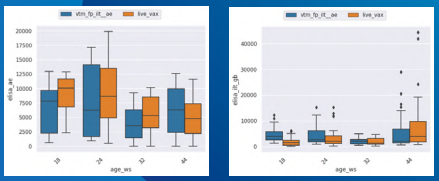

Fig. 1 AE and ILT gB titer boxplots.: Vectormune FP ILT+AE group and standard group tested at age of 18, 24, 32, 44 weeks of age

Vaccine price VTM vs. Std.	+15 €
Egg price	0.30 €
Eggs/hen housed VTM vs. Std.	+ 4
Mortality rate VTM vs. Std.	+ 0.2 %
Slaughter price per kg	0.60 €
Body weight at slaughter	4.50 kg
Surplus VTM vs. Std. due to egg revenue (per 1,000 birds)	+ 1,200 €
Surplus VTM vs. Std. due to meat revenue (per 1,000 birds)	- 5.40 €
Return of Investment	1: 80

Fig. 2 Economical ROI calculation at 55 weeks of age comparing Vectormune FP ILT+AE (VTM) and standard (Std.) vaccinated groups.

Materials and Methods

40,000 broiler breeders were vaccinated with Vectormune FP ILT+AE at 9 weeks of age via wingweb. Pox scoring was done at 13 dpv in 100 animals. A control group of 40,000 birds was vaccinated according to the standard protocol against FP (wingweb), ILT (double dose of an attenuated live vaccine) via drinking water and two times against AE (attenuated live vaccine via drinking water). Birds from both rearing farms were transferred to one production farm where they were still kept in separated groups. Seroconversion was tested in ELISA for ILT (IDVet gB) and AE (Idexx) at the age of 18, 24, 32 and 44 weeks or at 18 and 24 weeks of age only (ILT BioChek). Production parameters (mortality; egg production) were collected over the whole production cycle. Economic performance was calculated period assuming the following: 0.30 €/hatching egg, slaughter weight of 4.5 kg, 0.60 €/kg live weight. Vaccine prices were considered.

Pict. 1: vaccine pox 13 dpv

Conclusion

Vaccination with Vectormune FP ILT+AE in broiler breeders per wingweb leads to homogenous seroconversion against ILTV and AEV at beginning of and during the entire production period. AE AMT titers are not significantly different between groups, despite the fact that the control group has been vaccinated twice. Production parameters were better in the Vectormune FP ILT+AE group, especially at the beginning of production. This might be ascribable to better homogeneity in the Vectormune FP ILT+AE group thanks to reduced vaccination reactions as attenuated live ILT vaccines were omitted. Return of investment is 1:80 compared to standard group considering German economical parameters. The ELISA systems (BioChek and IdVet gB) offer an easy applicable DIVA-tool to differentiate between vaccinated and infected birds when Vectormune FP ILT+AE vaccinated.

References:
Lozano F., Alvarado L., Tatar T., Palya V., Lesceu S., Forero R. (2019): Use of an Infectious Laryngotracheitis Virus gB Protein Elisa kit to Assess the Serological Response to Different LT Vaccination Régimes. AAAP annual meeting, Washinton D.C.

Koutoulis K., Horvath-Papp I., Papaionnou N., Evangelou K., (2018): An outbreak of Avian Encephalomyelitis in broilers in Greece. J Hellenic Vet Med Soc. 66(2): 91-98

Matos, M., Bilic, I., Palmieri,N., Nitsch, P., Sommer, F., Tvarogová, J., Liebhart, D., & Hess, M. (2022): Epidemic of cutaneous fowlpox in a naïve population of chickens and turkeys in Austria: Detailed phylogenetic analysis indicates co-evolution of fowlpox virus with reticuloendotheliosis virus. Transbound Emerg Dis, 1–11.

Comparison of infectious bronchitis vaccine virus recovery: spray versus gel administration at day old in the hatchery.

P. Abbey[1], K. K. Koutoulis[2]
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Introduction

Infectious bronchitis virus (IBV) vaccines are licensed for administration via coarse spray from day old, with this widely being applied in the hatchery, often in addition to the administration of other products in a gel solution[1]. The gel is topically applied to chicks, that ingest it by preening one another. Hatchery staff have raised concerns about the volume of liquid being sprayed onto chicks, particularly smaller chicks from young parent flocks, and the possible detrimental effect this could have on lowering their body temperature. With some hatcheries already choosing to apply IBV vaccines via the use of gel, a field trial was designed to compare the two administration methods.

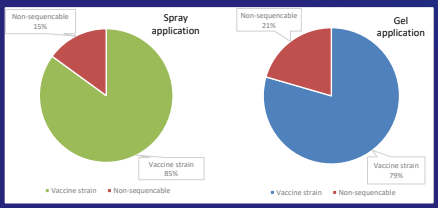
Materials and Methods

Eight broiler farms were placed over a period of four weeks. Each week one farm was placed with chicks receiving IBV vaccines, Cevac IBird® and Cevac Mass L®, administered by spray and one farm with chicks receiving IBV vaccines administered in gel, at day old n the hatchery. These farms were visited when the birds were five days old to collect oropharyngeal swabs from 10 randomly selected chicks for real-time polymerase chain reaction (RT-PCR) testing and subsequent genome sequencing. Serology samples were also collected from 10 randomly selected chickens at 34-35 days of age for IBV antibody titres testing using enzyme-linked immunosorbent assay (ELISA) BioChek kits.

Results

All the samples tested at 5 days old were positive for IBV and the subsequent sequencing of vaccine strain (1/96 or Massachusetts B-48) was achieved in 85% and 79% of samples respectively. The remaining 15% and 21% of positive samples were not sequencable.

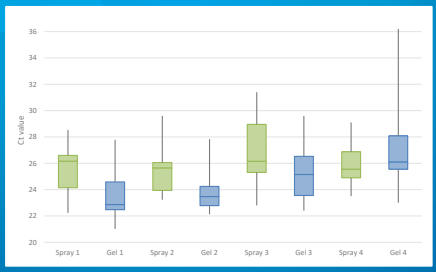
Fig 1: *Vaccine virus recovery at 5 days old*



[1]Tucciarone C.M., Franzo G., Bianco A., Berto G., Ramon G., Paulet P., Koutoulis K.C., Cecchinato M. (2018) Infectious bronchitis virus gel vaccination: evaluation of Mass-like (B-48) and 793/B-like (1/96) vaccine kinetics after combined administration at 1 day of age. Poultry Science 97:3501–3509.

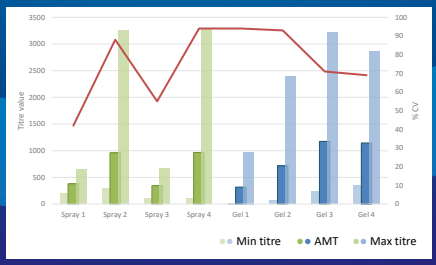
The RT-PCR cycle threshold (Ct) values obtained from all IBV positive samples (average 25.94 and 24.97 for spray and gel administration respectively) were not significantly different when analysed by ANOVA.

Fig 2: *Box and whisker plot of IBV vaccine virus recovery at 5 days old*



Serological analysis of the blood samples collected at 34-35 days of age showed that for spray administration the average mean IBV antibody titre (AMT) across all farms was 663 (min 104, max 3297, CV% 70) and for gel administration, the AMT was 839 (min 21, max 3214, CV% 82).

Fig 3: *Serology results at 34-35 days old (min, AMT, max)*



Finally, the IBV seroconversion observed following the the two administration methods, although slightly higher for gel administration, was not statistically different as demonstrated by ANOVA analysis.

Conclusion

Following both spray and gel application of IBV vaccines in the hatchery, vaccine strain was equally recovered at 5 days of age and suitable to stimulate a strong and protective immune response.

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Efficacy of a new generation immune-complex IBD vaccine in Broiler Breeders

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Introduction

Infectious Bursal Disease (IBD) remains as one of the major threats for poultry producers worldwide and its outcomes can vary from high mortality to performance decrease due to immunosuppression. A combination of strict biosecurity procedures and vaccination may prevent the disease and minimize its consequences. In recent years, different technologies have been used to maintain the disease under control, including the conventional attenuated live, killed vaccines, vector vaccines and immune-complex vaccines. Immune-complex technology has proven its efficacy through years in broilers and broiler breeders[1]. A next generation of this type of vaccines has been developed lately bringing up new elements in the fight against the disease. The new immune-complex vaccine (Nextmune®) is a frozen IBD immune complex vaccine consisting of the Winterfield 2512 strain linked to specific antibodies called Virus Protecting immunoglobulins and can be administrated by In-Ovo or subcutaneous route, in day old chicks in the hatchery. The aim of this study was to evaluate the benefits of using the aforementioned immune-complex vaccine in broiler breeders under field conditions as measured by antibody response and molecular detection of the vaccine strain in the Bursa of Fabricius (BF).

Material and Methods

A novel immune-complex IBD vaccine (Nextmune®, Ceva Animal Health) was applied in the hatchery by subcutaneous injection to 52000 Ross 308 broiler breeders.

Blood samples (n=20) and Bursa of Fabricius (n=12) were analysed for serology (BioChek and IDEXX) and RT-PCR respectively according to the following scheme:

Days of Sampling											
ELISA	1	7	15	21	29	35	42	71	189	287	371
RT-PCR	x	x	15	21	29	35	42	x	x	x	x

No live IBD vaccines were applied in the field, apart from an inactivated IBD vaccine at the end of rearing (17w) by intramuscular injection.

Results

The pattern of antibody response was very similar when assessed by different ELISA kits. Both IBD ELISA results showed a marked serological conversion from 35 days of age sampling onwards (Fig. 1) with BioChek showing higher titers when compared to IDEXX. As anticipated, after the inactivated vaccine application, a further increase in titers was noted.

The molecular tests showed a consistent bursa colonization by the vaccine strain (W2512) from 29 days of age sampling onwards (Fig.2). In addition, from 35 days old sampling, 100% of sampled birds showed vaccine colonization in the bursa, confirming an optimun vaccine intake.

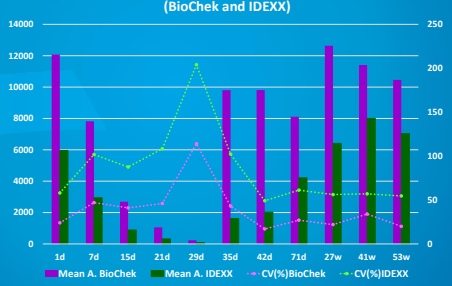

Fig. 1. Serological results at different ages

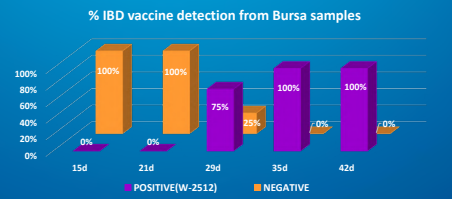

Fig. 2. RT-PCR results at different ages

Conclusion

The efficacy of this new generation frozen immune-complex IBD vaccine in broiler breeders was confirmed. The mechanism of action of the vaccine allows colonization of bursa concurrently with the individual decrease of maternal immunity, as evidenced by the detection of colonized bursas (75% of sampled birds) from 29 days of sampling. Due to sampling schedule, samples were not taken between 21 and 29 days old, so it is safe to assume that an even earlier detection of the vaccine strain, coinciding with the individual reduction of maternal derived antibodies, it may have occurred.

The strong BF colonization is the first step to provide protection and trigger a serological response that it can be detected a few days later (from 35 days old sampling onwards). It should be noted that even before the serological response, by the colonization of BF, the vaccine provides the necessary protection. In addition, a successful hatchery vaccination provides the optimum vaccine intake, conferring protection to the flock (100%) as the current study indicates. The use of an immune-complex vaccine provides optimal and effective vaccination IBD of meat type breeders, without the need for subsequent live on-farm vaccinations.

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XXII nd CONGRESS OF THE WVPA
World Veterinary Poultry Association
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Safety of a H5N1 RNA vaccine in ducks in field conditions

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Introduction

Highly pathogenic avian influenza (HPAI) of the Gs/Gd/96 lineage is a widespread devastating disease to the poultry industry, backyard flocks, as well as wild birds. For the last five to six years this virus showed an increasing ability to expand and persist. In 2022, outbreaks reached an unprecedented amount in Europe, including in France. French authorities have decided to assess which benefits vaccines could provide in addition to biosecurity, monitoring and stamping out, thanks to the new European Regulation (2023/361). Novel RNA-based vaccines offer promising avenues in disease prevention (Covid-19), including in animal health (Meurens F. 2020; Hundt S. *et al*., 2022; Xu S. *et al*., 2023). Safety was assessed in commercial ducks in field conditions.

Materials and Methods

Three duck farms were selected in different municipalities in Southwest France; a repetition was done in the 1st site; hence 4 field experiments were conducted throughout 2022. A total of 6,200 one-day old male mule ducks (hybrid of male Muscovy x female Pekin duck) were delivered on the same day of hatch. Ducklings were free from H5 maternal immunity. Table 1 summarizes the rearing sites, number of ducks (V=vaccinated, C=controls, always kept in separate, but similar houses), and stocking density. Ducklings were purchased from the genetic company A (sites 1 & 3), and B (site 2).

A self-amplifying H5 RNA (2.3.4.4b clade) vaccine (Respons® AI H5, Ceva Animal Health, Libourne, France) was used as follows: a priming at one day of age, and a booster at 4 weeks of age, by intramuscular injection of 0.2ml/bird in the thigh. Unvaccinated controls did not receive anything. Ducks were raised according to the routine protocols in place in the farms. Mortality, feed conversion, and growth were monitored: 40 ducks (exception: 80 in site 3) were randomly weighed at weekly intervals in each group until 10 weeks of age (7 weeks in the last site). Statistical analysis was done using the Student's t test (body weight) at $p<0.05$ level of significance.

Table 1: summary of sites, quantity of ducks per group (Vaccinated [V] / Controls [C]), type of farm, and birds' density.

Sites #	Number of ducks	Type of farm	Density (/m²)
1	800 V / 400 C	Experimental farm, with covered outdoor area	5
2	1800 V / 2000 C	Commercial farm with open outdoor area	6.6-7
3	700 V / 300 C	Experimental farm, fully indoor	4
1 (repetition)	100 V / 100 C	Experimental farm, with covered outdoor area	5

Results

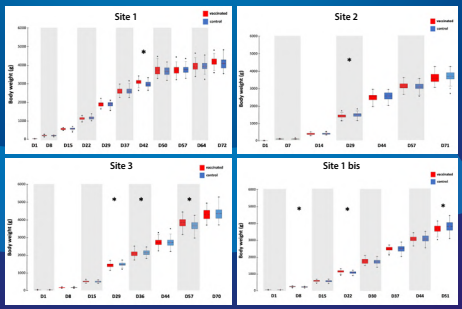
No lameness was observed after the injections. Mortality remained lower than 2% for the 10 weeks of rearing in all sites; this complies with the genetic line's requirements. The cumulative feed conversion ratio (FCR) was very similar between the two groups in all experiments. Table 2 summarizes the mortality rates and the cumulative FCR.

Table 2: Mortality rates (%) and cumulative FCR in the two groups.

Site (final age in days)	Mortality rate (%)		cumulative FCR	
	Vaccinates	Controls	Vaccinates	Controls
1 (72 d)	1.1%	2.0%	3.6	3.6
2 (71 d)	2.1%*	0.6%	2.9	3.2
3 (70 d)	0.8%	1.5%	3.1	2.9
1 (repetition, 51 d)	0%	0%	2.7	2.5

** Extra mortality in this group was due to heat stress (Summer 2022).*

Body weights were similar between vaccinates and controls, in all experimental sites. Statistical significance between groups was infrequently observed (see*) and without any trend. Growth curves in the four experiments are displayed in the following four figures.



Conclusion

These four field experiments in various rearing conditions, in several areas of France, in various duck genetic lines demonstrated the satisfactory safety of Respons® AI H5 vaccine in mule ducks, according to the monitoring criteria which included mortality rate, FCR and body weight.

Ref: Convention Cadre CC-2022-002 (April 15, 2022). The authors would like to acknowledge the other stakeholders: DGAL (French Directorate for Food), Anses (French Agency for Food, Environmental and Occupational Health & Safety), and ENVT (Veterinary University of Toulouse). The authors are grateful to the field veterinarians, as well as to F. Lavigne, L. Perrin, and B. Prépoint-Figuière for their technical help. Respons® AI H5 vaccine is licensed in France (ATU no.90053).

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Hundt S., Szczodrok S, Deutskens F, Vecsei AL, Gyuro Z, Kamrud K, de Foucauld J, Penzes Z. New tools to fight avian influenza. 10th Riems' Diagnostic Days, Greifswald, Germany, November 24-25.
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XXII nd CONGRESS OF THE WVPA
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ASSESSMENT OF THE ANTIBODY RESPONSE ELICITED BY VECTORMUNE® ND BEFORE AND AFTER NDV CHALLENGE IN COMMERCIAL BROILERS IN VIETNAM

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Introduction

Vectormune ND® is a vector rHVT-NDV vaccine that expresses the F gene from the D26 strain of Newcastle disease virus (NDV) and elicits both humoral and cellular immune responses. Since the F protein is the protein expressed in the rHVT, the Hemagglutination Inhibition (HI) test has certain limitations to show adequate antibody response to vaccination. In a challenge study, we compared the individual antibody response to ID Screen® Newcastle Disease Indirect ELISA (*IDVet, France*) and to the Hemagglutination Inhibition (HI) test, assessing seroconversion before and after the challenge.

Materials and Methods

Fifty (50) day-old chicks with maternally derived antibodies against NDV were divided into 2 groups: 30 birds vaccinated subcutaneously with rHVT-ND at 1 day of age and 20 birds unvaccinated as control group. Both groups were challenged at 33 days of age, intranasally with a $5\log_{10}$ dose of a local Vietnamese NDV Genotype VIII. The birds were monitored for 9 days post-challenge (dpch) and blood samples were collected before (32 days) and after (42 days) to compare the individual antibody response.

Results

At 9 days post-challenge (dpch), all birds from the control group had succumbed already at 4-5 dpch with typical lesions attributable to ND, while 100% of the birds from the vaccinated group survived the challenge. From those, 97% (29/30 birds) showed positive ELISA titers. Even birds with negative titers survived the challenge (*Figure 1*).

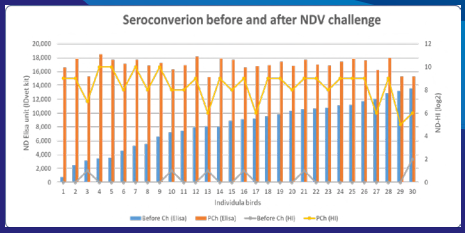

Figure 1: Individual ND titers before and after NDV challenge in broilers vaccinated with Vectormune® ND by both ND-HI and ND ELISA tests.

The ND ELISA mean titers were 8,283 (773 – 13,538) units and 17,015 (15,198 – 18,472) units before and after the challenge, respectively (*Figure 2*). Meanwhile, the HI mean titers remained negative at 32 days of age (0.23 $\log_2$) and 8.3 $\log_2$ at 42 days (*Figure 3*).

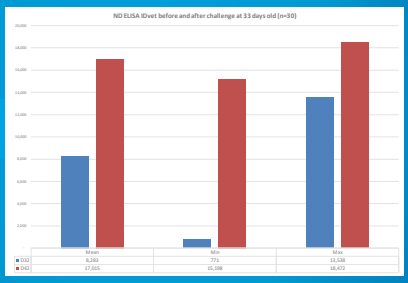

Figure 2: ND ELISA titers before and after NDV challenge in broilers vaccinated wih Vectormune® ND.

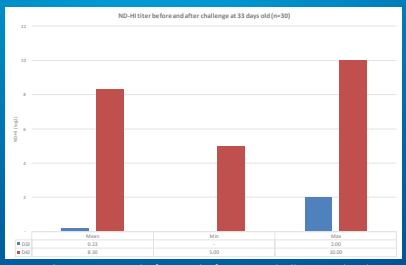

Figure 3: ND-HI titers before and after NDV challenge in broilers vaccinated with Vectormune® ND.

The results demonstrate that Vectormune® ND induced 100% of clinical protection against virulent NDV challenge at 33 days of age. Interestingly, the HI test did not detect the antibody response before the challenge, while the indirect ELISA did.

Finally, the variation between the individual antibody response before and after challenge shows that birds with higher ELISA titers at 32 days had a much lower delta at 42 days, indicating a stronger protection against challenge virus replication. In some birds, there was no noticeable change in the ELISA antibody response before and after challenge. This might suggest a stronger cellular immunity which enabled the bird to counteract the challenge.

Conclusion

These results confirm that a single dose of Vectormune® ND can induce sufficient protective titers against a NDV challenge. The ND ELISA titers (IDVet Indirect) over 15,000 units, could be derived from the challenge.

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1. Scientific information Vectormune HVT-ND book 2. The American Association of Avian Pathologists, Avian Disease Manual Thirteenth Edition, Newcastle disease.

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Verona (Italy)
September 4-8, 2023

Evaluation of the efficacy of a single administration of a bivalent parvovirosis vaccine (Cevac Duoparvo) in day-old ducklings, by parvovirus challenge at seven, 21 or 35 days after vaccination

B. Huyghe[1], E. Moreau[2], L. Joudou[1], R. Mellet[1], F. Beilvert[1]
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[2] Ceva Santé Animale, 10 avenue de la Ballastière, 33501 Libourne Cedex, France

Introduction

Parvovirosis is a highly contagious and deadly disease in Muscovy ducklings up to 5 weeks of age. Parvoviruses are very resistant, consequently most barns are still positive to parvoviruses before setting up day old ducklings. Cevac Duoparvo is introducing a new vaccine scheme for parvovirosis with only one administration, as soon as 1 day of age. Poor cross-protection was reported between Muscovy duck parvovirosis (MDPV) and Derzsy's disease (goose parvovirosis-GPV) (1). As a bivalent vaccine, Cevac Duoparvo protects Muscovy ducklings against both diseases. The aim of the study was to demonstrate the efficacy of Cevac Duoparvo vaccine against a MDPV challenge 7, 21 or 35 days after vaccination.

Material and methods

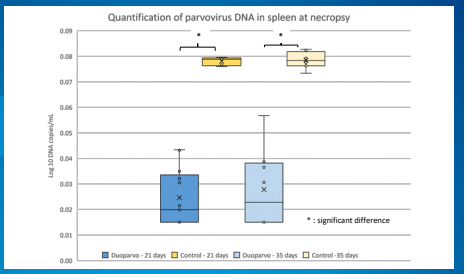
Forty-nine seronegative day-old Muscovy ducklings hatched from three consecutive hatching batches (each fourteen days apart) were included in the study. Within each hatching batch, the ducklings were randomly distributed in two experimental groups as follows:

Groups	Challenge day after vaccination		
	7 days	21 days	35 days
Control (phosphate buffer)	n=4	n=8	n=9
Cevac Duoparvo	n=4	n=16	n=8

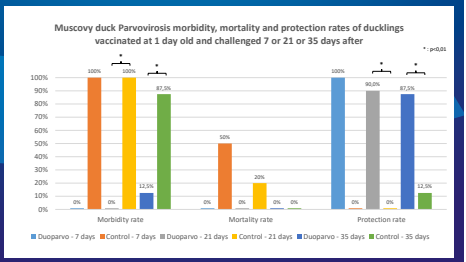
Vaccinations were performed subcutaneously at the base of the neck as per standard hatchery procedures. All animals were challenged with a virulent heterologous MDPV strain. The animals were observed and scored for clinical signs of parvovirosis for 3 to 4 weeks following challenge and then humanely euthanized for necropsy. Body weights were recorded weekly and compared to a reference growth curve (per sex) to evaluate the occurrence of parvovirosis-associated stunting. At necropsy, parvovirosis lesions were scored and individual spleen sample were collected for quantification of parvovirosis virus DNA by qPCR. Animals without clinical or necropsy signs of parvovirosis as well as without stunting were considered as protected from the MDPV challenge. Animals with clinical or necropsy signs of parvovirosis or stunting were considered as affected by the MDPV challenge.

Results

Based on the parvovirosis signs observed in the control group, the challenge with the MDPV strain was considered suitable for the evaluation of the efficacy the vaccine. The quantification of MDPV DNA in the spleen from the Cevac Duoparvo groups were significantly lower than those quantified for the control groups with p-values <0.001 for the groups challenged at 21 and 35 days (no statistical analysis was performed for the groups challenged at seven days considering the low number of animals). Therefore vaccination with Cevac Duoparvo did prevent the replication of the challenge strain in this target organ 21 and 35 days after vaccination.



At the end of the observation period, morbidity, mortality and protection rates were determined. In the Cevac Duoparvo group challenged at seven days, morbidity, mortality and protection rates were 0%, 0% and 100%, respectively, while in the control group these rates were 100%, 50% and 0% respectively. These results were in line with statistically significant different results observed four days after vaccination in another study. Morbidity rates in the control groups were 100% (group challenged at 21 days) and 87.5% (group challenged at 35 days), while these rates for the Cevac Duoparvo groups were 0% (group challenged at 21 days) and 12.5% (group challenged at 35 days). High protection rates were achieved in the Cevac Duoparvo groups (90% for the group challenged at 21 days and 87.5% for the group challenged at 35 days). The difference in protection rates with the control groups were all statistically significant, with p values <0.01 for both the 21 and the 35 days challenged groups.



Conclusion

Overall, Cevac Duoparvo induced a high protection rate, whatever the age of the ducklings at challenge, for at least up to five weeks after vaccination of day-old ducklings.

References

(1) : Palya, V.; Zolnai, A.; Felföldi, B. Immunogenic Cross-Reactivity between Goose and Muscovy Duck Parvoviruses: Evaluation of Cross-Protection Provided by Mono- or Bivalent Vaccine. Vaccines 2022, 10, 12552022, 10, 1255.

XXII nd CONGRESS OF THE WVPA
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Evaluation of the efficacy of a single administration of a bivalent parvovirosis vaccine (Cevac Duoparvo) in day-old ducklings, by parvovirus challenge four days after vaccination

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[2] Ceva Santé Animale, 10 avenue de la Ballastière, 33501 Libourne Cedex, France

Introduction

Parvovirosis is a highly contagious and deadly disease in Muscovy ducklings up to 5 weeks of age. Parvoviruses are very resistant, consequently most barns are still positive to parvoviruses before setting up day old ducklings. Cevac Duoparvo is introducing a new vaccine scheme against parvovirosis with only one administration, as soon as 1 day of age. Poor cross-protection was reported between Muscovy duck parvovirosis (MDPV) and Derzsy's disease (goose parvovirosis-GPV) (1). As a bivalent vaccine, Cevac Duoparvo protects Muscovy ducklings against both diseases. The aim of the study was to demonstrate the efficacy of Cevac Duoparvo vaccine against a MDPV challenge 4 days after vaccination.

Material and methods

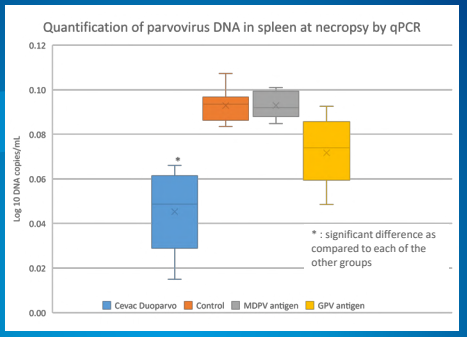
Fifty seronegative zero to three day-old Muscovy ducklings were randomly distributed into four different experimental groups as follows:

Group	Vaccine	Ducklings
Control	Phosphate buffer	n=12
Control	Cevac Duoparvo	n=13
MDPV antigen	MDPV antigen (inactivated) and adjuvant of Duoparvo	n=13
GPV antigen	GPV antigen (live) of Duoparvo	n=12

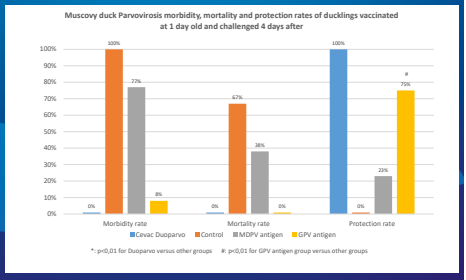
Vaccinations were performed subcutaneously at the base of the neck as per standard hatchery procedures. Four days after vaccination, all animals were challenged with a virulent MDPV strain (heterologous to the vaccine strain). The animals were observed and scored for clinical signs of parvovirosis for 3 weeks following challenge and then humanely euthanized for necropsy. Body weights were recorded weekly and compared to a reference growth curve (per sex) to evaluate the occurrence of parvovirosis-associated stunting. At necropsy, parvovirosis lesions were scored and individual spleen samples were collected for quantification of parvovirosis virus DNA by qPCR. Animals without clinical or necropsy signs of parvovirosis as well as without stunting were considered as protected from the MDPV challenge. Animals with clinical or necropsy signs of parvovirosis or stunting were considered as affected by the MDPV challenge.

Results

Based on the parvovirosis signs observed in the control group, the challenge with the MDPV strain was considered suitable for the evaluation of the efficacy the vaccine. The quantification of MDPV DNA in the spleen from the Cevac Duoparvo and GPV component groups were significantly lower than those quantified for the control or MDPV component groups ($p<0.001$). In particular, the quantification of MDPV DNA was 4.4log10 lower in the bivalent Cevac Duoparvo group as compared to the control group. Therefore, vaccination with Cevac Duoparvo four days before MDPV challenge did prevent the replication of the challenge strain in this target organ.



At the end of the three-week observation period, the morbidity, mortality and protection rates were 100%, 67% and 0% in the control group, respectively. In the group vaccinated with Cevac Duoparvo, the morbidity, mortality and protection rates were 0%, 0% and 100%, respectively. The difference in protection rates was statistically significant ($p<0.01$). Vaccination with each individual valence of Cevac Duoparvo, i.e., GPV and MDPV antigens alone, induced an intermediate protection as compared to the administration of the bivalent vaccine Cevac Duoparvo.



Conclusion

The results of this study demonstrate the efficacy of vaccination with Cevac Duoparvo shortly after hatching, against a MDPV challenge performed only four days after vaccination.

References

(1) : Palya, V.; Zolnai, A.; Felföldi, B. Immunogenic Cross-Reactivity between Goose and Muscovy Duck Parvoviruses: Evaluation of Cross-Protection Provided by Mono- or Bivalent Vaccine. Vaccines 2022, 10, 1255.

XXII nd CONGRESS OF THE WVPA
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Efficacy of a new-generation immune-complex vaccine (Nextmune®) in broilers: first field results in Italy

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[1] Ceva Animal Health, Italy / [2] Ceva Animal Health, Libourne, France

Introduction

Infectious bursal disease (IBD), also known as Gumboro disease, is an immunosuppressive condition in chickens caused by a double-stranded RNA, non-enveloped and highly resistant virus of the genus Avibirnavirus, family Birnaviridae (IBDV).

As an RNA virus, IBDV has a high mutation rate leading to the emergence of new viruses with a modified antigenicity and pathogenicity. In the last couple of years, several publications have reported the discovery of a new genotype and the circulation of new IBDV reassortant strains in Europe, where they currently represent the main field threat. These IBDV reassortant strains are linked more with sub-clinical forms and subsequent economical losses rather than to high mortality outbreaks, leading to an underestimation of their circulation and importance.

In Italy, the A3B1 Italian-Russian & Middle Eastern clade, separated from the main A3B1 circulating in Western Europe, has been recently identified in layers and broilers flocks in the North-East regions. In order to protect the birds against IBD, the strict application of biosecurity measures in synergy with a proper vaccination and the use of an efficacious vaccine are crucial.

In this evolving context, a new generation of immune-complex vaccine has been developed for broilers (Nextmune®) aiming to induce an even earlier than before onset of immunity against IBD by blocking the colonization of Bursa of Fabricius (BF) from early wild field IBDV strains.

The scope of this study was to observe the kinetics of Nextmune® in broilers under field conditions as depicted by the molecular detection of the vaccine strain (W2512) in BF and the antibody response after vaccination.

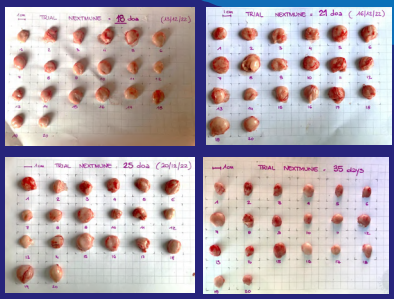

Fig. 1-2-3-4. Bursa sampling at 18-21-25-35 days of age

Materials and Methods

Therefore, a broiler farm of 40.000 birds was selected in the area of Emilia-Romagna and was vaccinated with Nextmune@ subcutaneously at day old in the hatchery. Quality of the vaccination in the hatchery was closely audited to guarantee the adequate application of the vaccine.

20 blood samples were collected at 14, 18, 21, 25, 28 and 35 days of age and sera were analyzed for antibody response to IBD vaccination using two commercial Elisa kits (IDEXX, BioCheck).

Also, 20 BF were collected at the same sampling points (Fig. 1, 2, 3, 4) for IBD virus detection and identification by RT-PCR.

Results

Molecular results showed that BF colonization by the vaccine strain started as early as at 21 days of age with 45% (9/20) positivity rate, reaching 100% (20/20) positivity at 25 days of age and remaining at this level till the end of sampling points (Fig.5).

Serological results were differed according to the commercial kit used in terms of positivity rates and mean titers with BioChek showing faster seroconversion and higher titers when compared to IDEXX.

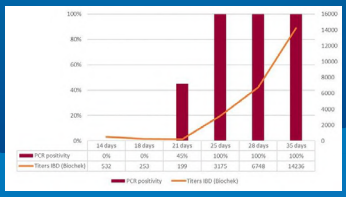

Fig.5: PCR and serology results according to sampling time

Conclusion

Due to a new balanced formula, Nextmune® colonizes earlier the bursa of Fabricius, thus supporting a faster onset of immunity against IBD in vaccinated birds.

The replication of the vaccine strain in the bursa, through a mechanism of competitive exclusion, prevents the shedding of wild field IBDV strains, reduces their pressure in the farm environment and minimizes the subsequent risk of challenge in the flock.

Aknowledgements

We acknowledge Dr Muccioli and Martini group to provide us access to field sampling.

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Efficacy of a vector r-FP ILT+ AE vaccine in Italy

F.R. Tonellato [1], M. Magrini [1], A. Cioffi [1], P. Paulet [2], J. Sarabia [2], K. Koutoulis [2]

[1] Ceva Animal Health, Italy / [2] Ceva Animal Health, Libourne, France

Introduction

Infectious laryngotracheitis (ILT) and avian encephalomyelitis (AE) are viral diseases affecting chickens worldwide. Biosecurity measures and vaccination are essential tools to control these diseases and different vaccination protocols can be adopted.

A Fowlpoxvirus vector vaccine expressing the glycoprotein B (gB) of ILT in association with the AE Calnek 1143 strain (Vectormune® FP ILT + AE) was launched in 2021 in Italy for the immunization of layers and breeders against ILT and AE.

The aim of this study was to monitor the serological response for ILT and AE in layers and breeders' flocks vaccinated with Vectormune® FP ILT + AE in Italy during 2021-2022.

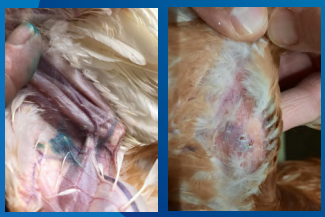

Fig. 1-2. Granuloma reaction 7 day PV with Vectormune ® FP ILT + AE

Materials and Methods

Blood samples were collected at 3,4,5 and 6 weeks post-vaccination (PV) with Vectormune® FP ILT + AE from 4 breeders and 6 layers farms. Birds were also sampled at 20 weeks PV in one broiler breeders farm and 37 weeks PV in one layers farm.

A total number of 674 blood samples (344 layers and 330 breeders) were tested by ELISA. Immunization for ILT was evaluated through a commercial ELISA kit especially developed for the gB insert of the FP vector vaccine (IDVet gB Indirect), while AE immunity was evaluated with two commercial ELISA kits (IDEXX and Biocheck).

Additional blood sampling was performed on the vaccination day with VTM FP ILT+ AE (T0) and granuloma reactions were also observed in all the farms 7 days PV to assess the quality of the vaccination (Fig. 1-2).

Results

Regarding ILT serology, 3 weeks post-vaccination the percentage of positive samples for ILT-gB was 95% and 76% for layers and breeders respectively, with an increasing trend reaching 6 weeks PV a positive rate of 96% and 92% respectively. No statistical difference was found in ILT-gB titers distribution between the two groups.

Positivity rate for ILT-gB was equal to 87% in layers 37 weeks PV, while 20 weeks PV 94% of the tested breeders sera were positive in ELISA (Table 1-2).

ELISA ILT-gB trends and titers distribution according to birds commercial type are respectively described in Figure 3 and 4.

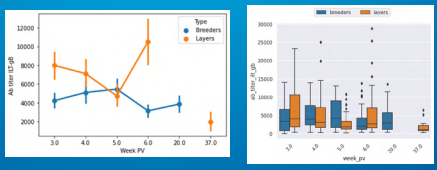

Fig. 3-4. ELISA ILT-gB trends and titers distribution in breeders and layers

Analysing AE serological results, 3 weeks post-vaccination the positive rate in ELISA was equal to 71% in layers and 30% in breeders, with an increasing trend reaching 6 weeks PV 100% of positive sera for layers and 82% for breeders. Positivity rate for AE was equal to 100% in layers 37 weeks PV, while 20 weeks PV 90% of the tested breeders sera were positive for AE in ELISA. (Table 1-2). Higher mean titers and a significant higher number of positive samples were assessed with Biocheck.

Breeders _wk PV	ILT_gB (%)	AE (%)
3 ws PV	76%	30%
4 ws PV	97%	81%
5 ws PV	94%	75%
6 ws PV	92%	82%
20 ws PV	90%	90%

Layers _wk PV	ILT_gB (%)	AE (%)
3 ws PV	95%	71%
4 ws PV	90%	90%
5 ws PV	93%	85%
6 ws PV	96%	100%
37 ws PV	87%	100%

Table 1-2. Positivity rates for ILT-gB and AE according to birds commercial type

Conclusion

Birds properly vaccinated with Vectormune FP ILT + AE rapidly developed humoral immunity against ILT and AE.

Acknowledgements : We acknowledge all the vets and their Companies to provide us access to field sampling

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Field evaluation of two ibd immune complex vaccines in male layer in Thailand

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Introduction

Infectious Bursal Diseases (IBD) is one of the most important poultry diseases. The Layer-type and the slow-growth genetic lines (e.g. Label Rouge) are usually more susceptible to the IBD virus than meat-type birds. As in Broilers, the Maternally Derived Antibody (MDA) is the first line of defence of layer pullets during the first few weeks. However, the decay of MDA in this type of bird is slow and uneven, making designing a field vaccination program very challenging.

Therefore, selecting the strain to be used as a vaccine and the delivery system must be carefully evaluated for this type of bird to fulfil the most important criteria: Safety, Efficacy and adjustment to the MDA decay. The advantages of IBDV Immune-complex (Icx) vaccines over other Gumboro vaccine types in achieving the above criteria are well known.

The objective of this study was to assess the performance of two different immune-complex (Icx) vaccines in male - layers raised as broilers.

Materials and Methods

In this study, 4 male layer flocks (Lohmann Brown) were vaccinated with two different IBD immune-complex vaccines. Flocks were reared under the same management conditions.

Group 1 (2 flocks) received an Icx containing the strain SYZA-26 and group 2 (2 flocks) received a Icx constructed with V877 strain.

Each flock had 20,000 birds and were commercially fattened to be sold as slow-growing free-range birds.

Serological monitoring of both groups was performed at day 21, 28, 35 and 56 post-vaccination using the IBD ELISA (Synbiotics) commercial kit.

At 35 days of age, 5 bursas were collected and a PCR test was performed to detect IBD vaccine-take. The Bursa of Fabricious was collected at day 33 (on group 1) and at day 39 (on group 2) for histological scoring (Muskett et al 1979).

Production parameters such as mortality, FCR, ADG and mortality were analyzed at slaughter age (65 days) in both groups.

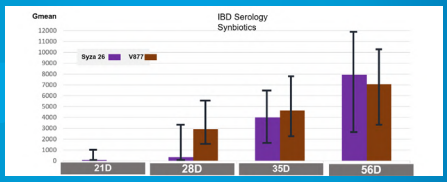

Fig. 1. Serological results.

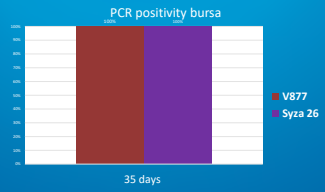

Fig. 2 IBDV detection from bursal samples, 28 days.

	Performance at 65 days of age				
	Livability	FCR	BW	ADG	EPEF
V877	97,80	2,39	1,07	16,46	67,36
V877	97,70	2,42	1,12	17,23	69,56
Syza 26	95,50	2,33	1,22	18,77	76,93
Syza 26	99,20	2,19	1,29	19,85	89,90

Fig. 3. Performance results.

Results

Serological values are compatible with a correct vaccine in both groups. Serological differences were observed at 28 and 35 days of age, which could be caused by different levels of maternally derived antibodies at day old which can affect the time for IBD vaccine take. No significant differences were observed at 56 days of life regarding the serological titers between groups. PCR results have shown the presence of the corresponding vaccine strain for each group, demonstrating a good vaccine take. Histological lesions were more severe, according to Muskett et al. at different ages in birds from group 2 compared to group 1. Regarding productive parameters (FCR,ADG, EPEF), group 1 showed better when compared to group 2.

Conclusion

Due to the higher susceptibility of layer-type birds to IBD virus and slow and uneven MDA decay, the IBD strain selection to be used is crucial. In this study, data has shown that the group that received a vaccine designed specifically for layers-type birds containing the SYZA-26 strain had better Livability, Body weight and lower FCR when compared to an Immune-complex vaccines containing a more invasive strain.

Development of a selective one-step real-time RT-PCR assay for the screening for avian encephalomyelitis viruses in chicken and turkey flocks

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Introduction

Avian encephalomyelitis (AE) is an infectious viral disease affecting young chickens, turkeys, quails and pheasants. Clinical signs of the disease in young turkeys are similar to those in young chickens: ataxia, incoordination, rapid tremors, especially of the head and neck, with high morbidity and variable mortality. The causative agent of the disease is avian encephalomyelitis virus (AEV), a member of the *Picornaviridae* family, which is highly resistant in the environment. The positive-sense, single-stranded RNA genome comprises a 5'-untranslated region (5'-UTR) followed by a large open reading frame encoding a polyprotein, which includes four structural proteins (VP4, VP2, VP3, VP1) and seven non-structural proteins. The mostly used RT-PCR assay for AEV (Xie et al. 2005) is unable to detect the turkey origin viruses, therefore Goto et al. (2018) developed a 5'UTR-based RT-PCR assay to cover the turkey AEV strains as well. Using this approach, viruses from outbreaks in turkey flocks in Hungary could be detected and their partial 5'UTR sequence could be determined.

The aim of our work was to develop a suitable methodology for screening both turkey and chicken flocks for the presence of AEV, which could be best reached by the use of real-time RT-PCR. Comparative sequence analysis of 5'UTR sequences revealed remarkable differences according to the host of the virus, which triggered the development of a TaqMan real-time RT-PCR assay that detects both the AEV from chicken and turkey origin, and at the same time can separate them by the two selective probes included in the assay.

Materials and Methods

Samples: Four turkey AE virus isolates, 2 chicken AE virus isolates and the Calnek vaccine strain were included in the testing of the assay. Turkey isolates originated from four flocks of different origin between 2004 and 2011, Hungary. The clinical signs in the acute phase included diarrhoea and difficulties in movement. Turkey poults that survived the acute phase developed nervous symptoms, various stages of ataxia, fine tremors of the head and neck. Chicken origin viruses were isolated in 1987, in Hungary. Due to rare diagnosis of AE recently, the availability of strains for the validation of the assay was limited.

Design of one-step real-time RT-PCR assay for AE detection: Real-time PCR primers for detection of AE virus from both origins were designed based on 5'-UTR region using Calnek strain from GenBank as a chicken origin and sequence of turkey AEV field isolates in our laboratory.

Primer and probe sequences were aligned to sequences from the Genbank for the verification of proper expected binding in silico. The one-step real-time RT-PCR assay amplifies an 89-bp region of 5'UTR spanning from 213 to 301 nt (Fig.1.).

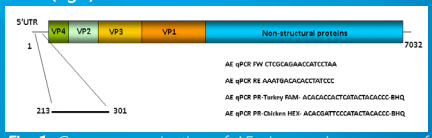

Fig. 1. Genom organization of AE virus and sequences of primers

RNA was extracted by MagMax Viral RNA Isolation kit (Applied Biosystems) and one-step real-time RT-PCR was performed using QuantiNova Probe RT-PCR Kit (Qiagen). qRT-PCR started with RT stage at 50°C for 30 min, initial denaturation stage at 95°C for 15 min, followed by 40 cycles of denaturation at 94°C for 15 sec, primer annealing and template amplification at 60°C for 45 sec .

Results

All the four turkey AEV isolates were positive by turkey specific TaqMan probe and negative for chicken specific probe. The two chicken AEV isolates and the Calnek vaccine strain were positive by chicken specific TaqMan probe and negative for turkey specific probe (Fig. 2).

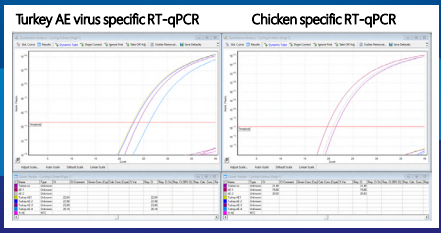

Fig. 2. Results of specific one-step real-time RT-PCR of turkey origin AEV and chicken origin AEV samples

Conclusion

Based on the available results, this TaqMan real-time RT-PCR method is suitable for fast routine diagnostic investigation of AE suspect cases not only in chickens, but in turkeys as well. This methodology can be included in the differential diagnosis of diseases with nervous clinical signs.

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Reassortant Infectious Bursal Disease Virus presence in Central and Eastern Europe

Tamás Mató[1], Timea Tatár-Kis[1], András Medvecki[1], Tomasz Kwiatkowski[2], Vilmos Palya[1] and István Kiss[1]

[1]Ceva Phylaxia Biologicals Co Ltd, Hungary; [2]Ceva Animal Health Polska Sp.z o.o., Poland

Introduction

Infectious bursal disease virus (IBDV) is one of the major threats to the poultry industry globally, represented by a variety of genetic, pathogenic, and antigenic strains. It is important from both epidemiological and control strategy point of view to have precise information about the prevailing IBDV strains in a given production unit, country or geographical area.

In recent years new reassortant strains of IBDV were detected in several commercial poultry flocks in the Netherlands, Belgium, Denmark, Sweden, Germany, France, United Kingdom, Portugal and Czech Republic (Mató *et al.* 2020, 2022 , Legnardi *et al.* 2022) . These flocks either did not receive any IBD vaccination but displayed strong sero-conversion to IBDV and reduced bursa sizes at slaughter or a survey in vaccinated flocks identified the presence of these field strains. In both cases, no IBDV related clinical symptoms were observed. Genetic analysis, based on the hypervariable region of the VP2 gene allocated them into genogroup 3 (very virulent strains) within a separate genetic cluster from the typical very virulent strains. Further analysis revealed that the VP1 gene of these strains was not related to very virulent strains, but showed the closest relation to sequences of vaccine viruses, therefore they are typed as genotype A3B1.

Similar reassortant strains with A3B1 genotype were detected and genetically characterized from further Central and Eastern European Countries as Slovakia, Poland and Ukraine based on partial VP2 sequence covering the hypervariable region and partial VP1 sequence in our study.

Materials and Methods

Samples: Bursa samples collected from a number of flocks with bursa atrophy and high IBDV ELISA titers (Biochek) at slaughter age without vaccination and clinical signs of infectious bursal disease (IBD) were submitted to our laboratory for IBDV detection and characterization. Altogether we received more than 100 bursa samples from 24 different flocks: 18 from Poland, 5 from Ukraine and 1 from Slovakia, between 2019 and 2023.

Molecular detection and identification of IBDV:

Methods of molecular detection and identification of the viruses as well as the phylogenetic analysis of partial VP2 and VP1 genes was done described previously (Mató et al. 2020)

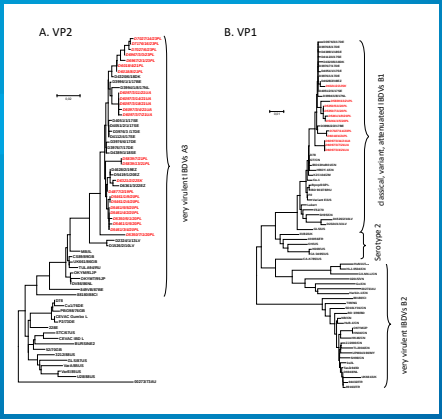

Fig. 1. A. Phylogenetic analysis of partial genome segment A B. Phylogenetic analysis of partial genome segment B.

Results

Genetic characterization

– Based on partial VP2 nucleotide sequence:

• All isolates grouped with the very virulent IBDV strains, but formed a unique branch compared to the referent very virulent strains (A3 genotype) and grouped together with previously described Western European low pathogenic reassortant strains.

– Based on partial VP1 sequence:

• All tested isolates grouped together with classical virulent-attenuated-variant strains (B1 genotype), and form separate group with Western European low pathogenic reassortant strains

• All isolates included in this investigation proved to be reassortants A3B1 genotype.

Due to the limited number of available samples in our laboratory, no calculation on prevalence could be made.

Conclusion

These results draw attention to the rapid spread of genotype A3B1 reassortant IBDV strains in Europe affecting not only Western, but Central and Eastern Europe as well.

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SEROSURVEILLANCE OF INFECTIOUS BRONCHITIS DISEASE IN COMMERCIAL BROILERS IN INDIA DURING 2022

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Introduction

Infectious bronchitis virus (IBV) affects the respiratory, reproductive, and renal systems of chickens, causing major economic losses to the poultry industry. In India, Infectious Bronchitis is not a major concern for broiler producers hence vaccination is not always practiced. The aim of this study is to assess the antibody response of the IBV live vaccine strains used in broilers.

Materials and Methods

In 2022, 10,621 serum samples from 599 broiler flocks with harvesting age above 33 days raised in different parts of country, irrespectively of their IBV live vaccination program, were collected. All samples were tested using a commercial IB ELISA kit (BioChek).

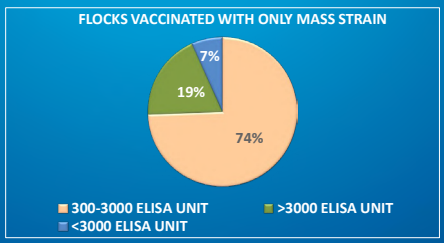
Overall, 81% of monitored flocks (484) were vaccinated with IBV live vaccine at the hatchery or during first week of age. 19% farms were not vaccinated with IBV live vaccine. 92% of vaccinated flocks used a Massachusetts strain only while the other 8% used the combination of the 1/96 (793B group) and Massachusetts strains.

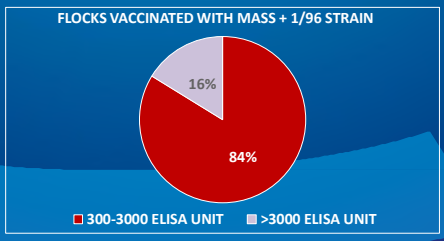
Results

Based on the BioChek guideline, 343 flocks (76.5%) vaccinated with only a Mass strain showed the expected mean titres (300 – 3,000 ELISA units), 87 flocks (19.5%) with suspected titres (>3,000 ELISA units) and 18 flocks (4%) with low titres (<300 ELISA units). Alternatively, 31 flocks (84%) vaccinated with the combination of 1/96 and Mass strains were in the expected range and only 6 flocks (16%) showed suspected titres. There were no flocks that failed to respond to this vaccination program. Finally, out of 115 non-vaccinated flocks, 92 of them (80%) had IBV antibody response suggesting the flocks had exposure to field IBV virus.

Type of flocks	No. of flocks
Total monitored flocks	599
IBV vaccinated flock	484 (81%)
Non- IBV vaccinated flock	115 (19%)

Type of flocks IBV vaccinated flock	No. of flocks	ELISA Unit	No. of flocks (%)
Mass strain	445 (92%)	300 – 3,000	343 (76.5%)
		> 3,000	87 (19.5%)
		<300	18 (4%)
Mass + 1/96 strain	37 (8%)	300 – 3000	31 (84%)
		> 3,000	6 (16%)
Non- IBV vaccinated flock	115 (19%)	> 3,000	92 (80%)
		< 300	8 (20%)





Conclusions:

This serosurveillance showed high prevalence of IBV in non-vaccinated flocks indicating that commercial broilers need to have proper immunization against this disease. Additionally, the flocks vaccinated with the association of the 1/96 and Massachusetts strains had higher percentage of mean ELISA titres within the expected range as compared to flocks vaccinated with only a Massachusetts strain, indicating that this combination of vaccine strains will be a good option to reduce the losses associated to IBV in India.

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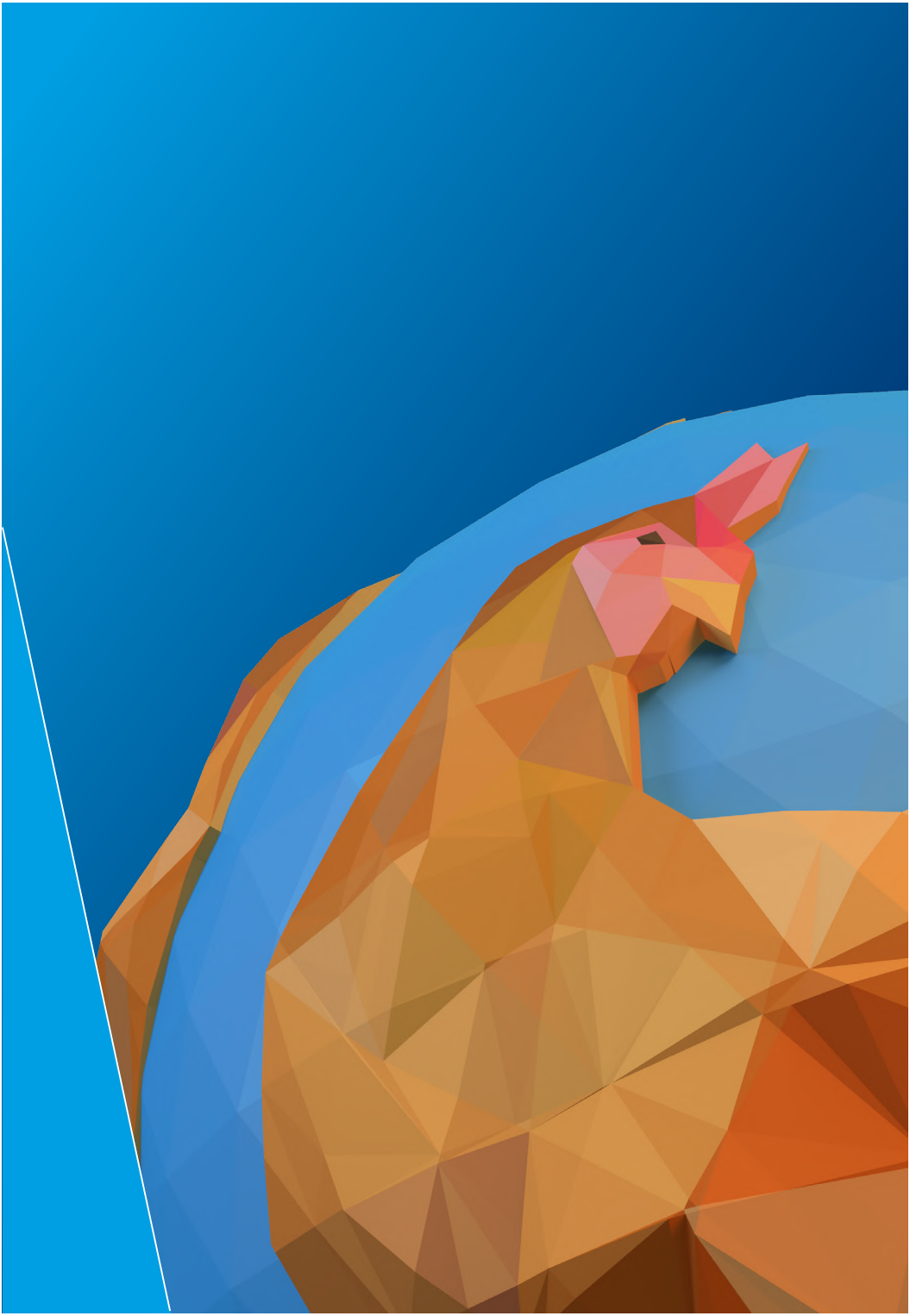
Together, beyond animal health



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