AI H5 INFECTIONS IN BIRDS, UNPRECEDENTED INCREASE IN OUTBREAKS SINCE 2021

In the last decade, the world faced **two of the most devastating HPAI outbreaks in poultry.**

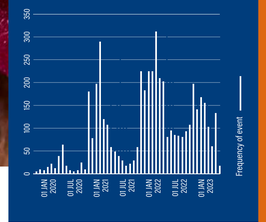
During 2014-2017 period, a Gs/GD lineage virus, H5N8 subtype, 2.3.4.4 C clade, caused a tremendous increase in the numbers of outbreaks in different parts of the globe.

Since 2021, a GS/GD lineage virus, H5N1 subtype, 2.3.4.4b clade, is causing an unprecedented increase in the numbers of outbreaks globally.

In Non-enzootic areas, the control measures based on biosecurity and stamping out policy was not able to alleviate the situation anymore. Vaccination is now accepted as an important component of the HPAI control strategies.

Evolution of weekly number of Avian Influenza confirmed outbreaks

Source : https://empres-Lapps.fao.org/



NEWCASTLE DISEASE:

A HIGHLY CONTAGIOUS, POTENTIALLY DEVASTATING DISEASE.

Newcastle Disease (ND) is a worldwide disease in chickens caused by the avian paramyxovirus serotype 1 (APMV-1), a single-stranded RNA virus that can cause devastating losses to the poultry industry. ND has different degrees of pathogenicity: it can be apathogenic (lentogenic forms), moderately pathogenic (mesogenic forms) or highly pathogenic (velogenic forms).

The most virulent form of the disease can cause up to 100% mortality and the main clinical signs are respiratory signs, depression, watery greenish diarrhea and head swelling. Young birds are the most susceptible to this form. Laying hens might experience partial or complete cessation of egg production, and eggs can be abnormal in color, shape, surface or have watery albumen.

Given the high contagiousness of Newcastle Disease, once the virus is introduced into a susceptible flock, virtually all the birds will be infected within two to six days. Since no real treatment exists, it's fundamental to provide the birds with a reliable clinical protection through a safe and effective vaccine.

- BROAD CROSS-CLADE PROTECTION
- DIVA
- REDUCTION OF AI VIRUS SHEDDING
- STRONG ND IMMUNE RESPONSE
- SAFE AND EASY TO ADMINISTER

The subunit inactivated vaccine to protect against Avian Influenza H5Nx & Newcastle infections

References:

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2- M.Radwan, L.Vianello, L. Baraldo, F. Bonfante, S, Lemiere, F. Prandini4 & E. Mundt. A single shot of VOLVAC™ B.E.S.T. AI+ND provides clinical protection in broilers against an H5N8 (clade 2.3.4.4b) highly pathogenic avian influenza virus (HPAI) also in presence of H5-specific Maternally Derived Immunity. WVPAC, Verona, September 2023.

3- Grasland, A. Schmitz, E. Niqueux, R. Busson, N. Morin, C. Guillemoto, A. Orosco, F. Souchaud, , S. Bougeard, FX. Briand, C. Martenot, M. Cherbonnel, P. Massin, N. Rose, K. Louboutin, I. Pierre, M. Amelot, M. Delpont, L. Pouvelle, S. Soubies, A. Keita, J-L. Guérin, N. Eterradossi. Experimental vaccination of mule ducks under field conditions against highly pathogenic avian influenza A (H5N1) virus of clade 2.3.4.4b

4- Study number : 14-027. Immunogenicity of the ND component and safety in SPF chickens. Final report

Boehringer Ingelheim

MAKE SURE TOMORROW PEOPLE **CAN STILL COUNT ON THE EGYPTIAN POULTRY INDUSTRY**

Volvac™ B.E.S.T. AI+ND

The recombinant inactivated vaccine to protect against Avian Influenza H5Nx & Newcastle infections

- Wide H5Nx cross-clade protection
- Reduction of mortality and clinical signs caused by H5Nx and ND
- Reduction of shedding
- D.I.V.A. Vaccine

Boehringer Ingelheim

AVIAN INFLUENZA:

A GLOBAL THREAT TO POULTRY INDUSTRY

Avian influenza (AI) is a disease caused by a type A influenza virus. AI was first identified in 1878 and the influenza virus was first described in 1955.

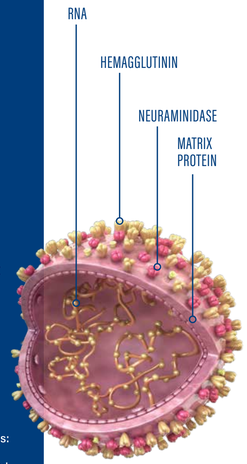
Influenza A viruses come in many **different subtypes** based on differences in their proteins. Each subtype has many different strains. **New subtypes and strains arise when the virus undergoes genetic mutations.**

AI virus has two large surface proteins: **hemagglutinin (HA)** a protein that mediates binding of the virion to target cells and entry of the viral genome, and **neuraminidase (NA)**, involved in the release of progeny virions from infected cells.

Highly Pathogenic AI (HPAI) is caused by two types AI virus, H5Nx and H7Nx. **Both type of viruses can circulate under low pathogenic or highly pathogenic forms.**

The H5 Goose Guangdong Eurasian lineage has almost a global distribution,it has evolved into different clades including the highly predominant 2.3.4.4.b

HA is currently the main protein that triggers neutralizing immune response to the virus.



8 RNA SEGMENTS RESPECTIVELY ENCODED

HA (hemagglutin)
NA (neuraminidase)
NP (nucleocapsid)
Two matrix proteins (M1 and M2)
P (RNA polymerase
sub-units PA, PB1 & PB2)
NS1, NEP (Non-structural proteins)

VOLVAC™ BEST AI+ND, AN INNOVATIVE SUBUNIT INACTIVATED VACCINE AGAINST AI AND ND IN BIRDS

The Hemagglutinin (HA) protein is one of the surface proteins of the AI virus. It is important for the attachment and fusion of the virus with the cell wall, initiating the infection process. It is also the target of neutralizing antibodies. Once HA neutralized by antibodies, the virus is not able to attach to the host cells anymore and infection is avoided.

B.E.S.T.

B.E.S.T. stands for Baculovirus Expression System Technology. The Baculovirus Expression System Technology is used for the large scale production of biologically active and functional recombinant proteins.

SUBUNIT

The AI component in Volvac™ B.E.S.T. AI + ND was specifically engineered at the R&D facilities of Boehringer Ingelheim by inserting the hemagglutinin (HA) viral sequence of the AI (H5 type) virus into the Baculovirus genome. The insert was generated using recombinant vaccine technology in order to highly resemble the HA protein of currently circulating H5 viruses, thus resulting in a product that provides the wide cross protection against circulating clades.

BROAD

The similarity of the aminoacids sequences between the H5 antigen in Volvac™ B.E.S.T. and the H5 of most prevalent AI viruses is one of the main reasons behind such a broad spectrum of protection.

An engineered H5 antigen for an improved protection:
Hemagglutinin gene based on HPAI H5 modified by insertion of defined amino acids on defined epitopes.

Two steps for genetic sequence design:

INSERTION 1 **Step 1: modification made the virus** more **immunogenic**.

INSERTION 2 **Step 2: an insert allows a more efficent cleavage of the HA to HA subunits HA1 and HA2 for proper folding forming Trimeric shape of the antigen.**

- Leading to excellent presentation of HA to the immune system of the vaccinate
- for a fast onset of immunity
- and a high level of neutralizing antibodies
- against different circulating H5 strains.

The specifically engineered gene sequence for the HA of AI H5 is included in the baculovirus genome by means of a transfer vector.

The inserted virus sequence results in the expression of a perfectly structured HA trimmer with high antigenic characteristics.

VOLVAC™ BEST AI+ND, AN OPTIMIZED H5 ANTIGEN FOR WIDE CROSS-PROTECTION

In different experimental studies[1,2,3] SPF chickens were vaccinated subcutaneously (SQ) at different ages with 0,5ml of Volvac™ B.E.S.T.

The efficacy of the vaccine was evaluated against different H5Nx challenges, the reduction of mortality and viral shedding were used as primary in order to assess the efficacy. For each trial, a positive control (unvaccinated and challenged) group was added in order to validate the challenge model.

Country of origin of challenge virus	Species of origin of challenge virus	Year of isolation	Virus Clade	Protection against mortality at 21 D.P.V.	Maximum shedding in infectious birds up to D.P.C. (positive/total)
Mexico (H5N2)[1]	Chicken	2004	0	100 %	NT
Vietnam[1]	Duck	2005	2.3.2	100 %	3 DPC (4/10)
Spain[1]	Chicken	2006	2.2	100 %	4 DPC (3/12)
Egypt[1]	Chicken	2008	2.2.1.1	90 %	3 DPC (4/10)
Egypt[1]	Chicken	2010	2.2.1.1	80 %	7 DPC (2/8))
Egypt[1]	Chicken	2010	2.2.1.1	100 %	7 DPC (2/10)
Egypt[1]	Chicken	2010	2.2.1	100 %	0/10
Egypt[1]	Chicken	2010	2.2.1.1	100 %	5 DPC (1/10)
Egypt[1]	Chicken	2012	2.2.1	100 %	4 DOPC (2/10)
Vietnam[1]	Duck	2014	2.3.2.1C	95 %	10 DPC (5/19)
Germany[1]	Chicken	2014	2.3.4.4	100 %	4 DPC (3/10)
Korea[1]	Duck	2015	2.3.4.4	100 %	5 DPC (3/9)
South Africa[1]	Pigeon	2017	2.3.4.4	100 %	4 DPC (3/20)
Egypt[2]	Chicken	2020	2.3.4.4B	100 %	5 DPC (3/20)
France[3]	Duck	2023	2.3.4.4b	100 %	4 DPC (2/9)

VOLVAC™ BEST AI+ND, THANKS TO ITS OPTIMIZED AI H5 ANTIGEN IS PROVIDING **ABOVE 80% PROTECTION AGAINST CONSISTENTLY PREVALENT ACTUAL CLADES**[1,2,3]

NEWCASTLE DISEASE:

ANOTHER IMPORTANT CHALLENGE FOR THE POULTRY INDUSTRY

Volvac™ B.E.S.T. AI + ND includes conventional inactivated ND virus for a convenient vaccination of chickens against AI (H5) and ND at the same time.

The efficacy of the ND component of an inactivated vaccine can be evaluated by Protective Dose 50 test (PD 50 test) as established in the European Pharmacopeia and WOAH methodology.

Protection after vaccination with Volvac™ B.E.S.T. AI+ND evaluated by means of the Protective Dose 50 test (PD^{50} test)[4]

SPF birds 3 weeks of age	Number of birds	Dose/bird	% protection p.c. 3 weeks p.v. with NDV Herts 33
Group 1	20	1/25	100%
Group 2	20	1/50	100%
Group 3	20	1/100	90%
Group 4	10	-	0%

Antibody levels (2Log, HI Test*) after vaccination with Volvac™ B.E.S.T. AI + ND vaccine

A high PD value is an indication for a highly potent inactivated ND vaccine.
Volvac™ B.E.S.T. AI+ND resulted in a PD value dose (0,5ml) of > 131, far much higher than the minimum requirements established for this type of products >'50 pd dose).

VOLVAC™ BEST AI+ND, A D.I.V.A VACCINE

The birds vaccinated only with HA protein, like in the case of Volvac™ BEST AI+ND, don't develop anti-influenza nucleoprotein (NP) antibodies.

Two trials were assessed in order to evaluate the implementation of a DIVA approach after Volvac™ B.E.S.T administration.
All sera were collected at different ages before vaccination (DPV) and after challenge (DPC) and tested by HI test and specific NP Avian Influenza indirect ELISA commercial kit[1].

Challenge strain	Vaccine	Protection (Mortality)	NP ELISA		
			21 d.p.v.	14 d.p.c.	21 d.p.c.
a/ck/Egypt/1285/2012 (H5N1)	H5 B.E.S.T.	10/10	0/10	3/10	7/10
	H5 B.E.S.T.	9/9	0/9	4/9	NT
a/mallard/korea/k413-2/2015 (H5N8)	Reverse Genetics H5 vaccine	7/9	2/9	6/7	NT
	Reverse Genetics H5 vaccine	9/9	4/9	8/9	NT
	Reverse Genetics H5 vaccine	9/9	6/9	9/9	NT

DIVA STRATEGY APPLIED FOR HA AI VACCINES

Chickens are vaccinated with HA, No AIV challenge

Only HA specific antibodies

Chickens are vaccinated with inactivated AIV

Chickens are vaccinated with HA and subsequently AIV infected

Antibodies against all AIV proteins

Chicken HA-Ab Chicken NP-Ab Chicken Ab

BIRDS VACCINATED WITH VOLVAC™ B.E.S.T. DIDN'T SHOW ANY NP SEROCONVERSION AT 21 DPV. AFTER THE CHALLENGE, NP SEROCONVERSION IS SHOWN BY NP ELISA.
THIS RESULT CONFIRMS THE APPLICABILITY OF DIVA APPROACH AFTER VOLVAC™ BEST AI+ND VACCINATION