M.B. – for complete Gumboro disease control

Gumboro disease

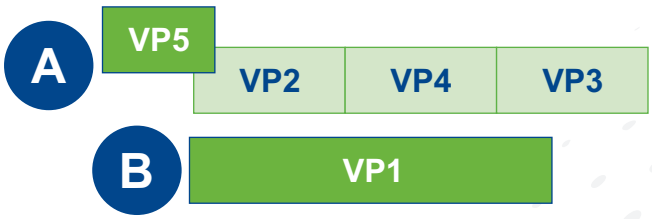
Infectious bursal disease virus (IBDV) is the aetiological agent of Gumboro disease, an acute and highly contagious disease in young chickens. Chickens infected with virulent classical IBDV between 3 and 6 weeks of age mostly show clinical signs and mortality accompanied with bursal atrophy. Chickens infected with virulent classical IBDV at less than 3 weeks of age usually have few or no clinical signs. The severity of infection and the disease profile depends on the strain virulence, the level of protective immunity and management factors.

The primary target organ for the IBDV virus is the Bursa of Fabricius where it targets, and destroys, immature IgM+ B-lymphocytes. The virus can also destroy T cells in the thymus and spleen. A serious consequence of both infections is immunosuppression of varying degrees that has a negative impact on the bird's responses to vaccination and makes them more vulnerable to a variety of secondary infections.

1. The IBD virus

IBDV is a double stranded RNA virus and a member of the *Birnaviridae family*. The intrinsic property of all RNA viruses is to evolve rapidly, leading to the development of many antigenic variants in the field and making it more difficult to control by means of vaccination.

The genome of the virus consists of two segments – A & B.

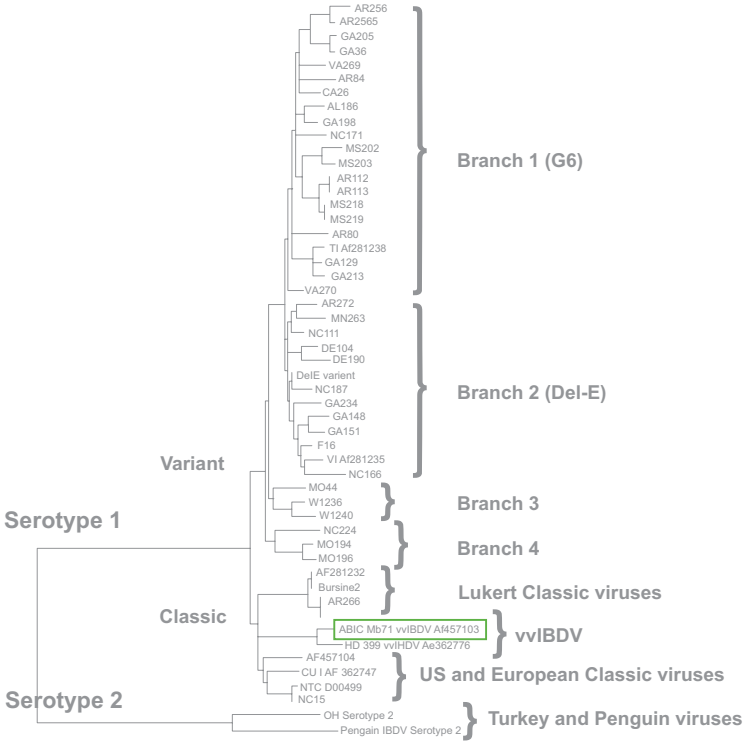


The genome codes for five main proteins – VP1 to 5. VP2 and VP3 are capsid proteins. VP3 is highly antigenic but the antibodies are non-neutralizing. VP2 is the main neutralizing antigen on the capsid of the virus and, therefore, stimulates the best protective immune response against the Gumboro virus. The hypervariable region within the VP2 protein appears to be the area where the most significant amino acid changes occur that impact the pathogenicity and antigenicity of the IBD virus.

Based on this fact, two main approaches are used to scientifically classify IBD virus strains.

1. Phylogenetic analysis of IBD viruses based on the VP2 genome:

(D. J. Jackwood AVIAN DISEASE 49, 220-226, 2005)



The variant strains that appeared in the 1980's in the USA did not cause clinical symptoms, but produced severe immunosuppression. Very virulent field strains were identified in Europe at the end of the 1980's and caused severe clinical symptoms and mortalities.

2. Genetic heterogeneity in the VP2 gene using RT/PCR-RLFP:

(D. J. Jackwood & S. E. Sommer AVIAN DISEASES 42, 321-339, 1998)

Results of the RT/PCR-RFLP assay for vaccine and laboratory strains of IBDV.

Molecular Group	IBDV Strains and Vaccines	Manufacturer	BstNI 424	BstNI 350	BstNI 209	BstNI 172	BstNI 154	BstNI 139	BstNI 119	MboI 480	MboI 403	MboI 362	MboI 234	MboI 229
1	Del-A (USA)	USA, Merial Select												
2	Del-E	USA												
3	Variant Vax_BO	USA, Schering-Plough												
3*	IBD BLEN	USA, Merial Select												
4	22BE	S. Africa, Intervet												
4	078	S. Africa, Intervet												
4	S706	USA, Merial Select												
4	Bur S706	England, Merieux												
4	TAD Cuim	S. Africa, TAD												
4	TAD Forte	S. Africa, TAD												
4	RP-Bur706	S. Africa, Merieux												
4	Gumborovax	S. Africa, TAD												
4	Univax-BD	USA, Schering-Plough												
5	Lukert (Lu-37-3-3)	USA												
5	Bursine	USA, Fort Dodge												
5	Bursine+	USA, Fort Dodge												
5	Bursine2	USA, Fort Dodge												
6	MB IBD Vaccine	Israel ABIC												
6	GM 97	USA, Intervet												
6	Bursavag	Australia, V877, Webster												

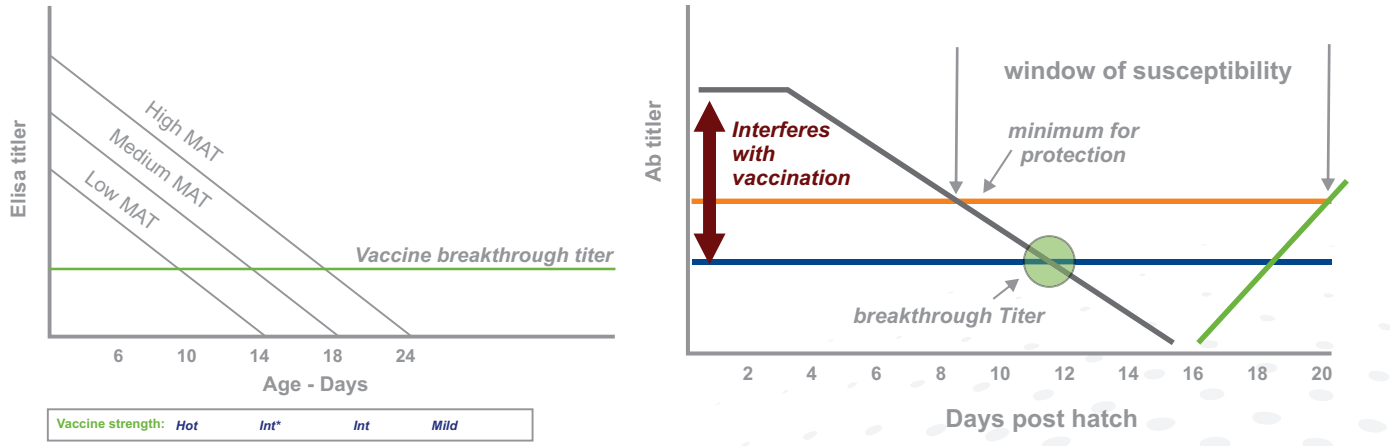
2 bands

Based on the above data, the majority of IBD virus strains tested have been allocated to 5 groups. Groups 1 and 2 contain the variant virus strains. Groups 3 and 4 encompass the majority of the classical field and vaccine strains. The Lukert type field and vaccine strains fit into group 5. A group of very virulent IBDV field strains has been allocated to Group 6.

2. Treatment and prevention of Gumboro Disease

There is no treatment for Gumboro disease. A key aspect of the control of Gumboro disease is the effective use of Biosecurity, vaccines and vaccination programs (The virus strain used must be able to protect against circulating field strains). Each bird must be protected from Gumboro infection from hatch to 6-8 weeks. Anti VP2 antibodies provide very good protection. Therefore maternally derived Ab are a key factor in prevention of Gumboro disease from hatch to 2-3 weeks while live vaccines complete the protection up to 6-8 weeks of age. In order to obtain this the following principles must be followed:

- Hyper-immunization of breeders (GPS or PS) with inactivated vaccines to pass on high levels of antibodies to their offspring.
- Timely application of live vaccines in offspring to ensure effective protection as the level of maternally derived antibodies declines.



Impact of maternal antibody titre levels on the type of vaccine and the age of application

Gumboro disease vaccination:

The key characteristics required for an effective Gumboro disease vaccine are:

- Rapid colonization of the Bursa to prevent colonization by field strain virus (Competitive Exclusion)
- Stimulation of high levels of humoral antibodies to help neutralize field viruses. Ab titer levels are correlated with the level of immunity.
- Allow the Bursa to maintain its functionality as an integral part of the young bird's immune system. In this regard it is important to take note that there is no direct link between bursa size and any disease or any vaccination. There is no standard of size for a given age, in a given breed. Bursal size (atrophy, normal, or enlargement) is therefore subjective. The bursa diameter also does not correlate with histopathology lesion scores nor is it correlated with immunosuppression.
- An ability to quickly spread among the birds in a house. This helps counter application problems and can shorten the time to onset of immunity and improve protection uniformity.
- Should be stable i.e. no reversion to virulence.
- The immune response stimulated by the vaccine should not cause immunosuppression. This would, typically, be measured by the bird's ability to mount an immune response to other vaccines e.g. Newcastle disease.

So, how can we choose the right vaccine?

Do we judge a product by its effect on the BF (size, weight), the ELISA antibody response, by the resistance to challenge with vvIBDV? How do we avoid immunosuppression?

The answer is in the careful and highly professional testing of all the mentioned parameters and the actual field experience amassed over time in different countries and management systems.

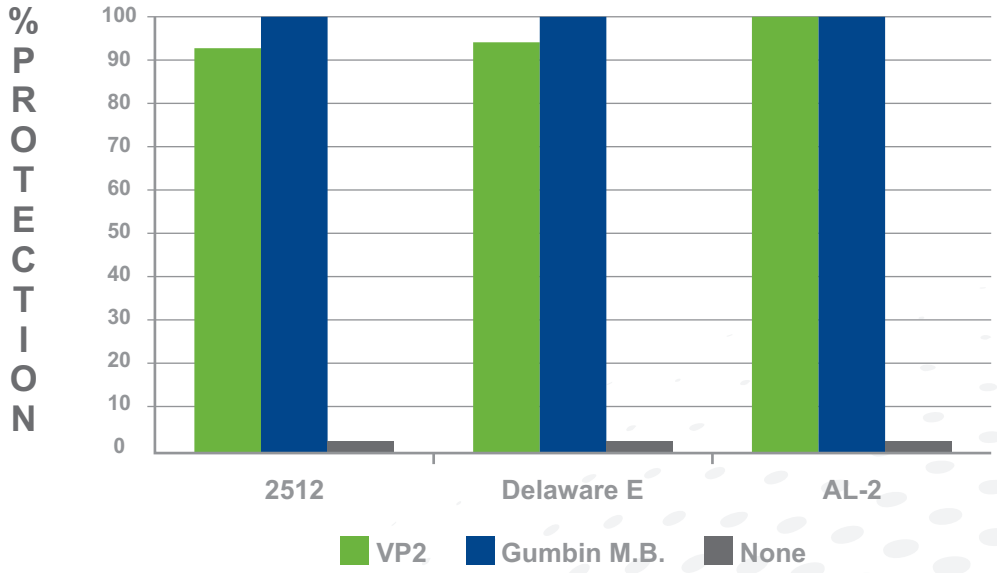
M.B. strain – the backbone of Phibro's IBD vaccine range

1. M.B. strain characteristics:

The M.B. strain was attenuated from the very virulent IBDV ks, Israeli strain, in 1989 by Abic scientists Drs B. Gutter and M.A M. Barbakov. The resulting M.B. strain showed the ability to confer immunity in the face of maternal antibodies and to protect chicks vaccinated at day 7 and older.

- RT/PCR-RLFP tests show that M.B. falls into molecular Group 6 of DJ Jackwood's classification method. This means that it is able to offer *high levels of protection against vvIBD field strains, classical strains and many variant strains.*

  Trial done by the Veterinary Diagnostic Pathology Lab in Auburn, Alabama, USA shows that antibody protection is stimulated by both a standard M.B bursa derived vaccine and the VP2 sub-unit vaccine derived from the M.B. strain protect again 3 variant field strains:



- Field-testing over the past 4 years in Southern Africa shows that in 80% of birds vaccinated with M.B., only M.B. strain was found during PCR surveys (compared to only about 30% where other live/ immune complex vaccines have been used). No classical or vvIBDV strains were seen in any flocks. The variant D1054/85 strain has, however, become increasing prevalent.

	M.B.	D78	228E	WF2512	ST12	HVT IBD
No. of samples	82	20	13	26	5	1
Vaccine only	80%	15%	23%	35%		
Vaccine & D1054/85	7%	15%		23%		
Other vaccine strain	2%		23%	8%		
D1054/85 only	6%	65%	16%	23%	100%	100%
Negative PCR	5%	5%	38%	11%		

A similar trend is seen in Brazil (Br.), where the use of M.B. was able to significantly reduce the incidence of variant field strains in a group of 312 farms that had been using an HVT vector IBD vaccine:

Group	Number of farms	NEG	Br Variant	Br Var + Live Vaccine	HVT-IBD	Live Vaccine
HVT-IBD Vaccine (2013)	312	123 / 312 (39.4%)[b]	167 / 312 (53.5%)[b]	0 / 312 (0%)[b]	27 / 312 (7%)[b]	0 / 312 (0%)[b]
M.B.-IBD ~~HVT-IBD~~ Vaccine (2014)	312	12 / 312 (3.8%)[a]	19 / 312 (6%)[a]	17 / 312 (5.4%)[a]	0 / 312 (0%)[b]	264 / 312 (84.6%)[a]

53.5% Br. Var positive in 2013
11.5% Br. Var positive in 2014

-78%

- **M.B. does not cause a reduction in the NDV vaccination response, showing that it is not immunosuppressive.**

  ND Serological response (HI Log_2) & protection from challenge with vvNDV (Heref Leet) strain. [M.B. was given at 10 days of age and V.H. at 17 days of age]

Age	Day 0		Day 10		Day 31		*Chall. Result
Titers / Group	Average	SD	Average	SD	Average	SD	
M.B.+ V.H.+	1.80	0.97	1.60	0.92	3.00	0.00	0/15
M.B.+ V.H.+					8.30	0.66	13/15
V.H.					8.37	1.12	14/15
Neg. cont					8.32	1.16	15/15

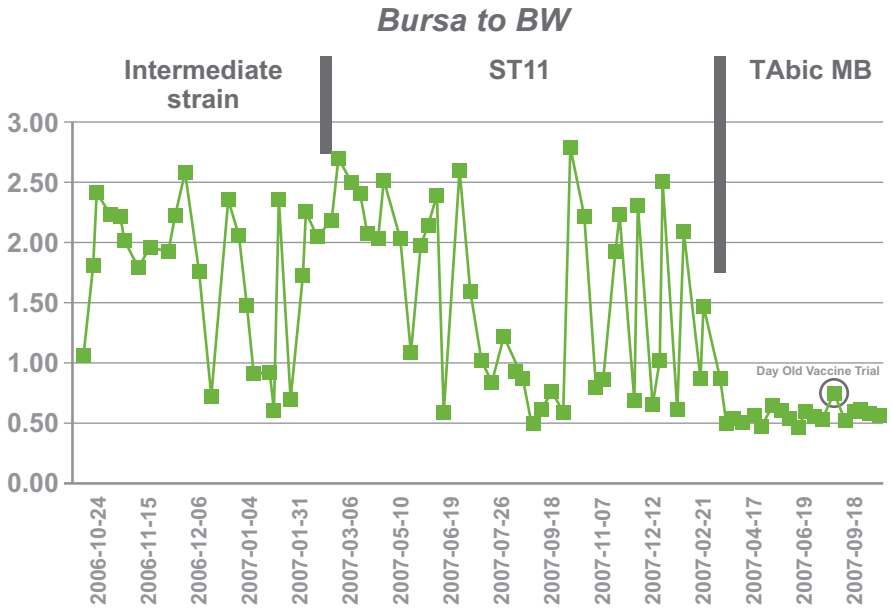
- Challenge trials done with M.B. during the development of the strain, show that while the virulence of M.B. decreased due to attenuation, the antigenicity of the neutralizing epitopes in VP2 did not change, enabling induction of a good response to virulent field strains.

- **M.B. elicits a very rapid bursal response and protection from field challenges**

Vaccinated DPV	Bursal AGP
3	0/10
5	0/10
7	0/10
11	0/10

Control DPV	Bursal AGP
3	10/10
5	10/10
7	10/10
11	10/10

- **M.B. stimulates a consistent response in the Bursa** – Post vaccination the Bursa will be small but functional



- **M.B. is able to break though Maternal Antibody levels of 800 – 1000 (IDEXX ELISA).**

Broiler chicks were vaccinated with M.B. at various ages: a single vaccination at 2, 7 or 14 days of age, or two vaccinations at 2 and 14 days of age. Maternal antibodies at 2, 7 and 14 days post-hatch were 11/11, 6/10 and 2/10, respectively (number of AGP-positive sera/ number of tested sera), or 2560, 1290 and 320, respectively (average titre by ELISA). Vaccination was via the drinking water, with $10^{3.35}$ EID_{50} per chick.

Table 2. Vaccination with attenuated IBDV M.B. strain: antibody response and resistance to challenges

Age at vaccination (days)	Serological response to IBDV (AGP)	Protection against vvIBDV challenge[a]	Body weight[b] (g)
2	1/14[c]	2/12	1077±92
14 + 2	13/14	14/14	1077±148
7	7/14	14/14	958±119
14	15/15	14/14	1114±83
Control	0/8	0/8	995±88

[a]Number protected/number tested, as determined by detection of virus in bursae 3 days post-challenge.
[b]Body weight at 28 days of age.
[c]Number positive/number tested.

- Has the ability to spread horizontally between birds in the same houses and between houses.
- M.B. is registered for use in Broilers, Breeders and Layers.

2. M.B. strain trial results:

1. Comparison of protection induced by M.B. and a Winterfield strain following challenge with a vvIBDV strain

(Broiler chicks at the age of 14 days were vaccinated with M.B. ($10^{3.75}$ EID_{50} per bird) or the Winterfield strain ($10^{4.28}$ EID_{50} per bird) via the ocular route. A control group was not vaccinated with IBDV. All groups were vaccinated with NDV vaccine at 1 and 17 days of age. At 31 days of age, all birds were bled and challenged with vvIBDV.)

Vaccination with	Antibodies against			
IBDV	NDV[b]	IBDV (AGP)[a]	NDV (haemagglutination inhibition)[a]	Protection against vvIBDV challenge[a,c]
M.B.	+	10/10[a]	5.2[a]	10/10[a]
Winterfield	+	0/10[b]	5.1[a]	0/10[b]
Unvaccinated	+	0/18[b]	4.7[a]	0/18[b]
Unvaccinated	-	0/20[b]	2.3[b]	0/20[b]

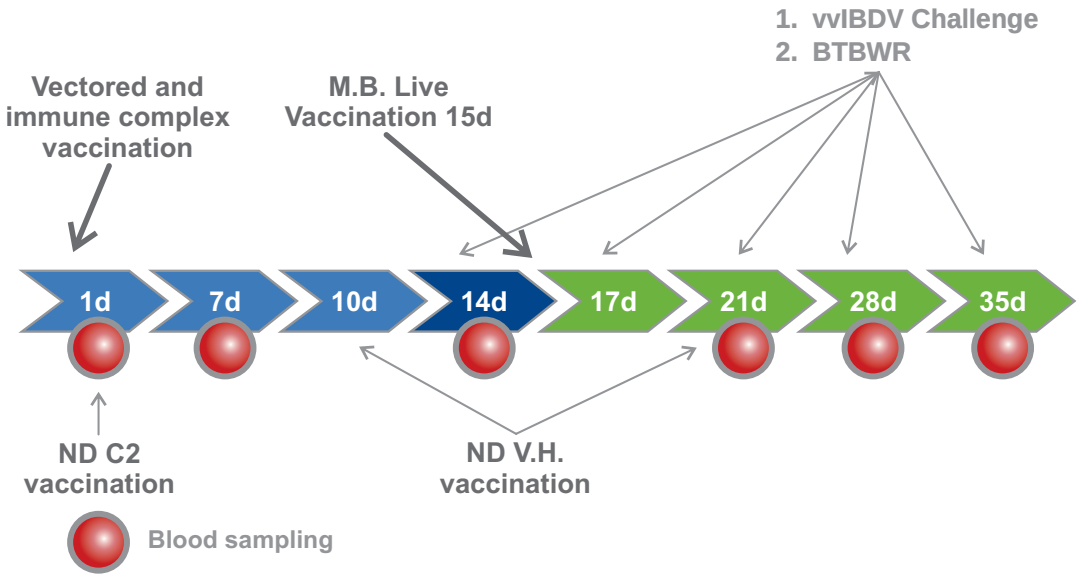
[a]Values followed by different superscript uppercase letters are significantly different (P <0.05).
[b]Live vaccine, VH strain (Abic, Israel)
[c]Number protected/number tested as determined by detection of virus in bursae 3 days post-challenge.

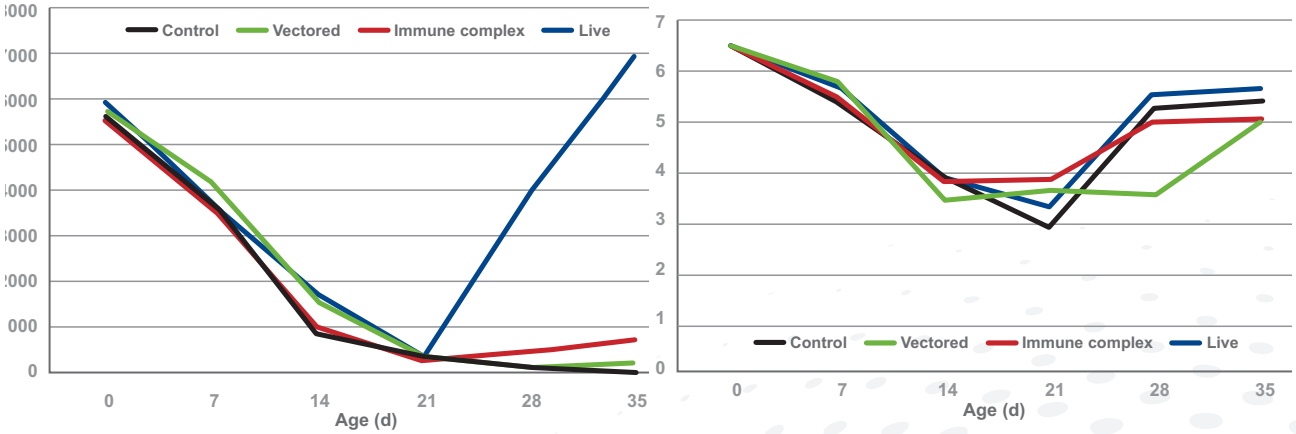
No protection against the very virulent wild-type isolate was achieved when the birds were vaccinated with the Winterfield strain, which is an attenuated form of a "classic" strain. This may be explained by the difference in VP2, the major neutralizing viral protein, between the Winterfield and very virulent strains.

Lazarus, D., Pasmanik-Chor, M., Gutter, B., Gallili, G., Barbakov, M., Krispel, S. and Pitcovski, J. (2008) 'Attenuation of very virulent infectious bursal disease virus and comparison of full sequences of virulent and attenuated strains', Avian Pathology, 37:2, 151 — 159

2. Elicits a good serological response post vaccination:

Broiler chicks were vaccinated with either M.B. or WF2512 at 15 days of age via drinking water.





4. Comparative performance data between birds vaccinated on farm with one dose M.B. or an in-ovo administered immune-complex vaccine (Brazilian data):

MM/YY / Parameter	10/12 IN-OVO	10/13 MB	Improvement
Survivability %	94.4	95.6	1.2%
BW (g)	2765	2722	-40g
DWG (g)	60.1	63.6	3.2g
FCR	1.86	1.71	0.15
Age (d)	46	43	3 (2.3)
Index	304	352	48

All performance parameters were dramatically improved

3. M.B. strain vaccines:

TAbic® M.B.

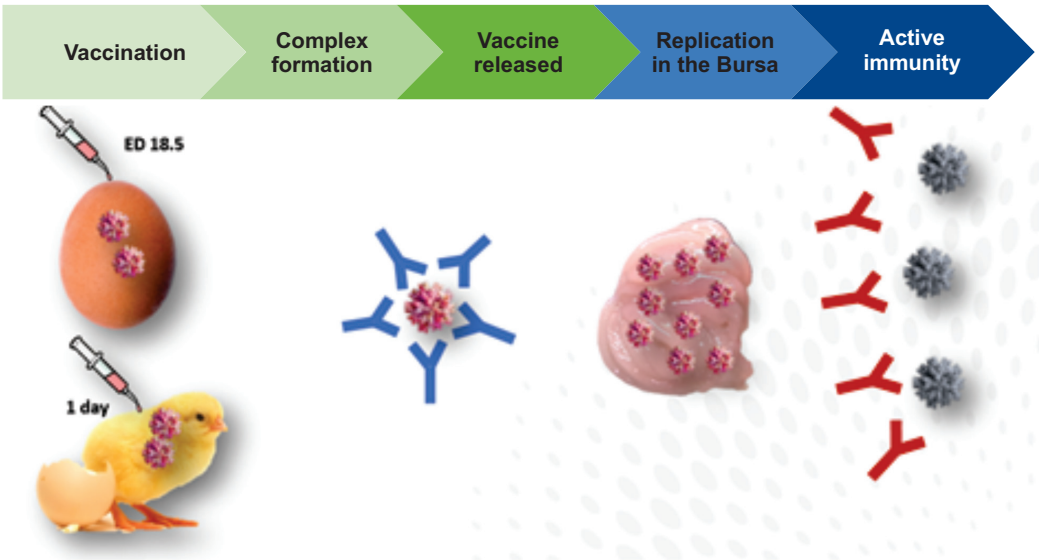
- Each effervescent tablet contains live, freeze-dried Infectious Bursal Disease virus, M.B. strain, of fowl embryo origin. Minimum titre per dose: $10^{2.7}$ EID_{50}
- TAbic® M.B. is intended for vaccination of chicks aged 10 to 15 days.
- The M.B. strain is able to break through Maternally Derived Antibody levels in broilers of between 500-800 (IDEXX ELISA).
- TAbic® M.B is administered via the drinking water.
- The age of application will be determined by the Day Old MAT levels but will be at approx. 14 days of age in broilers.

MB-1™

MB-1™ is a freeze-dried, live virus Gumboro disease vaccine containing the M.B. strain adapted for In-ovo or Subcutaneous injection in the hatchery that can be used in broiler, layer and breeder chicks.

- MB-1™ represents an evolution in hatchery vaccination for IBD control:
  - MB-1™ stimulates an earlier onset of immunity compared to other hatchery vaccines currently available in the field.
  - The development of immunity is individually adjusted according the level of maternal antibodies in the chicken. The M.B. virus is released in each bird at the optimal time to ensure a rapid development of immunity.
  - Once the vaccine virus is released it has the same ability to spread horizontally as the standard M.B. live vaccine applied on farm.
- MB-1™ can be reconstituted with either a Marek diluent or its own specific diluent.
- MB-1™ vaccine can be applied simultaneously with Marek's vaccines, Vector vaccines, Nectiv Forte, Pox vaccine (IN-OVO) or any spray vaccine.
- MB-1™ vaccine does not interfere with the immune response to other vaccines applied simultaneously or individually to any type of chicken

The mode of action of MB-1™:



- After injection, the MB-1™ vaccine viruses are coated with the chick's maternal antibodies (MAbs).
- These MAbs prevent the virus from replicating too early in the young chick's bursa.
- After the natural deterioration of the MAbs, the vaccine virus is released and can replicate in the bursa like any other regular live vaccine.

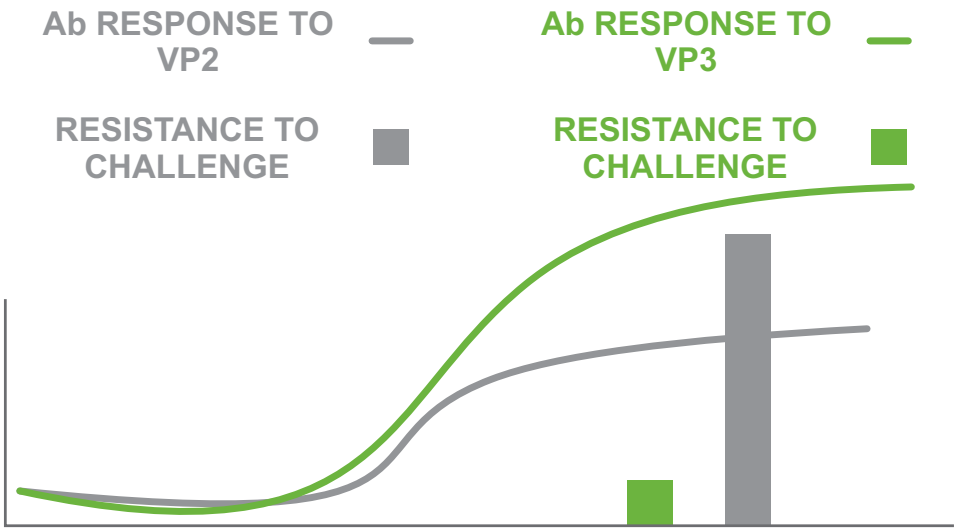
Based on numerous, global field trials, the key benefits seen with the use of MB-1™ are:

- It is effective across a wide range of Maternal Antibody titres. To date the range is between 4600 and 11 000 (IDEXX ELISA).
- The onset of immunity is at least 4 days earlier compared to the current hatchery IBD vaccines available in the market. The onset of immunity seen with MB-1™ is very similar to that seen with TAbic® M.B. applied on farm.
- The use of MB-1™ does not lead to immunosuppression or severe bursal damage.
- MB-1™ given In-ovo has no effect on hatchability or chick quality.
- Performance parameters in birds vaccinated with MB-1™ are equal to, or in many cases better than, those seen with the use of other hatchery and on farm IBD vaccines.

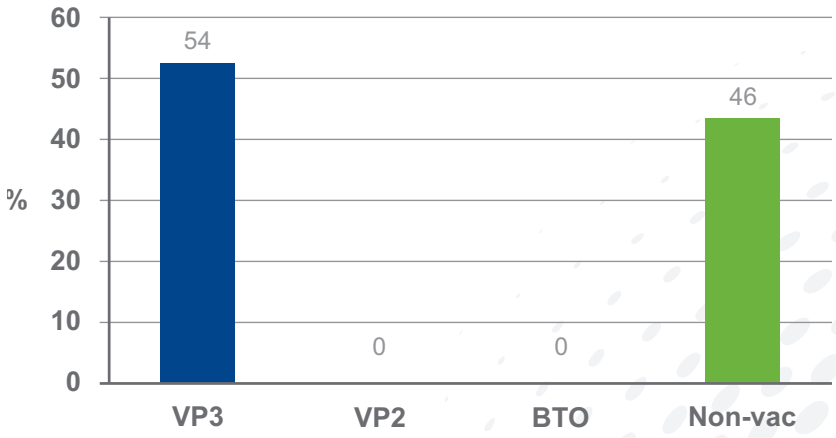
VP2 sub-unit vaccine

As mentioned earlier, antibodies targeting the VP2 protein of the IBD virus are the main neutralizing antibodies. The VP3 capsid protein stimulates much higher levels of antibodies than VP2 but they are not neutralizing i.e. they do not contribute to protecting the bird against field challenges.

The graph below illustrates the differences between the VP2 and VP3 responses.



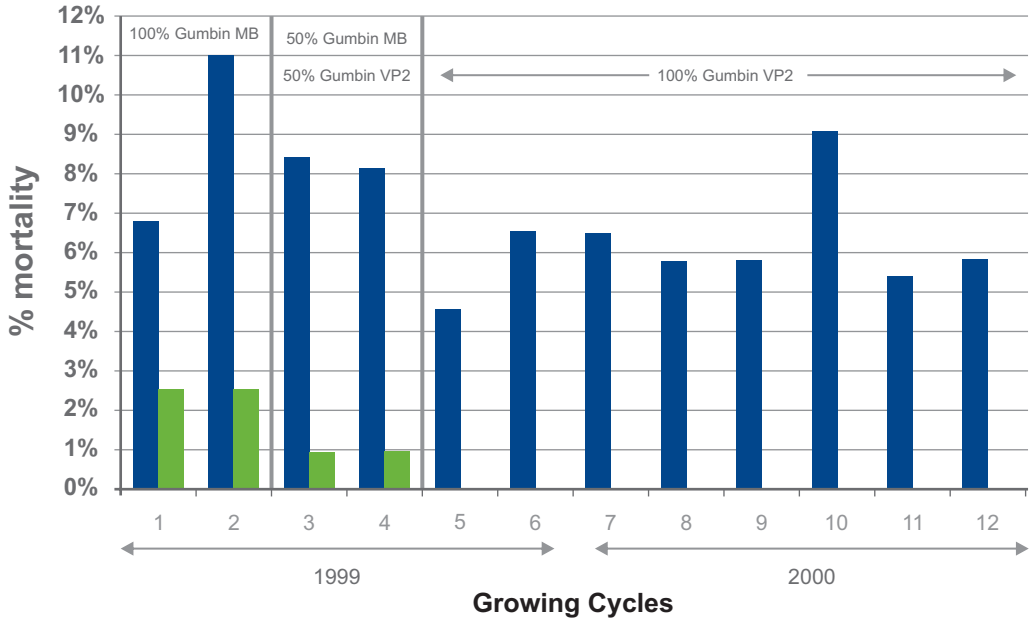
The graph below shows the levels of protection against a virulent field strain challenge in birds vaccinated with a VP3 sub-unit vaccine, a VP2 sub-unit vaccine and a normal Bursa tissue derived (BTO) vaccine (all vaccines are derived from the M.B. vaccine strain):



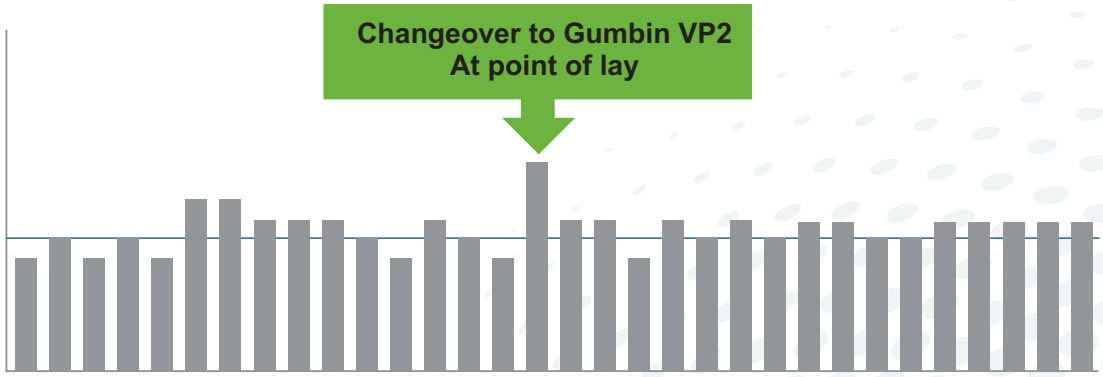
After much research, Phibro has perfected a way to produce a purified IBD VP2 protein, in a biotechnology process, for inclusion in our range of inactivated vaccines. When injected in a bird, it stimulates the production of only the protective VP2 antibodies and, therefore, also a highly specific immune response in the bird that ensures good protection against field challenges.

The graph below shows the impact of the better protection seen with the use of a VP2 vaccine compared with a BTO vaccine - measured by the % of IBD related mortalities on a farm over time:

Total Mortality Gumboro mortality (%) in one farm in Israel (Ross) 1999-2000



Results from a Broiler Breeder producer in South Africa show that the use of the VP2 sub-unit vaccine over time has also helped to stabilize their Gumboro titers when compared to the titers seen with the use of a normal commercial BTO vaccine:

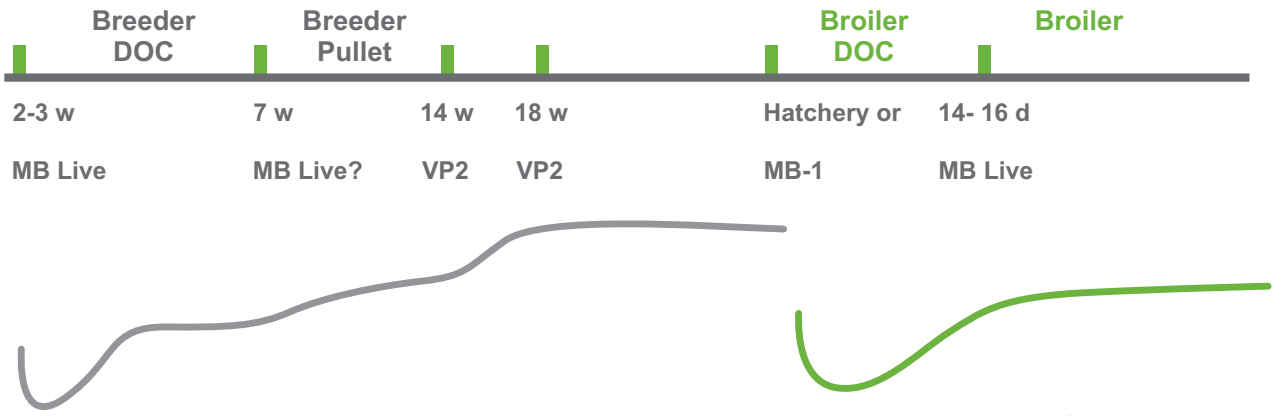


Currently in South Africa, the VP2 sub-unit vaccine is only available in combination with the ND V.H. strain and marketed as Gumbin VP2

In summary, therefore, M.B.:

- **Offers broad spectrum protection against virulent and variant IBD field strains.**
- **Multiple routes of application make it suitable for use on-farm and in hatcheries.**
- **Provides a rapid onset of protection.**

Vaccination program options



TAbic M.B. Reg. no.: G3402 (Act 36/1947)
MB-1 Reg. no.: G4209 (Act 36/1947)
Gumbin VP2: Reg. no.: G3696 (Act 36/1947)

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