Innovative diagnostic and prevention tools for infectious bursal disease

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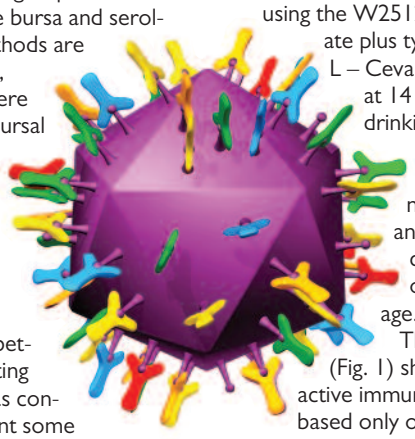
Any effective prevention program starts with a proper diagnosis of the disease and it applies to infectious bursal disease (IBD) as well. For several years, IBD diagnosis was based mostly on the evaluation of clinical signs, post-mortem, histological lesions of the bursa and serology. Although these methods are useful in some situations, mainly in those ones where very virulent infectious bursal disease virus (vvIBDV) is involved, they present clear limitations to diagnose more subtle presentations of this disease.

Moreover, the poultry industry has looked for better solutions for vaccinating their flocks against IBD as conventional vaccines present some shortcomings. They can be neutralised by maternally derived antibodies (MDAs) against IBD and there are also the known limitations regarding the method of vaccine administration in the farms.

In recent years, new tools have been made accessible for better diagnosis of IBD and new vaccines are now available for better prevention of this disease. This article discusses these issues.



Schematic representation of immune-complex vaccine.

Innovative diagnotic tools

Serology has been used for many years as a tool to monitor the seroconversion after vaccination against IBD. In fact, it assesses indirectly the replication of the virus in the bursa by measuring the antibodies induced by IBD virus. However, serology has its limitations as it is not able to unquestionably distinguish between the immunity induced by a vaccine virus or a field infection.

With the use of molecular techniques, such as Reverse Transcription Polymerase Chain Reaction (RT-PCR) together with analysing the PCR product by sequencing or by Restriction Fragment Length Polymorphism (RFLP), it is possible to identify which virus(es) has been present in a certain flock and triggered the immune response.

The importance of the use of these new techniques is clearly demonstrated in a field trial conducted by Gardin and co-workers. Six broiler flocks, identified as SF, AC, AB, EC, LB and CZ, were vaccinated against IBD using the W2512 strain-based intermediate plus type vaccine (CEVAC IBD L – Ceva Santé Animale – France) at 14 days of age through drinking water.

One flock, identified as PM, was left unvaccinated against IBD. Serum and bursa samples were collected weekly from one day to 42 days of age.

The serological results (Fig. 1) show that all flocks had an active immune response. However, based only on these results, it would not be possible to distinguish the vaccinated flocks from the unvaccinated one.

By analysing the bursas using molecular tools (Table 1), it was shown that the seroconversion detected in PM flock was actually induced by a field virus. There was, however, no clinical evidence of the infection since the virus (G15) detected caused only subclinical disease.

Farm	Laboratories	IBDV strain
PM	Lab S	G 15
	Lab P	NT
SF	Lab S	G 3
	Lab P	W 2512
AC	Lab S	G 3
	Lab P	W 2512
AB	Lab S	G 3
	Lab P	W 2512
EC	Lab S	G 3
	Lab P	W 2512
LB	Lab S	G 3
	Lab P	NT
CZ	Lab S	G 3
	Lab P	W 2512

G3: includes the W2512 strain
G15: Brazilian subclinical IBDV variant typre
NT: Not tested

Table 1. Results of molecular biological tests.

More importantly, based on these findings, it is possible to extrapolate that the active immunity observed in vaccinated flocks is not always induced by the applied vaccines.

In some cases, the seroconversion is induced by field virus and ultimately it means that the vaccination failed. The use of mole-

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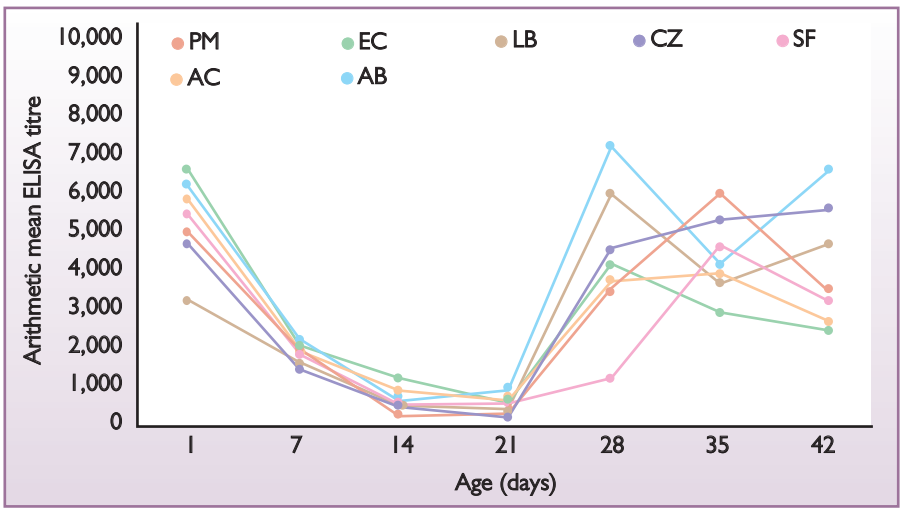
Fig. 1. Serological results.



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cular tools can identify such cases and they can help veterinarians and technicians to improve the vaccination program and procedures.

These molecular tools can also be used to investigate the mechanism of protection of IBD vaccines. Palya and co-workers evaluated the time needed for the development of protection against vvIBDV infection after the vaccine take.

Three week-old SPF chickens were vaccinated with one full dose of an intermediate plus type vaccine (CEVAC IBD L, Ceva Santé Animale, France).

Challenges were carried out at 2, 3 and 4 days post-vaccination with $10^{5.0}$ EID_{50}/dose of a vvIBDV strain (D407/2/04TR) per os.

At challenge, blood and bursa samples were taken for serology (virus neutralisation) and histology, respectively.

At four days post-challenge, blood and bursa of Fabricius were collected for serological, histopathological and PCR-RFLP analysis. The results are summarised in Table 2. For those chickens challenged at two days post-vaccination, the histological results showed that IBD virus was already replicating in four out of five bursa of Fabricius and the serology was positive for only one out of five birds.

Challenge date	At challenge		4 days post challenge	
	Histopathology (positive/ tested)	Serology (positive/ tested)	PCR (positive/ tested)	RFLP (vaccine/ vvIBDV)
2 days post vacc.	4/5	1/5	5/5	5/0
3 days post vacc.	5/5	3/5	5/5	3/0*
4 days post vacc.	4/4	4/5	5/5	5.0

**only three samples were tested by RFLP*

Table 2. Results of serology, histopathology and PCR-RFLP.

At three and four days post-vaccination, 100% of the bursas were affected by the IBD virus replication and the serology was progressively turning positive. Four days post-challenge, the PCR was positive for all samples and the RFLP reaveled that it was the vaccine virus which was replicating in the bursa.

As a result, the vaccine protected all animals from death/clinical signs as soon as vaccine-take was detected (two days post-vaccination) even in those chickens in which the serology was still negative. Moreover, it is interesting to observe that intermediate plus vaccine virus can actually inhibit the replication of vvIBDV right after it has entirely colonised the bursa.

These findings explain a common doubt among producers. Whenever a flock is monitored by ELISA weekly, it is not unusual to find very low titers between the third to fourth weeks of age even in properly vaccinated flocks. This situation could lead less experienced technicians to believe that the flocks are not protected. In fact, the chickens are protected as soon as the vaccine virus replicates in the bursa of Fabricius (vaccine take) even without detectable ELISA antibodies.

New vaccines against IBD

Along with the introduction of more accurate diagnostic tools, the poultry industry has continuously looked for better solutions of protecting their flocks against IBD since conventional vaccines can be neutralised by MDAs and the limitations of the drinking water method of administration can affect their efficacy.

The hatcheries are considered the ideal place to administer vaccines against IBD. However, conventional IBD vaccines would be promptly neutralised by the MDAs. In order to overcome this problem, two distinct and sophisticated types of IBD vaccines were developed: immune complex and vectored vaccines.

Immune-complex vaccines are based on a well balanced combination of an IBD vaccine virus and its homologous hyper-immune serum in order to limit and postpone the virus's effects, thus ensuring that the vaccine

virus is harmless to chicks with low level of MDA and, at the same time, to protect the vaccine virus from being neutralised by the MDA. In the case of vectored or recombinant vaccines (rHVT-IBD), the genes which encode the VP2 protein of the IBD virus (donor) are inserted into a non-essential region of the turkey herpesvirus (HVT) DNA (vector) which is the most widely used vaccine against Marek's disease. When the HVT replicates, it will express the VP2 protein and the immune system of the chickens is stimulated by the immunologically relevant antigens of both viruses, HVT and IBD.

There are several differences between these two concepts. However, the most important point is related to the way the protection is built up by each one of them.

For immune-complex vaccine, the antigen-antibody complexes enter the bird's blood circulation and, upon reaching the primary lymphoid organs (bursa and thymus) and spleen, these complexes are captured by the follicular dendritic cells present in those organs.

However, the antigen-antibody complexes bound to the follicular dendritic cells are not permanently stable. With time, the specific antibodies will detach from the complex and the viral particles are then released intact into the blood circulation. If there are still circulating maternal antibodies, the vaccine virus will be rapidly identified and neutralised by them.

As time passes, the levels of MDA diminish through natural metabolisation. In this way, the vaccine virus released from the antigen-antibody complexes will be free to reach the bursa of Fabricius where it starts replicating and initiate the production of protective antibodies against Gumboro disease.

In the case of vectored IBD vaccine, the protection is directly related to replication of the vector virus as the VP2 protein of the IBD virus is expressed during the replication of the rHVT-IBD. After the inoculation, the recombinant virus infects the targeted cells in different tissues and replicates at those sites, thus stimulating both cellular and humoral immune responses.

As a consequence, the protection induced by rHVT-IBD vaccine increases gradually in all injected chickens as a consequence of the vector virus replication.

These different mechanisms of action lead to distinct ways to protect the flocks against IBD. Basically, immune-complex vaccine adjusts itself to the level of MDA of each day-old chick.

It means that the replication of the vaccine virus in the bursa of Fabricius, and consequently the protection, will take place at the precise moment when the MDA drops to the level which allows the vaccine virus to reach the bursa.

On the other hand, the immunity developed by vectored vaccines increases gradually in all chickens depending upon the replication of the rHVT-IBD without taking into account the level of MDAs.

In common, these highly sophisticated vaccines, immune-complex and vectored vaccines, do not face any interference with MDAs and therefore they are appropriate for being used in hatcheries.

These products, administered either by in-ovo route or subcutaneous injection, afford life-long protection for broilers against IBD without any revaccination in the farms.

Conclusions

In an industry with rather narrow margins, any misdiagnosed condition can lead to unacceptable losses. Therefore, the use of more sophisticated diagnosis tools has become more common as their costs become accessible. Without a doubt, the proper use of molecular tools increases the chances of reaching a correct and conclusive diagnosis and therefore the right countermeasures can be taken by the technicians.

More importantly, in order to increase the chances of reaching a proper immunisation of the flocks, the poultry industry has largely adopted both immune-complex and rHVT-IBD vaccines.

These vaccines are not neutralised by MDAs and hence they are suitable for being used in the hatcheries. ■

References are available from the author upon request