Review

Recombinant vaccines and infectious bursal disease virus

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Abstract

Infectious bursal disease virus (IBDV) is the aetiological agent of infectious bursal disease (IBD), an immunosuppressive and highly contagious disease that affects young birds causing important economic losses in the poultry industry worldwide. Currently, vaccination programmes with inactivated and live-attenuated viruses have been used to prevent IBD. However, these vaccines present a number of disadvantages, mainly because of their viral nature. Consequently, in the last two decades, many studies have been conducted in order to replace conventional virus-based vaccines by new, rationally designed vaccines that are safer as well as effective. In this review, we will present a background on the disease and its causative agent, and focus on the development of new generation vaccines against this significant poultry disease.

Basic Concepts in Infectious Bursal Disease Virus (IBDV) and its Impact on the Chicken

Production of poultry products have increased significantly in the last few years as the demand for avian meat has gained ground over other meat types worldwide. The chicken is an economically relevant livestock animal, and poultry meat accounts for almost 40% of global meat consumption. In this context, sanitary conditions in commercial flocks are of utmost importance. Almost all chicken viral diseases are prevented by vaccination and infectious bursal disease (IBD) is not an exception.

IBDV belongs to the *Birnaviridae* family. It is a non-enveloped, icosahedral, double-stranded RNA virus and its genome is presented in two segments, A and B [1–3]. The segment A of the IBDV genome has two open reading frames (ORFs): one ORF encodes a polyprotein, pVP2-VP4-VP3, which undergoes cleavage by the viral protease VP4; and the other, encodes a small protein called VP5 of controversial function. The autoproteolytic processing of the polyprotein yields pVP2, VP3 and VP4 products [4]. Then, the precursor pVP2 (512 aa) matures to generate VP2 (441 aa) and four peptides that remain associated with VP2 within the virion [5]. The last step of maturation of VP2 also requires interaction with VP3 and it is supposed to occur during viral particle assembly [6].

IBDV is the aetiological agent of IBD, a highly contagious, worldwide-spread immunosuppressive chicken disease, also known as 'Gumboro disease'. The severity of the disease depends, in general, on the age of the bird infected and the virulence of the infecting virus. The clinical manifestation of IBD usually occurs in chickens between 3 and 6 weeks of age and has a sudden onset, with a rapid increase in the mortality rate of the affected flock. Clinical signs of the disease include dehydration, trembling, ruffled feathers, vent pecking and depression.

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More importantly, the disease can cause severe, long-lasting suppression of the immune system. Chickens that are immunosuppressed by early IBDV infections do not respond well to vaccination [7, 8] and are more susceptible to other diseases, including those that do not normally affect healthy chickens. On necropsy, the principal lesions are found in the bursa of Fabricius (BF). This organ is found exclusively in birds and it is a specialized and essential organ for the amplification and differentiation of B-cells.

Two serotypes of IBDV (1 and 2) have been described, being serotype 1 viruses the only ones pathogenic for chickens [9]. Serotype 2 viruses are generally isolated from turkeys and are non-pathogenic [10–12]. In increasing order of virulence, different pathotypes of IBDV within type 1 viruses were characterized as mild, intermediate, classical virulent and very virulent strains. Antigenic variant strains are also described; typically, they do not cause clinical signs of disease but they can cause a marked immunosuppression and they are eventually able to infect vaccinated chickens, because these strains are generally serologically different from the classic pathotypes [13–16].

After oral infection or inhalation, IBDV replicates in gut-associated macrophages and B-cells, causing a primary and transient viraemia. Subsequently, virus travels to the bursa by the blood stream, and massive virus replication occurs in this target organ shortly after inoculation, leading to a secondary and pronounced viraemia and causing lesions in other organs [17]. As the predominant feature of the pathogenesis of IBDV, infection results in extensive lymphoid depletion in the medullar and the cortical regions of the follicles of the bursa. In a lesser degree, damage of caecal tonsils and spleen can be seen. The arrival of the virus to the bursa and its replication is accompanied by a high T-lymphocyte (CD4+ and CD8+) infiltration that begins at 4 days post infection (d.p.i.) and reaches a peak at 7 d.p.i. Before influx of T-cells into the bursa, a strong up-regulation of pro-inflammatory cytokines such as interleukin (IL)-1b, IL-6, nitric oxide synthase and the chemokine IL-8 occurs at 3 d.p.i.

Infection with IBDV frequently results in immunosuppression, even in a subclinical course of the disease. This immunosuppression often provokes secondary infection of the respiratory tract, and unresponsiveness to live-attenuated vaccines against respiratory diseases such as infectious bronchitis, Newcastle disease and avian influenza [18–20].

Currently, when maternal antibodies have disappeared, vaccination against IBD with the inactivated and live-attenuated intermediate virus (used for commercial birds such as broilers/layers) serves to create an immunological state in the flock against virulent strains. However, these vaccines present a number of disadvantages because of their viral nature. Live-attenuated vaccines can revert to virulence by the recombination of genomic segments [21]; they usually produce a state of immunosuppression in young chickens (although it is a short period from which birds can recover); and, they can be, eventually, inefficient in protecting animals from very virulent strains and variant strains [13–16]. On the other hand, inactivated vaccines are costly and lack efficient immunogenicity unless they are adjuvanted and administered in multiple inoculations, or delivered as a boost after priming with a replicating antigen [22]. Generally, their use is constrained to breeder birds just before laying in order to induce high levels of transferred maternal immunity in the progeny. Thus, there is a genuine need for replacing the conventional virus-based vaccines by new ones that could solve these concerns, having higher efficacy and fewer side-effects.

A new technology being used in the field is immune complex vaccines, which are obtained by mixing a certain amount of specific antibodies obtained from the serum of hyperimmunized chickens with live IBD vaccine virus [23, 24]. Since it has been shown that these vaccines were effective in the presence of maternally derived antibodies (MDA) [25, 26], they are suitable for *in ovo* vaccination of 18–day-old embryonated chicken eggs with commercial egg-injection machines or delivery by subcutaneous injection in one-day-old chickens [27]. Both methods of administration present some advantages over conventional live vaccines, which are usually given via drinking water: they allow earlier immunity and more automated, uniform and systematic administration process [22]. Additionally, *in ovo* vaccination offers reduced labour cost and reduced stress to birds compared with the subcutaneous route.

New generation vaccines act upon the immune system in different ways depending on the type of vaccine. The election of the production system of a recombinant vaccine will depend on the immune response that needs to be elicited. The development of recombinant vaccines includes the study of the immune response against the pathogen for which it is designed. In this way, it would be possible to design an immunogen that fulfils the requirements for rendering the best performance against IBDV. Given its importance in eliciting the antibody response, VP2 has been extensively used to develop recombinant vaccines against IBDV, as will be seen in the following section.

Recombinant Subunit Vaccines

Advances achieved in the fields of molecular biology, genetic engineering, crystallography and immunology, have allowed the genetic and structural characterization of several pathogens, identifying the epitopes or protein regions responsible for inducing a protective immune response. This knowledge promoted the development of new pathogen-free vaccines named recombinant subunit vaccines, where only the immunogenic element of a pathogen is delivered to the host for stimulating a specific immune response. In this context, the new generation

vaccines to combat Gumboro disease are based on: protein immunogens, live vectors and nucleic acids [22, 28]. Table 1 recapitulates some of the most important properties of each strategy, as well as the major immunological response elicited by each system. As can be seen, each strategy presents particular features that have to be taken into consideration when selecting a method for a new development. However, a common feature is that all recombinant subunit vaccines have a defined chemical composition and low risk of pathogen escapes because of manipulation during the production stage.

Table 2 summarizes almost all of the studies found in the literature where the immunogenic properties of recombinant VP2 are assayed. Recombinant vaccines are organized as follows: DNA vaccines, live delivery system (viral and bacterial vectors) and protein immunogens, where VP2 is present in the soluble form or forming particles.

Naked plasmid DNA and live viral or bacterial vectors carrying either *vp2* or *vp2-vp4-vp3* genes have been assayed as vaccines against IBDV since they present a number of advantages as cost-effective production and are capable of eliciting both humoral and cellular immune responses (Table 1). However, the levels of protection produced by these vaccines have been variable, ranging from partial to complete protection against IBDV challenge. DNA vaccines are based on DNA encoding the target gene that is then injected into an animal. Animal cells capture these plasmids and incorporate them into the cell nucleus, allowing the expression of the foreign gene and the production of the desired protein [73]. The possibility of inducing an effective immune response by this strategy has been demonstrated, although with variable success [31–39]. The *in ovo* inoculation with DNA vaccine without a boost was insufficient to evoke the protective immunity [39].

Live vector vaccines are based on the use of a live micro-organism that acts as a vector for the expression of heterologous genes. An interesting application of vectored vaccines is the possibility of dealing with two diseases using the same sanitary tool. This is the case, for example, of the use of recombinant turkey herpes virus (HVT) as a vectored vaccine for viral avian diseases. HVT has the ability to confer protection against Marek's disease (MD). A recombinant HVT carrying the *VP2* gene of IBDV was constructed and successfully assayed as a live vaccine against both illness, MD and IBD. Vaxxitek HVT+IBD (Merial) [49, 50] and VECTORMUNE® HVT IBD (CEVA Santé Animale) are two commercially products based on HVT serotype 3 that are already being used in the field and they have proved to be safe and efficient. These vaccines do not show interference with MDA and in consequence, they are indicated to be applied in one-day-old chickens by the subcutaneous route or in 18-day-old embryonated chicken eggs. Remarkably, chickens first immunized with HVT vaccines should not be vaccinated again with these recombinant vaccines because the former vaccination has a major inhibitory effect on the efficacy of the later ones [49]. Nowadays, the number of eggs and chickens inoculated with these viral vaccines is increasing and the new technology for the *in ovo* vaccination supports the scale up.

Protein immunogens have also been used as subunit vaccines against IBDV where soluble or non-soluble VP2 is delivered to the host. The most commonly used expression systems to produce VP2 at large scale are *Escherichia coli,* yeast and baculoviruses. While *E. coli* has the advantage of being the fastest, cheapest and high-yielding expression system, post-translation modifications and correct folding of proteins can be problematic to obtain and this may affect the immunogenicity and protective efficacy of the product. Hence, other expression system, although more costly, have been employed to produce recombinant proteins. However, vaccination with the isolated antigen obtained by these expression systems supposes the use of adjuvants and more than one inoculation as the protein itself is a poor immunogen. Nevertheless, industry has taken profit of the technology. An example that arrived to the market is the commercial vaccine Gumbin® VP2 (Phibro Animal Health Corporation), a water in oil bivalent vaccine, that contains high concentrations of IBD VP2 produced in yeast [58]. This vaccine is intended for vaccination of chickens after priming with live Newcastle disease and live IBD vaccine, although it can be used in broiler, layer and breeder chickens any age starting from day one.

As can be observed in Table 2, delivery of non-soluble VP2, whether it is displayed in a vector or forming particles, have proved to be more efficient in eliciting an immune response than the soluble protein. VP2 is present in the capsid of the virion forming trimmers in which each subunit folds into three domains named base (B), shell (S) and projection (P) [74]. In the P domain, four loops harbouring the conformational epitopes responsible for eliciting the neutralizing response (loops P_{BC} and P_{HI}) and the regions involved in cell tropism and virulence (loops P_{DE} and P_{FG}), are identified [75–77]. It was reported that recombinant bamboo mosaic virus displaying loop P_{BC} region on the coat protein was successful in eliciting a protective response with a 100% survival rate of chickens challenged 28 days after the intramuscular immunization [68].

The expression of VP2 or the polyprotein (pVP2-VP4-VP3) in a heterologous system may lead to the generation of subviral particles (SVP) and virus-like particles (VLP), respectively, which basically differ in size but conserve the immunogenic properties. VLPs mimic the conformational structure of the infectious virus from which they derive but they are highly safe as they are non-infectious because they lack the viral genome. As these particles present a redundant antigenic structure they are highly immunogenic, being a promising candidate for the development of alternative vaccines. The baculovirus and

Table 1 Comparison of crucial properties of different vaccine types1

Vaccine type	Composition	Pathogenicity	Cost	Immune response elicited	Vaccination scheme	Other characteristics
Whole cells vaccines						
Live, attenuated vaccines	Undefined	Risk of reversion to virulent strain. Certain risk of transmission	Low	Both humoral and cellular responses	One or few doses normally required. No need for adjuvants	Long-lasting immunity. Controlled attenuation normally required
Killed, inactivated vaccines	Undefined	No risk of reversion to pathogenicity. No risk of transmission	Moderate	Mainly humoral responses	Multiple doses typically required. Adjuvants normally needed.	Less powerful than live vaccines
Recombinant subunit vaccines						
DNA vaccines	Defined	No risk for pathogenicity	Lower than conventional vaccines	Both humoral and cellular responses.	Multiple doses typically required.	*In vivo* amplification systems. Inefficient transfection. Risk of integration into genome not completely excluded.
Live vectors vaccines	Defined	Risk of reversion to virulent forms when using attenuated pathogens as carriers	Moderate	Both humoral and cellular responses	One or few doses normally required. No need for adjuvants	Possibility to develop oral vaccines with live bacterial vectors
Protein immunogens	Defined	No risk for pathogenicity	Generally expensive. Cost depends on production system	Mainly humoral responses	Multiple doses typically required. Adjuvants needed	Soluble proteins are poor immunogens. Immunogenicity can be enhanced by VLPs or antigen display delivery systems

1Adapted from [29, 30].

Table 2 Developments in recombinant IBDV vaccines

Recombinant vaccine	Expression system	IBDV antigen	Delivery route	Protective efficacy against challenge	Reference
DNA vaccines					
		vp2	Intramuscular	52% protection was defined as the percentage of chickens with no or mild BF lesions	[31]
		vp2-vp4-vp3	Intramuscular	90–100% when chickens were vaccinated three times with 7.5 or 10 mg of plasmid. Lower doses or fewer immunizations could not elicit protection against challenge. Protection was evaluated by bursal weight/body weight (B/B) ratio and gross and histopathological bursal lesions examination	[32]
		vp2 and vp2-vp4-vp3	Intramuscular	15 and 30% when chickens were vaccinated three times with VP2 or VP243 plasmids, respectively. Protection was evaluated by mortality, gross and histopathological bursal lesions and B/B ratio	[33]
		vp2	Intramuscular	64% protection was evidenced by absence of clinical signs, atrophy of BF and mortality	[34]
		vp2-vp4-vp3, vp2, vp2-vp4, vp4, vp3, vp3-vp4	Intramuscular	80% with VP243, VP2 and VP24 plasmids and 0% with VP4, VP3 and VP34 plasmids. Protection was evaluated by mortality, gross and histopathological bursal lesions and B/B ratio	[35]
		vp2-vp4-vp3 and vp2	Intramuscular	66.6 and 20% with 200 µg of VP243 and VP2 plasmids when homologous virus was used in challenge and 40 and 0%, respectively, when heterologous virus was used in challenge. Protection rate was initially calculated based on gross bursal lesions and further confirmed by bursa histopathological examination	[36]
		vp2 and vp2-vp4-vp3	Intramuscular	50 and 70% survival rate and less bursal damage compared with unvaccinated group, with VP2 and VP243 plasmids, respectively	[37]
		vp2	Intramuscular	80% survival rate and 0% morbidity and gross bursal lesions observation	[38]
		vp2-vp4-vp3	In ovo	100 and 20% survival rate when in ovo prime with DNA vaccine was followed or not with killed-vaccine boost, respectively	[39]
Live delivery system					
Viral vectors	Adenovirus	VP2	Different delivery routes were evaluated	100% protection when CELO-VP2 vector under the CMV promoter was used and following intradermal or subcutaneous double (or single) inoculation(s) of chickens. Other vectors, delivery routes and vaccination schemes were evaluated yielding lower protection rates	[40]
	Adeno-associated virus	VP2	Intramuscular	80% survival rate and absence of IBD clinical signs in surviving chickens	[41]
	Fowlpox virus	VP2	Wing-web puncture	78% protection was defined as the percentage of chickens with no or mild BF lesions	[42]
	Fowlpox virus	VP2	Wing-web puncture	60–100% protection against different challenge titres in various chicken lines. Protection was evaluated based on the degree of bursal damage	[43]
	Fowlpox virus and MD virus	VP2	Subcutaneous	0 and 13% protection against gross bursal lesion and 33 and 42% protection against histopathological BF lesion with recombinant Fowlpox virus (rFPV) and recombinant Marek's Disease virus (rMDV), respectively. Co-administration of both viral vectors yielded 14 and 53–73% protection against gross and histopathological bursal lesions respectively	[44]
	MD virus	VP2	Subcutaneous	100% survival rate and 55% protection defined as the percentage of chickens with no or mild BF lesions	[45]

Table 2 (Continued)

Recombinant vaccine	Expression system	IBDV antigen	Delivery route	Protective efficacy against challenge	Reference
	MD virus	VP2		87 and 100% survival rate, and 53 and 73% protection defined as the percentage of chickens with no or mild BF lesions after vaccination with 10^3 and 10^4 PFU, respectively	[46]
	MD virus	VP2	Subcutaneous	50, 60 and 80% when chickens were vaccinated with 1000, 2000 and 5000 PFU of rMDV, respectively. Protection ratio (%)=(mortality of unvaccinated rMDV-challenged chickens − mortality of vaccinated rMDV-challenged chickens)/mortality of unvaccinated rMDV-challenged chickens×100%	[47]
	Newcastle disease virus	VP2	Eyedrop	80–100% protection was determined by the absence of viral antigen in bursa 3 days post challenge	[48]
	HVT	VP2	Subcutaneous	100% when chickens were raised in experimental conditions. Chickens raised in field conditions had lower protection. Protection was determined based on the absence of gross bursal lesions and B/B ratio	[49]
	HVT	VP2	Subcutaneous	93% protection was defined as the percentage of chickens with absence of bursal atrophy	[50]
	HVT	VP2	Subcutaneous	Protection based on the absence of bursal atrophy and mild histopathological bursal lesions was observed in specific pathogen free (SPF) chickens and commercial broilers	[51]
	HVT HVT	VP2 VP2	Intramuscular Subcutaneous	100% protection was evaluated by gross bursal lesions examination 100 and 58% when chickens were vaccinated with rHVT-pecVP2 or rHVT-cmvVP2, respectively. Protection was evaluated by gross bursal lesions examination	[52] [53]
Bacterial vectors	E. coli	VP2	Oral	73, 91 and 95.4% when chickens were vaccinated with 10^7, 10^8 and 10^9 CFU, respectively. Protection was assessed on the basis of gross bursal lesions and confirmed by bursa histopathological examination	[54]
	E. coli	VP2	Intramuscular	85.7% protection was defined by the percentage of chickens no or mild BF lesions	[55]
	S. typhimurium	VP2-VP4-VP3	Oral	73.3% protection was initially calculated based on gross bursal lesions and further confirmed by bursa histopathological examination	[56]
Protein immunogens Soluble protein	Fowlpox virus	VP2	Intramuscular	17–100% depending on vaccine concentration and vaccination scheme. Protection was defined as the percentage of chickens with no or mild BF lesions	[57]
	Pichia pastoris	VP2	Intramuscular	100%. Protection was determined by the absence of IBDV in bursa 3 days post-challenge and the degree of bursal atrophy	[58]
	E. coli	VP2	Intramuscular	90–100%. Protection was determined by the absence of IBDV in BF 4 days post-challenge	[55]
	E. coli A. thaliana	rVP252–417 VP2	Intramuscular Subcutaneous and oral	100%. Protection was defined as the percentage with no or mild BF lesions 60 and 80% with subcutaneous and oral vaccine respectively. Protection was determined based on B/B ratio	[59] [60]

	Expression system	Protein	Route	Results	Reference
	Saccharomyces cerevisiae	VP2/VP4/VP3 and VP2	Intramuscular	100% passive protection. All chickens injected i.p. with chicken antisera to native VP2a/2b were protected against challenge. Protection was assessed by the absence of viral antigen in the bursa 3 days after challenge.	[61]
	E. coli	VP2	Intramuscular	20 and 57% survival rate when chickens were vaccinated with 50 µg of purified VP2 protein or 150 µg of crude VP2 protein, respectively	[62]
	E. coli	Multi-mimotope peptide 5EPIS	Intramuscular	100 and 40% survival rate when 5EPIS was administered with or without adjuvant, respectively. Surviving chickens did not show clinical signs and pathological lesions of IBDV infection	[63, 64]
Non-soluble protein	Rice	VP2	Oral	16.6, 33.3 and 83.3% after vaccination with 1, 3 or 5g of transgenic rice seeds, respectively. Protection was assessed based on the absence of gross bursal lesions and BF histopathological examination	[65]
	Baculovirus display	VP2	Intramuscular	100% survival rate and 90% protection defined as the percentage of chickens that remained healthy and had no or mild gross bursal lesions	[66]
	T4 bacteriophage display	VP2	Subcutaneous priming and intramuscular boost	100% survival rate and no clinical signs of IBD	[67]
	Bamboo mosaic virus	VP2 P domain loop P_{BC}	Intramuscular	100% survival rate	[68]
	Semliki forest virus	VP2/VP2-VP4-VP3	Intramuscular	No challenge	[69]
	Baculovirus	N5-452H SVP	Intramuscular	Neither mortality nor presence of virus in bursa after challenge with very virulent IBDV	[70]
	Baculovirus	VP2, VPX or VP234 VLPs	Subcutaneous	100% survival rate and no severe clinical signs after vaccination with 25 µg of the purified VLPs. At lower doses, VP2 VLPs had higher protective efficacy than VP234 VLPs	[71]
	E. coli	VP2-VP4-VP3 VLPs	Intramuscular	91% survival rate although bursal atrophy similar to the unvaccinated group was observed	[72]
	E. coli	HBc-5EPIS, VLPs	Intramuscular	100% survival rate. Surviving chickens did not show clinical signs and pathological lesions of IBDV infection	[64]

E. coli expression systems have also been used to obtain these particles as vaccines. Baculoviruses are able to invade mammalian or avian cells; however, they are not known to initiate a replication cycle and producing infectious virus in these hosts [78, 79]. Baculoviruses are viruses that can infect insects and replicate in insect cells, resulting in a very safe system for producing proteins to be used in superior organisms. The presence of any trace of baculovirus in the formulation has no harmful effect on the host receiving the vaccine; on the contrary, residues of baculovirus can even work as immunomodulators or as antivirals in chickens [80, 81]. These newly discovered features of baculoviruses may have also contributed to the success of such recombinant vaccines.

Among the different strategies to generate vaccines against the disease, the construction of recombinant virus is the most widely-used approach. This may be because of the advantage of obtaining bivalent vaccines, the auto-replicative attribute of the viral vectors, or even because of the type of immune response (cellular response) that viruses are able to elicit. Nevertheless, they must be inoculated by the subcutaneous route, and they must be stored in liquid nitrogen. Conversely, the method exploited to a smaller extent is the plant expression system. Molecular farming, which refers to the use of transgenic plants for the expression of different antigens has been increasingly employed for the production of experimental immunogens. For different reasons, the design of efficient edible vaccines by means of transgenic plants represents a challenging alternative to the conventional ones [82]. The simplicity of their production, handling and administration makes them an attractive option for developing affordable vaccines. In addition, products from transgenic plants are unlikely to be contaminated by animal pathogens, microbial toxins or oncogenic sequences [83, 84]. Several authors have reported antibody response to parenteral or oral administration of plant-derived antigens [85–91]. The use of this expression platform was also described for IBD, where transgenic *Arabidopsis thaliana* [60] or rice seeds [65] expressing VP2 were assayed as oral vaccines. Results show a dose-dependent efficacy, although a 100% of survival rate was not achieved and several doses of the immunogen were needed. It is worthy to notice that VP2 was resistant to gut degradation.

Concluding Remarks

The main issues that the development of recombinant vaccines needs to tackle are basically: safety, cold chain and differentiation between infected and vaccinated animals. New generation vaccines act upon the immune system in different ways, depending on the type of vaccine but the most important advantage of all of them is that they lack the infectious agent. This feature not only eliminates the possibility of infection but also makes possible the differentiation between infected and vaccinated animals, since diagnostic strategies can be approached for the detection of antibodies against specific proteins that allow this discrimination, which is of utmost importance during disease eradication campaigns. Subunit and synthetic peptides vaccines do not require cold chain as conventional vaccines do. This advantage makes it possible to reduce costs at the time of vaccine storage and distribution.

In this context, IBDV recombinant vaccines that include VP2 antigen have been successful in providing protection against IBDV infection, depending on the delivery route and dose of the vaccine, without the risk of reverting to virulence. Nevertheless, the cost-effectiveness and commercial viability of these vaccines is still an issue. We hope to witness an increase in the number of commercially available IBDV recombinant vaccines and the actual adoption of this so useful technology by the poultry industry in the near future.

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