SciMedCentral

Journal of Veterinary Medicine and Research

Original Article

Comprehensive Evaluation of Infectious Bursal Disease Vaccine Candidate According To the Current Industry Standards

Anto Vrdoljak[1*], Olga Zorman Rojs[2], Gert Jan Boelm[3], and Lana Ljuma Skupnjak[1]

[1]*Genera Inc., Rakov Potok, Croatia*
[2]*University of Ljubljana, Veterinary faculty, Ljubljana, Slovenia*
[3]*GD Animal Health, Deventer, The Netherlands*

***Corresponding author**

Anto Vrdoljak, Genera Inc. Svetonedeljska cesta 2, Kalinovica, HR-10436 Rakov Potok, Croatia, Tel. +385-1-3388635, Email: anto.vrdoljak@dechra.com

Submitted: 23 November 2018

Accepted: 12 December 2018

Published: 13 December 2018

ISSN: 2379-948X

Keywords

• *Infectious bursal disease; Vaccine; Pharmacopoeia*

Abstract

Vaccination of chickens using live attenuated vaccines is still the principal method used for control and prevention of infectious bursal disease (IBD) in chickens. All aspects of development, production, quality control and use of modern vaccines are comprehensively regulated by the national and trans-national guidelines. Detailed in vitro characterization, safety and efficacy of a new live attenuated vaccine against IBD was evaluated in this work in compliance with the current European Union regulations. In vitro characterization confirmed that the vaccine strain belongs to intermediate strains with high degree of homology with the existing vaccine strains of the same virulence. General safety of the vaccine was demonstrated in specific pathogen free (SPF) and seropositive chickens. No significant immunosuppression against immunization with live Newcastle disease vaccine was detected in birds previously vaccinated with the IBD vaccine candidate. Vaccine virus was able to spread at least 3 times from vaccinated to naive chickens, without inducing morbidity and mortality. A single amino acid change at position H253Q was detected in the VP2 gene after 5 in-vivo passages. Complete protection against challenge with very virulent IBD virus was demonstrated in laboratory trials using SPF and seropositive chickens. Field trials on commercial broilers and layers confirmed the satisfactory safety and efficacy profile of the vaccine. These data demonstrate the safety and efficacy of the novel vaccine candidate but also illustrate by an example the regulatory pathway and complexity of the contemporary vaccine development process in the regulated environment.

ABBREVIATIONS

IBDV: Infectious Bursal Disease Virus; MDA: Maternally Derived Antibodies; LAV: Live Attenuated Virus; EMA: European Medicines Agency; Ph.Eur: European Pharmacopoeia; EC: European Commission; BBR: Bursa to Body Ratio; BLS: Bursa Lesion Scores; SPF: Specific Pathogen Free; $TCID_{50}$: Median Tissue Culture Infective Dose; DPI: Days Post Inoculation; NDV: Newcastle Disease Virus.

INTRODUCTION

Infectious bursal disease virus (IBDV) causes an immunosuppressive disease of young chickens that has lymphoid tissue as its primary target with a special preference for the bursa of Fabricius. IBD has been responsible for major economic losses in the poultry industry worldwide as virulent strains cause increased mortality and prolonged immunosuppression. Chickens immunosuppressed by early infection with infectious bursal disease virus (IBDV) do not respond well to vaccination and may be susceptible to infections with normally non-pathogenic viruses and bacteria [1].

Protection of young chicks from early infection is usually accomplished by transfer of maternally derived antibodies (MDA) to the newly hatched chick. As level of MDA will decrease over time, older birds need to acquire active immunity by vaccination. Vaccines that are available commercially include the live attenuated (LAV), inactivated, immune complex and live viral-vector vaccines. For their ease of use and affordability, most commonly used vaccines against IBD are LAV which may be classified according to their virulence as mild, mild intermediate, intermediate, intermediate plus, or "hot" [2]. The major problem with active immunization of young chicks with LAV is determining the proper time of vaccination due to the interference with MDA and potentially the transient immunosuppression which may be induced by the vaccine virus [3].

The regulatory requirements for registering veterinary vaccines have grown considerably over the past decades. Nevertheless, the result is the steady increase in the availability of vaccines of high quality with good safety profiles and proven efficacy. In the EU, the regulatory framework for vaccines is defined by the applicable documents of European Medicines Agency (EMA) [4], European Pharmacopoeia (Ph.Eur.) [5] and European Commission (EC) [6]. Consequently, the cost and time needed for new vaccine development, production, quality control, registration and marketing is increasing but the process also assures that newly registered products are safer and more efficacious than predecessors.

In this study an outline of the molecular characterization, as well as results of safety and efficacy studies performed using Avishield® IBD INT, new live attenuated vaccine against IBD based on the intermediate strain VMG91, are presented. The goal of the project was to develop the new intermediate IBD vaccine and characterize it according to the current European requirements. In addition, the complexity of the new vaccine development and levels of safety and efficacy which new vaccine candidates must accomplish to be granted with the marketing authorization is demonstrated.

MATERIALS AND METHODS

Vaccines and viruses

Avishield® IBD INT vaccine was provided by Genera Inc. (Croatia), part of Dechra Pharmaceuticals PLC (UK). The vaccine, based on intermediate attenuated IBDV strain VMG91, is commercially available and therefore complies with the OIE and Commission Decision 93/152/EEC concerning the virulence of vaccine strains, and with the requirements of the European Pharmacopoeia and Directive 2004/28/EEC concerning the quality, safety and efficacy of live poultry vaccines. For vaccination experiments, vaccine was reconstituted in sterile water and applied via either oral or eye-drop route in a dose specific for each experiment (see below). Vaccine strain VMG91 was passaged in vivo five times and produced material was designated as VMG91p. Commercially available live attenuated IBDV vaccine with intermediate strain was used as a comparator in field studies. The efficacy of Avishield® IBD INT was assessed using vvIBDV strain DV86. To assess the potential immunosuppression of Avishield® IBD INT as described in the Ph.Eur. monograph for live IBD vaccines, chickens were vaccinated with a Hitchner B1 strain of ND and challenged with very virulent ND virus strain Herts33/56 [7].

Nucleotide sequencing and analyses

Virus RNA was extracted using High Pure Viral RNA Kit (Roche) following manufacturer`s protocol. One-step RT-PCR was carried out using AffinityScript One-Step RT-PCR Kit (Agilent) according to the manufacturer's instructions. Forward primer IBDV-576s (5'-GCCAACATCAACGACAAAATTGGG3', nucleotides 576-599) and reverse primer IBDV-R (5'-ATCCTGTTGCCACTCTTTCGTAGG-3', nucleotides 1212-1189) were selected to amplify highly conserved part of VP2 genome region [8,9]. Sequencing of the PCR products was outsourced to Macrogen Europe (the Netherlands). The obtained chromatograms were verified using ChromasLite version 2.6.5 (Technelysium Pty Ltd.).

The nucleotide sequences obtained from IBDV strains in this report were deposited in GenBank with accession numbers MK109005 for VMG91 and MK109006 for five times in vivo passaged VMG91 (VMG91p). Obtained sequences were compared against previously published sequences for strains D78 (EU162087.1 and EU162090.1), F52/70 (D00869.2), DV86 (AJ878899.1), UK661 (X92760.1), Var E (AY819703.1), 228E (AF457104), Cu1 (D00867.1) and Lukert (FJ497057.1). The nucleotide and deduced amino acid sequences were aligned using T-Coffee online alignment software [10].

Phylogenetic tree was constructed based on 417 nt long sequences of the hypervariable VP2 regions of selected IBDV strains using neighbour joining algorithm.

In-use stability testing after reconstitution of freeze dried vaccine

In-use stability testing after reconstitution has been performed as described in the EMA Guideline [11].

Two lots of 2500 doses per vial and one lot of 1000 doses per vial presentation were tested at ambient temperature after reconstitution of the vaccine. The samples were reconstituted with sterilized tap water. Virus titre and appearance of reconstituted vaccine were tested up to 3 hours after reconstitution.

Animal studies

A set of laboratory and field animal studies required for registration of live IBDV vaccine in EU countries was performed in order to assess vaccine`s safety and efficacy. Studies were designed and conducted following specific and general guidelines outlined in relevant Ph.Eur. and EMA monographs and chapters. Details on the design of each specific study may be found in the documents cited in Table 1, with specific details briefly described in the Results section where applicable.

Animal experiments were conducted following national and European Union regulations regarding the use and protection of animals used for scientific purposes.

Statistical analysis

Differences in ELISA antibody titers, weight, and bursa to body ratio (BBR) were assessed using ANOVA. Differences in bursa lesion scores (BLS) were assessed using the Kruskal-Wallis test. Differences in morbidity and mortality were assessed using a proportion test.

RESULTS AND DISCUSSION

Molecular characterization of the IBDV VMG91 strain

Sequencing of the hypervariable region of VP2 gene (nucleotide positions 599-1189) was performed for the vaccine strain VMG91 and five times in vivo passaged VMG91 strain (VMG91p). Amino acid sequences were compared against 8 other relevant vaccine or field IBDV strains (Figure 1).

The nucleotide homology in the analysed hypervariable region of VP2 protein of the vaccinal strain VMG91 was 100% identical with the vaccine strain D78. In vivo passaging of VMG91 resulted in a single amino acid substitution His (H) → Gln (Q) at position 253. This particular position is known to be related to target-cell tropism and degree of virulence, in addition to other important positions [12,13]. Literature data suggest that a single change at position 253 alone is not sufficient to change the virus` degree of virulence but it requires at least one other change at position aa284 [13,14].

High homology was found between VMG91 and other attenuated/vaccine strains ranging from 98% similarity with Cu-1 (attenuated tissue culture-adapted strains) to 94% with vvIBDV strains DV86 and UK661.

Table 1: Summary of in vivo studies performed using VMG91 vaccine strain.

Study type	Laboratory or field study	Chicken breed	Design according to
Safety in SPF chickens	Laboratory	SPF layers	Ph.Eur. 04/2013:0587[7]
Safety: Damage to the bursa of Fabricius			
Safety: Increase in virulence			
Safety: Immunosuppression			
Safety: Dissemination and spreading			Ph.Eur. 04/2013:50206[25]
Efficacy in SPF chickens			Ph.Eur. 04/2013:0587[7]
Efficacy: Onset of immunity		Commercial layers	Ph.Eur. 04/2013:0587[7] Ph.Eur. 04/2013:0062[26] EMEA/CVMP/682/99[27]
Efficacy: Duration of immunity		Commercial broilers	
Safety and efficacy in the field conditions	Field	Commercial broilers	EMEA/CVMP/852/99[28] EMEA/CVMP/VICH/359665/2005[29] EMEA/CVMP/VICH/595/98[30]
Safety and efficacy in the field conditions		Commercial layers	

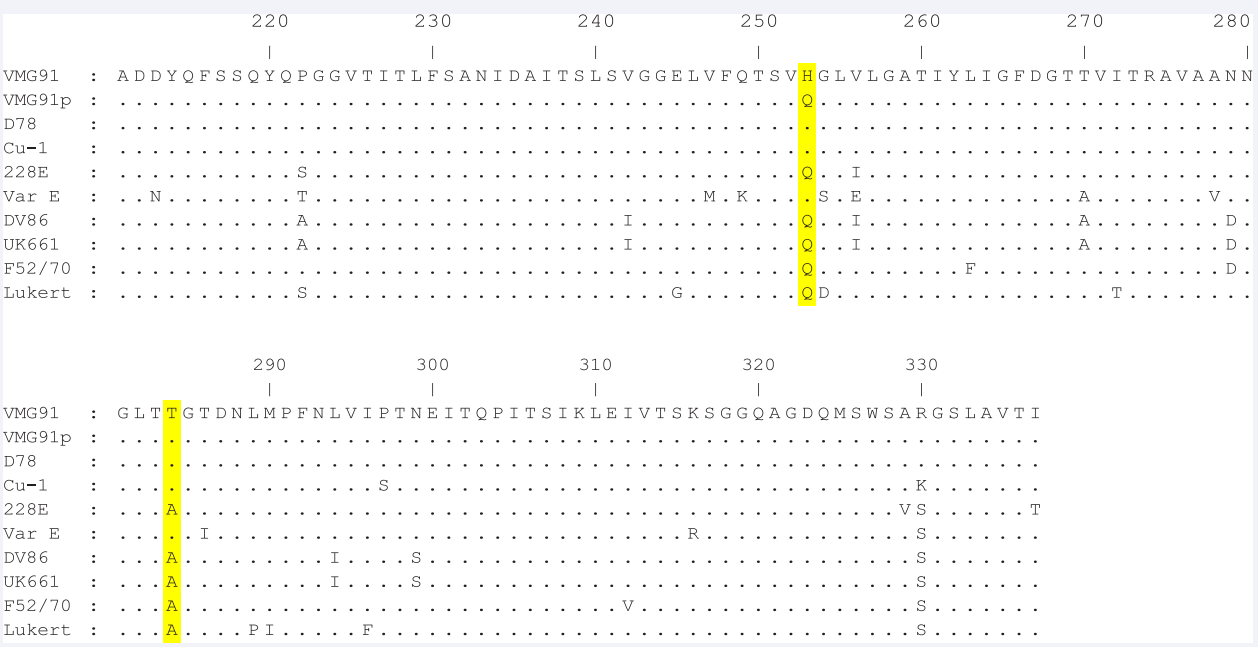

Figure 1 Alignment of the predicted amino acid sequences of VP2 hypervariable region (positions 212-338) of the vaccine strain VMG91, five times passaged strain VMG91 (VMG91p) and selected vaccine (D78, 228E, Cu-1) or field (F52/70, DV86, UK661, Var E, Lukert) IBDV strains. Highlights indicate positions 253 and 284 important for pathogenicity of IBDV and adaptation to growth on cell cultures[12].

To further characterize the vaccine strain, VP1 gene was partially sequenced. Region 131-624 nt, segment B VP1 gene, was compared for the VMG91, in vivo passaged VMG91p and existing vaccine strain D78. No changes in the nucleotide sequence were observed in the passaged material and 100% identity in the said region was confirmed between the samples (data not shown).

The phylogenetic tree based on nucleotide sequences (Figure 2) shows that VMG91 strain clusters together with related intermediate vaccine strains with high homology against similar classical attenuated vaccine strains [12].

Stability in solution

Vaccine stability in solution was tested using three representative batches of the vaccine at ambient temperature (20-25°C). Decrease in titer was measured during the period of three hours after reconstitution (Table 2), which is the recommended period during which vaccine should be applied in the field conditions.

Results show that the virus titer remains relatively stable during the observed period of 3 hours using sterilized tap water.

Live viral vaccines are inherently unstable in a solution and degradation starts immediately after reconstitution or thawing of a vaccine. Vaccine producers are therefore required to provide evidence-based information on the stability of the reconstituted vaccine, optimum time frame and conditions which ought to ensure the delivery of the vaccine in a dose not less than the minimum recommended [7]. For reasons of practicability and rapid delivery, mass application by means of drinking water is therefore much preferred over individual vaccine application,

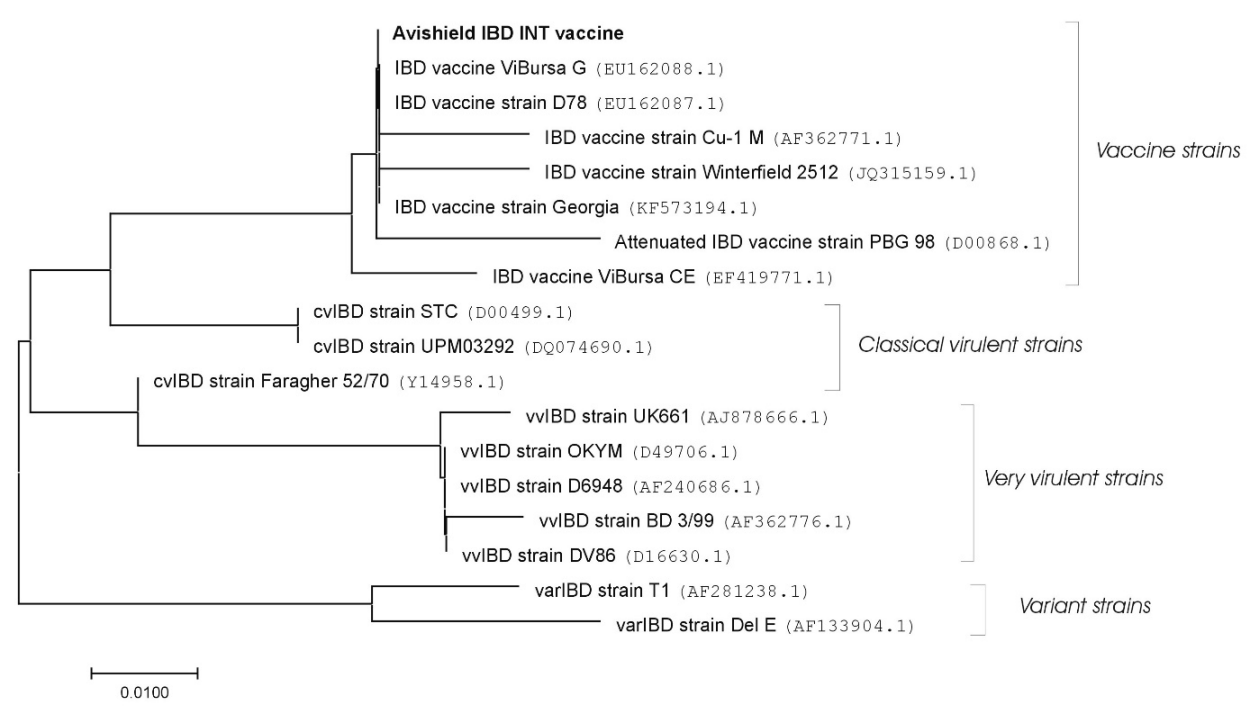

Figure 2 Phylogenetic tree based on the alignment of VP2 protein partial amino acid sequences of selected vaccine or field IBDV strains.

Table 2: In-use stability testing at ambient temperature (20-25°C) after reconstitution of vaccine.

Time/h	Virus titre/$TCID_{50}$/dose		
	Batch A	Batch B	Batch C
0	$10^{4.5}$	$10^{4.3}$	$10^{4.2}$
2	$10^{4.4}$	$10^{4.2}$	$10^{4.2}$
3	$10^{4.3}$	$10^{4.2}$	$10^{4.2}$

Table 3: Bursa to body weight ratios and bursa lesion scores for SPF layers inoculated with VMG91 or five times in vivo passaged VMG91 strain. Bursa lesion scoring was performed 7 – 28 days post inoculation of VMG91 or VMG91p, according to Ph.Eur. method [7].

Days post inoculation	Mean bursa to body weight ratio		Mean bursa lesion score*	
	VMG91	Five times in vivo passaged VMG91	VMG91	Five times in vivo passaged VMG91
7	3.3	1.5	2.4	5.0
14	4.7	1.7	0.8	3.6
21	3.7	2.0	0.8	2.4
28	7.6	4.5	0.2	1.8

* Scores are assigned progressively from 0 for normal bursae to score 5 for bursae showing complete lymphoid depletion and loss of follicular structure.

albeit at the cost of a less uniform vaccination and uneven vaccine uptake by individual birds [15].

The rate of reduction of a vaccine titer depends on number of physico-chemical and environmental factors. In addition to the inherent instability, the most important factors known to affect the virus` stability in solution are water temperature and quality (in terms of presence of chlorine, heavy metals, pollutants, pH) [15]. While IBDV is known to be a fairly robust and stable virus [2], still most of the available live IBDV vaccines are recommended to be used within 2 hours after reconstitution [16], which should provide sufficient amount of time for preparation and uniform application of the vaccine in most cases.

Safety in SPF chickens

General safety of the vaccine based on VMG91 strain was demonstrated in SPF chickens inoculated via either oral or eye-drop route with a 10-fold maximum dose ($10^{6.0}$ $TCID_{50}$). No clinical signs or deaths were observed during an observation period of 21 consecutive days after vaccination.

Dissemination and spreading

Dissemination of VMG91 strain within the body of vaccinated

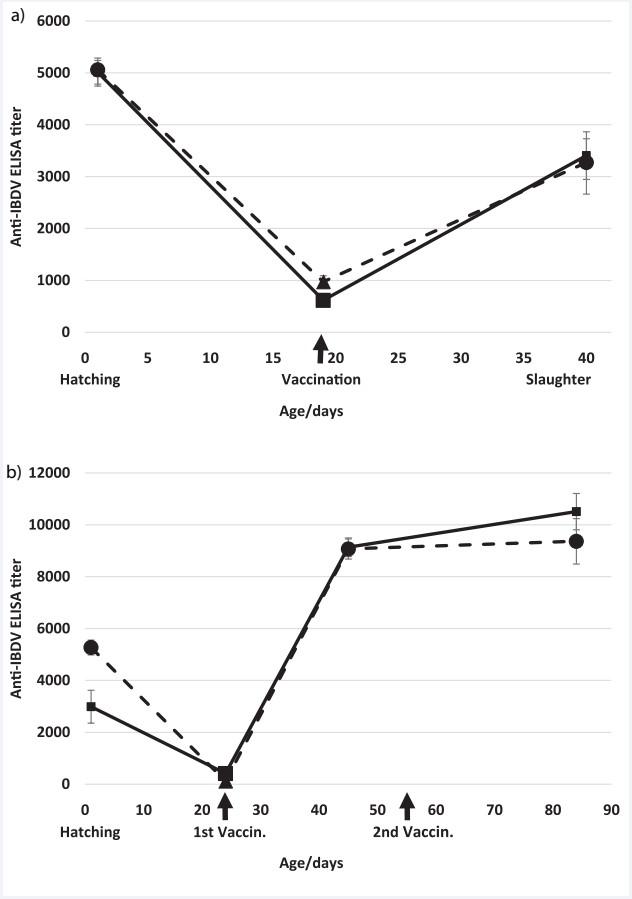

Figure 3 ELISA antibody titers to IBDV in commercial broilers (a) or layers (b) vaccinated in the field conditions using vaccine strain VMG91 (full line) or commercially available intermediate IBDV vaccine (dotted line). Arrows indicate time(s) of vaccination. Error bars indicate standard deviations.

chicks was assessed by inoculating 8-day-old SPF-chickens with $10^{5.0}$ $TCID_{50}$/per dose and examination of organs for presence of IBDV by PCR 5 days later. It was shown that VMG91 may be found in thymus, spleen, kidney, bursa, caecal tonsils and proventriculus / gizzard of the infected chickens, all without inducing pathological changes to these organs.

While it is well known that lymphoid tissue is the primary target for IBDV with a special predilection for the bursa of Fabricius, observed dissemination over a number of organs was expected given the fact that VMG91 and related strains are known to be well adapted to propagation on non-lymphoidal cells, such as chicken embryo fibroblasts and its derivatives [17], but also to other avian cells such as QT35 [18], and mammalian lines such as Vero (monkey), NIH3T3 (mouse), chimpanzee liver cells, and others [19]. The absence of pathological changes on PCR-positive organs confirms the low virulence of the strain.

Natural spreading of VMG91 over SPF chickens was examined by introducing non-inoculated hatch mates to groups of chickens infected 5 days earlier. The procedure started with inoculation of the chickens with Avishield® IBD INT vaccine and repeated another 2 times using VMG91 virus from the previous passage. It was shown that VMG91 was able to spread at least 3 times between groups of IBDV naive SPF-chickens, without inducing morbidity and mortality. Virus was readily detected in cloacal swabs 5 and 10 days after exposure to the vaccine while oropharyngeal swabs were weakly positive only in the second group.

Cloacal excretion is known as the most important route of transmission of IBDV between co-housed chickens [2]. The virus is excreted in droppings which might be ingested by naive chickens resulting in the uptake of vaccine virus. This may explain the presence of the virus in some oropharyngeal swabs. The ability to transmit horizontally without returning to virulence is desirable for vaccine viruses as chickens which were missed during vaccination, are highly able to take virus excreted by chickens which took the vaccine.

Increase in virulence

VMG91 strain was passaged successfully five times over 8-day-old SPF-chickens by inoculation of the bursa homogenate of infected chickens to the next group of SPF birds. No clinical signs indicating reversion of virulence of the VMG91 strain occurred during the passages over chickens.

Safety of the vaccine strain VMG91 in respect to the risk of reversion of virulence was assessed by comparing bursal lesions, clinical signs and bursa to body weight ratios between SPF chickens inoculated at 8 days of age with VMG91 and chickens inoculated with the strain passaged 5 times over SPF-chickens (VMG91p). Eight-day-old birds received $10^{6.0}$ $TCID_{50}$ doses of either strain, which is equivalent to ten times the maximum recommended vaccine dose. Chickens were observed for 28 consecutive days after inoculation. No abnormal clinical signs were observed in neither of the groups after inoculation. Mean bursa to body weight ratios increased during the 28 day observation period for both groups, and were consistently higher for the group of chickens inoculated with VMG91. Mean bursa lesion scores decreased during the 28 day observation period for both groups, and were consistently lower for the group of chickens inoculated with VMG91 (Table 3). At 28 days post inoculation (DPI) of the VMG91, complete repopulation and restoration of the bursas were observed. Following initial depletion of the bursal follicles after inoculation of the passaged virus VMG91p, repopulation and restoration of bursal structure was observed, evidenced by increasing size of follicles along with a lower cellular density and presence of lymphocytes. Infiltration with heterophils of the upper epithelial layer of the bursas was observed 14 days post inoculation.

Although the said horizontal spread of an attenuated virus may enhance the vaccine`s efficacy within the flock, it also increases the probability for mutations and potentially reversion to more virulent states. Indeed, potential reversion of virulence is the main environmental concern over field use of disseminating live viral vaccines. It is generally accepted that for the safety reasons, ideal live vaccine is ought to be sterile i.e. non-transmittable horizontally.

While in-vitro attenuation of virulent isolates imposes genetic pressure toward less virulent subspecies (but ideally still immunogenic), the in-vivo application of a vaccine may revert the trend and offer the chance that suppressed virulent subspecies

emerge again. As the probability of unwanted mutations correlate with the number of generations, the horizontal spread of the vaccine virus should be minimized. To achieve this, the ideal vaccination should ensure simultaneous application of the virus to all birds within the flock to minimize the average number of generations of a vaccine virus within the vaccinated flock. In the case of IBDV virus, achieving the high vaccine uptake within the flock should effectively prevent the horizontal spread. The reason is that bursa which is already populated with IBDV virus becomes less susceptible to re-infection with new viral particles. This is the consequence of the reaction of immune system to the initial infection with IBDV which induces strong anti-viral response reflected in the production of proinflammatory and Th1-like cytokines, especially from day 3 post infection onward [20]. This reaction effectively decreases the likelihood of the repeated infection of the already populated cells in the bursa. The interference phenomenon observed was attributed to competition for host receptor sites or production of cytokines [21]. This is further supported by our findings where bursae of vaccinated and then challenged chickens contained only vaccinal virus, while challenge strain was routinely found in bursae of the control (non-vaccinated) chickens (Table 6).

Decades of safe use of live attenuated IBDV vaccines have shown that the likelihood of reversion to virulence of intermediate vaccine strains in the field conditions is minimal. Nevertheless, as the worst case analogue, current regulations still require the controlled laboratory test for spreading of the vaccine virus to be conducted for each new vaccine candidate [7]. While bursa lesions score for the vaccine strain was low and in accordance with the Ph.Eur. requirements [7], the passaged strain apparently exhibited increased virulence (Table 3). As discussed above, sequencing identified a single aa change in VP2 gene at position 253 H→Q which seems insufficient to fully explain the bursal lesions result [13,14] but clarifies the change in the susceptible cell-type preference from non-lymphoid to lymphoid cells [12]. Indeed, further investigation revealed that the amount of passaged VMG91p given in the test was significantly underestimated as its titer was measured by titration on no longer susceptible fibroblast-based culture. Consequently, the amount of VMG91p in the test was significantly higher than that of VMG91 which probably also contributed to the higher bursal lesions score for VMG91p. Worth mentioning is the observation that other comparable intermediate vaccine strains, such as D78 and related, may undergo similar mutations at the same position [14], while no conclusive data on the emergence of vvIBDV strains from intermediate vaccine strains in the field conditions is available.

IMMUNOSUPPRESSION

Along with chicken infectious anemia and Marek's disease, IBD is the major infectious disease that may result in immunosuppression [22]. Classical intermediate strains, such as VMG91 and related, may cause temporary lymphocyte depletion in the bursa of Fabricius within 7 days of vaccination (Table 3). Nevertheless, it is the requirement to provide an empirical evidence and risk-benefit analysis that vaccination against IBD will not cause substantial immunosuppression and reduced response to other vaccines [7]. In general, the higher the strain`s virulence, the more risk of the immunosuppression may be expected, but also the better protection against vvIBDV [2,12].

Risk of immunosuppression which may occur after vaccination with live IBDV vaccines was assessed according to the standard Ph.Eur. immunosuppression test [7]. The test evaluates seroconversion and protection against challenge with virulent Newcastle disease (ND) virus in birds vaccinated first with IBDV vaccine candidate and then with Hitchner B1 strain of ND vaccine. The test is designed to represent the worst case scenario where ND vaccine is applied during the period when BLS index is the highest.

The protection against challenge with virulent NDV and antibody response following vaccination with live attenuated vaccine against ND containing Hitchner B1 strain (ND B1 vaccine) was compared between two groups of SPF chickens. One group of chickens was first vaccinated against IBDV with VMG91 strain and then 7 days later received ND B1 vaccine, while control group received only ND B1 vaccine. VMG91 strain complied with the requirements as neither seroconversion nor protection against NDV challenge was significantly altered when test group was compared with the control chickens vaccinated with NDV vaccine only (Table 4).

Mean ELISA antibody titers of 4.4 and 3.9 were found in chickens vaccinated with ND Hitchner or VMG91 + ND Hitchner B1 respectively, indicating that in both groups vaccine was taken well. None of the vaccinated chickens developed clinical signs of ND while all control non-vaccinated chickens either died or showed severe signs of ND.

Differences in serology and clinical signs between the non-vaccinated and vaccinated groups were significant ($P<0.05$), whereas the differences between vaccinated groups were not significant.

Efficacy in SPF chickens

Efficacy of the vaccine based on VMG91 strain was first demonstrated in SPF chickens vaccinated with a minimum recommended dose following Ph.Eur. protocol [7]. ELISA titers against IBDV were measured 14 days after vaccination. Following challenge with vvIBDV on the 14th day post vaccination, bursa lesion scores and morbidity/mortality were observed 10 days after challenge. None of the vaccinated chickens showed any clinical signs of IBDV. All non-vaccinated chickens showed severe clinical signs of IBDV or were euthanized. No difference was observed between the recommended vaccination routes (oral and eye-nose drop). Bursa lesions were mild with average values below score of 1. Efficacy on SPF chickens was clearly demonstrated as 100% of birds were both seropositive and protected against challenge 14 days post vaccination (Table 5).

Efficacy on MDA-positive chickens

Vaccination of commercial birds is complicated by the passive transmission of MDA from hens to the offspring via the egg. MDA provide passive immunity which may protect chickens from an early infection but it can also interfere with vaccination [1]. It is therefore of crucial importance to determine the vaccination time properly to ensure both the good vaccine uptake and short time span during which level of protection may be suboptimal. The

Table 4: ELISA antibody titers to ND 14 days after vaccination and protection rates after challenge of chickens vaccinated with ND B1 vaccine only, VMG91 + ND B1 vaccines or left non-vaccinated. Challenge strain: ND Herts33/56. n=13 SPF chickens per group.

Group	Mean ELISA ND antibody titer (log_{10})	% chickens responding serologically to ND vaccine	Protection against challenge with ND**
Non-vaccinated control	<1.0^{A*}	0%^{A}	0%^{A}
Vaccinated with ND B1	4.4^{B}	70%^{B}	100%^{B}
Vaccinated with VMG91 and ND B1	3.9^{B}	62%^{B}	100%^{B}

*Different letters in each column indicate that the difference between groups is significant (P<0.05).
**Chickens without clinical signs of ND at the end of observation period are considered protected

Table 5: Laboratory study of efficacy of IBDV vaccine with VMG91 strain using SPF chickens. Chickens received $10^{4.0}$ $TCID_{50}$/dose which is the minimum recommended vaccine dose. Fourteen days later birds were challenged with vvIBDV and bursa lesions scored 10 days later.

Group*	ELISA titer before challenge (14 days post vaccination)		Bursa Lesion Score**	Morbidity**	Mortality**
	Mean arithmetic titer**	% chickens responding***			
Oral	2347^{A}	100%	0.90^{B}	0%^{A}	0%^{A}
Eye-nose drop	1945^{A}	100%	0.80^{B}	0%^{A}	0%^{A}
Non-vaccinated	41^{B}	0%	n.d.	90%^{B}	100%^{B}

*20 chickens in vaccinated groups and 10 in control group
**means within a column with a common superscript letter are not significantly different (P>0.05)
*** samples with an SP-ratios of > 0.2 (titer>396) were considered positive

Table 6: Laboratory study of efficacy of IBDV vaccine with VMG91 strain on MDA-positive chickens. Thirty eight days old commercial layers received $10^{4.0}$ $TCID_{50}$/dose which is the minimum recommended vaccine dose. Fourteen days later birds were challenged with vvIBDV and bursa lesions scored 10 days later.

Group*	Mean arithmetic titer before vaccination (D38)*	Mean arithmetic titer 14 days after vaccination (D52)*	Bursa Lesion Score 14 days after vaccination (D52)*	Bursa Lesion Score 10 days after challenge (D62)*	Morbidity**	Mortality**	Presence of IBDV virus 10 days after challenge (D62)
Oral	236^{A}	6784	1.0	1.8^{A}	0%^{A}	0%^{A}	100% Vaccinal strain
Non-vaccinated	349^{A}	n.d.	n.d.	4.8^{B}	75%^{B}	45%^{B}	100% challenge strain

*20 chickens in vaccinated group and 10 in control group
**means within a column with a common superscript letter are not significantly different (P>0.05)

common practice is to use serological monitoring to determine the optimal timing for vaccination, e.g. the Deventer formula [23].

Efficacy of VMG91-based vaccine on MDA positive birds was tested under laboratory conditions using commercial layers and broilers. Targeted breakthrough titre was 125 anti-IBDV ELISA units. Optimum vaccination day was determined using Deventer formula and it was calculated to be day 38 of the study, as estimated from ELISA titers obtained from sera collected on days 1 and 21.

Seroconversion was high among vaccinated chickens with a mean titer of 6784 ELISA units at 14 days post vaccination, despite the fact that average titer on the vaccination day was significantly higher than targeted 125 ELISA units: 236 and 349 ELISA units for vaccinated and control group respectively (Table 6). No morbidity or mortality was observed among vaccinated chickens, while non-vaccinated chickens were severely affected by the challenge virus with mortality reaching 45% by the end of the 10-days observation period after challenge. Bursa lesion scores were low/moderate in the vaccinated group while control chickens experienced almost complete lymphoid depletion and loss of follicular structure.

Sequencing of IBDV samples found in bursae after challenge showed that in vaccinated chickens only vaccine VMG91 strain was present. This confirms the abovementioned finding that infected bursae become less susceptible to re-infection with field IBDV thus preventing propagation and shedding of the non-vaccine virus. It also demonstrates the importance of the uniform flock vaccination where proportion of non-vaccinees is low.

Efficacy on commercial broilers and layers in field studies

Safety and efficacy of the vaccine strain VMG91 in the field conditions was tested on commercial broilers and layers using commercially available intermediate IBDV vaccine as a comparator. Studies were designed according to applicable guidelines and regulations (see Table 1).

In the study using commercial broilers, four flocks of Hybrid Ross 308 chickens with approximately 20 000 birds per house were used. Two flocks were vaccinated with vaccine strain VMG91 while remaining two flocks received comparator vaccine,

a commercially available intermediate vaccine based on the similar IBDV strain. Day of vaccination was determined for each flock independently using Deventer formula taking the titre of 1:250 as the targeted break through titre, as recommended by the ELISA kit producer (Biocheck) for intermediate vaccines.

Birds were vaccinated on SD 18-19. ELISA results showed that at the vaccination days an average anti-IBDV titer was 615 and 972 ELISA units for two experimental groups, which is significantly higher than the targeted titre of 250 units. However, ELISA test of sera collected at the end of the cycle at day 40 showed good seroconversion with average titres well above 3000 ELISA units, confirming the good vaccine uptake (Figure 3a). No significant differences in antibody levels, or mortality was found between the groups at the end of the trial (P>0.05). Likewise, average body weight, feed conversion, total animal loss and incidence of general clinical signs were comparable between the groups (Table 7).

In the study using commercial layers, four flocks of commercial hybrid line Lohmann brown chickens with approximately 20 000 birds per house were used. Flocks were vaccinated two times via drinking water with either vaccine strain VMG91 or commercially available vaccine based on the similar IBDV strain. The day of first vaccination was determined using Deventer formula taking the titre of 1:250 as the targeted break through titre. The second dose was administered on day 56, as this was the common practice of this particular producer.

The results of serological testing revealed that the time of vaccination was determined properly in all flocks and no significant difference in ELISA antibody titer among flocks was observed on the day of the first vaccination (day 24, P>0.05). Seroconversion was monitored 3 weeks after the first vaccination and 4 weeks after the second dose (Figure 3b). Antibody response to the first vaccination was good and uniform in both groups reaching an average titer of over 9000 ELISA units. Four weeks after the second vaccination the slight increase in antibody response was detected in all flocks reaching the average titer of 10512 ELISA units for VMG91 groups and 9367 ELISA units for the comparator group. Statistical analyses confirmed no significant differences between the compared groups (P>0.05).

No significant difference was found between the groups at the end of the observation period in antibody levels, mortality or body weight (P>0.05). The overall production results as well as incidence of general clinical signs were as expected and comparable between the groups.

It is important to stress the benefit and rationale behind the repeated vaccination which may be given to layers. As it is not justified to wait until all animals become susceptible to vaccination (this would create an immunity gap in the birds with low MDA) the repeated vaccination serves to vaccinate birds with initially high MDA at the time when their MDA titer had further declined to an acceptable level. At that time such birds become susceptible to the chosen vaccine and thus this second vaccination contributes to herd immunity and more homogenous high titres in the flock [24].

Table 7: Average production results in commercial broilers in field conditions. Commercial broilers were vaccinated via drinking water with VMG91 strain or comparator IBDV vaccine at the age 18-19 days.

Production parameter	VMG91	Comparator
Average age at slaughter	37.715	38.155
Average body weight (kg)	2.28	2.42
Feed conversion	1.808	1.781
Production index - PI	325	342.5

CONCLUSION

Complete in vitro and in vivo characterization of the IBDV Avishield® IBD INT vaccine according to the current Ph.Eur. and EMA requirements was performed for the purpose of the registration of the product in EU countries. Safety and efficacy using SPF and MDA-positive chicks was demonstrated in both laboratory and field trials. The vaccine is therefore safe and suitable for protection against field infection in areas with moderate risk of vvIBDV outbreak.

CONFLICT OF INTEREST

Anto Vrdoljak and Lana Ljuma Skupnjak are employed by Genera Inc., now part of the Dechra Pharmaceuticals PLC Group, manufacturer of Avishield® IBD INT vaccine. Olga Zorman Rojs is employed by the University of Ljubljana, Veterinary faculty, Slovenia, which performed field trials with the Avishield® IBD INT vaccine. Gert Jan Boelm is employed by the GD Animal Health, the Netherlands, which performed laboratory trials with the Avishield® IBD INT vaccine.

REFERENCES

1. Berg TPVD. Acute infectious bursal disease in poultry: a review. Avian pathology. 2000; 29: 175-194.
2. Eterradossi N, Y Saif. Infectious bursal disease. Diseases of poultry. 2008; 12:185-208.
3. Jackwood DJ. Advances in vaccine research against economically important viral diseases of food animals: Infectious bursal disease virus. Veterinary microbiology. 2017; 206: 121-125.
4. European Medicines Agency. Veterinary medicines: regulatory information. https://www.ema.europa.eu/en/veterinary-medicines-regulatory-information. Accessed 17.10.2018.
5. Council of Europe. European Pharmacopoeia. 9th ed. https://www.edqm.eu/en/european-pharmacopoeia-ph-eur-9th-edition. Accessed 17.10.2018.
6. European Commission. EudraLex - Volume 4 - Good Manufacturing Practice (GMP) guidelines. https://ec.europa.eu/health/documents/eudralex/vol-4_en. Accessed 17.10.2018.
7. Council of Europe. European Pharmacopoeia. 7th ed. Avian infectious bursal disease vaccine (live) 04/2013:0587.
8. Zorman Rojs O, U Krapež, B Slavec, S Mankoč, R Jurišič-Cizerl, D Barlič-Maganja, et al. Molecular characterisation of infectious bursal disease viruses isolated in recent acute outbreaks in Solvenia. Acta Veterinaria Hungarica. 2008; 56: 255-264.
9. Cao Y, W Yeung, M Law, Y Bi, F Leung, B Lim. Molecular characterization of seven Chinese isolates of infectious bursal disease virus: classical, very virulent, and variant strains. Avian diseases. 1998; 340-351.
10. T-Coffee Online Alignement Tool. http://tcoffee.crg.cat/apps/tcoffee/do:mcoffee. Accessed 17.10.2018.

11. European Medicines Agency. Committee for medicinal products for veterinary use. Data requirements to support in-use stability claims for veterinary vaccines. EMEA/CVMP/IWP/250147/2008. .

12. Tamás M. Genetic diversity of infectious bursal disease virus strains and the molecular basis for change in virulenceDoctoral thesis. Veterinary Medical Research Institute of the Hungarian Academy of Sciences Budapest. 2009.

13. Qi X, H Gao, Y Gao, L Qin, Y Wang, L Gao, X Wang. et al. Naturally occurring mutations at residues 253 and 284 in VP2 contribute to the cell tropism and virulence of very virulent infectious bursal disease virus. Antiviral research. 2009; 84: 225-233.

14. Olesen L, R Dijkman, R Koopman, R van Leeuwen, Y Gardin, R Dwars, et al. Field and laboratory findings following the large scale use of intermediate type infectious bursal disease vaccines in Denmark. Avian Pathology. 2018.

15. Vermeulen B, P De Backer, JP Remon. Drug administration to poultry. Advanced drug delivery reviews. 2002; 54: 795-803.

16. The PoultryMed Website. http://www.poultrymed.com. Accessed 7.5.2016. 2016.

17. Lim BL, YCao, T Yu, CW Mo. Adaptation of very virulent infectious bursal disease virus to chicken embryonic fibroblasts by site-directed mutagenesis of residues 279 and 284 of viral coat protein VP2. Journal of virology. 1999; 73: 2854-2862.

18. Cowen B, M Braune. The propagation of avian viruses in a continuous cell line (QT35) of Japanese quail origin. Avian diseases. 1988; 282-297.

19. Jackwood DH, Y Saif, J Hughes. Replication of infectious bursal disease virus in continuous cell lines. Avian Diseases. 1987; 370-375.

20. Yasmin A, S Yeap, M Hair-Bejo, A Omar. Characterization of chicken splenic-derived dendritic cells following vaccine and very virulent strains of infectious bursal disease virus infection. Avian diseases. 2016; 60:739-751.

21. Ashraf S, G Abdel-Alim, M Al-Natour, Y Saif. Interference between mild and pathogenic strains of infectious bursal disease virus in chickens. Avian diseases. 2005; 49: 99-103.

22. Hoerr FJ. Clinical aspects of immunosuppression in poultry. Avian Diseases. 2010; 54: 2-15.

23. De Wit J. Year. Gumboro disease: estimation of optimal time of vaccination by the Deventer formula. Pages 21-28 in Proceedings of the 3rd meeting of working group.

24. 2016. OIE Terrestrial Manual. 2.3.12. Infectious Bursal Disease (Gumboro disease) 655.

25. Council of Europe. European Pharmacopoeia. 7th ed. 5.2.6. Evaluation of safety of veterinary vaccines and immunosera 04/2013:50206.

26. Council of Europe. European Pharmacopoeia. 9th ed. Vaccines for veterinary use 01/2017:0062.

27. European Medicines Agency. Committee for medicinal products for veterinary use. Note for guidance: Duration of protection achieved by veterinary vaccines. EMEA/CVMP/682/99.

28. The European agency for the evaluation of medicinal products. Committee for veterinary medicinal products. Note for guidance: Field trials with veterinary vaccines. EMEA/CVMP/852/99. 2001.

29. European Medicines Agency. Veterinary Medicines and Inspections. GUIDELINE ON TARGET ANIMAL SAFETY FOR VETERINARY LIVE AND INACTIVED VACCINES. EMEA/CVMP/VICH/359665/2005.

30. The European agency for the evaluation of medicinal products. Veterinary medicines and information technology unit. Guideline on good clinical practices. CVMP/VICH/595/98. 2000.

Cite this article

Vrdoljak A, Rojs OZ, Boelm GJ, Skupnjak LL (2018) Comprehensive Evaluation of Infectious Bursal Disease Vaccine Candidate According To the Current Industry Standards. J Vet Med Res 5(10): 1164.