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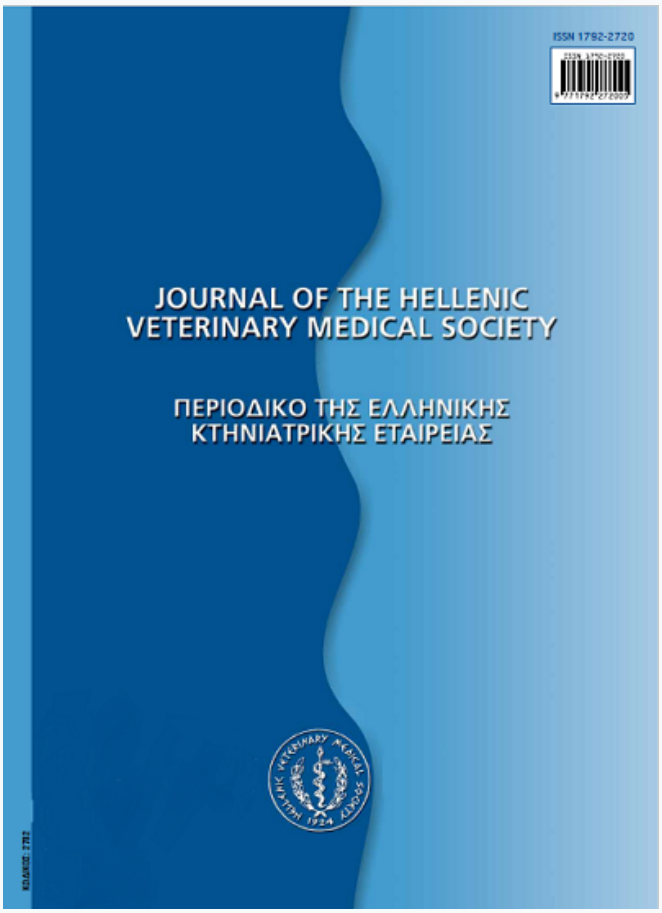

Efficacy of recombinant VAXXITEK HVT-IBDv vaccine against very virulent Infectious bursal disease virus (vvIBDv) challenge in layer chicks: A pilot study

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Efficacy of recombinant VAXXITEK HVT-IBDv vaccine against very virulent Infectious bursal disease virus (vvIBDv) challenge in layer chicks: A pilot study

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ABSTRACT. The infectious bursal disease virus (IBDv) is widespread in poultry flocks all around the world. Various biotypes have emerged and because of that, adequate management practices and vaccination of chicks are of paramount importance for the protection against field strains. One day old Lohmann Brown chicks were vaccinated with intermediate vaccines and the recombinant VAXXITEK HVT-IBDv vaccine formulation, and challenged at 48 days of life with the very virulent IBDv (vvIBDv) strain CH/99. The best protection (100%) was achieved with the recombinant vaccine administered by the subcutaneous or intramuscular route at a day old, while intermediate and intermediate plus vaccines protected 80% of birds from clinical symptoms. The highest bursa body ratio (5.33, 3.50 and 4.12) was accomplished in non- vaccinated and non-challenged birds and birds vaccinated with the VAXXITEK HVT-IBDv vaccine. The recombinant VAXXITEK HVT-IBDv vaccine has provided protection for commercial chicks against challenge with the vvIBDv strain in this experiment. Under field conditions, additional vaccination is possibly needed with supplementary application of live attenuated vaccines. However, the recombinant vector vaccines are providing significant aid against clinical signs and immunosupression caused by the vvIBDv.

Keywords: poultry, IBDv, intermediate vaccines, recombinant vaccines, challenge

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INTRODUCTION

The infectious bursal disease virus (IBDv) causes health problems in poultry flocks all around the world. This virus is capable of spreading across a long distance and survives in a poultry farm environment, even if the farms were thoroughly cleaned and disinfected between production cycles. Once the farm is contaminated with the IBDv it is almost impossible to prevent the infection of chicks. Vaccination of chicks against IBDv is often inefficient due to the interference of maternally derived antibodies with vaccine viruses (Chettle et al., 1989). Studying molecular genetics of the virus and various vaccination approaches substantially contributed to understanding the biology of the IBDv and has improved control strategies (Müller et al., 2012).

The IBDv is a double stranded RNA virus with a bisegmented genome and it belongs to the genus *Avibirnavirus* of the family *Birnaviridae* (Müller et al., 1979). The larger segment of the virus encodes the polyprotein, which is autocatalytically cleaved to VP2, VP3 and VP4 proteins, while a small overlapping open reading frame fragment encodes the protein VP5, which has a role in cell lysis and the apoptosis process. The smaller segment encodes an RNA dependent RNA polymerase (Mundt et al., 1995). The most studied is the capsid protein VP2, since it is exposed at the surface of the virus and possesses conformational epitopes with different amino acid arrangements in various biotypes (Bayliss et al., 1990). Therefore, the VP2 protein has become an important target for genetically engineered vaccines, some of which are based on the recombinant technology (Darteil et al., 1995).

The turkey herpes virus (HVT) is an avirulent, cell associated virus and has been used for decades for the vaccination of chicks against Marek's disease (MD). As the Marek's disease virus is ubiquitous, all commercial chicks have to be vaccinated in hatcheries subcutaneously at the first day of life or *in ovo* (Gimeno, 2008). Because of the cell associated nature of the HVT, it has become an attractive target for the development of vector vaccines. The basic concept for the vaccination of chicks with rHVT-VP2 depends on the expression of the VP2 at the surface of the cell which in turn induces immune responses. The vector virus (VAXXITEK HVT-IBDv) was found in the feathers follicles, which means that the pathogenesis of the vaccine strains was well established in the recombinant formulation (Gelb et al., 2016). The partial aid, when it comes to the protection from MD, is also possible with some of the HVT recombinant vaccines (Aly et al., 2012). However, the results from research studies are different and the success of field vaccination depends on multiple factors such as management practices, and virulence and antigenicity of the field strains.

Outbreaks of vvIBDv still occur in broiler and layer chicks in Serbia. Most commonly the vaccination with the intermediate plus vaccines takes place at approximately 10 days of age and second vaccine is given to chicks 7 to 10 days apart. In cases when for a longer period of time new outbreaks are not recorded, the intermediate vaccines are continuously used. Such control has shown some benefits but in farms with poor management practices it is still highly risky to replace vaccination with the intermediate vaccines only. In such circumstances the option to vaccinate chicks as early as at day one or *in ovo* with the recombinant rHVT-VP2 seems to be promising. Hence, a challenge experiment was done to evaluate several vaccination protocols in order to gain experience with the rHVT-VP2 in Serbia. The goal was to perform an experimental infection of chicks vaccinated with the intermediate or intermediate plus vaccines *per os* and with the rHVT-VP2 vaccine subcutaneously or intramuscularly, in order to determine the level of protection against challenge with the vvIBDv strain.

MATERIAL AND METHODS

General description

The following work was conducted at the Faculty of Veterinary Medicine in Belgrade. The facilities where the experiments were carried out were separated by concrete walls. The walls and concrete floors were easy to disinfect. The commercial feed produced by technology for Lohmann brown provenience was prepared in a local feed factory according to HACCP quality assurance system and ISO 22000 standards implemented. Water was provided ad libitum. The facility is the only establishment certified

for experiments involving poultry and as such it has permit issued by the Ministry of Agriculture of the Republic of Serbia, Veterinary Directorate (permit number 323-07-02263/2014-05/2). The chicks from all the experimental groups were vaccinated in the hatchery against MD, infectious bronchitis (IB) and Newcastle disease (NDV). The type of MD vaccines used in this study is presented in experimental infection. During the experiment, the complete vaccination program against poultry diseases commonly applied in Serbia was also performed. The vaccines included those against Newcastle disease virus and infectious bronchitis virus i.e. Nobilis Ma5-Clone30, Nobilis ND clone30, IB Bioral H120 (MSD Animal Health, The Netherlands).

Vaccines against IBDv

Three types of commercial live IBD vaccines were used in the study: the intermediate (D78) and intermediate plus vaccines (228E), (MSD Animal Health, The Netherlands) and the recombinant vector vaccine VAXXITEK HVT-IBDv (Merial-Sanofy, France).

Challenge virus

For the challenge experiment, the local field vvIBDv strain CH/99 was used. The CH/99 IBDv is the standard challenge strain used in Serbia. It was not titrated on chickens but it causes mortality of 50% layer chicks which are free of maternal antibodies. According to the amino acid sequences of the hypervariable domain of the VP2, this virus belongs to the very virulent biotype (GeneBank accession number KF439863), (Dobrosavljević et al., 2014).

Experimental infection

Sixty one-day old commercial Lohmann Brown chicks were held in isolation units and provided with feed and water ad libitum. Each vaccinated group and two control groups consisted of 10 chicks. Groups G1 and G2 had received the VAXXITEK HVT-IBDv vaccine subcutaneously or by intra muscular route, respectively. They were also vaccinated with the CVI988 vaccine. Chickens in group G3, G4 and G5 were vaccinated at one-day of age, with the Cryomarex (CVI 988-Rispens+HVT) vaccine (Merial-Sanofi, France). No interference between rHVT and CVI988 has been established so far (Hein et al., 2011). G3 group of chicks received the intermediate vaccine at 28 days of age, while G4 group received the intermediate plus vaccine at 26 days of age using the per/os method (water mixed with skim milk). The timing for the vaccination against IBD was estimated using Deventer formula based on an ELISA antibody titer of day old chicks, in order to avoid the interference with maternally derived antibodies (MDA). After seven days, groups G3 and G4 were vaccinated one more time against IBDv with the intermediate vaccines. Groups G5A and G5B were not vaccinated against IBDv. Group G5A was a challenge control, while group G5B was not vaccinated nor challenged during the experiment. Challenge with the CH/99 virus (10 birds from each group except G5B) was done at 48 days of age by oculo/nasal administration of 50µl of the crude bursal homogenate which was prepared as described previously (Dobrosavljević et al., 2014). Clinical symptoms, mortality (Le Nouen et al., 2012) and bursa/body weight ratios (Sharma et al., 1989) were used to evaluate the success of the vaccinations. Bursas were collected from six chicks from each group sacrificed at 59 days of age. The number of samples for statistical analysis was determined according to the following formula, for the minimal size of the sample in population.

$$n = \left[\frac{z \cdot \sigma}{G}\right]^2$$

z- Confidence level

σ - Basic standard deviation

G – The maximum permissible error

Chicks were sacrificed according to the EU Directive 2010/63 of the European Parliament and the Council on the protection of animals used for scientific purposes (Directive 2010/63/EU). The Committee of animal welfare of the Republic of Serbia has provided the permit (permit number 323-07-07812/2014/05/1) for the challenge experiment.

STATISTICAL ANALYSIS

The statistical analysis was done by using descriptive statistical parameters (analysis of variance-ANOVA and Tukey test). The established statistical sig-

nificance was at the level of 5 and 1% and was further elaborated using software GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, California USA, www.graphpadHYPERLINK "http://www.graphpad.com/".com and MS Excel programs.

RESULTS AND DISCUSSION

During the experiment the health status of the birds was recorded on a daily basis until the termination of the experiment. The symptoms of the acute IBDv (such as depression, ruffled feathers and watery diarrhoea) were recorded 48 hours after challenge in all birds from the non-vaccinated challenged group of chicks (group G5/A) and the mortality was 50%. In the groups of chicks vaccinated with the intermediate and intermediate plus vaccines (groups G3 and G4 respectively) the symptoms of acute IBDv were noticed in two chicks from each group, within two days from the challenge control chicks and those birds had succumbed to the infection. In necropsy discrete bleedings on pectoral muscle were seen and the bursa was swollen in gelatinous edema. The IBDv was confirmed in the bursa applying immunodiffusion test. At the time of challenge, residual MDA in four chicks from groups G3 and G4 respectively, may have still been high at 26 and 28 days of age and active immune responses may have been delayed or even absent at the time of challenge. Hence, the second vaccination with the intermediate vaccine did not provide sufficient protection for the chicks which was also observed in the field (Aliyu et al., 2016) and experimental conditions (Massi et al., 2008). However, in chicks that received the recombinant vector vaccine formulation (G1, G2) and in the control group unvaccinated and unchallenged (G5B), no clinical symptoms or mortality were recorded.

Table 1

The mean bursa/body weight ratio was significantly higher ($p<0.01$) in the control non-challenged group and chicks that were vaccinated with the recombinant vector vaccine, compared to non-vaccinated challenged birds and birds that received the intermediate and intermediate plus vaccines. Also, the mean bursa/body weight ratio (5.33+1.02) was significantly higher ($p<0.05$) in the control non-challenged group (G5/B) compared with that in the chicks vaccinated

Table 1: Clinical symptoms, mortality rate and bursa/body index in vaccinated and challenged chickens at 59 days (11 days post challenge)

Group	Vaccination against IBDV	Clinical symptoms*	Mortality**	Mean bursa weight/g	Bursa/body index
G.1	vHVT13 s/c.	0/10	0/10	2.3	3.50
G 2	vHVT13 i/m.	0/10	0/10	2.85	4.12
G.3	"Intermediate"	2/10	2/10	1.0	1.55
G.4	"Intermediate plus"	2/10	2/10	0.75	1.22
G5/A	Non-vaccinated/ infected	10/10	5/10	0.45	0.69
G5/B	Non-vaccinated/ non-infected	0/10	0/10	3.73	5.33

**number of chickens with clinical symptoms/total number of chickens in experimental group,*
***Number of chickens that succumb infection/total number of chickens in experimental group*

with the recombinant vector vaccine in group G2 (mean B/B weight ratio of 4.12+0.55), and was significantly higher ($p<0.01$) compared with that in the chicks vaccinated with the recombinant vector vaccine in group G1 (mean B/B weight ratio of 3.50+0.20), while there were no significant differences between groups G2 and G1 ($p>0.05$). The mean bursa/body weight ratio in group G1 (3.50+0.20) and G2 (4.12+0.55) was significantly higher ($p<0.01$) in comparison with those in groups G3, G4 and G5/A.

Table 2

The results of the challenge experiment are in agreement with the results obtained by Massi et al., 2008. In their experiment**,** 100% protection was established after the subcutaneous application of vHVT13 in chicks that had been challenged with the vvIBDv. Our experiments are also in agreement with Darteil et al., (1995), who accomplished 100% protection against IBDv with 105 plaque forming units (pfu) of vaccine dose and 60% with 104 pfu vaccine dose per bird at one day of age, with the vHVT002 recombinant vector vaccine. In this vaccine, the open reading frame of the VP2 was inserted at the deleted locus of the glycoprotein gene *gl* under the control of the human cytomegalovirus immediate early promoter. It was established that the efficacy of the vector vaccines depends on the potency of the promoter (Tsukamoto et al., 2002) as well as the proper target site used for insertion of the foreign gene (Darteil et al., 1995).

In the field situation (or in experiments with the commercial chicks), the titers of the maternal antibodies are high at the first day of age and the interference with live-attenuated IBDv vaccine strains is plausible but not with the recombinant IBDv-HVT vaccines. The bursa of experimental birds that were examined 11 days post challenge showed clear differences between the groups of chicks in our study. The size of the bursa and spleen was larger in the non-challenged and the birds vaccinated with the recombinant vaccine, compared to challenged control and groups vaccinated with the intermediate vaccines.

Figure 1

Vaccination with live IBD vaccines is controversial since the occurrence of immunity depends on many attributes even in controlled experiments. In commer-

Table 2: Data on descriptive statistic analysis of the B/B weight ratio at 59 days of age (11 days post challenge)

Groups of chickens	N	$\bar{x}$	SD	Sy	CV (%)	X max	X min
G1	6	$3,50^{dhij}$	0,20	0,0982	5,61	3,75	3,27
G2	6	$4,12^{Aefg}$	0,55	0,2714	13,17	4,59	3,65
G3	6	$1,55^{ceh}$	0,16	0,0780	10,05	1,75	1,37
G4	6	$1,22^{bfi}$	0,09	0,0460	7,54	1,35	1,15
G5/A	6	$0,69^{agj}$	0,30	0,1489	43,12	1,04	0,35
G5/B	6	$5,33^{abcdA}$	1,02	0,5094	19,11	6,74	4,36

Different superscript letters indicates statistical significance between experimental groups of chickens: a, b, c, d, e, f, g, h, i, j, p<0,01; A, p<0.05, N, total number of chickens per group; $\bar{x}$ *the arithmetic average, SD, standard deviation; Sy, standard error; CV, coefficient of variation; X max, maximal value of the bursa/body weight ratio; X min, minimal value of the bursa/body weight ration.*

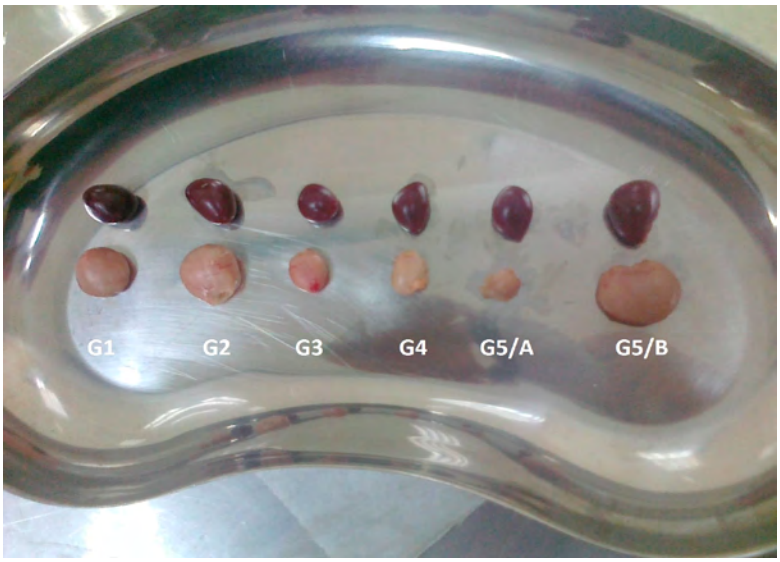

Fig 1: Bursa and spleen of chicks which have been sacrificed at 11 days post infection with vvIBDv (CH/99 challenge strain)

cial broilers the vaccine viruses could cause transient destruction of the bursa and it was postulated that a delay in bursa recovery influences the decrease of the number of target cells in the bursa which are then less available for the pathogenic virus (Rautenschlein et al., 2005). However, in experiments presented here, 2 out of 10 birds in groups G3 and G4 had symptoms of acute IBD, and had succumbed to the infection

It has been established recently that even in the presence of high titers of maternal antibodies, the recombinant vaccine (vHVT13) was efficiently protecting chicks against classical, very virulent and variant IBDv (Bublot et al., 2007, Perozo et al., 2009, Prandini et al., 2016). A few experiments with the rHVT-IBDv have been done in commercial broilers and a good antibody response to IBDv was obtained in the research work conducted in Italy (Le Gros et al., 2009), Slovenia (Zorman-Rojs et al., 2011) and Jhenaidha (Rashid et al., 2013) after a subcutaneous application and when the *in ovo* vaccine delivery system was used (Roh et al., 2016). It was also shown that the recombinant VAXXITEK HVT-IBDv vaccine provided a high maternal antibody titer in progeny from parents vaccinated with a single recombinant vaccine or if the rHVT-IBDv vaccine was combined with the inactivated vaccine, compared to a single inactivated vaccine. The protection of broiler chicks originating from parents vaccinated with the recombinant VAXXITEK HVT-IBDv which have been vaccinated with rHVT-IBDv *in ovo* was superior comparing to chicks originating from parents vaccinated with a single inactivated vaccine, even in the face of high levels of MDA (Lemiere et al., 2013). Authors concluded that the clinical protection of broilers under field conditions could be achieved after vaccination of parent flocks and their progeny with the rHVT-IBDv vaccines. Gelb et al., (2016) has shown that the recombinant VAXXITEK HVT-IBDv vaccines offer clinical protection of broiler chicks with MDA against challenge with homologous and heterologous IBDv strains. These birds were protected based on the incidence of microscopic lesions in the bursa even if interference with the active immune response was observed. Active protection in specific pathogen free chicks was achieved at 18 DPV, onwards in their experiments. However, it is still not known whether the application of the recombinant HVT-IBDv vaccine alone at one day of age, in the circumstances where vvIBDv persist on poultry farms, is sufficient for the clinical protection of birds**.**

In situations when broilers are not routinely vaccinated against MDV, the HVT type of vector vaccine may provide some convenience for the protection against both diseases or to minimize the immunosuppression caused by both viruses (Aly et al., 2012). Therefore, the successes of the application of recombinant vaccines under field conditions and the experience gained from various epidemiological situations will determine the application of genetically engineered vaccines for the present and future in Serbia.

In conclusion, the protective ability of the recombinant VAXXITEK HVT-IBDv vaccine against the challenge with vvIBDv (strain CH/99) was established in this experiment. However, in the future, a more detailed investigation should be performed using virus titration, immunohistochemistry detection of the IBDv antigen in lymphoid organs of chicks, serology testing and PCR in order to gain more experience with the recombinant HVT-IBDv vaccines. Good management practice on poultry farms has to

become imperative as much as vaccination, especially in developing countries, where vvIBDv still causes significant economical losses.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests.

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