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Review Article

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Q&A on the Paper of Kurukulasuriya et al. (2017) on IBD Vaccine Efficacy Against a Canadian Variant IBDV Strain in Broiler Chickens

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Abstract

Kurukulasuriya, et al. (2017) are reporting the efficacy of two IBD vaccines against an early (6 days post-hatch) challenge with a variant Canadian IBDV strain in broilers. A modified live vaccine(UNIVAX BD) administered by SQ route at 1 day-of-age delayed infection whereas an HVT-IBD vector vaccine (VAXXITEK HVT+IBD) administered in ovodid not protect. Furthermore, the authors suggested that the HVT-IBD vector induced immunosuppression responsible for an earlier IBDV challenge strain replication in the bursa.

The data presented in the paper showed no evidence of VAXXITEK HVT+IBD vaccine take since the mean IBD ELISA antibody titer at D35 in the vaccinated/non-challenged group was not significantly different from that of the non-vaccinated group. It wasmuch lower than the expected one based on previous studies performed in the same conditions : in ovo vaccination of broilers [1,2]. Since there is no evidence of vaccine take, the other potential effects (immunosuppression and earlier IBDV replication in the bursa) observed in that group cannot be attributed to the vaccine.

Since its launch in 2006 in Brazil, VAXXITEK HVT+IBD has been licensed in more than 75 countries and more than 80 billion birds have been vaccinated. VAXXITEK HVT+IBD is protecting against a wide variety of IBDV strains including the classical, the very virulent and different variant strains. To our knowledge, noabsence of efficacy nor bursa depletions have been so far officially reported as long as the vaccine has been administered properlyto healthy embryonated eggs or to healthy one-day-old chicks.

Introduction

The modified live vaccine (MLV) UNIVAX-BD(MSD), administered by the subcutaneous (SQ) route at 1-day-of-age, delayed the infection of neonatal broiler chickens (6 days post-hatch) with the variant SK09 IBDV strain, whereas the HVT-vectored VAXXITEK HVT+IBD (Boehringer Ingelheim Animal Health) IBDV vaccine, administered by the *in ovo* route,had no protective effects. In contrast, birds vaccinated with VAXXITEK HVT+IBD had an earlier IBDV replication in the bursa, an inhibition of cytotoxic CD8+ T-cell response (CD44-downregulation) and decreased splenic lymphocyte counts compared to the unvaccinated ones, suggesting an early immunosuppressive effect induced by the HVT-IBD vector vaccine.

UNIVAX-BD vaccine

The UNIVAX-BD vaccine is a MLV vaccine that contains the mild strain (ST-12) of Infectious Bursal Disease (Gumboro) virus grown in tissue culture and combined with stabilizing agents and gentamycin as preservative. The product is supplied as a lyophilized vaccine contained in vials sealed under vacuum. The vaccine can be administered to 18 - 19 day old embryonated fowl eggs by the *in ovo* route using an *in ovo* system or subcutaneously at 1 day of age and / or by drinking water at 1 week or older(Univax®-BD is a registered trademark of Merck Animal health).

Surprisingly, this vaccine did not induce bursal lesions in the vaccinated/unchallenged group questioning the way it worked in this study.

IBDV epidemiological situation in Canada

Different published papers indicated that the most common Canadian IBDV field strains were North-American variant viruses (varIBDV), the majority (including the challenge SK09 strain) being close to the NC171 U.S.Aand to the South African 05SA8 isolate; others are related to Delaware E or strain 586 U.S.A. isolates. There is apparently no vvIBDV in Canada [3-5].

Origin of the broiler eggs used in this study

The broiler eggs were obtained from a local hatchery (Prairie Pride Chick Sales Ltd., Saskatchewan, Canada), where broiler-breeders undergo routine IBDV (classic strains) hyper-immunization [6]. Based on the latter reference, the broiler breeder parent flocks of those broiler chicks had been vaccinated against IBDV at 14 days of

age (Bursin 2, Zoetis, Kirkland, Quebec), 21 days of age (Bursimune, Ceva Animal Health, Cambridge, ON), 8 weeks of age (Bursa Blen M, Merial, Gainesville, GA), 10 weeks of age (Matimavac) and 18 weeks of age (Maximune Avi-Pro 432 ND-IB2-BD3 REO, Lohmann Animal Health International, Winslow, ME). These breeders did not receive inactivated IBD vaccines containing variant IBDV strains. The age of breeders at the time of egg collection was not mentioned.

The program of vaccination of the breeders for the other diseases is not known. In particular, it would have been interesting to know if the breeders were correctly vaccinated against the chicken infectious anemia virus (CAV), since it is known that early CAV infection in broilers is inducing immunosuppression and interference on HVT-vectored vaccine take. The immune status of the breeder flock relative to other viruses such as fowl adenoviruses (FAdV) and avian adeno-associated virus (AAAV) which are frequently found in Canada was not mentioned as well [6].

Overall, there are many unknowns relative to the health status of the chicks and their status relative to early infection with different agents that may potentially interfere with vaccination.

Design of the study relative to the administration of the two tested vaccines

Although both HVT vectored and MLV IBD vaccines can be administered by SQ or *in ovo* route, the HVT vectored vaccine was administered by the *in ovo* route and the MLV IBD vaccine was administered by the SQ route. It is quite surprising to use these two routes in the same study since in hatcheries, only one route will be used whether the hatchery is equipped or not with the *in ovo* administration device. Since *in ovo* administrationis the most common administration route to vaccinate broilers in North America, it would have been logical to use this route for both vaccines.

VAXXITEK HVT+IBD vaccine was administered to the birds

The VAXXITEK HVT+IBD vaccine was said to have been administered in the amniotic cavity of 18-day-old embryonated eggs. Several comments can be made relative to vaccine preparation and administration:

A. The source of this vaccine is not mentioned; authors did not give guarantees that the cold chain has been preserved from the manufacturing site to the laboratory where the vaccine preparation was made.
B. It is not specified if the vaccine was prepared correctly and if commercial diluent for Marek's disease vaccine was used for the dilution. In addition, the time between the dilution of the vaccine and administration to the birds is not mentioned.
C. The vaccine has not been back-titrated at the time of administration and therefore, the real titer of vaccine administered to the embryo remains unknown. In addition, no virological test (virus isolation and/or PCR) was performed on samples (spleen or feather tips) from vaccinated birds to confirm the good vaccine take.
D. 18-day-old is too soon to get a good vaccine take; embryo should be at least 18 days and 18 hours to get optimal vaccine take.
E. It is not specified if a specific *in ovo* administration device (either individual in-ovo injector or an *in ovo* machine with multiple injectors) was used or if the administration was performed manually. For such research work, it was probably a manual administration.
F. There is no data proving that the vaccine was administered in the amniotic cavity as stated in the paper. The *in ovo* administration, whether automatic or manual, is not easy to perform reliably and needs to be adapted to the size of eggs and embryos.

Overall, there are many unknowns relative to the administration of VAXXITEK HVT+IBD vaccine and the real dose the embryos received. Furthermore, the age of the embryo was likely too low to get an optimal vaccine take.

Origin of the SK09 varIBDV strain and how the birds were challenged?

The SK09 varIBDV strain was isolated from broiler chicken farms in Saskatchewan, Canada. This strain has 98.3% nucleotide sequence identity to the varIBDV U.S.A. strain NC171 and was selected to represent circulating Canadian varIBDV strains. Indeed, a recent epidemiological study demonstrated that the majority of circulating strains in Canada have high sequence identity to NC171 [4]. This strain was shown to induce early (6 days post-hatch) infection and immunosuppression in broiler chickens with high levels of maternally-derived antibodies (MDAs) [6]. The batch of this challenge strain was a bursal homogenate from infected SPF chickens that was titrated in embryonated eggs. However, it was not specified in this paper if the challenge strain batch has been tested for the presence of undesired extraneous agents such as chicken anemia virus (CAV), fowl adenovirus (FAdV) and avian adeno-associated virus (AAAV) which are frequently found in Canada [4].

Birds were orally challenged with $3x10^3$ EID_{50} of the varIBDV SK09 strain at day 6 post-hatch, which is very early.

Antibody response induced by VAXXITEK HVT+IBD

The IBDV antibody response in serum was evaluated using the PROFLOCK Plus Synbiotic Corp. (now belonging to Zoetis). This IBDV ELISA kit is able to detect the anti-VP2 IBDV antibodies induced by VAXXITEK.

At hatch, the mean IBD MDA titer was 8144 (± 3423), a relatively hightiter but which may not be as high as the titer sometimes observed in Europe [1]. The mean IBDV antibody titer in the VAXXITEK unchallenged birds did not differ from that of non-vaccinated/non-challenged controls: at about 1500-2000 at D19 and below 1000 (threshold of positivity of the test) at D35. These titers are in sharp contrast to what is generally observed after *in ovo* vaccination with VAXXITEK in the field : for instance, mean titer of *in ovo* vaccinated broilers with a mean antibody titer of about 10,000 at hatch was at about 7000 at D21 and reached 10,000 at D42 [1]. In another study done with *in ovo* vaccination in broilers (Rautenschlein *et al*., 2011), the ELISA titers were about 6400, 2200 and 9400 at 0, 21 and 35 day-of-age, respectively.These results strongly suggest that the birds were not correctly vaccinated and/or the vaccine did not induce the expected immune response in this study.

No other test, such as detecting the vaccine virus in the spleen or in the feather pulps, was done after vaccination and therefore there is no guarantee of vaccine take in these birds.

Effect of VAXXITEK on IBD replication and on immune parameters

At 9 day-of-age (3 days post-challenge), the varIBDV virus load in bursa of VAXXITEK vaccinated birds was much higher than

in the unvaccinated/challenged control, suggesting that HVT-IBDV vaccination facilitated varIBDV replication. Furthermore, flow cytometric analysis of splenic lymphocytes at day 8 of age showed, among CD3+ T cells, a reduction of both the percentage of CD8+ (from 23.5% to 16.8%) and of CD4+ (from 28.6% to 23.4%) T lymphocytes in HVT-IBD vaccinated birds relative to unvaccinated birds. The CD8+ T cells of VAXXITEK HVT+IBD birds had a reduced CD44 (adhesion molecule and activation marker) expression compared to unvaccinated birds. Based on these results, the authors concluded that HVT-IBD is causing immunosuppression in vaccinated chicks.

Although authors indicated in the text a significant decrease in the number of total CD4+ and CD8+ T cells, figures 4A and 4B showed a decrease of percentages of CD4+ and CD8+ T cells (T cells being the CD3+ cells). The total CD3+ T cell concentration was not shown, and therefore it is not possible based on the presented data to evaluate the decrease in the absolute number of total CD4+ and CD8+ T cells. It is unclear if statistical analysis was performed on these flow cytometric analysis data. Similarly, it is not clear if the down-regulation of CD44 expression on CD8+ cells compared to unvaccinated chickens shown on figure 4C was statistically significant or not.

Since VAXXITEK HVT+IBD vaccine did not induce the expected anti-IBD antibody ELISA titers, it cannot be confirmed that these observed effects in that group are due to the vaccine.Nevertheless, it should be mentioned that a change in percentage of the different T lymphocytes in spleen induced by a vaccine does not mean that this vaccine is inducing immunosuppression. For instance, HVT is replicating in spleen and therefore, it is not surprising if slight changes in the lymphocyte subpopulations occur as already detected [7]. To our knowledge, HVT vaccination has not been shown so far to impair the immune response to an antigen.

HVT vaccine has been widely used as a Marek's disease vaccine since the early 1970s with no detected immunosuppression. It is considered as one of the safest avian vaccine in the field. Furthermore, recent paper indicated that the HVT vector administered by the *in ovo* route could hasten maturation of chicken embryo immune responses in specific-pathogen-free chickens [8]. The significantly higher IBDV load found at day 9 (3 days post-challenge) in bursae of VAXXITEK HVT+IBD-vaccinated birds is puzzling. Although VAXXITEK HVT+IBD administration was not confirmed (see question 8.), the difference for this aspect suggested that this group received something different from the unvaccinated control group that was mock-vaccinated with a saline solution administered*in ovo*. The absence of seroconversion induced by VAXXITEK HVT+IBD suggested that the vaccine take was not optimal likely due at least in part to a suboptimal administration but maybe also to a factor that interfered with vaccine take. Early chicken anemia infection could be such factor since previous field observation showed that it could significantly impaired VAXXITEK HVT+IBD vaccine take (Boehringer Ingelheim, internal unpublished data).

Major reasons why VAXXITEK did not induce protection in this study

There was no evidence of VAXXITEK HVT+IBD vaccine take in this study, and therefore it is not surprising that the vaccine did not protect. The reasons for this lack of immunogenicity may be multiple including a problem in the *in ovo* administration of the vaccine and a potential factor (maybe an infectious agent) interfering with the vaccine take.

Onset of immunity induced by VAXXITEK HVT+IBD

VAXXITEK HVT+IBD has the claim of onset of immunity in the European product at 14 days of age. However, experimental studies done in broilers in the USA with both the standard (STC) and Variant E IBDV challenge have shown that a protective effect can be observed as soon as at 7 days of age. However, in the field the onset of immunity may sometimes not be as good as in laboratory conditions. We therefore continue to recommend breeder vaccination against IBDV using the IBDV inactivated vaccines before lay in order to transfer a high MDA titer to the progeny [9]. This high MDA level should protect the young broiler chicks during the first day of life. In areas where varIBDV are known to breakthrough high levels of MDA in young broiler chicks, it may be recommended to use an inactivated vaccine containing this varIBDV antigen in breeders.

Protection data of VAXXITEK against different varIBDV challenge

VAXXITEK HVT+IBD showed heterologous protection of the bursa of Fabricius against North America variant IBDV challenges [10]. Protection induced by VAXXITEK HVT+IBD against USA variant AVS-SU and Delaware E IBDV challenges was tested using SPF eggs set for incubation and divided into 9 groups at day 18 of embryonation for vaccination. At 14 and 28 days of age respectively, groups of vaccinates and controls were challenged with variant E IBDV and AVS-SU intra-ocularly with 103.5 EID50/bird. Protection was assessed using the bursa to bodyweight ratio 7 days after challenge. The protection threshold was established using the average ratio on the negative control minus two standard deviations. Interpretation of the percentage of protection used the following criteria: poor < 20%, fair = 30–40%, good ≥ 50%. Fixed bursal tissues from the same samples were examined by histology. Serum samples for IBDV antibodies were collected on the days of challenge, 14 or 28 days. Protection against challenge at 14 or 28 days of age with AVS-SU and Delaware E was good (> 50%) in all the vaccinated groups (Merial Study #09-107 MS. Data on file).

Protection against classical ST-C and variants Del E, DMV/5038/07 or FF6 strains were also recently reported [11].

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