

IN OVO VACCINE APPLICATIONS IN CHICKENS SURPASS THE CONVENTIONAL APPROACH

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Important developments with relation to poultry production in many countries are an increasing consumption, the demand for microbiological safety and disease incidence (new as well as well-known diseases). Therefore it is understandable, that poultry production needs a strong (company) organised health care program with regard to prevention and control of poultry diseases.

Always and everywhere the following is important:

- to improve hygiene management on poultry farms
- to increase the level of immunity.

This article will focus particularly on the possibility to evoke immunity in chickens by means of vaccine administration.

The objective of vaccination is the administration of the correct dose of vaccine to a maximum of susceptible birds, knowing in advance that it is almost impossible to immunize 100 % of the birds. One of the most important reason for this impossibility with regard to conventional vaccination is that a number of chickens involved do not receive vaccine at all or receive an insufficient dose. A second reason is due to the biological variation and the general health condition of the birds.

During many years it has proven to be very difficult to vaccinate broilers effectively through spray- and drinking water administration or manual injection. Evident reasons for these difficulties are:

- Many poultry caretakers and/or broiler farmers are involved
- A variety of vaccination equipment is used on different farms
- Re-use of containers with viable vaccine residues
- Blocked spray nozzles
- Incorrect spray pressure resulting in defective distribution patterns
- Poor water quality (pH, minerals)
- Use of hot water to reconstitute freeze dried vaccine pellets
- Mixing heat and time sensitive vaccines for a complete day of vaccinations
- Incorrect dosage of vaccine for reconstitution
- Incorrect dosage of diluent for dilution
- Water lines contaminated with bacteria and/or carrying heavy biofilm loads
- Too long or too short time within waterlines
- Blocked needles or tubing
- Defective vaccine reservoirs
- Use of improperly handled diluents
- Incorrect calibrated syringes.

The threat of decrease in biosecurity due to vaccination crews is an additional problem linked to mass application of vaccines in hatcheries or on poultry farms. Because of

failing precautions or carelessness deviations occur easily and can result in a large percentage of badly vaccinated or missed birds. So the question is: What is the objective of vaccine administration? To just "vaccinate" or to really immunize a flock? Finally to just "vaccinate" will cost more! And one has to keep in mind: *Without challenge every "vaccination" is "successful"*.

If the objective of vaccine administration is to apply exactly one dose of vaccine to each bird, and at the same time one has to meet demands like quick, careful, and safe it is obvious that one has to focus on the combination of a low number of skilful people and the availability of a sophisticated and reliable automated system. Therefore in more and more countries in ovo vaccination is being rapidly adopted as the method of choice for immunizing chickens against Marek's Disease (MD) and other poultry diseases. The INOVOJECT® system allows the poultry industry to vaccinate in ovo by "automated-mass-application". At the same time this mass application is an individual application, in contrast to vaccinations through drinking water or spray administration. Originally the INOVOJECT® system is developed because of MD. But the availability of this application method enables the poultry industry to improve prevention and control of an increasing number of poultry pathogens.

In all countries where vaccination through in ovo route is introduced discussions started or will start about safety and efficacy of this approach. Since this discussion also started in Western Europe, field trials were performed with regard to in ovo vaccination against MD, Infectious Bursal Disease (IBD) and Newcastle Disease (ND).

Conventional and in ovo administration of MD vaccines

Marek's Disease is a lymphoproliferative disease of chickens and is prevalent world wide. The incidence of infections ranges widely (none to 80 %), depending on the geographic location of the flock, the genetic susceptibility of a particular strain of chickens and the virulence of an endemic virus strain (BIGGS, 1982).

Economic loss from MD is caused by mortality, condemnations, loss of egg production, and cost of vaccine and application. In spite of the development of very effective vaccines against MD, MD virus (MDV) infection is still ubiquitous. Losses from MD, though much smaller than they were before vaccines were developed, still occur world wide. In several parts of the world there appeared to be a resurgence in losses from MD.

Under intensive husbandry there is often a considerable residue of waste, dust, and feathers left in the growing house between crops of birds, resulting in the early infection of young chicks. Chicks are in close proximity to one another, facilitating contact spread. Also there is a limited free exchange of air, particularly in the winter, resulting in a high concentration of MDV-infected dander in the air. These conditions lead to an earlier infection with a larger dose of virus than under extensive conditions.

The development of new, more effective vaccines such as bivalent vaccines, and of new methods of application,

promises to control these increased losses. There is little or no possibility of ever eradicating MD from a country. It is hoped that continued research will keep pace with the appearance of new, more virulent strains of MD and thus keep losses from MD worldwide to minimum (PURCHASE, 1985)

MDV fieldstrains of low up to high pathogenicity levels induce immunodepression in chickens. Vaccine against MD is (was) routinely administered through manual injection in the hatchery. Prehatch vaccine administration - in ovo - does not adversely affect hatchability of survival of chicks. There is ample evidence that in hatched chicks, protection by HVT against MDV challenge is optimum only if Herpes Virus of Turkey (HVT) has several days of lead time before chicks are exposed to pathogenic MDV (SHARMA, 1987). Therefore in ovo vaccination is being rapidly adopted as the method of choice for immunizing commercial broilers against MD.

Recently, during field trials in Europe the protection against MD challenge as result of in ovo vaccination with protection after manual injection in broiler breeders as well as in layers was compared. The results are shown in Table 1.

These trials show that simultaneous MD-Rispens + MD HVT in ovo vaccination in broiler breeders as well as in layers has evoked a level of protection against challenge with virulent MDV which is at least equal or higher than protection as a result of the same vaccines administered through manual injection.

Conclusions of this trial (collaboration Embrex Europe and Utrecht University):

- Hatchability not influenced negatively by in ovo application
- Quality of one day old chickens not influenced negatively by in ovo application
- Simultaneous in ovo vaccination of MD vaccines Rispens and HVT proves to be a good alternative compared to manual i.m. injection.

NB: On request additional information will be available in due time.

Conventional and in ovo administration of IBD vaccines

Infectious Bursal Disease is a contagious disease of fowls caused by a double stranded RNA virus. IBD virus (IBDV) has been shown to infect ostriches, ducks, pheasants, chickens and turkeys, and is of major importance in all poultry producing regions of the world. IBDV is highly infectious in young chickens and causes severe damage to the bursa, resulting in suppression of the immune system. The virus is very stable. IBDV has been shown to remain infectious for 122 days in a chicken house and for 52 days in food and water. The most important characteristics of IBD are sudden morbidity, a high morbidity rate, ruffled feathers, prostration, dehydration, and depending on the virulence of the IBDV a high mortality rate is possible (up to 50 % in layer pullets and 30 % in broilers). Experiences in the past decades have shown that in most circumstances the high maternal antibody levels will not protect broilers up till slaughter time. The assumed long lasting maternal immunity in broilers, together with the application methods in the field underlie the IBD problems in many countries.

Inquiries in the field have revealed at least three successful methods to compromise vaccinations with live IBD vaccine:

- Thirst period too short,
- Shortage in drinking water and
- Vaccination in presence of a too high maternal antibody level.

The prevention and control of IBD asks a high hygiene standard on each broiler farm, every day, every week, every month, every fattening period, every year! Moreover it proved to be rather difficult, if not impossible, to estimate the right time of vaccination through the drinking water route because of the range in maternal immunity in each flock. The challenge concerning IBD prevention and control in chickens is increased because of the reemergence of highly virulent IBD strains in Europe, the Middle East and elsewhere. Despite extensive vaccinations of breeders and progeny, these virulent IBDV strains still cause economic losses.

To help prevent IBD, a new technology has been developed. Utilizing IBD antibodies in combination with an IBD

Table 1: MDV challenge in broiler breeders and layers after in ovo vaccination with MD-Rispens combined with MD-HVT (Summary of results at 19 weeks of age)

Broiler Breeders			Layers		
RIS ¹ + HVT ² in ovo	RIS + HVT i.m. (Inj. autom.)	Control	RIS + HVT in ovo	RIS + HVT i.m. (Inj. manual)	Control
1000 TCID ³ RIS 1000 PBE ⁴ HVT	1000 TCID RIS 1000 PBE HVT		1000 TCID RIS 1000 PBE HVT	1000 TCID RIS 1000 PBE HVT	
99 chicks	94 chicks	96 chicks	56 chicks	106 chicks	96 chicks
Challenge GA i.m.	Challenge GA i.m.	Challenge GA i.m.	Challenge GA i.m.	Challenge GA i.m.	Challenge GA i.m.
Mortality due to MD 7.1 %	9.6 %	69.8 %	Mortality due to MD 8.9 %	23.6 %	86.5 %
Protective Index 89.8	86.2		Protective Index 89.7	72.7	

¹ RIS = Rispens ² HVT = Herpes Virus of Turkey ³ TCID = Tissue culture infective dose ⁴ PFU = Plaque forming units

⁵ Protective Index [PI] = $\frac{(\% \text{ MD lesions in non vaccinated control}) - (\% \text{ lesions in vaccinated group})}{\% \text{ MD lesions in non vaccinated control}} \times 100$

Claim for efficacious MD vaccines PI = 80; a publication about these trials is in preparation

vaccine virus to form a complex, the antibody + IBD complex is used as a vaccine that is administered in ovo via the INOVOJECT® system, only once during the life of the broiler. This complex vaccine has been shown to be safe and efficacious for in ovo administration to birds with no maternally derived immunity (SPF birds) and to broilers that have high maternal antibody titers. This complex vaccine has undergone efficacy and field safety trials and were shown to be safer and more efficacious than administration of IBD vaccine alone. The complexing of virus with the correct ratio of antibody allows for safe in ovo administration because viral replication in the bursa - of SPF birds as well as commercial broilers - is delayed.

As a result of the aforementioned research this vaccine (commercial name Bursamune IN OVO® will be approved for use in the field in due time in the UK. Bursamune IN OVO® is used in several field trials in Europe. In Table 2 and 3 results are shown of a paired trial on 7 broiler farms.

Table 2 shows that the treatment group demonstrated comparable or better hatchability than the control group (both groups are progeny from the same breeder flocks).

Table 2: Hatchability of inoculated and non inoculated eggs

Farms	Treatment: BURSAMUNE <i>in ovo</i> ™			Control: Uninoculated		
	No Inoc.	No Hatched	% Hatch	No Set	No Hatched	% Hatch
E-F	41100	34168	83.13	51239	41921	81.72
G	15900	13560	85.28	15750	13503	85.73
H	24300	20735	85.33	24507	20673	84.36
Total	81300	68463	84.21	91496	76097	83.17

Table 3 shows that the level of immunity in the treatment group is significantly higher compared to the control alone.

Table 3: Means of 2 log Elisa antibody titre to IBD virus and proportions of broilers with a positive AGP¹ titre to IBD virus (paired trial on 7 broiler farms)

OUTPUT VARIABLE	Week 3		Week 4		Week 5	
	BIOV ² [n=168]	CV ³ [n=168]	BIOV [n=168]	CV [n=168]	BIOV [n=168]	CV [n=168]
² log titre (Elisa)	3.80 (0.58) ⁴	3.47 (0.58)	6.48 (1.15)	4.15 (1.15)	9.7 (1.14)	5.7 (1.14)
	P = 0.68		P = 0.08		P = 0.03	
% broiler with titre (AGP)	0.6	0	48.8	12.0	81.5	42.2
	P = 0.99		P = 0.03		P = 0.03	

¹ AGP = Agar gel precipitation
² BIOV = in ovo vaccination with BURSAMUNE
³ CV = vaccination with conventional live IBD vaccine in drinking water
⁴ standard error of the mean
Differences in means are statistically significant if p < 0.05

Conventional and in ovo administration of ND vaccines

Newcastle disease is a very contagious disease of the respiratory tract of chickens and turkeys. ND outbreaks may cause economic disasters to the international poultry industry. In the early nineties this was emphasized in

Europe by extensive outbreaks. These outbreaks could be explained by:

- Import of virulent ND virus (= NDV); e.g. backyard poultry, petbirds
- Spread of virulent NDV (without doubt the greatest potential for spread of NDV is by humans and their equipment, including spray- and aerosol vaccination machines)
- Aversion of many broiler farmers against ND vaccinations, because of reactions of the respiratory system after conventional ND vaccination. Consequently the immunity level of vaccinated broilers was often too low.

As long as it is possible to import pet birds and backyard poultry in Europe, the poultry industry is obligated to ascertain a sound vaccination policy (the paucity of knowledge concerning the presence of poultry pathogens in backyard poultry is a continuous economical threat for the poultry industry).

At present ND vaccinations in the broiler industry have to be performed at each farm using a spray, or aerosol machine (after the first spray vaccination on the hatchery). This means under practical field circumstances that many different persons are involved in prevention and control of ND. Therefore it is rather difficult to implement a uniform vaccination policy with regard to all broiler farms within a region.

Necessary goals to improve prevention and control of ND in broilers around the world:

- To improve the application method to ensure that every broiler will receive the necessary dose of vaccine at the right time.
- To look for the possibility to choose a better application method for use in hatcheries which will exclude the necessity to have involved a lot of people in the field with their spray and/or aerosol machines.
- To try to decrease the level of post-vaccinal respiratory reactions and at the same time to reach and maintain a sound protection level against challenge with virulent NDV.

At present it is still common practice to vaccinate broilers (at least) twice in those regions where NDV is prevalent. For example: At day 1 by means of spray in the hatchery and around 3 weeks of age through spray (or aerosol). Experiences in the past have taught that vaccination of commercial broilers in the field against ND through spray methods will often result in resistance against challenge from 40 up to 80 % of the vaccinated broilers per flock. This is also shown in Table 4. These results were produced after challenge of broilers sampled at random from Dutch broiler farms in 1993, one year after outbreaks of virulent ND. The intention of these challenges was to assess the protection against virulent NDV- Herts 33. Between these challenges and the first outbreaks it was made compulsory to perform at least one booster vaccination on the farm after the spray vaccination at the hatcheries.

Because it is very difficult to implement a uniform vaccination-policy on broiler farms, a promising approach to control ND proves to be through in ovo application. The results of recent experiments with an ND in ovo vaccine for broilers as shown in Table 5 are very encouraging, especially compared with earlier results from challenge experiments as shown above in Table 4.

Table 4: Protection after challenge with NDV Herts 33 ($10^{6.5} \text{EID}_{50}$ per bird¹) of broilers vaccinated twice with live ND vaccines during growing period

Group ²	Day of vacc.	Method of adm.	Day of vacc.	Method of adm.	Challenge age	% protection	Average % protection
1	1	S ³	14	S	4	67	
2	1	S	14	S	4	92	
3	1	S	14	S/D ⁴	4	75	78
4	1	S	14	S	6	75	
5	1	S	16	S	6	42	
6	1	S	21	S	6	67	
7	1	S	17	S	6	75	
8	1	S	14	S	6	83	
9	1	S	13	S/D	6	67	
10	1	S	14	S	6	100	
11	1	S	14	S/D	6	75	73

Animal Health Service and Utrecht University (1993)

¹ EID_{50} = Egg infectious dose 50 %² Each group of challenged broilers consisted of 10 -15 birds³ S = ND vaccination through spray application⁴ D = ND vaccination through drinking water administration

100 % of non protected control SPF birds died within 5 days pc.

Table 5: Protection of broilers vaccinated in ovo with Poulvac® OVOline™ ND after challenge with NDV Herts 33 ($10^{6.8} \text{EID}_{50}$ per bird¹)

Group ²	Day of vacc.	Method of adm.	Challenge age	% protection
1	ED18 ³	IN OVO	2	96.9
2	ED18	IN OVO	4	96.7
3	ED18	IN OVO	6	81,2 ⁴

Fort Dodge, Embrex Europe, ID Lelystad and Utrecht University (1998)

¹ EID_{50} = Egg infectious dose 50 %² Each group of challenged broilers consisted of 32 birds³ ED18 = day 18 of embryo development⁴ 9.4 % (3 birds) died and 9.4 % (3 birds) were ill

100 % of non protected control SPF birds died within 5 days pc.

Because ever more broilers are grown up to 5 weeks (even only 4 in some countries) one may expect that possibly in many cases one ND vaccination in ovo will prove to be adequate, if we are able to repeat these results on a larger scale.

Conclusion

In ovo administration of vaccines gives the possibility to aim very effectively at universal poultry health care where possible and tailormade health care were necessary.

Literature

Available on request from the author.