Republic of Iraq Ministry of Higher Education & Scientific Research University of Al-Qadisiyah College of Veterinary Medicine



In ovo poultry vaccination

A Graduation Project Submitted to the Department Council of the Internal and Preventive Medicine-College of Veterinary Medicine/ University of Al-Qadisiyah in a partial fulfillment of the requirements for the Degree of Bachelor of Science in Veterinary Medicine and Surgery.

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لِمُ لِلَّهِ ٱلرَّحْمَدِ ٱلرَّحِيمِ بْسُ

فَنَعَنَّكَ ٱلَّهُ ٱلْمَلِكُ ٱلْحَقُّ وَلَا تَعَجَلُ بِٱلْقُرْ إِلَى اللَّهُ اللَّهُ الْمَعَلَى لَلْعُ لَقُونُ من سورة طه إِلَيْكَ وَحْيُهُ وَقُل رَّبِّ زِدْنِي عِلْمَا ٢

Certificate of Supervisor

I certify that the project entitled (-----HawraaAbbas Hayawan-----) was prepared by ------Hawraa Abbas Hayawan------ under my supervision at the College of Veterinary Medicine / University of Al-Qadisiyah.

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-- / -- / 2020

Certificate of Department

We certify that -----hawraa Abbas Hayawan----- has finished his/her Graduation Project entitled (---In Ovo poultry vaccination) and candidate it for debating.

Instructor

-- / -- / 2020

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-- / -- / 2020

Dedication

To, my God and my nation to the Messenger Muhammad (God bless him and God and peace), My family and my friends and my professors and All those who supported me in completing my Graduation Search ,Thank you from the bottom of My heart for all the support and assistance and to Pray. I wish you achieve the satisfaction of God And achieve Wish list.

Hawraa

Vaccine

It is a sensitive, soluble and perishable product that contains one or more types of (germs) Viruses, parasites or antigens (which stimulate the body to make and produce Immune bodies, and the vaccine may be live, weakened or killed.

Type of vaccines

- Vaccines Recombinant
- Vaccines Toxoid
- Nucleic Acids vaccines
- Vaccines attenuated Live
- Killed vaccines

ABSTRACT

The various methods employed for the in ovo administration of different moting the health and productivity of poultry are dis-cussed in this review article. The amnion has proven to be an effective site for injection and the timing of In ovo injection has commonly occurred at transfer However, the volumes and dosages or concentrations of the materials administered vary depending on bird type, egg size, timing and site of injection, incubation system and regimen, and the type of material. Both manual and automated injections have been shown to be effective. Nevertheless, commercial application man- dates automation. Materials described in the literature over the past 20 years or more for in ovo use in avian species include vaccines, drugs, hormones, competitive exclusion cultures and prebiotics, and supplemental nu trients. Vaccines approved for in ovo delivery include trients. Vaccines approved for in ovo delivery include trients. Some of the materials listed above have been shown to be viable

candidates for enhancing immunity and for promoting embryonic and posthatch development. Several reports have indicated that probiotics may be effectively used to fight intestinal bacterial infections, and folic aid as well as egg white protein and various amino acids including L-arginine, L-lysine, L-histidine, HMB, and threonine alone or in combination, have been shown to benefit embryonic development or posthatch perfor mance. Furthermore, CpG oligodeoxynucleotides, vita mins C and E, and thyme and savory have the potential to enhance immunity, carbohydrates can be used to in crease tissue glycogen stores, and creatine can be used to promote muscle growth. Trace minerals and vitamin D3 have shown potential to improve bone strength and potassium chloride may be an effective alternative electrolyte in vaccine diluent. The in ovo application of these and other materials will continue to expand and provide further benefits to the poultry industry.

Key words: injection, in ovo, nutrients, poultry, vaccinevaccine

<u>Chapter</u> one (Introduction)

Introduction

The vaccination in the eggs is done by machines. These machines take a number of measures to ensure that the chicks are properly fertilized inside the egg. The benefits of oocyte vaccination include avoidance of bird stress, controlled health conditions, and early immunity with less interference from the maternal antibodies <u>.</u>

The in ovo vaccination was first proved to be efficient for Marek's vaccine by experiments performed by Sharma and Burmester in 1982. These authors demonstrated that chicks vaccinated in ovo at 18 days of embryo development against Marek's Disease had better protection against virulent MD challenge carried out at 3 days of age as compared to those chicks vaccinated at hatch. At seven days of age, the both groups (vaccinated at hatch or in ovo) induced equivalent level of protection .

At that time in ovo vaccination was only a new idea, but today it is being used in commercial applications worldwide in the poultry industry. The basic laboratory concept of this vaccination, initially used for vaccination against Marek's disease, has expanded and today we can find machines capable of inject up to 60,000 eggs per hour.

Benefits of vaccines

- Safe, consistent and accurate method of vaccine application
- Significant labour costs reduction vs subcutaneous or field vaccination
- No Post-vaccination reaction
- · Improvement of the chick quality of your flocks
- Early access to food and water. Day-old-chicks are not stressed after hatch
- Better development of the immune system at hatch.

In ovo vaccination can be used for Marek's disease, Gumboro disease, Newcastle disease or Avian Influenza disease.

Vaccined

Marek's Disease

Sharma and Burmester (1982) were the first to con- ceive, through a basic laboratory experiment, that in ovo vaccination could be used to effectively protect birds against MD. Since that time, in ovo vaccina-tion has also been used for Gumboro or IBDV, New-castle disease, and avian influenza, and has expanded as a standard commercial practice in hatcheries world- wide. In the article by Sharma and Burmester (1982), it was reported that chickens that had been vaccinated as 18-d embryos with strain FC126 of herpesvirus of turkey (HVT) had a much greater resistance to an intra-abdominal challenge of pathogenic MD virus at 3 d posthatch than did those that were vaccinated sub- cutaneously in the back of the neck on the day of hatch. The embryonated eggs were inoculated with a 0.1 mL volume of HVT using a 1-inch-long, 22-gauge needle. The entire length of the needle was inserted through a small hole that was punched either into the large end or halfway on the long axis of the egg. When injected in the side of the egg, inoculum was delivered 55, 25, and 20% of the time in the embryo body, amniotic fluid,

and yolk sac, respectively. When injected in the large end of the egg, inoculum was delivered 11, 89, and 0% of the time in the embryo body, amniotic fluid, and yolk sac, respectively. While not affecting hatchability, the in ovo application allowed the chicks to be persistently viremic from hatch through 8 wk of age posthatch. Fur- thermore, recoverable virus titers were higher in chicks that had been vaccinated as embryos in comparison to those that were vaccinated posthatch. It was concluded that not only can prenatal vaccination protect chick -ens against a subsequent naturally occurring infectious viral disease, but that the vaccine virus needs at least 6 d to confer the chick with maximum resistance to a

Challenge.Succeeding similar studies were conducted by Sharma And Witter (1983) and Sharma et al. (1984) confirm- Ing that in ovo vaccination with serotypes 1 and 2 of The MD virus (Sharma and Witter, 1983) and HVT (Sharma et al., 1984) at 18 d of incubation provided The bird a marked increase in immunological compe- Tence. Specifically, Sharma and Witter (1983) showed That in ovo vaccination rather than at hatch, better Protected birds against a virulent MD challenge at 3 d Posthatch, and Sharma et al. (1984) demonstrated that HVT vaccination at 18 d allowed the virus to quickly Propagate in the embryonic tissues, particularly in the Lung. More recent work by Wakenell et al. (2002), in Which an HVT/SB-1 vaccine that was likewise injected By manual injection, using an 18-gauge needle, showed That MD vaccines administered by in ovo injection must Be delivered into the amnion or body proper of SPF And broiler embryos for optimum performance to be re-Alized In recent studies, it was shown that the automated in Ovo injection of 50 µL of the MD vaccine in the amnion Or body of the embryo at 18 d of incubation did not Negatively affect the early posthatch quality of Ross 708 Broilers whether or not they experienced a 4- or 18-h Holding time before placement (Peebles et al., 2016). It was also shown that that same vaccine regimen did Not exacerbate the effects of the 4- to 18-h increase In holding time on their performance through 48 d of Posthatch age (Peebles et al., 2017).

Infectious Bursal Disease (Gumboro disease)

Giambrone et al. (2001) were the first to investigate The safety and efficacy of 3 intermediate IBDV vaccines, Administered by in ovo injection, that are routinely used To

protect birds against standard and antigenic variant IBDV challenges. The vaccine was injected manually Through the air cell at 18 d of incubation into eggs con-Taining specific-pathogen-free (SPF) or broiler chicken Embryos using a 1-inch-long 21-gauge needle. Birds were Challenged by eye or nasal routes with virulent IBDV Viruses at a 103 mean chicken infective dose at 3 wk of Age. It was found that although all the vaccines caused Severe microscopic lesions in the bursae of the birds at 1 and 3 wk posthatch, they resulted in a minimum of %87protection against a standard and 60% protection Against a variant IBDV challenge. A full dose of any of The vaccines increased embryonic and 3 wk posthatch Mortality, whereas a half dose had no adverse effect on Mortality at either time. Using half of the commercial Doses of IBDV and MD vaccines upon dilution should, Therefore, protect birds against immunosuppression and Subsequent mortality when administered by in ovo in-Jection Also, in a later study, in which SPF and broiler chick- Ens were likewise used, Moura et al. (2007) tested the Efficacy of a recombinant attenuated vaccine for its abil- Ity to protect the birds against a subsequent IBDV Challenge. It was observed that vaccine doses of either 2.3×103 or 5.6 \times 103 pfu, delivered at 18 d of incu-Bation, protected both types of birds against mortality And bursal damage after an STC strain IBDV challenge At 2 wk of age posthatch. Furthermore, both doses gen-Erated high antibody titers and had no effect on hatch-Ability or wk 1 survival in both the SPF and broiler Birds. However, the lower dose fully protected the SPF Chickens while the higher dose was required to fully pro-Tect the broilers. The means of vaccine delivery and the Tissue area targeted in the egg were not indicated, but It is suspected that the vaccine was introduced into the Amnion through the large end of the egg by manual Injection

<u>NSA (Best Jumboh Vaccine): -</u> - Immunosaway to prevent injury and not only protect against Jamburu's day in the lamp. (Jomborough control needs to prevent an incidence of protection). - Protection against Jamburu's disease at one dose of a day in the lab of hatching without any doses activities during the education cycle. - Does not conflict with illiteracy. - Comprehensive protection against all blinds of Jamburu (Classic - Maghaira - severe essential). - Reduce the epidemic load on farms by reducing the re-secretary and thus prevents Jamburu disease during the following courses. - Immunization of the Saifa International Immunization Group in the Hatching and Al-Wahid Lab at the Certificate of

Chick Program, which ensures the optimal application and good practices for immunization within the hub.

Newcastle Disease

The effects of various volumes and antigen con-Centrations of an inactivated oilemulsion Newcastle Disease vaccine, administered by in ovo injection at 18 d of incubation, on the hatchability and immunity Of White Rock and White leghorn chickens were evalu- Ated by Stone et al. (1997). Using needles between 16-And 22-gauge, the vaccine was delivered at a 1.5-inch Depth in the small end of the egg so that the vaccine Was deposited either subcutaneously, in the yolk, or in The coelomic cavity. Serum hemagglutination-inhibition Titers were first identified at 2 wk of age posthatch, se-Roconversion of chickens vaccinated as embryos ranged From 27 to 100%, and vaccination provided protection Against morbidity and mortality in response to a vis-Cerotropic velogenic Newcastle disease virus challenge At 53 d of age. When using 18, 20, or 22-gauge needles, The injection of 100 μ L of the vaccine did not signifi-Cantly affect hatchability in either the White Rock or White Leghorn chickens. Stone et al. (1997) concluded That if the Newcastle disease vaccine contains suffi-Cient antigen and is administered properly, that pro-Tective immunity and acceptable seroconversion rates And hatchability can be achieved.

Avian Influenza

The effects of an inactivated oil-emulsion avian in-Fluenza vaccine administered by in ovo injection at 18D of incubation on the hatchability and immunity of White Rock and White Leghorn chickens were also Evaluated by Stone et al. (1997). The procedures em-Ployed by Stone et al. (1997) for vaccine administration Were the same as those used for the Newcastle disease Vaccine. Serum hemagglutination-inhibition titers were Likewise first identified at 2 wk of age posthatch, se- Roconversion of chickens vaccinated as embryos ranged From 85 to 100%, and vaccination provided protection Against morbidity and mortality in response to a highly Pathogenic avian influenza virus challenge at 34 d of Age. As for the Newcastle disease vaccine, Stone et al. 1997) found that when 18-, 20-, or 22-gauge needles Were used, the injection of 100 μ L of the vaccine

did not significantly affect hatchability in either the White Rock or White Leghorn chickens. Stone et al. (1997)

Concluded that if the avian influenza vaccine, like the Newcastle disease vaccine, contains sufficient antigen And is administered properly, that protective immunity And acceptable seroconversion rates and hatchability Can be achieved.

Mycoplasma Gallisepticum

The F-strain of Mycoplasma gallisepticum is a vaccine strain used in the commercial layer industry for protec- tion against field-strain infections. Elliott et al. (2017) delivered a 50 μ L volume of F-strain Mycoplasma gal-lisepticum vaccine by in ovo injection into the amnion of layer embryos at 18 d of incubation. After swabbing, F-strain Mycoplasma gallisepticum was found in the tra- chea, mouth, esophagus, yolk sac membrane, and duo-denal loop of the subsequent hatchlings.Based on those

Findings and the effects of various dosages on hatcha-bility, it was indicated that vaccinating layer embryos with lower dosages (not greater than 102 CFU/dose) of F-strain Mycoplasma gallisepticum has the potential for practical application in the commercial table egg industry.

Sites of injection

We can consider that there are 5 basic compartments on an egg during the final stage of incubation: Air Cell, Allantoic sac (waste), Amniotic sac, the Embryo itself and the Yolk sac.

1-The air cell which is basically filled with gas.

2-The allantoic sac which is filled with fluid containing embryo development byproducts.

3-Amniotic sac which is composed by amniotic fluid and the embryo body.

4-The embryo itself which is located inside the amniotic sac.

5-The yolk sac which is also inside the amniotic sac

Any one of those compartments can be accessed by the needle of the machine. However, in order to achieve and maximize immune response by in ovo vaccination, it is essential to assure that the correct compartment inside the egg is accessed.

Due to its fast development in this last stage, specially when we consider the in ovo injection window (as mentioned before, between 17.5 and 19.2 days of incubation), it is important to realize that these compartments can change fast as they are utilized by the embryo. It is also essential to recognize that the specific compartments are responsible for different support during the development of the embryos, and the placement of vaccines and/or other compounds into those compartments may allow or limit their absorption by the embryo.

The position of these compartments inside of the egg, and more specifically, the position of the embryo depend on its stage of development.

Age	vaccine	Method of vaccine	Туре
1days	New castle	Spray	B1
	IB		Massachusetts
14 days		Water Drinking	Intermediate
	Gumboro		
21days	New castle	Drinking or	Lasota
	IB	spraying water	Massachusetts
28 days	Gumboro	Water Drinking	Intermediate

Meat chicken vaccination program

The vaccination program in hatching eggs

type of vaccine	Method vaccination
Maric (Risp+HVT)	Intramuscularl
double	
IBHI20	Coarse half-dose spraying (1000 doses, 50 ml distilled water / 2000 sauces)
Newcastle oil+E_coli	A needle to a muscle in the thigh or chest

Newcastle (Clone 20 or	Coarse half-dose spraying (1000 doses, 50 ml distilled water / 1000 sauces)	
Hitcherba)		
Gumboro	In drinking water 1000 doses in 10 liters of	
	disinfectant-free water / 1000 sauces	
Newcastle La Sota	fine spray sauce 1000 doses in 500 ml distilled water / 1000 sauces	
Gumboro	in drinking water 1000 doses in 15 liters of disinfectant-free water / 1000 sauces	
Newcastle La Sota	fine spray 1000 doses 300 ml distilled water / 1000 birds or drinking water	
Avian influenza	Intramuscular or Subcutaneous	
Newcastle oil+E_coli	Intramuscular or Subcutaneous	
Fowl Pox	Wing prick	
Newcastle La Sota	fine spray 1000 doses 400 ml distilled water / 1000 birds or drinking water	
Avian influenza	Intramuscular or Subcutaneous	
Infectious	Eye drop	
Laryngotracheitis(ILT)		
Avian	Drinking water	
Encephalomyelitis		
(AE)		
IBHI20	fine spray 1000 doses 400 ml distilled water / 1000 birds or drinking water	

Conclusions

In ovo injection has become an important tool to administer vaccines in the hatcheries. As it was aforementioned, everything started around 25 years ago with Marek's Disease vaccine and today there are several other products to be injected through this equipment. Products like Transmune®, Vectormune® IBD, Vectormune® ND, Vectormune[®] AI are already commercially available and many others are being developed, clearly demonstrating an even brighter future for this technology. Nevertheless, some basic pre-cautions must be taken into account in order to achieve the best results with this tool. Good sanitation of the hatchery, proper disinfection of the hatching eggs are among these special cares. Furthermore, precise maintenance of the machine is compulsory. By ensuring that these procedures are properly followed, companies can sure benefit from this interesting technology.

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