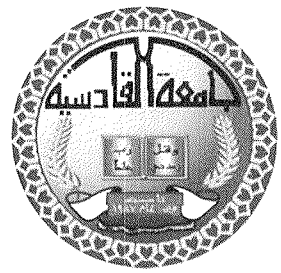
Republic of Iraq

Ministry of Higher Education

And Scientific Research

University Of Al-Qadisiya

Collage of Veterinary Medicine



Study Effect of Gumboro Vaccine on other Vaccines Programs .

A graduation project submitted to

The college Board of veterinary medicine Al-Qadisiya university , as a partial requirement to get B.S.c. in medical and surgery of veterinary medicine .

By

Mohammed Saad Rasol

Supervised

By

Ass.lect.

Dr. Nafea S. Jassim

2015 A.D **1436 A.H**

بسم الله الرحمن الرحيم

((فتعالى الله الملك الحق ولا تعجل بالقرآن من قبل أن يقضى أليك وحيه وقل رب زدني علم))

صدق الله العلي العظيم

سورة طه:من الآيه114

إقرار المشرف

أشهد بان اعداد هذا البحث قد جرى تحت إشرافي في جامعة القادسية كلية الطب البيطري وهو جزء من متطلبات نيل درجة البكالوريوس في الطب والجراحة البيطرية.

أ.م.د. نافع صبيح جاسم

٢٠١٥/٣/

بناءاً على التوصيات المتوفرة ارشح هذا البحث للمناقشة

Certificate of Instructor

We certify that Mohammed Saad has completed the fulfillment of her graduation project for the year 2014 /2015 under our constraction

Head of depart. Of Int.&prev.med

Dr. Asaad J .A.

Instructor

Dr. M. H. Hussain

الاهداء

الى من سكن قلبي...

ابي...امي

الى من هم سندي في هذه الدنيا...

الى من هم رفاق مشواري...اصدقائي صحبتي...

الى من هم لهم الفضل في انارة طريقي..

أساتذتي الافاضل...

شكر وتقدير

أتقدم بجزيل الشكرو التقدير لعمادة كلية الطب البيطري وخاصة السيد عميد الكلية المحترم لتوجيهاته السديدة وتشجيعه المستمر للبحث العلمي.....

ولا يفوتني ان أتقدم بالشكر والعرفان لفرع الامراض وامراض الدواجن والاسماك لتوفير جميع مستلزمات البحث.....،

كان لأستاذي المشرف أ.د .نافع صبيح جاسم عظيما في تصميم وأنجاز البحث فله مني كل الحب والتقدير لما بذلته من جهد لن انساه ابدا....

كما أتقدم بجزيل الشكر والعرفان لأخي محمد عبد الله لما بذله من جهد في خدمتي.

محمد

List of Contents

Summray

Infectious bursal disease (IBD) (Gumboro disease) has been described throughout the world, and the socio-economic significance of the disease is considerable world-wide. Various forms of the disease have been described, but typing remains unclear, since antigenic and pathotypic criteria are used indiscriminately, and the true incidence of different types is difficult to determine. Moreover, the infection, when not fatal, leads to a degree of immunosuppression which is often difficult to measure. Finally, the control measures used are subject to variations, and seldom follow a specific or standardised plan. In the context of expanding international trade, the authors provide an overview of existing knowledge on the subject to enhance available information on the epidemiology of IBD, the identification of reliable viral markers for diagnosis, and the implementation of specific control measures to ensure a global and co-ordinated approach to the disease. (T.P. van den Berg ,*etal.* 2000)

The destruction of immature B lymphocytes in the bursa creates an immunosuppression, which will be more severe in younger birds. In addition to the impact on production and role in the development of secondary infections, this will affect the immune response of the chicken to subsequent vaccinations which are essential in all types of intensive animal production (Haddad E.E., etal,. 1997).

Different consanguineous lines of poultry show highly variable susceptibility to experimental infection with the same strain of IBDV. The results of crosses between resistant and susceptible lines show that resistance is a dominant hereditary characteristic. However, the genes responsible for resistance have not been identified, and genetic selection for resistance has not yet been practised (Kouwenhoven B .2000) .

In addition to strict compliance with rules of hygiene and disinfection, the success of vaccination depends on the choice of the vaccine strain and on the vaccination schedule. These must take account of the existence of certain pathotypes and the presence of antigenic variants in certain regions (T.P. van den Berg ,*etal.* 2000) .

The deleterious effect of IBD vaccine on antibody levels against ND vaccine was low when IBD vaccine was administered at 14 days of age as compared to 7 days of chicken age. No great variation in the antibody titers when chicks were administered ND vaccine containing LaSota or Hitchner B1strain of the virus were observed, although slight better antibody responses were noted for LaSota over HitchnerB1 strain. Vaccination of chicks with ND vaccine of LaSota strain at 7 days followed by vaccination with IBD vaccine at 14 days yielded better antibody titers than Hitchner B1.

Chapter One

1- Introduction

Infectious bursal disease virus (IBDV) is an acute and highly contagious viral disease of young chicken. It belongs to the genus Avibirnavirus, family Birnaviridae (El-Yuguda A. D. 2000). IBDV is the causative agent of acute or immunosuppressive disease in chickens because of the resulting morbidity and mortality as well as the immune suppression. The disease affects primarily bursa of Fabricious and other lymphoid organs to lesser degree. The virus could be classified into tow serotypes serotype 1 and serotype 2. Serotype 1 strains are pathogenic, with the target organ being bursa of Fabricius (BF) while studies on serotype 2 strains demonstrated that they do not cause disease or protect against infection [Haddad, etal . 1997.].

Serotype 2 antibodies are very widespread in turkeys and are sometimes found in chickens and ducks. There are no reports of clinical disease caused by infection with Serotype 2 virus (Lasher H.N. & shane S.M. 1994).

Infectious bursal disease (IBD) is caused by a virus that is a member of the genus Avibirnavirus of the family Birnaviridae. Although turkeys, ducks, guinea fowl and ostriches may be infected, clinical disease occurs solely in chickens. Only young birds are clinically affected. Severe acute disease of 3–6-week-old birds is associated with high mortality, but a less acute or subclinical disease is common in 0–3-week-old birds. This can cause secondary problems due to the effect of the virus on the bursa of

Fabricius. IBD virus (IBDV) causes lymphoid depletion of the bursa, and if this occurs in the first 2 weeks of life (Lasher H.N.& shane S.M. 1994).

1-2 History

The first case of IB was diagnosed in 1931 in the USA. At that time it was a disease affecting chickens but in the 1940s it was already causing significant losses within the laying industry and in the 1960s the first cases of nephropathic syndrome was observed. The virus was isolated in 1936 and in 1956 the first report of multiple serotypes was published. The first commercial vaccine appeared in the 1950s (Charlton BR. 1996).
There has been intense research concerning the disease and its prophylaxis since the discovery, but it is still one of the most important poultry diseases in the world with a virus constantly changing to new serotypes and strains requiring the development of new empirical vaccines. At least since the 1970s the disease has been prevalent in Sweden (Engström B, *etal* .2003) with major outbreaks in the 1990s with vaccination commencing in 1997 (Farsang A, *etal.* 2002). Today it is to be reported to the Swedish authorities upon.

IBDV is very stable and resistant to many disinfectants, and therefore vaccination is considered as the best way to control the disease (Van den Berg TP and Meulemans G, .1991). Breeders are hyperimmunized with live and inactivated vaccines in oil suspension in order to protect the progeny by the passive transfer of antibodies (Sharma JM, *etal.* 2000). reported that young chicks with high titers of maternal antibodies and vaccinated at 3 days of age with an intermediate vaccine did not develop a humoral mmune response, even though these birds resisted to a later

challenge with a classic strain of IBDV. evaluated three vaccines available in Japan and observed that one of them (intermediate type) protected almost 100% of the birds vaccinated at 20 days of age and challenged 10 days later with a very virulent IBDV strain. The authors suggested that the high levels of maternal antibodies play an important role in protection and that all flocks should be vaccinated according to adequate schedules. In practice, although different schedules of vaccination are recommended and used in Brazil, outbreaks outbreaks have been frequently reported.(Sharma JM, *etal.* 2000)

Chapter two

2-1Types of immune

2.1.1.Passive Immunity

It is immune acquired body bird moms as well as through injections and is not for the body bird role in the composition of which varies on natural immunity (passive immunity), which the body produces against antigens that have the ability to stimulate the immune system in the body of the bird (lethonen & wiljanen, 1980).

Is a maternal immune antibodies transmitted from mothers to chicks by the egg yolk, and vary the levels of antibodies acquired from the herd to another as well as between the chicks in the same herd, depending on the immune status of mothers (Grimes, 2002).

Equalized the amount of antibodies in chicks aged one day with the amount of antibodies in mothers but primitive decline and fall after (2-3) on the estimated rate of decline for Ugartm one every four days and a half, and even after 21 days disappear if the mothers chicks fertilized vaccine neighborhood and more than 40 days if mothers were vaccinated with killed vaccine (Chandral etal, 200).

2.1.2. (Cell Mediated Immunity)

The cellular immunity important role in resistance to disease in the early stages of bird life (Mast & goddeeris, 2000), and can stimulate cellular immunity by re-vaccination stimulates cellular immunity when Vaccinated drip eye (McGinnes etal., 2002), the cellular immune consists stimulate lymphocytes (T cell), and also a section of lymphocytes stimulate immune cell type B (B cell) and cells (macrophage) or the rest

of helper cells (T helper) working there lymphocytes another type (T cell) are inhibiting the effectiveness supperssors cells (mebastion *etal*,. 2001)

2.1.3. Humoral Immunity

These include HIV antibodies (immunoglobulins), which produces the B lymphoid cells emerging from the **bursal**, that these antibodies have the ability to help or neutralizing the equation in the equation of specific infectious agents (zander etal., 1997)

Depends opposites produce a strain of the type of disease, as well as virulence in addition to the immune status of the birds, and age, and the type of bird, and nutrition (Obrdorfer etal., 1999), that the immune humoral of antibody and cell constituents are speciálized where adhere opposites virus (playa, 1991)

2.1.4.Local Immunity

Researchers proved (Palya & Rey Imamu, 1992) that the initial protection may notice the presence of a small level of measured antibodies in serum or its absence is due to the presence of local immunity in the respiratory tract is gland Harder are the factory for most specialized eye protection, which are sensitive to infection for topical opposites through the air, spray (Partadiredja etal., 1978).

Plasma cells gathering begins after hatching and evolve within 4 weeks of age and be a high percentage in Harder gland and then the lacrimal glands (peters etal., 1999), and stimulates the production of immune globulin IgA high rate and IgM and IgG by less with secretions Harder gland, and be transmission of this globulins Harder gland to mucous secretions and tears through the circulatory(Powell, 1982).

The constituent cells for IgG and IgM and IgM in Harder They gland (1% 0.3% 0.1%) respectively Is age a week and to increase the number (32% .12% .36%) four weeks old (Quarles etal., 1970).

2-2 Vaccines Type

Divide the vaccines that are used for the prevention of viral diseases into two types: - live vaccines and vaccines revoked, or the killed (Snyder etal., 1999)

2.2.1: - Live vaccines

2.2.1.1 Vaccines live weak virulent *(Live Lentogenic Vaccine)*

using few strain pathogenicity and capable of generating sufficient immune response when given (OIE, 2004; spradbrow, 2002) and is giving it in different ways, such as distillation or eye nostril, and spraying, and feed and have an impact in the prevention of losses caused by diseases (ston etal., 1997)

Vaccines are given small reconstruction has standard antibodies after vaccination up to a good standard (Jordon, 1990), and stimulates the vaccine inoculation live all forms of immune response (Grimes, 2002), the live vaccine to immunize the most is the most virulent and cause complications after vaccination (spradbrow, 1988).

Efficiency depend reluctance vaccine live on the type of vaccine used, and the strain, and the method of suppuration (symbiotic corporation, 1993).

2.2.1.2 Vaccines medium virulent (*Live Mesogenic Vaccines*)

Often used in the second vaccination enhanced the initial vaccination by weak virulent strain, is one of the vaccines of high immune efficiency,

contributing outstandingly to control the disease (Alexander, 2000), from vaccines given this kind by strain either way and injected into the dermis in Fold the wing or the muscle or under the skin and can be given via drinking water (Jordon, 1990).

2.3 - Vaccines revoked or the killed (*Inactivated virus vaccines*)

The preparation of vaccines killed processing means Allantoise liquid containing the virus with chemicals such as substance formalin or material Beta propoilacton or physical ways, such as exposure to radiation or temperature (Grimes, 2002), and from these hydroxide of aluminum materials, alum, and the oily salt, and vitamin E, and liquid paraffin (Liquid Paraffin) and material Aveidine (Wakenell and Sharma, 1998), and vaccines oily emulsion (Emulsion Water in Oil) are the most efficient in the events immune response to strain vaccine after a single vaccination (Weraer etal., 1986)

And continues opposites level about 3 weeks, and up to the age of zero (25) days (Rahman et al, 2002).

The vaccines killed more efficient in immunization live vaccines (Wineland, 1996) and increases the efficiency if previously vaccinated neighborhood (OIE, 2005).

2-4 Methods of vaccination

2.4.1Vaccinated by spraying

Stimulates this type of vaccination (Mucosal Immune System) effective pattern (Anjum etal., 1993), This is the way of one of the widest roads vaccination prevalent for being easy to implement, inexpensive addition to efficiency in the generation of fast immune response, and homogeneous within three days after inoculation and efficiently (4) times the way of vaccination with drinking water (Anderson etal., 1999).

2.4.2 Vaccinated by drinking water

This is the way of the easiest ways, and the least expensive, and which is in common use and exports are really give a few different immune response of the reduced efficiency (Alexander etal., 2004) may give sometimes convincing results and preferably give day-old one after hatching because of overlap with the immune illiteracy in addition to the lack of water consumption ((Cho & Edgar, 2003, be immune response in this way non-uniform (Chui & Thorsen, 1999).

2.4.3 Vaccinated by injection

This method is used to give the killed vaccines prepared from weak or medium virulent strains or fierce injected vaccine intramuscularly in the wing or under the skin and chest area of the man in the neck area (Bell, 2001).

The medium virulent strains gets her prints (Adptation) embryos developing eggs constant injected into the brain (ICPI) is equal to 1.4 given by injection under the skin or muscle

(Chandral, 2001).

2.4.4 Vaccinated by distillation eye nostrils

This gives the kind of vaccination localized immunity by stimulating Alhardr Harderian Gland gland)) to configure immune globulin IgA (Bramble, 1999), in addition to the formation the (IgG, IgM) in tears and blood serum (Brandt, 2001).

Reflect this method of ways individual immunization, give good and homogeneous immune response during the longer period of time compared to methods other (spray, drinking water), where this method is the largest four times the way the spray and drinking water (Becht etal., 2002), and the negative aspects of the way the vaccination drip difficult to apply in the fields of intensive education to the high cost of implementation as well as the effort (Cheville, 2005).

2.4.5 Vaccinated by feed

You can use this method in places that are sources of water which is appropriate, but generated immune few in comparison to a way Vaccinated by drinking water or other methods, and is one of the simple ways of Vaccinated, which does not need a big effort to vaccinate a large number of poultry in a short time (Young etal ., 2002)

The vaccine is given with the feed wet Boluses after starving chicks (Alexander, 2004).

2.4.6 Vaccinated by embryos

Is a modern and practical way in economic terms are used to give many of the vaccines for various diseases, viruses (Eterradossi, 1992). On the benefits of a unified doses of vaccine per egg using automatic syringe itself, and also that this type of vaccination gives immunity up to 85%, but the disadvantages of this method is that giving the live vaccine, and

even debilitating lead to the destruction of embryos and then drop hatchability (Fareeda , 1999 and Jordon, 1990) was completed this type of vaccination debut researcher (Frnandez-Arias, 1997), and used this technique successfully in Iraq and researchers Jafar (2002) and Zahid (2005) .

2-5 Definition

Infectious bursal disease is a viral infection, affecting the immune system of poultry. The disease is highly contagious, affects young chickens, and is characterised by the destruction of the lymphoid organs, and in particular the bursa of Fabricius, where B lymphocytes mature and differentiate. The target cell of the virus is the B lymphocyte in an immature stage, and the infection, when not fatal, causes an immunosuppression, in most cases temporary, the degree of which is often difficult to determine.(T.P. van den Berg, etal., 2000)

2-6 Pathology and lesions

Although the other lymphoid organs are affected (Sharma J.M., etal. 1993), the principal target of the virus is the bursa of Fabricius (Kaufer I. & Weiss E. 1980). which is the reservoir of B lymphocytes in birds. Indeed, the target cell is the B lymphocyte in active division, for which the infection is cytolytic (Burkhardt E. & Müller H. 1987). Cell sorting studies have demonstrated that the B lymphocyte is susceptible in the immature stage, during which immunoglobulin M is carried on the surface of the lymphocyte (Hirai K,, Funakoshi T., Nakai T. & Shimakura S. 1981). This accounts for the paradoxical immune response to IBDV, - in which immunosuppression co-exists with high anti-IBDV antibody titres. The mature and competent lymphocytes will expand as a result of

stimulation by the virus whereas the immature lymphocytes will be destroyed

2-7 Immunosuppression

The destruction of immature B lymphocytes in the bursa creates an immunosuppression, which will be more severe in younger birds (Faragher J.T.,. & Wyeth C.J. (1974). In addition to the impact on production and role in the development of secondary infections, this will affect the immune response of the chicken to subsequent vaccinations which are essential in all types of intensive
animal production (Giambrone J.J., & Kleven S.H. 1976).

The most severe and longest-lasting immunosuppression occurs when day-old chicks are infected by IBDV (Allan W.H., Faragher J.T. & Cullen G.A. 1972). In field conditions, this rarely occurs since chickens tend to become infected at approximately two to three weeks, when maternal antibodies decline. Evidence suggests that the virus has an immunosuppressive effect at least up to the age of six weeks (Wyeth P.J. 1975). Immunosuppression is most often demonstrated using experimental models based on the measurement of humoral responses induced by different antigens such as *Brucella abortus* (Hopkins I.G., & Thornton D.H. 1979), sheep red blood cells, or Newcastle disease vaccines (Allan W.H., Faragher J.T. & Cullen G.A. 1972) . The best assessment is clearly the measurement of vaccinal protection against a challenge infection by the Newcastle disease virus, as described in the OIE *Manual of Standards for Diagnostic Tests and Vaccines* (Office International des Epizooties (OIE) 2000), since this constitutes a measurement of both humoral and cellular immunity. Unfortunately, these techniques are time-consuming, tedious, costly, and require the use

of animals. Thus, they are usually confined to IBD vaccine registration procedures.

2-8 Vaccination

In addition to strict compliance with rules of hygiene and disinfection, the success of vaccination depends on the choice of the vaccine strain and on the vaccination schedule. These must take account of the existence of certain pathotypes and the presence of antigenic variants in certain regions.

2-9 Types of vaccines used

Attenuated live virus vaccines and oil-emulsion inactivated virus vaccines are used against IBDV (Thiry G., & Colau D. 1994). The general principles governing the choice and the use of these vaccines were presented by Thornton in 1977 (Thornton D.H. & Pattison M. 1975) and remain valid. The ideal vaccine must offer the correct balance between efficacy and innocuity (Guittet M. & Bennejean G. 1982); the vaccine must not cause disease or bursal lesions, must not be immunosuppressive or excreted, and must confer long-lasting immunity even in birds with a high level of maternal immunity. Unfortunately, such a vaccine does not exist (McFerran J.B. 1993).

2.9.1 Live virus vaccines

Live virus vaccines are very widely used. These are made from strains of virus that have been attenuated by serial passages in embryonated eggs. Depending on the degree of attenuation, the vaccine strains cause histological lesions of varying severity to the bursae of SPF

chickens, and are classified as mild, intermediate or hot (Office International des Epizooties (OIE) 2000). The hot strains induce histological lesions in SPF chickens which are comparable to those caused by pathogenic strains, the only difference being that the hot strains do not cause mortality. The mild strains are used chiefly for the vaccination of breeder flocks. These are very sensitive to interference by homologous maternal antibodies, and are administered when these antibodies have disappeared, i.e. between the fourth and eighth week of age, depending on whether the grandparent flocks have or have not been vaccinated with an oil-emulsion inactivated vaccine before lay. Intermediate vaccines are used for vaccinating broilers and pullets (Mazariegos L.A., Lukert P.D. & Brown J. 1990). These are also administered to chicks in breeder flocks which are at risk of challenge by highly pathogenic strains at an early age. Although intermediate vaccines are also sensitive to neutralisation by passive antibodies, these vaccines may be administered at day-old by nebulisation in order to protect a chick that may not have a sufficient level of specific antibodies. Another reason for such early vaccination is to bring about replication of the vaccine virus in the chicks, and the dissemination of the virus within the farm; this would, at least partially, provide indirect vaccination to the other chicks at a time when they become sensitive to the infection. In high-risk farms, two vaccinations are generally performed. The age at vaccination depends on the maternal antibody titres present in the chicks at hatch. Vaccines are usually administered through drinking water, although nebulisation is also possible.

Live IBDV vaccines are compatible with other avian vaccines. However, the strains that cause serious lesions to the bursa of Fabricius may also provoke immunosuppression, exacerbate the pathogenicity of other immunosuppressive viruses (Marek's disease virus [MDV] and chicken

anaemia virus [CAV]) and jeopardise the immunisation of poultry against other diseases. Registration procedures for these vaccines must include tests to verify the absence of interference with other vaccinations as well as the absence of reversion to virulence in the course of serial passages in three- to six-week-old SPF chickens. A vaccine for *in ovo* vaccination of embryos has recently been developed. The vaccine is a mixture of virus and specific antibody, and is injected into eighteen-day-old embryos. Broiler chicks hatched from these eggs are immunised against IBDV throughout the growing period. This method avoids interference by parental antibodies (Haddad E.E., & Wakenell P.S. 1997). Various vaccines using recombinant viruses expressing the VP2 protein of IBDV have been described, and have proven efficacy in laboratory tests. The advantages of these vaccines are the absence of residual pathogenicity, sensitivity to maternal antibodies and risk of selection of mutants, as well as the possibility of use in *ovo* and of differentiation between infected and vaccinated animals (Darteil R., & Riviere M. 1995). No commercial version of these vaccines is currently available.

2.9.2 Inactivated vaccines

Inactivated vaccines are essentially used to produce high, uniform and persistent antibody titres in hens prior to lay that have been vaccinated with a live virus or have been naturally infected through exposure to the virus on the farm (Cullen G.A. & Wyeth P.J. 1976). These vaccines are administered by the subcutaneous or intramuscular route at the age of sixteen to twenty weeks. Progeny of hens that have been vaccinated in this way have protective antibodies until the age of approximately thirty days (Wyeth P.J., & Mohepat A.R. 1992). The chicks are thus protected during the period of susceptibility to the IBDV strains that only provoke immunosuppression. However, the chicks are not protected from other

highly pathogenic strains that may inflict high mortality rates at later stages (Wyeth P.J. & Cullen G.A. 1979). The decision to use an inactivated vaccine will thus depend on the epidemiological context, namely: presence or absence of highly pathogenic strains requiring vaccination of broilers with live virus vaccines. Where no risk of infection with vvIBDVs exists, boosting of laying hens with an inactivated vaccine just before lay is fully justified. However, the duration and uniformity of the immunity thus conferred upon chicks will, to a great extent, depend on the concentration and the antigenic specificity of the virus present in the vaccine. These vaccines are obtained either from bursal homogenates of infected chicks, or from viral cultures on embryonated eggs or fibroblasts, which are then inactivated by formaldehyde and presented as oil emulsions. Sub-unit vaccines produced in yeast (Marquardt W.W. & Schlotthober B.A. 1980) or insect cell cultures (Vakharia V.N. & Mengel-Whereat S.A. 1993) have also been described, but are not currently in use.

2-10 Vaccination failure

The causes of failure of live-virus vaccinations are numerous. The most trivial causes are non-observance of the expiry date, inappropriate storage, non-observance of recommended doses, and incorrect or deficient vaccination techniques. Freeze-dried live vaccines must be rehydrated immediately before use in distilled water. The use of distilled water to dilute the vaccines is compulsory when the spray technique is used. When the vaccine is administered in drinking water, is particularly important to deprive the birds of water for two to three hours before distributing the vaccine solution (Burkhardt E. & Müller H. 1987) .Only fresh water, with no organic matter, chlorine or heavy metals, may be used. Adding powdered milk at a concentration of 2 g per litre helps to

stabilise the vaccinal virus. Interference from parental antibodies is one of the most frequent causes of failure. The date of vaccination of the offspring must therefore be determined on the basis of the immune status of the chicks, and thus of the vaccination protocol used for the parents. Vaccination failure with inactivated vaccines is rare, but may occur, either due to the absence of previous contact of some of the birds with a live virus (a vaccine virus or otherwise), or to the existence of antigenic variants not present in the vaccine. All suspected cases of antigenic variation in the field should be tested in isolation units on SPF birds after vaccination with classical strains (Darteil R., & Riviere M. 1995) .

2-11 Evaluation of Maternal Immunity.

The injuries found in inoculated SPF chickens have demonstrated that the field sample used to challenge the birds was able to induce typical IBD injuries. Circulating antibodies before challenge reduced progressively both in vaccinated and unvaccinated birds until 22 days of age, corroborating results from other studies that have reported a similar behavior. Knoblich et al. (2000), Alam et al. (2002), Knezevic et al. (1999) concluded that vaccination does not accelerate the decrease in maternal antibodies f chicks are vaccinated at one day of age. Similarly to results reported by Knezevic et al. (1999), chicks with passive immunity vaccinated with an intermediate IBDV strain in the first day of age showed no increase in antibody titers. Nevertheless, when vaccination was performed at 14 days, an increase in titer was observed. Kumar et al. (2000) reported similar conclusions using a quantitative agar gel precipitation test. Ahmed & Akhter (2003) described a decrease in maternal antibodies and suggested an equation to estimate maternal antibody levels and establish the age until which the birds would be

protected against field virus samples. In the experiment, these authors reported no mortality in El-Yuguda A. D. (2000). Effects of material antibodies and vaccine interactions on specific antibody response of Village chickens to single or combined Newcastle disease and infectious bursal disease vaccines. M.V.Sc. dissertation, University of Maiduguri group of birds challenged until 14 days of age, suggesting that the maternal antibodies provided some level of protection up to that age. However, the degree of histological injury in the bursa of Fabricius was not mentioned. The results of the present study showed that diameters varied from 2 to 6 according to the age of birds. Considering that there were similar histological injuries between control and challenged birds, independent of vaccination status and company, it seems that there is no relation between bursa diameter and presence of injuries caused by disease or vaccination. These findings corroborate results previously reported by Pereira (2002). In a previous study performed in our lab, broilers were vaccinated with different intermediary IBD vaccines and injury indexes could not be attributed to disease or vaccination based solely on bursa diameter; therefore, histological evaluation was also performed to give appropriate injury scores (Pereira, 2002). Based on these observations, it can be concluded that BF diameter measurement should not be used as a single parameter for disease diagnosis or in decisions related to the evaluation of vaccination programs. The measurement of BF diameter must be seen with caution and used only as an auxiliary method, rather than a definitive one. When the relative weight of the bursa was analised, the data showed that the bursas of Fabricius had their relative weight equal or heavier after the challenge, until the beginning of the histological injuries considered as disease. After, it turns into equal or lighter than the controls. As the relative weight was calculated 5 days after the each challenge, it could be

supposed that until the fourth challenge, the level of maternal antibodies decreased the infectious rate, increasing the inflammatory process time, making the bursas apparently heavier, since they were still in the oedema phase. After the fourth challenge, the virus would overcome the antibodies barrier and cause the injuries in a shorter time, presenting the atrophy phase, 120 hours after the challenge, and, therefore, reducing the bursa of Fabricius weight. Chickens challenged with El-Yuguda A. D. (2000). Effects of material antibodies and vaccine interactions on specific antibody response of Village chickens to single or combined Newcastle disease and infectious bursal disease vaccines. M.V.Sc. dissertation, University of Maiduguri very virulent sample of the IBD virus isolated in Japan showed bursa atrophy as soon as 3 to 4 days after inoculation (Tsukamoto et al., 1992). These observations are similar to the findings of the present study experiment. However, Mazariegos et al. (1990) stated that the relative weight of the bursa of Fabricius was not a good indicator of IBD. The same conclusion is true for the bursa diameter. Thus, the use of one of these criteria alone cannot establish if the bird has IBD or is healthy, and whether it was vaccinated or not. After the birds were challenged with the GAR-1 sample, it was considered as IBDV infection sign if the birds had average histological scores higher than 3 after the challenge and showed macroscopic alterations (edema). Vaccinated birds after the third and the fourth challenges showed scores higher than 3, which were similar to the scores from unvaccinated birds. Mazariegos et al. (1990) assessed the effects of intermediate vaccine samples and reported lesion scores ranging between 1.4 in controls, and up to 4 in the vaccinated birds. Therefore, it was considered that the animals were protected until a histological score of 3, which corresponded to the period between 7 and 10 days of age. Histological injuries were seen even when antibody titers were considered protective. In these situations, the existing

injuries were less intense than the injuries seen in sick birds, results similar to those reported by Maas et al. (2001). Chicks with high titers of maternal antibodies (9 log2) measured by virus neutralization were challenged with either a classic viral strain (52/70) or with a very virulent strain of IBDV. The high titers of maternal antibodies prevented the clinical signs of the disease until 14 days of age, but not the damage in the bursa of Fabricius (Maas et al., 2001). If the birds were protected between the third and the fourth challenge, measured by histology, it is possible to use the mean of the ELISA titres, in these birds.age, as an indicator of the protection titre. The titre, in this occasion, was about 3,4 log10 to vaccinated and unvaccinated birds. This way, it could be applied the regression equation, where in one of them the ELISA titre is an independent variable and the age the dependent one. The protective titre was chosen for being the highest (3,21; 3,30; 3,43 e 3,31 log10) capable of protecting the birds according to the experiment's working conditions. In the other, the titre is the dependent variable and the age the independent one, to establish until which age the animals would be protected or what titres the birds would have at El-Yuguda A. D. (2000). Effects of material antibodies and vaccine interactions on specific antibody response of Village chickens to single or combined Newcastle disease and infectious bursal disease vaccines. M.V.Sc. dissertation, University of Maiduguri determined age. Solving the equation and establishing 3,4 log10 as the protective titre, the birds would be protected against the virus challenge until 6-7 days of age in the Company A and until 11-12 days of age in the Company B. These results demonstrate that the vaccination in the first day of life is not necessary; moreover, they show that the animals were protected during the first week of age. Therefore, the breeder vaccination program used in Company B protects the progeny longer than the program used in Company A. The difference

between the two vaccination programs clearly shows that failures may result if the age of the first vaccination is defined based solely on general models, as suggested by Kouwenhoven (1995) and Witt (2001), such as using the Deventer formula: Age= [square root (mean titer) . square root (target titer)]/2.82. When the values found in the present study are used in the Deventer formula, the expected values were not found. Factors such as correct breeder management practices, variations within flocks, and different vaccination programs and vaccine strains may also interfere in the results (Witt, 2001). ELISA results were different between the two companies, which is according to expected, since they use different breeders. vaccination programs. Similar results were reported by Maas et al. (2001), who evaluated two inactivated oil vaccines against IBD and showed differences in relation to the maternal antibody levels and progeny protection against challenge with classic samples and very virulent samples of the IBD virus. The data from the present study permit to conclude that vaccinated and unvaccinated birds with antibodies titers higher than 3.4 log10 in the first day of life were protected against the disease after challenge with the a very virulent strain of IBD. Besides, the histological findings in the bursas of Fabricius were compatible with the injuries shown previously to be induced by vaccination (Mazariegos et al., 1990). Chicks from hyperimmunized breeders do not need to be vaccinated on the first day of life. The age of vaccination must be determined using a regression equation specific for each company, because of differences between breeder vaccination programs and husbandry practices. In earlier studies, Salle (1989) challenged commercial chicks showing maternal antibodies with a classic sample of the virus (52/70) and also observed protection until 14 days of age, whereas Moraes et al. (1998) stated that vaccine strains were not antigenically different from field strains, and therefore other factors must

be responsible for the difficulty in controlling the disease. Based on those results, the authors recommended that the vaccination schedule should follow an adequate planning, cleaning and disinfection of the environment, the choice of the best vaccine sample (Moraes, 2004), the field strains. typing (Sharma et al., 1989), the presence of mycotoxins (Azzam & Gabal, 1998) and/or other diseases and the level of maternal antibodies. These factors are prone to influence the performance of the breeders vaccinations, as well as the broilers.

2-12 EVALLUTION OF VACCINATION SCHEDULES FOR ND AND IBD

feeding and roosting in the same place they serve as source of infection to each other . guinea fowl, which has been reported to be very susceptible to infectious diseases of poultry, such as Newcastle disease, egg group syndrome- 76, infectious bursal disease, etc (Agoha *etal.*, 1992). Guinea fowls rank second to chickens in terms of population and acceptability to farmers in Nigeria (Nawathe and Lamorde, 1982). It is observed in this study that IBD virus infection or vaccination reduces the response of guinea fowls to ND "LaSota" vaccine. This agrees with the findings of other workers (Rao and Rao, 1992; El- Yuguda, 2000) who observed significant depression of primary antibody of chickens to ND vaccine when administered one week after IBD infection or vaccination. Trautwein (1992) also reported that chickens infected with IBD virus become susceptible to opportunistic secondary infections and respond poorly to immunization against other pathogens. This could be due to the effect of the virus on the lymphoid organs, such as the bursa of Fabricious of the infected birds. The virus causes necrosis of the lymphocytes in the

medullary area of the lymphoid organs resulting in the suppression of both humoral and cell mediated immune responses (Ritter, 1982; Fenner *et al.*, 1986; Lukert, 1992). The poor response of the IBD virus infected or IBD vaccinated guinea fowls to ND "LaSota" vaccine and their seroconversion to the IBD virus with no apparent clinical signs observed in this study shows that the guinea fowls could serve as source of IBD virus infection to chickens and other birds. This is serological evidence has shown that free flying feral birds serve as source of spread of ND and other viruses to chickens, even when they do not come down with the clinical disease (Martin 1992). This may hamper the success of the ND control program in the village poultry (Spradbrow, 1987; Martin, 1992) .

2-13 Efficacy of IBD Vaccine Receiving Different Vaccination Programs

The efficacy of IBD vaccination program was related to the level of MDA (maternally derived antibody) in the chickens. The MDA of chickens can impede the virus in vaccine infected to the target cells and also reduce the ability of virus in vaccine to stimulate the chicken's immune system (Chansiripornchai and Wanasawaeng, 2009; Naqi et al., 1983). Anyhow, the MDA is of benefit to IBDV infection in chickens at the age of 1-4 weeks (Al- Natour et al., 2004). Kreider et al. (1991) The formula of determining the appropriate age of chickens for IBD vaccination was the mean square root titer of MDA at 1-day-old – the mean square root of target titer/2.82 (Kouwenhoven and van den Bos, 1995). For intermediate plus vaccine, an MDA equal to 334 is the suitable time for vaccination. According to the formula, the appropriate date of intermediate plus vaccination in the experiment is 22-day-old. Therefore,

vaccination at 1 or 16-day-old may be affected by MDA resulting in the partial neutralization of the vaccine virus by MDA before the vaccine virus can stimulate chickens' immunity against IBDV infection, that subcutaneous vaccination of IBDV vaccine at 1-day-old, would not accelerate the reduction of antibodies and antibodies would not be detected at 28-day-old. Also, the antibodies of these chickens would be higher and longer than the antibodies of non vaccinated chickens. Moreover, the detection of antibodies against IBDV after challenge revealed that subcutaneous injection at 1-day-old would stimulate the active immunity of chickens (Haddad et al., 1997). the bursa of all groups of chickens was reduced and histopathological damage, indicated that the experimental chickens were susceptible to the challenge virus. The results accorded with the no detection of antibodies against IBDV at 30-day-old (before challenge). Also, at 30- day-old, no active immunity had been developed due to the effect of the intermediate or high level of MDA (Winterfield and Thacker, 1978; Tanimura et al., 1995). After challenge, The challenge virus resulted in the high level of HLS. Anyhow, the survival rate of chickens in each group was more than 95%. The heavy breed chickens reveal lower mortality than the light breed chickens (Bumstead et al., 1993; Chansiripornchai and Wanasawaeng, 2009).

From the experiment, the efficacy of IBDV vaccine in broilers was related to the level of MDA against IBDV at the vaccination date. Vaccination at 1- day-old, 1 and 16-day-old and 16-day-old of chickens that have the ELISA titers of MDA of more than 6,000 at 1-day-old may not be effective enough to elicit the antibodies at 30-day-old(Chatchai Sarachai1 & etal,.2010) More research should be performed. Further study in low or intermediate MDA chickens should be done. Experiments on virus protection from challenge and the availability of the virus in

infected organs are required for evaluation of the efficiency of a vaccination program .

Chapter Three

3.Conclusion

1.The study suggests that vaccination by high virulence vaccines or medium virulence to severe injury bursal, claim to about 90% depletion of lymphocytes, so you must consider the degree of virulence of the vaccine before use .

2.The vaccination process is basically the process of events injured slightly in the animal in order to stimulate the immune system to produce antibodies (and note the high degree of bird heat after vaccination process), so that vaccination against Gumboro disease causing Immunosuppression at the birds and because of that virus Gumboro disease *Birna virus* infects the bursal as a target organ for virus, since Busal is responsible for the production of cells, B cells and that these cells are responsible for the production of acquired Immune for the birds, the vaccination process, leading to destroy these B-cells lead to the vaccination process that preceded the failure of Gumboro disease vaccination

3.The method of vaccination important role in the success of the vaccination process where we must apply the appropriate method of inoculation and the creation of a controlled program for all local spread of disease

4.A vaccination by (method vaccination by drinking water) as well as the food that method in the early days (from 7 to 10 days) because these methods lead to neutralization an immune with Maternal immune Thus, the bird will become weak immune from making it susceptible to various diseases.

5.The vaccination process against Gumboro disease must be applied with a time lag for other programs at a rate of 2 to 4 days to avoid the immune interaction in bursal who destroys a number of lymphocytes cells and by the way of vaccination

6. A second dose after stimulus of vaccination against Gumboro disease, to re-stimulate the lymphatic cells in the bird .