THE DECAYING PATTERN OF MATERNALLY DERIVED ANTIBODIES AGAINST INFECTIOUS BURSAL DISEASE VIRUS IN BROILER CHICKS

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DEDICATION

To my mother, father, sisters and brothers
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ABSTRACT

This study was conducted to assess the decaying pattern of maternally derived antibodies (MDA) in broiler chicks against infectious bursal disease virus (IBDV) and the immune response for D78 vaccine via different routes of administration. For this purposes hundred and fifty chicks were used, the chicks were divided into vaccinated and non-vaccinated groups with IBD vaccine. The study revealed that the maternally derived antibodies (MDA) against Infectious bursal disease virus in unvaccinated chicks persisted up to the 6th week as determined by ELISA. However the protective level of these antibodies expired by the 4th week. Generally failure of vaccination by D78 at the 18th day via different route of administration was observed in this study but the second vaccination dose at 25 day of age gave good results. So we suggest that for appropriate vaccination day, the level of MDAS must be evaluated while the chicks are growing at 10 –14 days of age. The declining pattern of maternally derived antibodies titres in unvaccinated group was compared with those given IBD intermediate vaccine (D78) via different routes namely drinking water, nasal drops, spray and subcutaneous. The results obtained indicated that immune responses against D78 vaccine given via different routes of administration were good and the difference between their S/P ratios was not significant (P> 0.05). The result also revealed that with regard to coefficient variation (vaccine take) there were significant (P<0.05) differences between the four routes of the vaccine and this significances were clear between drinking water and nasal drops and between nasal drops and spray and between spray and subcutaneous injection. However the vaccine take via nasal drops at various ages was the best among others routes with
regard to coefficient variation. It is recommended to check serum for vaccination at day 10-14 day of age and one week later for vaccine take.
ملخص الأطروحة

أجريت هذه الدراسة في كتاتيب لأحم تقييم نمط اضمحلال المناعة الأمية ضد فيروس مرض جراب فابريشس المعدي وتم قياس الاستجابة المناعية للفحص (D78) عبر اعطاءه بطرق مختلفة. تم استخدام مانه وخمسون كتبوت لهذا الاغراض وقسمت الى مجموعات ملقحة وغير ملقحة بوسطة لقاح القمبرو. أظهرت هذه الدراسة أن المناعة الأمية لممرض جراب فابريشس المعدي تبقت في الطيور غير الملقحة حتى الأسبوع السادس من عمر الكتاتيب بعد أن تم تحديثها بواسطة اختبار الاليسا. مع أن كمية الأجسام المضادة الكافية للمحافظة على هذا المرض قد خلصت للإسبوع الرابع عموما فشل عملية تلقيح الكتاتيب في اليوم الثامن عشر بواسطة (D78) عبر الطرق المختلفة قد تمت ملاحظته في دراستنا. ولكن الجرعة الثانية لللقاح عند اليوم الخامس والعشرين أعطت نتائج جيدة. لذلك نقترح أن من أجل الحصول على اليوم الثالي لللقاح يجب أن تفحص المناعة الأمية أثناء نمو الكتاتيب مثل من اليوم العاشر الى الرابع عشر من عمر الكتاتيب.

معيار نمط اضمحلال المناعة الأمية في الطيور غير الملقحة تمت مقارنته مع تلك التي أعطيت لقاح مرض جراب فابريشس (D78) عبر طرق مختلفة هي ماء الشرب، التقطير بالبنف، الرش و الحقن تحت الجلد. أظهرت النتائج أن الاستجابة المناعية لكل طرق اعطاء اللقاح كانت جيدة وان الاختلاف بين معاييرهم لم يكن ذا معنى (P > 0.05). أظهرت النتائج أيضا بالنظر الى معامل الاختلاف (أخذ اللقاح) لطرق اعطاء اللقاح المختلفه كان هناك اختلاف ذا معنى بينهم (P < 0.05) وكان هذا الاختلاف ظاهر بين ماء الشرب والتقطير بالبنف وبين الرش والتقطير بالبنف وبين الرش والحقن تحت الجلد. بالرغم من أن درجة أخذ اللقاح عبر التقطير بالبنف للكتاتيب كان الأحسن بين الطرق المختلفة عند كل الأعمر. نوصي بأن يتم فحص السيرم من أجل التلقيح في اليوم 10-14 من عمر الكتاتيب وبعد أسبوع احتسابا لمعرفة درجة أخذ اللقاح.
Introduction

Infectious bursal disease (IBD), also known, as Gumboro disease, is an acute, highly contagious viral infection of 3 to 6 weeks susceptible chickens caused by an avibirnavirus genus. The causative virus has two serotypes 1 and 2. Both serotypes are present in commercial chickens although serotype 2 is most prevalent in turkey. The disease is characterized by pathological effects on the lymphoid organs of the chickens, primarily the bursa of Fabricious, thus immunosuppression is a common consequence of the infection. The disease gets its importance in poultry industry due to its significant economic losses resulting from high mortality and immunosuppression. The disease was firstly reported in Sudan when an outbreak occurred at El-Obied in Northern Kordufan state in 1980.

Many previous studies stated the role of maternally derived antibodies (MDA) in the chicks in protection against infectious bursal disease virus (IBDV). The MDA is acquired by chicks through the passage of IgG from hen serum to the embryo and remained protective for certain period of time and then start to decay. The amount and duration of these maternally derived antibodies (MDA) were variable in progeny chicks. In practice, different vaccination schedules have been recommended and used but still outbreaks are reported. The time of vaccination, maternal derived antibodies in chicks, type of vaccine, routes of administration and pathogenicity of the IBDV challenge are important factors determining the efficacy of Infectious bursal disease vaccination.
The objective of the present work:

The present study was conducted to investigate the decaying pattern of maternally derived antibodies against IBDV and the immune responses of four administration routes of D78 vaccine namely drinking water, nasal drops, spray and subcutaneous injection in commercial broiler chickens. D78 vaccine was chosen because most poultry producers in Sudan use it for routine vaccination against Gumboro disease and mostly via drinking water.
CHAPTER ONE

LITERATURE REVIEW

Infectious bursal disease (IBD)

1.1. Definition

Infectious bursal disease is an acute, highly contagious viral disease of young chickens. The signs of the disease as described by Cosgrove (1962) include soiled vent feathers, ruffled feathers, whitish watery diarrhea, anorexia, depression, dehydration and death. The causative virus primary target is the lymphoid tissue with special predilection for the bursa of Fabricius (Lukert and Saif, 1991).

1.2. History

The disease was known in 1957, and observed firstly by Cosgrove in 1962 in Gumboro, Delaware, USA. This is why the disease is called Gumboro. Also the disease used to be called (avian nephrosis) due to the kidney damage detected in infected birds. The disease was also named infectious bursal disease when Wilter and Hitchner (1970) isolated the causative virus and differentiated it from certain infectious bronchitis variant virus, which gives similar kidney lesions.

1.3. Economic Importance

Infectious bursal disease became a big problem in poultry industry in many parts of the world since when it was first reported in 1962 by Cosgrove. The disease has a potential hazard to poultry industry due to the change of clinical picture to the sub clinical form of the disease manifestation (Survache, 1987). The disease economic impact is affected by strains of the virus; flocks breed susceptibility, intercurrent, primary and
secondary environmental factors. The infection influence seen at the level of flocks livability, weight gain, feed conversion and reproductive efficiency (Shane et al., 1994). Beside mortality IBD is an immunosuppressive disease (Rosenberger and Cloud, 1986). Both broilers and pullet flocks infected with IBD frequently have reduced antibody response to live attenuated vaccine against respiratory diseases such as infectious bronchitis (IB) and Newcastle disease (ND) (Snyder et al., 1986), increased susceptibility to respiratory viruses including Newcastle disease (Faragher et al., 1974), and infectious bronchitis (Pejkoveski et al., 1979). A chronic form of the disease was associated with gangrenous dermatitis, coccidiosis, chronic respiratory disease and hepatitis with varying mortalities and adversely affected broilers flock performance (Mcllory, 1994). In addition to death and immunosuppression, losses from IBD including depressed growth rate, feed conversion inefficiency were recorded in affected broilers flocks. Variability in flocks uniformity and maturity occurred in affected pullet replacement flocks. Costs incurred as a result of more thorough disinfections, poor quality of eggs and carcasses are further reducing revenue and profit (Shane et al., 1994). Mcllory (1994) had also confirmed that subclinical IBD has economic effects on broilers production.

1.4. IBD in Sudan

The disease was first reported in late December 1980 and early 1981 at El-Obied government poultry farm in the Kordofan Region, Western Sudan. The mortality rate was 36% in 6-weeks old chicks and 22% in one day old, a new batch introduced in the same premises. In October of the same year, mortality was 17.8% in the first generation of chicks from parent flocks that had the disease earlier and which were kept in the same area (Shauib et al., 1982). The outbreak-involved chicks of mixed layers breeds (local,
crosses, Fayoumi, New Hampshire and White Leghorn) (Salman et al., 1983). Since then the disease has been reported regularly from most part of the country and became a serious problem to the poultry industry. In 1988, an outbreak of the disease was reported in Kassala state (Gaffer et al., 1988). Later studies have shown that Kassala virus strain and that of EL-Obied outbreak were similar, which were both isolated from chicks imported from abroad. The authors suggested that the disease has been introduced to the country through these imported chicks. Serological studies concluded that IBDV antibodies could be detected in local and exotic breeds. The figures were 14.5% in Kordofan Region and 44.21% in Khartoum State, Obeid and Kassala towns (El-Hassan et al., 1989; Mahasin, 1998). Mahasin (1998) confirmed the existence of subclinical IBD in Sudan. A serotyping study of representative local field IBDV strains was conducted in order to identify the most suitable strain for vaccine production and it was proved that Sudan IBDV isolates belongs to serotype 1 standard strain (Mahasin, 1998).

1.5. Etiology

1.5.1. Classification and structure

Infectious bursal disease virus (IBDV) is a member of Birnaviridae family, avibirnavirus genus. The other genus in that family is aquabirnavirus, which include the pancreatic necrosis virus of fish (Murphy et al., 1999). Virions are non-enveloped, hexagonal in outline with icosahedral symmetry, 60 nm in diameter. The genome consists of two molecules of linear double stranded RNA (Murphy et al., 1999). The smaller genomic segment encodes viral protein (vp1) 98,000 daltons and the viral polymerase. The larger segment encodes three proteins namely vp2, vp3 and vp4; of which vp2 and vp3 are structural proteins while vp4 is viral protease (Fahey et al., 1991). Neutralizing
monoclonal antibodies (Mab) have been shown to bind to vp2 whereas vp3 doesn’t carry neutralizing epitopes (Fahey et al., 1991; Synder et al., 1992).

1.5.2. Physiochemical Properties

The virus is stable, resistant to ether, chloroform, heating at 70 Cº for 30 minutes and was unaffected by exposure to 0.5% phenol and 0.125% thimorsal for one hour at 30 Cº, but there was a marked reduction in virus infectivity when exposed to 0.5 formalin for 6 hours (Benton et al., 1967). Definitely the strong nature of the virus explains its long survival in poultry houses even when thorough cleaning and disinfection procedures are followed (Lukert and Saif, 1991).

1.5.3. Serotypes and Variants

Two serotypes of IBDV, 1 and 2, have been reported (McFerran et al., 1980; Jackwood et al., 1982). The two serotypes can be differentiated by virus neutralization test (VN) (Lukert and Saif, 1991). The two serotypes can be found in chickens but only serotype 1 is pathogenic while serotype 2 is detected in turkeys (Ismail et al., 1988). McFerran et al. (1980) reported antigenic variation among IBDV isolates and showed only about 30% correlation between several strains of serotype 1 and designated them as prototypes of that serotypes. The two serotypes share common antigens demonstrated in agar gel precipitation test (Synder et al., 1992).

In the United State of America, variant viruses were isolated from specific pathogen free (SPF) chickens, which were vaccinated with classical vaccines and mixed with naturally infected broiler chickens. These viruses were found to differ from the standard strains both antigenically and in terms of their pathogenicity for broilers and SPF leghorns (Rosenberger and Cloud, 1986). These strains were breaking through maternal immunity
against "standard" strains, and they also differ from standard strains in their biological properties (Rosenberger et al., 1985). Jackwood and Saif, (1987) found that of 13 serotype 1 strains tested by VN test six different IBDV subtypes were identified. Also Saif and Jackwood (1987) isolated a virus from broiler chickens, which had bursal lesions in spite of the presence of high maternal antibodies. Later, strain proved to be antigenically different from those isolated before hence it was designated as variants, whereas viruses isolated before 1984 were considered as classical viruses. McFerran et al. (1980); Saif et al. (1987) reported failed vaccination program and failure of maternal immunity to protect chicks due to antigenic differences between subtypes of serotype 1 IBDV. Using neutralizing monoclonal antibodies (Mabs) directed at vp2 of IBDV in antigen capture enzyme linked immunoassay (Ac-ELISA), it was evident that variant IBDVs had altered or deleted neutralization sites previously found on the standard or classic IBDV strains and that neutralizing Mabs prepared against the classic IBDVs would no longer bind to or neutralize the variant strains (Snyder et al., 1988). The very virulent strains of IBDV were isolated from broilers and replacement layers in 1986 in Europe (Chettle et al., 1989). These strains caused up to 70% mortality in laying pullets and 30% in broilers and caused typical lesions of IBDV. These strains break through maternal antibodies, which were protective against classical strains (Chettle et al., 1989; VoB and Vielitz, 1994). Within few years, this highly pathogenic IBDV strains spread over Europe, Middle East, South Africa and south East Asia (VoB and Vielitz, 1994). In USA, highly virulent strains isolated were defined as variants by the fact they has been isolated from vaccinated flocks and by specific reaction pattern with Mab (Synder et al.,
1988). In Sudan, the highly virulent strains were detected within the virus isolated since 1994 onwards (Mahasin, 2001).

1.6. Susceptible age

The clinical picture is most commonly recognized in 3-6 weeks old susceptible chicks, birds younger than 3 weeks have subclinical infection (Lukert and Saif, 1991). The disease outbreaks were also reported in 14 and 15-week white leghorns chickens (Ley et al., 1997) and in 16 and 20 weeks old broilers (Durojaiye et al., 1984).

1.7. Transmission of IBD

The feces are the main source of the infection, the virus can be found there for up to 2 weeks post infection. Aerial spread is not important nor is there evidence for egg borne spread or virus carriers (Wiebe van der Sluis, 1994). Benton et al. (1967) found that the infection persists in contaminated buildings for as long as 122 days after removal of infected birds. Naturally infected wild birds, beetles (grass insects), sparrows, other free-living birds and rodents can transmit mechanically the infection (Weibe van der Sluis, 1994). The *Aedes vexans* mosquitoes and meal worms (*Alphitobius diaperinus*) have been incriminated as even more important vectors (Howie and thorsen, 1981; Snedeker et al., 1967). Parede et al. (1996) isolated infectious bursal disease virus from darkling beetles (*Carcinopes pumilio*) and their larvae, which have been suspected to act as vectors.

1.8. Incubation period

The disease incubation period is very short about 2-3 days (Lukert and Saif, 1991). Detection of histopathological lesion in the cloacal bursa could be during 24 hours post infection (Helmboldt and Garner, 1964). Muller et al. (1979) detected gut cell
associated virus within 4-5 hours using immunofluorescence technique post-oral infection. Viruses infected cells present in the cloacal bursa by 11 hours after direct application of the virus to the bursa.

1.9. Morbidity and Mortality

The type and virulence of the infecting virus determine the morbidity and mortality rates. Morbidity may reach 100% in highly susceptible birds (Lukert and Saif, 1991). Mortality starts after 3 days from the infection and rises to peak and reduce within 5-7 days. Mortality ranges from zero to 30% (Lukert and Saif, 1991). However with very virulent IBDV strains mortality can reach 70-80% (Chettle et al., 1989; Murphy et al., 1999).

1.10. Pathogenesis

Oral route is usually the natural way for infection of IBDV but the upper respiratory tract and conjunctiva probably play also role in the transmission. The virus travels to the bursa by blood, although direct spread from the gut cannot be excluded. Once arriving the bursa there is massive virus replication, with strong immunofluorescence reaction from 11 hours post infection onwards detected (Wiebe van der Sluis, 1994). Histologic lesions in the cloacal bursa resemble an arthus reaction (necrosis, hemorrhage, and large number of polymorph nuclear cells). This reaction is type of localized immunologic injury induced by antigen –antibody complexes and complement (Skeels et al., 1979; Ivany and Morris, 1976). The hemorrhagic lesions, which were observed with the disease, were suggested to be due to an increased clotting time in chicks infected with IBDV (Skeels et al., 1979; Skeele et al., 1980).
1.11. Gross lesions

The gross lesions of IBD includes dehydration, dark discoloration, petechial hemorrhages of the pectoral, thigh muscles and in the junction between proventriculus and gizzard, increased mucus in the intestine and renal changes (Cosgrove, 1962). The primary target organ for IBDV is the bursa, which was found on the third day post infection increased weight and size due to edema and hyperemia and the normal white color changed to cream. By the fourth day it was double its normal weight then starts to decrease in size. By the fifth day it returned to normal size and weight and became gray but continued to decrease, and on the eight day onwards it was approximately one-third its normal size (Cheville, 1967).

1.12. Immunosuppression

Is a state of temporary or permanent dysfunction of the immune response resulting from insult to the immune system and leading to increased susceptibility to diseases (Dohms and Saif, 1983).

Immunosuppression due to IBD is due to direct lyses of B-cells or their precursors (Rosenberger, 1994). It was found that the degree of virulence of IBDV is an important factor in exertion of immunosuppression (Higashihar et al., 1991). The immunosuppressive effect was found to be lower when chicks were infected with IBDV at 21 days than at one day of age (Souza et al., 1987). In another study, it was shown that immunosuppression could be produced even when exposure to IBDV was at one day old but depending on the virus strain (Minta et al., 1986).

Faragher et al. (1974) in their first report on immunosuppression by IBDV stated that suppression to Newcastle (ND) disease immunity was greatest in chicks infected at day
old, moderate at 7 days and negligible when infection was at two or three weeks. Furthermore, Meulemans et al. (1977) mentioned that the immunosuppression due to IBDV infection depends on the age of the birds, the time of ND vaccination and strains of IBDV used.

To study the immunosuppressive effect of IBDV on vaccination against ND the response was compared in 2,3 and 4 weeks old chickens inoculated with the highly virulent IBDV field isolate 90-11 and the reference serotype 1 strain GBF-1. And then vaccinated with ND Lasota vaccine. In all age groups, isolate 90-11 severely lowered antibody response to ND vaccination on challenge. In contrast, birds inoculated with reference strain GBF-1 and vaccinated with ND vaccine were protected from ND challenge (Nakamura et al., 1992).

The influence of IBD infection on vaccination to ND in broiler chicks was greatly dependent on the age of birds and time of vaccination (Rosendale et al., 1987).

IBD vaccine was reported to have adverse effect on the ND vaccine whereas the reverse was not true (Ali et al., 2004).

The immunosuppressive potential and pathogenicity of field isolates in commercial broilers were also investigated by Rosales et al. (1989) who showed that field isolates of IBDV reduced the serological response to NDV vaccine.

It was reported that IBDV affects the response to many antigens. In a study by Dohms and Jaeger (1988) broiler chickens infected at 3 weeks of age, were given Brucella abortus (BA) or sheep red blood cells (SRBC) antigens intranasally and intraocularly during and after the acute phase of the infection. Glands of Harder (GH) extract and serum samples were used to measure systemic and local antibody titre to each antigen seven days after antigen administration. Antibody titre to both BA and SRBC antigen were less in GH extracts and serum on IBDV –infected chickens than uninfected controls. Subclinical IBD was proved to have adverse effect also on ND vaccination of broilers chickens. Okoye and Shoyinka (1983) inoculated 4500 broiler chicks with NDV vaccine introcularly (strain B1) at ten days of age. The chicks showed clinical signs of the disease 19 days later with 71% mortality rate and characteristic NDV lesions on post mortem
examination. Newcastle disease virus was isolated and identified by haemagglutination (HA) test. In addition, histopathological examination showed that follicles of the bursa were adapted for lymphocytes with many large activities. Both the acute and convalescent sera samples were positive for IBD antibodies in agar gel precipitation test (AGPT). It was concluded that the vaccination failure might be due to the subclinical IBD or the highly pathogenic character of the wild virus.

Immunosuppression resulting from IBD infection weakens response to vaccination and makes chickens more susceptible to a number of other infections including coccidiosis, Marek’s disease, gangrenous dermatitis, infectious laryngotracheitis, infectious bronchitis, chicken anaemia agent, salmonellosis and colibacillosis (Anon, 2000). IBDV has been shown to compromise the ability of chickens to react immunologically to ND, *Mycoplasma synoviae* and *Haemophilus gallinarum* (Wiebe van der Sluis, 1994). The earlier in life a chick is infected the greater the immunosuppression. It has been suggested that the longer period a bird retain an intact bursa, the smaller the chance of immunosuppression (Wiebe van der Sluis, 1994).

### 1.13. Diagnosis of IBD

Diagnosis of IBD in chickens is historically based on clinical signs and gross lesions seen at necropsy and by histopathology; confirmation of the diagnosis is based on detection of viral antigens, detection of antibodies and isolation of the virus (Liiticken *et al.*, 1994).

#### 1.13.1. Detection of viral antigens
Viral antigens can be detected in the bursa of Fabricius on 3-4 days post infection using an agar gel immunodiffusion (AGID) test in which homogenized bursal tissue antigens are precipitated by known specific positive antisera (Mekkes and Wit, 1994). A direct immunofluorescence smears of bursa of Fabricius and direct electron microscopic examination of bursa specimens had been compared with virus isolation in chick embryo fibroblast and embryonated eggs for diagnosis of IBD (Allan et al., 1994). It has been indicated that immunofluorescence was, overall, more sensitive method than virus isolation and direct electron microscopy, and gave a good correlation with histopathological diagnosis of IBD.

Antigen capture (Ac)-ELISA had been used for detection of IBDV antigens directly from infected tissues (Snyder et al., 1988). The same method also used to differentiate IBDV stains. Virus neutralization (VN) assay has been considered as only reliable technique for differentiation of IBDV strains into antigenic serotypes and subtypes (Jackwood and Saif, 1987). However the test is time consuming and expensive to be conducted. Kataria et al. (1998) applied a more recent method; a reverse transcriptase (RT) polymerase chain reaction (RT/PCR) to differentiate IBDV antigenic serotypes and subtypes. Furthermore, Jackwood and Nielsen (1997) tested bursa samples for the presence of IBDV using the reverse transcriptase polymerase chain reaction –restriction endonuclease (RT-PCR-RE) assay. The results of their studies demonstrated that the (RT/PCR-RE) assay can be used to diagnose IBD in chickens and that IBDV strains exist in commercially reared chickens that have restriction endonuclease (RE) patterns different from known IBDV strains. Counter immuno electrophoresis (CIEP) technique was used for detecting viral antigen 4 hours after infection. This method was found more sensitive than agar gel precipitation.
test (AGPT) (Dzhavador et al., 1988). For qualitative and quantitative estimation of IBDV specific antigen, Raj et al. (2000) used the rocket electrophoresis by which IBDV – specific antigen was detected in bursa of experimentally infected chickens up to 7 days post infection, and in a significantly great number of samples collected from field outbreaks, than the conventional AGPT. Immunoperoxidase and immunofloourescence technique had been used for detection of IBDV in formalin fixed paraffin embedded sections of the bursa of Fabricius of infected chickens (Cruz-coy et al., 1993) they assumed that the immunoperoxidase test was more useful than the immunofloourescence test, since the same section could be examined by immunoperoxidase and examined for microscopic pathology. Pereira et al., 1998 applied a western blot for diagnosis of IBDV infection, they found that by the use of blocking western blot test, two distinct viral proteins (VP2) and (VP3) were detected. The use of this methodology for detection of infection was then suggested in animals suspected of having IBD reinfection and a chronic subclinical form of the disease. Immunorheophoresis technique was applied for detecting IBDV antigen in infected bursa, the precipitin lines appear 3-5 hours in the immunorheophoresis technique, whereas that of AGID appear 14-24 hours (Raj et al., 1998). Rao and kumar, (1994) evaluated double sandwich ELISA, immunoperoxidase, fluorescent antibody and radial immuodiffusion tests for detection of IBDV in the bursa of Fabricius, they found that double sandwich ELISA, is specific, sensitive and the results are reproducible with each sample and easy to perform. The reverse passive haemagglutination test (RPHA) was used to detect IBDV antigen in various organs of experimentally infected chickens and field cases (Nachimuthu et al., 1995). They
detected IBDV antigen in 86.4%, 80.4% and 80.5% of different organs by RPHA, latex agglutination and AGID test respectively.

**1.13.2. Detection of antibodies**

The agar gel precipitation test (AGPT) and viral neutralization test (VN) tests are the most immunological methods used to detect chickens exposure to IBDV (Weisman and Hitchner, 1978). The AGPT is both economical to use and simple to perform and the results could be obtained within 28 hours. Yet VN is laborious and time consuming than the AGPT, but is more sensitive and can detect antigenic variations between IBDV isolates (Lukert and Saif, 1991; Mekkes and De wit, 1994; Weisman and Hitchner, 1978). An indirect ELISA was used for measuring IBDV antibodies in chickens (Mekkes and Wit, 1994). Antibodies could be detected as early as 4 days post infection and the test is safe, highly reproducible, specific and sensitive. Counter immuno electrophoresis (CIEP) was also used to detect IBDV antibodies in chickens’ sera. The method was found to be more sensitive, simple, reproductive and rapid, detecting precipitin in 15 minutes (Ninna, 1982). In addition, results when compared well with that obtained with AGPT could be read visually without staining. Nakamura et al. (1993) used monoclonal antibody bounded to polystyrene latex microspheres for quantitative measuring of IBDV antibodies. The results were obtained within 5 minutes. The titre of this rapid assay showed a close relationship with that of VN test. Perera et al., (1996) developed an ultra –micro ELISA technique for the detection of antibodies to IBD. This technique had high sensitivity (99%) and specificity (95.6%) when compared to ELISA. It is concluded that the technique is suitable for serological diagnosis of IBD.

In comparing serological methods used for detection of IBD antibodies ELISA was found
to be useful in detecting the maternal derived antibodies (MDA). This was because in infectious bursal disease (IBD) the MDAS are mainly of immunoglobulin G (IgG) class (Rose and Orlans, 1981) and ELISA system mainly detects the IgG class (Solano et al., 1986).

1.13.3. Isolation of IBDV

1.13.3.1 Cell Culture
Virulent IBDV strains are very difficult to adapt to tissue culture and when adapted they become less virulent (Synder et al., 1994). It was reported that both classic virulent and very virulent IBDV strains cannot be propagated in vitro, hence should be propagated by in vivo method (Brown et al., 1994). Isolation of IBDV from field specimens of the disease was reported to be very difficult (Synder et al., 1994). Even in chicken embryo growth was not satisfactory. In addition, it was very difficult to isolate and serially passage the virus in cell culture of chicken embryo origin (McFerran et al., 1980). However, Hirai and Calnek (1979) propagated virulent IBDV in normal chicken's lymphocyte and in lymphoblastoid B-cell line derived from an avian leucosis virus induced tumor cells. Many strains of IBDV could be adapted to cell cultures of chicken embryo origin and cytopathic effect had been seen. Cell culture adapted virus is easy to assay by plaque production method (Lukert and Saif, 1991). Many scientist were able to culture egg adapted strains of IBDV in chick embryo fibroblast culture which was considered as the method of choice for virus propagation (Rinaldi et al., 1992). Wild type virus could also be adapted to grow successfully in culture prepared in chicken embryo bursa after four serial passages. Then this virus was propagated in chicken kidney cells and produced plaques under agar (Lukert and Davis, 1974). Mammalian continuous cell
lines known to be susceptible to IBDV include RK-13 derived from rabbit kidneys (Petek 
et al., 1973), Vero cells derived from African green monkeys (jackwood et al., 1987), and MA -104 cells from fetal rhesus monkey kidneys (Jackwood et al., 1987).

1.13.3.2. Egg Embryos

Initial isolation of the virus in egg embryo was difficult to maintain in serial passage (Lukert and Saif, 1991). When allantoic sac used as route of inoculation all inoculated embryos died after the first passage, 30% in second passage and no mortality was reported in third passage (Landgraf et al., 1967). Highest virus titre was obtained 72 hours after inoculation (Hitchner, 1970). The difficulty of virus isolation in egg embryos refer to two factors (Hitchner, 1970), eggs from hens with IBDV antibodies were highly resistant to infection, and in early passage all fluid had a very low virus titre chorio allantoic membrane (CAM) had higher titre of the virus.

The best route of virus inoculation in egg embryo was found to be CAM of 9–11 day old (Hitchner, 1970). Mortality of inoculated embryo 10 day old started from 3rd to 5th day post inoculation.

1.13.3.3 In vitro and in vivo methods

The amount of infective IBDV is determined by scoring mortality resulting in the chicken lethal dose 50 (CLD50) or determination of the chicken infective dose scoring clinical signs and/or bursal lesions (CID50)(Van Loon et al., 1994). In vitro methods are determining of the embryo lethal dose (ELD50), embryo infective dose (EID50) or tissue culture infective dose (TCID50) (Van Loon et al., 1994). Also Van Loon et al., (1994)
described a more recent diagnosis assay to determine the amount of IBDV based on embryonated eggs as the most effective substrate for the virus and on the application of Mabs directed against IBDV for titration. The authors' method: ten-fold serial dilution of IBDV challenge virus are inoculated on the CAM of ten to eleven day old fertilized specific pathogen free (SPF) eggs. Five days after the inoculation the CAMS were harvested and homogenized. The presence of virus in CAMS was then determined with indirect ELISA assay making use of Mab-8 which can recognize all serotype I viruses. Different viruses including challenge strains, classic and variants could be assayed by this method.

1.14. Control of IBDV infection

1.14. Control by vaccination and types of vaccines used

Application of various management strategies was used to control the virulent IBDV. They all proved to be of limited success (Kouwenhoven and Van Den Bos, 1994). These include use of disinfections, use of mild and intermediate vaccine strain and different vaccination programs such as day old application, multiple vaccination and vaccination based on ELISA determination of maternally derived antibody (MDA) status (Gardin, 1994). Immunity against IBDV is mainly antibody mediated and maternal antibodies are transmitted via the egg-yolk to the chick (Kouwenhoven, 1994). IBD is mainly controlled by vaccination of breeder flocks so as to confer parental immunity to their progeny. Such maternal immunity will protect the chicks from early immunosuppressive infections and naturally protect chicks for 1-3 weeks, but, by boosting the breeder flocks with oil adjuvant vaccines, passive immunity may be extended to 4-5 weeks. However, such maternal antibodies can neutralize the vaccine virus (V o B and Vielitz, 1994).
According to Lukert and Saif, (1991) a universal vaccination program against IBD cannot be offered and the major problem with active immunization of young maternally immune chicks is the determination of the proper time of vaccination, and this varies with level of maternal antibodies, route of vaccination and virulence with vaccine virus. Also monitoring of antibody level in the parent flock or its progeny can aid in determining the proper time of vaccination.

**Types of Vaccines**

There are two types of vaccines: live and inactivated vaccines.

1.14.1. **Live Vaccines**

The live vaccine strains could be classified into three groups by the determination of the bursa body weight index in vaccinated and unvaccinated control birds (Mazariegos et al., 1990). These are mild, intermediate and hot vaccine strains.

1.14.1.1 **Mild Vaccines**

They do not induce significant difference in bursa/body weight index in vaccinated and control birds (Mazariegos et al., 1990). They are mild field or attenuated strains. Mild highly attenuated strains are non immunosuppressive to the bursa and can easily be neutralized by maternal antibodies. They are suitable to use for prevention of early exposure in one-day-old susceptible chicks and in chicks with low MDA initially or after waning of MDA (Mazariegos et al., 1990).

1.14.1.2 **Intermediate Vaccines**

These induce temporary significant difference in vaccinated and control birds in bursa/body weight index. Here the bursa weight in vaccinated birds is less than half that in control unvaccinated ones (Mazariegos et al., 1990). They cause no lesion even in SPF
chickens. They include: CU, D78, IM, Lukert CK37, LZ228TC and Bursine 2. They are used for vaccination of replacement pullets and for primary vaccination before inactivated revaccination (Lohren, 1994).

1.14.1.3. Hot Vaccine Strains

These showed significant difference throughout the observation period, the bursa size in vaccinated birds was more than or three times less than in the control (Mazariegos et al., 1991) they include: LZ228E (intervet), Bursa plus (Solavy, Duphar, Sterwin) and TAD LC75 (Gumboro vac forte). Its use should be restricted to very severely affected areas where no other means of control exist. In Europe, its use needs permission from the authorities (Kouwenhoven et al., 1994).

1.14.2. Inactivated Vaccines

Killed vaccines in oil emulsion give a depot effect and provide along uniform protection and high antibody levels (Edison et al., 1980; Wyeth et al., 1992). However, it requires priming with live virus exposure for effectiveness and should be given late in the rearing period before lay (Abram, 1990).

1.15. Vaccination of broilers

Many European countries do not vaccinate broilers. They thought that by giving the breeder flock a booster dose of Oil adjuvant killed virus vaccines; the immunity transferred to the off spring was enough to protect them during their short life span (Lohren, 1994). Life long protection of broilers by maternal antibodies was reported to be
impossible (Gmeiner, 1983). The author’s study revealed that variation in maternal antibodies was higher than expected in commercial broilers.

Killed adjuvant virus vaccine used to control IBD in Britain until 1988 by vaccination primed parent chicks at 18-20 weeks of age (Chettle and Wyeth, 1994). The investigators reported that the immunity transferred to the progeny was enough for protecting them for the whole duration of their lives. But, since the emergence of the very virulent IBDV in England in 1988, it became necessary to vaccinate broiler and parent chicks (Chettle et al., 1989).

1.16. Vaccination failure

Many reasons were involved in IBD vaccination failure. Of these:

1) Improper timing of vaccination

The level of MDA at the time of vaccination is very important. When application is too early the vaccine virus was neutralized by MDA (Solano et al., 1986). When applied too late the virulent field virus had the chance to be the first to infect the chicks (Kreager, 1989). Serological evaluation of MDA determines the optimum time of the vaccine (Gullen and Wyeth, 1975; Kouwenhoven and Van Den Bos, 1992). Considering that MDA is related to the flock not individual birds and the vaccine application date must be forecasted at least one week earlier and ELISA should be the serological tool to be used (Kouwenhoven et al., 1994). The optimum age at which broilers coming from adjuvant killed virus vaccine parents could be vaccinated with intermediate vaccines was 14-21 days (Kouwenhoven et al., 1994). However, according to the investigator these vaccines were effective to some extent but they failed in situation of high infection pressure. More recent reports stated that serological examination of day old chicks obtained from
similarly vaccinated flocks revealed that 8-10 days old was the most optimum time for 
early vaccination (Lohren, 1994). Other reports stated that live vaccine were able to 
reduce the number of susceptible chicks but there was a time during which chicks were 
susceptible which corresponded to day 25 of age (Chettle et al., 1994). In fact the hardly 
challenge virus could break through MDA at least one week earlier than live vaccines 
could (Chettle et al., 1994).

2) The ability of the virulent field virus to break through high MDA levels i.e. to infect 
broiler chicks at a much younger age than the intermediate vaccine virus (Chettle et al., 
1994)

3) Variation of maternal antibodies titre and deficient proper vaccination timing 
(Kouwenhoven et al., 1994). However the problems of the less effective intermediate 
vaccines was over come by the use of hotter vaccines or by multiple repeated 
vaccinations by these intermediate vaccines, which in high infection pressure also failed 
(Kouwenhoven et al., 1994).

To avoid the variation of maternal antibody hatcheries were advised to avoid as much as 
possible combination of hatches from different breeder flocks and if possible to combine 
off spring from flocks of similar titres (Kouwenhoven and van den Bos, 1994).
CHAPTER TWO
MATERIALS AND METHODS

2.1.1. Experimental chicks

One hundred and fifty, one day old, Hy-line broilers chicks were obtained from El Gharia Company, Khartoum, Sudan. The broiler breeder parent flocks of these chicks were vaccinated against IBD using killed IBD virus vaccine.

2.1.2. Housing of chicks:

The chicks were reared in isolated rooms with open system at the poultry houses of Faculty of Animal Production, University of Khartoum. All chicks were fed, watered and kept under the same environmental conditions throughout the experiments. Before and after the experiment, the rooms were thoroughly cleaned, washed, disinfected and left for 4 weeks before being used for the experiment.

2.1.3. Preparation of D78 Vaccine:

This is an intermediate live vaccine strain (intervet). Every dose contains at least 4.0 log10 TCID50. It was obtained from Detasi Company, Khartoum. The manufacture instructions for vaccine application with the standard procedure were strictly followed.

2.1.4. Vaccination program:

The vaccine D78 was given twice times, primary dose on the 18th day and booster dose at day 25 of age. The vaccine was administered via drinking water, nasal drops, spray and subcutaneous injection according to the experiment design.

2.1.5 Blood collection and serum preparation:

The blood was collected from the heart of chicks and the wing vein of older birds. Sterile disposable syringe of 29 gauge and 1-millimeter length was used for blood collection. An
amount of 0.5 ml was collected from each bird. The blood was left for 2 hours at room
temperature, and then the clot was loosened from the surface of the syringe, kept
overnight at 4 degree centigrade. The serum was separated and clarified by centrifugation
at 2000 revolutions per minute (rpm) for 10 minutes. The serum was stored in test tube at
-20°C till use.

2.2. Enzyme-Linked Immunosorbent Assay (ELISA):

Infectious bursal disease antibody test kit was obtained from BioCheck B.V. Crabethstaat
38-C 2801 AN Gouda Holland.

The antigen coated plates (inactivated viral antigen on microtitre plates) and the ELISA
kit reagents were adjusted at room temperature prior to the test. The serum was diluted,
five hundred folds (1:500) prior to the assay with sample diluents provided. 100 µl of
diluted serum was then put into each well of the plate. This was followed by addition of
100 µl of undiluted negative control (Specific pathogen free serum in phosphate buffer
with protein stabilizers and sodium azide preservative (0.1% w/v)). 100µl of positive
control was also added. (Antibodies specific to IBD in phosphate buffer with protein
stabilizers and sodium azide preservative (0.1% w/v)). The plate was incubated for 30
minutes at room temperature. Each well was then washed 4 times with wash buffer, this
contains 0.05% Tween 20 in powdered phosphate buffered saline (300 µł per well). A
100 µl of conjugate reagent (Sheep anti-chicken: alkaline phosphatase in Tris buffer with
protein stabilizers, inert red dye and sodium azide preservative (0.15 w/v)) was added
into each well. The plate was incubated in room temperature for 30 minutes. Each well
was washed again 4 times with buffer (300 ml per well). 100 µl of substrate reagent (p-
Nitro phenyl phosphate was dissolved in Diethanolamine buffer with enzyme co-factors)
was dispensed into each well. The plate was then incubated at room temperature for 15 minutes. Finally 100 µl of stock solution (Sodium Hydroxide in Diethanolamine buffer) was dispensed into each well to stop the reaction. The absorbance values were measured and recorded at 405 nm wavelength using ELISA microtitre Plate reader.

2.3. Experimental design:

2.3.1 Experiment 1:

The objective of this experiment was to monitor the declining pattern of maternally derived antibody (MDA) against infectious bursal disease virus (IBDV) in commercial broiler chicks.

For this purpose, 25 broiler chicks of one day old were reared in separation. The chicks were given infectious bronchitis (IB) Vaccine and Newcastle (ND) vaccine (colon 30) as spray at day 3 of age and another dose of Newcastle vaccine (colon 30) spray at day 14 of age for protection from IB and ND. But were not vaccinated against infectious bursal disease (IBD).

Blood was collected from five chicks randomly selected at days 1, 18, 25, 32, 39 and 45 day old. The serum prepared as mentioned in section 2.1.5

2.3.2 Experiment 2:

Four administration routes of D78 live vaccine were adopted in this experiment to study the effect of IBD vaccine route on the depletion rate of IBDV maternally derived antibody (MDA) titres in the chicks, along with the immune response in the IBDV vaccinated birds with each route was examined.

The vaccine was administrated by four routes namely drinking water (A), nasal drops (B), spray (C) and subcutaneous (D) and the test group was left without vaccination (F).
125 birds were divided into five groups (25 chicks of each). All groups were vaccinated by infectious bronchitis (IB) vaccine and Newcastle (ND) vaccine (colon 30) as spray at day 3 of age and another dose of Newcastle vaccine (colon 30) spray at 14 day of age for protection against IB and ND.

The four groups were vaccinated separately by D78 on day 18 and 25 by either route (A, B, C and D). The remaining 25 acted as control (F). From each group 5 birds were selected randomly for blood collection every week starting from day 18 of age. The sera were separated and saved at –20 ºC till used for antibodies detection by ELISA.

2.4. Data analysis:

IBD antibody titre was calculated automatically, using software.

The ELISA data are presented as:

1- S/P ratio of the samples where (S) represented the absorbance value of the test serum divided by the absorbance Value of the positive control (P) serum.

\[
\frac{\text{Mean of Test Sample} – \text{Mean of negative Control}}{\text{Mean of positive control} – \text{Mean of Negative Control}}
\]

The following equation relates the S/P of a sample at a 1:500 dilution to an end point titre

\[
\log 10 \text{ Titre} = 1.1 \times \log (SP) + 3.361
\]

\[
\text{Antilog} = \text{Titre}
\]

<table>
<thead>
<tr>
<th>S/P value</th>
<th>Titre range</th>
<th>Antibody status</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.145 or less</td>
<td>284 or less</td>
<td>negative</td>
</tr>
<tr>
<td>0.150 – 0.199</td>
<td>285 – 390</td>
<td>suspect</td>
</tr>
<tr>
<td>0.200 or greater</td>
<td>391 or greater</td>
<td>positive</td>
</tr>
</tbody>
</table>
2- Coefficient of Variation (CV): is an indicator of individual value dispersal with regards to titre mean.

\[
\text{Coefficient of Variation} = \frac{\text{Standard deviation}}{\text{Arithmetic mean titre}}
\]

CV values is currently made according to the following threshold:

- < to 30% = Very homogenous
- 30 to 50% = Homogenous
- 50 to 80% = poorly homogenous
- > 80% = Heterogeneous
- > to 150% = Very heterogeneous

For statistical analysis stat graphics plus version 3.0 (1997) was employed for analysis. One-way ANOVA was used in order to find out the relationship between different vaccine administrations. With regard to S/P and CV%.
CHAPTER THREE

RESULTS

The declining pattern of maternally derived antibodies (MDA):

The decaying pattern of maternally derived antibody (MDA) against infectious bursal disease virus was observed as the age of chick’s progress. High S/P ratio (5.4) of MDA was obtained for day one, this level was almost stable up to the 7th day with S/P ratio of 5.06, and then the MDA started to decline rapidly. By the 18th day (S/P ratio = 0.62) and with very low level at day 45 of age (S/P ratio 0.04) (Figure 1).

![Figure 1: The levels of maternal anti-Infectious Bursal Disease antibodies in progeny chicks at various ages post hatch](image)

The effect of vaccine administration route on MDA:
Generally, the correlation between the four groups of vaccine administration routes with regard to S/P ratio was found ($F = 0.94$ and $P = 0.4519$) which is statistically not significant ($P>0.05$).

Following, the administration of the vaccine via drinking water, the result obtained showed that at day 25 of age the level of immunity was very low ($S/P$ ratio = 0.05). Detection of serum at day 32 (one week after the second vaccination) indicated that the $S/P$ ratio was increased to 1.67. Then the $S/P$ ratio was continuously increased up to day 45 (3.26) (Figure 2).

**Figure 2: Seroconversion of progeny chicks after vaccination with D78 via drinking water**

**NB:**
Day 18 the first vaccination dose of D78

**Day 25 the second vaccination dose of D78**
S/P ratio indicates the amount of antibodies as determined by ELISA.

Following the administration of the vaccine via nasal drops, the S/P ratio was 0.1 when the serum tested at day 25 of age, then the S/P ratio was increased at day 32 of age (S/P ratio = 1.19) and it remained high (S/P ratio = 1.86) until the last day of the experiment (Figure 3).

![Seroconversion of progeny chicks after vaccination with D78 via nasal drops](image)

**Figure 3: Seroconversion of progeny chicks after vaccination with D78 via nasal drops**

**NB:**
Day 18 the first vaccination dose of D78
Day 25 the second vaccination dose of D78
S/P ratio indicates the amount of antibodies as determined by ELISA.

The application of the vaccine through spray route revealed that the S/P ratio was low (0.19) at day 25 of age, and then the S/P ratio was raised up to day 45 of age (1.54) (Figure 4).

Figure 4: Seroconversion of progeny chicks after vaccination with D78 via spray.

NB:
Day 18 the first vaccination dose of D78

Day 25 the second vaccination dose of D78
S/P ratio indicates the amount of antibodies as determined by ELISA.
Following the administration of vaccine via subcutaneous injection, the level of antibodies was very low at day 25 of age (S/P ratio = .06). Then the S/P ratio began to increase up to the last day detection of serum at day 45 (S/P ratio = 3.29) (Figure 5).

**Figure 5: Seroconversion of progeny chicks after vaccination with D78 via subcutaneous injection**

**NB:**

Day 18 the first vaccination dose of D78
Day 25 the second vaccination dose of D78
S/P ratio indicates the amount of antibodies as determined by ELISA.

The homogeneity of the results was measured with regard to coefficient variation (CV %), which is an indication for vaccine take. The difference between the four different types of vaccine administration was statistically significant (F=6.95  P< 0.05). This significance was clear between the group of nasal drops and drinking water, and nasal drops and spray route, subcutaneous injection and spray route. The result of CV% is summarized in Table 1.

Table 1: The coefficient variation of MDA in chicks vaccinated via different routes

<table>
<thead>
<tr>
<th>Age of chicks (days)</th>
<th>CV% of MDA</th>
<th>CV% of drinking water</th>
<th>CV% of nasal drops</th>
<th>CV% of spray</th>
<th>CV% of subcutaneous injection</th>
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<tbody>
<tr>
<td>0</td>
<td>27</td>
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<td>78</td>
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<td>Percentage Range</td>
<td>Homogenity Description</td>
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<td>&lt; to 30%</td>
<td>Very homogenous</td>
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<td>30 to 50%</td>
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<tr>
<td>50 to 80%</td>
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<td>80%</td>
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<tr>
<td>&gt; 150%</td>
<td>Very heterogeneous</td>
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CHAPTER FOUR

DISCUSSION
Infectious bursal disease (IBD) or Gumboro is an old disease of chicks caused by a Birnavirus; which targets the bursa of Fabricius, most of the economic losses associated with IBD are due to its immunosuppressive effects. The disease is mainly controlled by vaccination. To achieve a successful vaccination program; an effective vaccine administered by an appropriate route is necessary. This study was conducted to assess the immune response of D78 vaccine administrated by drinking water, nasal drops, spray and subcutaneous injection; the declining pattern of maternally derived antibodies (MDA) was also investigated.

From the results obtained in the present study, it was clear that, the level of maternally derived antibodies (MDA) or passively transferred antibodies against infectious bursal disease virus (IBDV) were very high at day one of age as they hatched from vaccinated dams. Which indicates that, the parent's flocks of these chicks were hyper immunized. The dams transmit this high level of antibodies in the form of maternal derived antibodies to the progeny chicks. This was previously published by Sharma et al. (1989). These high levels of maternal derived antibodies protect the chicks from infection by IBDV in early ages, but will hinder vaccination against IBD as the vaccine will be neutralized by the circulating MDA and rendered infective. This fact was previously confirmed by (Solano et al., 1986).

In this study, the maternally derived antibodies was observed to decline rapidly after the first week and was noted to remain detectable in chicks up to 45 days of age with appreciable magnitude log low (S/P of 0.04). However the protective limit of these antibodies level expired by the 4th week. The finding of this study looks in agreement
with Kenzevic et al. (1987) who reported that the progeny antibodies persisted up to 6 weeks of age and diminished after the first week. While this result is somehow in disagreement with Zaheer and Saeed, (2003) who reported that the progeny antibodies persisted up to 4 weeks and the protective limit of them expired by the second week. The difference in the results of our study and above mentioned study could be due to the difference in the initial titer of IBD in chicks, which is a direct reflection of the immune status against IBD in the parent flock. This explains the individual variation among chicks to take the vaccine

The maternally derived antibodies declined rapidly after the first week of age, however were still detectable by the end of the study (45 days). This is not surprising since these chicks were not raised on specific pathogen free environment and were perhaps still exposed to low levels of antigenic challenge from external sources. Similar levels of maternally derived antibodies decay were observed in another study (Ahmed and Akhter, 2003). The reason for such rapid declining at this period of age attributed to the proteolytic degradation of antibodies or neutralization because of naturally occurring infectious bursal disease virus challenge. It is clear evident that an appropriate time for maternal derived antibodies level testing in chicks is while they are growing i.e. day 10-14. Since the rate of decrease in the level of MDA is affected by existence of the pathogen in the environment, metabolism and growth rate.

The second part of the study showed that the S/P ratios for all different routes of administration of the vaccine D78 was decreased post the first vaccination day and the increase to the protective level after the second vaccination was observed and remained high up to the 45th day of age. This seroconversion indicated that the immune response
for all different routes of administration of the vaccine is good, and the decrease was a result of neutralization of the vaccine by the high MDA at the first vaccination day hence the intermediate vaccine D78 can not break through high MDA like the hot vaccines. This phenomenon was reported in previous studies (Zaheer and Saeed, 2003) which suggested that a customized vaccination regime with regard to the type of vaccine and level of MDA is crucial for complete protection rather than the route of the vaccine administration which does not have great influence on the protection level of antibodies. And the increase in the immunity due to the second vaccination dose is observed and in fact because the level of MDA was not high enough to hinder the work of the vaccine at this time.

The difference between the S/P ratios of the birds against different routes of administration was not significant (P> 0.05) and this agreed with Nakamura et al. (1995) who confirmed the insignificance between vaccination routes with regard to immune response when he studied intratrachea, intranasal, per os, by crop gavage and intramuscular routes for vaccine administration. Giambrone and Hathcock (1991) reported similar findings when they studied coarse spray and dinking water routes for vaccination against IBD. This indicates that D78 vaccine can be administrated by all routes used in the study without detectable variation in their immune response elicited.

On the other hand the S/P ratio was seen useful as it allows to estimate whether the birds were immunized or not, but it doesn't allow us to compare the vaccine take. So the most significant criterion is to find out whether the batch as a whole is immunized or not, and this is established with homogeneity or coefficient variation and the differences between the four different routes of administration of the vaccine was statistically significant in
our study (P<0.05). This significance was clear between drinking water and nasal drops, and nasal drops and spray, spray and subcutaneous injection. And it was clear from Table 1; the nasal drops route of administration at various ages had the best interpretation with regard to the coefficient variation and this because this route gives the birds equal opportunities for vaccine antigens exposure.

In conclusion, this study revealed that the decay of maternal derived antibodies in chicks started rapidly after the first week and remained detectable to the end of the study under our experimental condition whereas the naturally occurring IBDV challenge played role in this decay. While the protective limit of antibodies expired by the end of the 4th week. It was also clear that improper vaccination time due to vaccination at early age (high MDA) leads to depression period of immunity between time of vaccine neutralization and protective antibodies resulting from the second vaccine dose. Concerning routes of vaccination our study revealed that there was no significant difference between drinking water, spray, subcutaneous injection and nasal drops as routes of administrations of the vaccine D78 with regard to the immune response. Although the vaccination take by nasal drops was the best.

So it is recommended to devise vaccination schedule in the light of prevailing maternal antibody titre while chicks are growing i.e. 10-14 day of age. And nasal drops is the best administration route of D78.

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