Cairo University
Faculty of Veterinary medicine
Department of poultry diseases

Studies on Gumboro disease in chickens

A Thesis Submitted

by

Mohamed Ahmed Sayed Ahmed

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Under supervision of

Prof. Dr. Mostafa Ahmed Bastami

Prof. Dr. of poultry diseases
Faculty of Veterinary Medicine
Cairo University

Dr. Khiled Sayed Shaban
Ass. Prof. of poultry diseases
Faculty of Veterinary Medicine
Cairo University

Dr. Mohamed Khalifa Hassan
Head of National Laboratory for veterinary quality control on poultry production
Animal Health Research Institute

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Cairo University
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Department of poultry diseases

Supervision SHEET

Prof. Dr. Mostafa Ahmed Bastami
Prof. Dr. of poultry diseases
Faculty of Veterinary Medicine
Cairo University

Dr. Khalid Sayed Shaban
Ass. Prof. of poultry diseases
Faculty of Veterinary
Cairo University

Dr. Mohamed Khalifa Hassan
Head of National Laboratory for veterinary
Medicin equality control on poultry production
Animal Health Research Institute

(2015)

Cairo University

Faculty of Veterinary Medicine

Poultry and Rabbit diseases Department

Name: Mohamed Ahmed Sayed Ahmed

Job Description: researcher assistant in National Laboratory for Veterinary Quality Control on Poultry Production.

Date and Place of birth: 25/09/1986, Cairo. **Nationality:** Egyptian

Degree: B.Sc 2008.

Supervisors:

Prof. Dr. Mostafa Ahmed Bastami, Professor doctor of poultry diseases, Faculty of Veterinary Medicine, Cairo University.

Dr. Khiled Sayed Shaban, assistant professor of poultry diseases, Faculty of Veterinary Medicine, Cairo University.

Dr. Mohamed Khalifa Hassan, head of National Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute.

Thesis title: Studies on Gumboro disease.

Abstract

Re-emergence of highly virulent forms of IBDV has been the cause of significant economic losses. In present study, bursal samples were collected from fifty broiler chicken farms across six different governorates during 2010-2012 which were showing IBDV clinical signs and postmortem findings. Fifty bursa homogenates representing the fifty investigated farms were assayed using reverse transcriptase-polymerase chain reaction (RT-PCR) for IBDV targeting VP2 gene. Out of the tested samples 9 were positives. IBDV-positive samples were selected for further isolation and characterization. The virus was isolated by inoculating bursa suspension into embryonated specific pathogen-free (SPF) eggs. Chorioallantoic membrane(s) (CAMs) were collected and tested by AGPT confirming the presence of IBDV. The virus was detected by RT-PCR and sequence analysis of PCR products of 9 selected samples was carried out. Four samples were characterized as very virulent (vvIBDV) and five samples were classic attenuated IBDV similar to classic attenuated (vaccine) strains. The genotyping of Egyptian vvIBDVindicate continuous evolution of IBDV in Egypt and they were closely related to previous isolated strains from Egypt.

Dedication

TO MY family:

My father: *who is always helpful for me and encourages to hard work.*

My mother: *I learnt with her to devote myself to high aims and consistently struggle for success.*

My little princess: Jessy*; the most blessing gift from Allah that shine my life.*

My wife: *who is source of happiness in my life, always push me forward and share me all my moments hand in hand.*

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1-Introduction

Infectious bursal disease (IBD) is an acute highly contagious viral disease of young chickens that has tropism to lymphoid tissues as its primary target with a special predilection for the bursa of Fabricius (cloacal bursa). The economic importance of the disease is manifested in two ways. First, some virus strains may cause up to 20% or more mortality in chickens 3 -6 weeks of age. The second and more important, manifestation is sever and prolonged immunosuppression of chickens infected at an early age. **(*Saif and Eterradossi, 2008*)**

The result that have been associated with immunosuppression induced by IBDV pathological conditions include gangrenous dermatitis **(*Sharma and Lee,* 1983)**, Marek's disease **(*Cho, 1970*)**, infectious laryngotracheitis**(*Rosenberger, et al., 1978*)**, infectious bronchitis **(*Pejkovski, et al., 1979*)**, chicken anemia agent **(*Yuasa, et al., 1980*)**, salmonellosis and colibacillosis**(*Takase, etal.,1996*)**, coccidiosis **(*Anderson, et al., 1977*)**, inclusion body hepatitis **(*Fadly, et al., 1975*)**, infectious proventriculitis (***Shaban, Kh.s.2004 Ph.D.*** Thesis).

IBD was first recognized as a specific disease entity by ***Cosgrove* (*1962*)** and was referred to as "avian nephrosis" because of the extreme kidney damage found in birds that succumbed to IBDV infection. Later, it was termed as infectious bursal disease referring to the specific lesions caused by the disease in cloacal bursa **(*Hitchner, 1970*)**. Since the first outbreak occurred in the area of **Gumboro**, **Delaware**, "**Gumboro disease**" was a synonym for this disease and is still frequently used.

IBDV is non-enveloped, icosahedral capsid belonging to genus Avibirnavirus of Family Birnaviridae **(*Hirai and Shimakure, 1974; Dobos, et al., 1979*)**. IBDV genome composed of 2 segments; A & B that codes for five viral proteins; VP1, VP2, VP3, VP4 and VP5. Due to neutralizing monoclonal antibodies against VP2 can be used to differentiate the serotypes and strains so it is consider the most important protein within the virus **(*Becht, etal., 1988 and Fahey, et al., 1989*)**. The VP2 is also responsible for antigenic variation **(*Brown, etal., 1996,Snyder, et al., 1988b, Vakharia, et al., 1994b*)** and virulence **(*Brown, etal., 1996, Yamaguchi,et al., 1996*)**.

The re-emergence of variant or highly virulent IBD forms has been the cause of significant economic losses. Until **1987**, the strains of virus were of low virulence causing less than 2% specific mortality, and satisfactorily controlled by vaccination. But in **1986** and **1987** vaccination failure were described in different parts of the world. The sudden onset of hyper-virulent IBDV created the need for a better characterization of the circulating strains so that, in the future, the vaccination schedule could be adapted faster to a new epidemiological situation. ***Van den Berg,*****(*2000*)**.

IBD was first reported in Egyptian flocks in the early seventies **(*El-Sergany et al., 1974*)**. However interest in IBDV antigenic characterization was triggered by the appearance of the very virulent IBDV in vaccinated Egyptian flocks **(*El-Batrawi and El-Kady 1990; Khafagy et al., 1990*)**. Several reports have classified the Egyptian IBDV isolates as classical IBDV **(*Khafagy etal., 1990; Bekhit, 1996*)**. On the other hand, some reports have provided partial evidence of the presence of antigenically variant IBDV strains in Egyptian flocks **(*El-Sanousi et al., 1994; Sultan, 1995; Shaban, K.S. Ph.D. Thesis 2004 and Maher, A. master thesis 2010*)**. Presently, evidence of circulating variant IBDV strains were isolated from flocks vaccinated using classical IBDV vaccines **(*El- Khiat, 2003; Hussein et al., 2003 and Metwally et al., 2003*)**.

There are currently three well-documented antigenic types. These are classic (often called standard) and variant serotype 1 and serotype 2 viruses. The classification according to pathogenicity based on experimental reproduction of acute IBD in specific pathogen free white leghorn chickens, "variant" IBDV induce little if any clinical signs and mortality, but marked bursal lesions, classical IBDV induce approximately 10-50% mortality with typical signs and lesions and very virulent IBDV induce approximately 50-100% mortality with typical signs and lesions **(*Saif and Eterradossi, 2008*).**

Very virulent IBDV is a pathotypic variant of IBDV, which was first detected in chickens in the Netherlands in 1986 **(*Chettle, et al., 1989*)**. The primary features of vvIBDV are the ability to induce high mortality ranges from 5-25% in broilers and 30-70% in layers. Surviving birds are severely affected condemnation rate. VVIBDV induces clinical signs similar to those induced classical virulent IBDV strains, except that the disease is more pronounced and acute in individual birds and generalized in flocks. Lesions are also more pronounced with hemorrhages in the cloacal bursa and muscular tissue with rapid bursal and thymic regression, while the microscopic lesions are similar to those seen with classical types of IBDV.

VVIBDV are indistinguishable from classical IBDV strains in some aspect and they differ at the genetic level from other IBDV strains in the hypervariable region of the VP2 gene. All vvIBDV isolates from different countries are genetically almost identical and have three unique amino acid residues at position **222(Ala)**, **256(lle)** and **294(lle)**. Detection of these three amino acid residues in a new isolated virus is indicative of a very virulent phenotype. Diagnosis of vvIBDVs is based on clinical signs, ability of isolate to induce high mortality in SPF chickens and genetic analysis **(*Ignjatovic, 2004*)**.

The present study was planned to fulfill the following:

1) Surveillance study on infectious bursal disease virus in chicken farms in Egypt.
2) Isolation of infectious bursal disease virus from suspected samples.
3) Characterization and Molecular identification of isolated IBDV strains.
4) Comparison with reference vaccinal and field strains.

2. Review of literatures

2.1.History of IBDV:

2.1.1.History of IBDV in the world:

Cosgrove, (1962) first recognized IBDV as a specific disease entity in a broiler flock near Gumboro town, Delaware, USA which appears to be infectious, contagious and characterized by ruffled feather, watery diarrhea, trembling and sever prostration. It seems to be spread from pen to pen within a poultry house and tends to recur in successive broods. It was referred to as "Avian nephrosis" due to extreme kidney damage found in birds that exposed to infection.

Winterfield et al., (1962) succeeded in isolating an agent in embryonating eggs from chicken flocks in USA, the mortality pattern was irregular and the agent was difficult to maintain in serial passage. The isolate was referred to as "infectious bursal agent" and was identified as the true cause of IBD while the Gray virus was identified as an isolate of Infectious bronchitis virus with nephropathogenic tendencies.

Hitchner, (1970) proposed the term "Infectious bursal disease" as the name of the disease causing specific pathognomonic lesions in the cloacal bursa.

Allan et al., (1972) reported that infection with the infectious bursal disease agent at an early age were immunosuppressive against immunization to Newcastle disease virus.

Ojo et al., (1973) recorded 7 outbreaks of Gumboro disease in Nigeria between 1966 and 1971. In such outbreaks, the morbidity rate was 60% while

the mortality rate was 12.5%. It was mentioned that the disease was introduced through the importation of one day-old chicks.

McNulty et al., (1979) and ***McFerran et al., (1980)*** demonstrated serotype 2 IBDV by isolation and serological studies on IBDV from fowl, turkeys and ducks in Northern Ireland.

Jackwood et al., (1982) recognized the presence of two serotypes of IBDV (I and II) in USA using virus neutralization test.

Rosenberger et al., (1985) recognized variant strains of IBDV in Delmarva poultry producing area that made the control of IBD viral infections have been complicated.

Rosenberger et al., (1987) found that the variant strains were breaking through maternal immunity against standard strains by using IBDV variant strain vaccines in broilers and broiler breeders.

Ismail et al., (1988) isolated IBDV variant strain from 39-43 day old commercial leghorn pullets for the first time in the USA. These chickens were vaccinated with a commercial live IBDV vaccine at 25 and 32 days old. The variant isolate termed IN, was recovered using SPF ECE and the BGM-70 established cell line. Experimental studies using SPF chickens vaccinated with either inactivated vaccine made from the vaccine strain used in the problem flock or a standard type vaccine indicated no protection against the IN isolate. Cross neutralization test indicated that the IN isolate differed antigenically from commercial vaccinal strains and was related to the variant IBDV strains recently isolated from broilers.

Giambrone et al., (1990) reported that vaccination against serologic standard IBDV or Delaware antigenic variant E strain can protect against STD

IBDV in the progeny but with variable protection percentage against the newly evolved variant strains by challenge test.

Nunoya et al., (1992) reported that there is extensive spread of vvIBDV throughout Japan and successful isolation of the virulent IBDV was done from field cases. The pathogenicity of the isolates was examined in specific-pathogen-free chickens which developed sever clinical case with high mortality rate. Histopathologically, infectious bursal disease was characterized by bursal and thymic necrosis, aplastic anemia, acute hepatitis with fatty changes and systemic inflammatory response.

Lasher and Davis (1997) stated that the disease emerged for first time in USA in 1957 as a clinical entity responsible for acute morbidity and mortality in broilers on the Delmarva Peninsula. The condition spread rapidly and was recognized throughout the U.S. broiler and commercial egg production areas by 1965.

Hon et al., (2006) investigated the phylogenetic origins of IBDV genome segments and estimated the time of emergence of their most recent common ancestors. Moreover, with recently developed coalescence techniques, they reconstructed the past population dynamics of vvIBDV and timed the onset of its expansion to the late 1980s. Their analysis suggested that genome segment A of vvIBDV emerged at least 20 years before its expansion, which argued against the hypothesis that mutation of genome segment A is the major contributing factor in the emergence and expansion of vvIBDV.

2.1.2. History of IBD in Egypt:

El-Sergany et al., (1974) reported for the first time IBD infection on the basis of histopathological examination.

Mousa etal., (1983) reported the presence of precipitating and neutralizing antibodies of IBDV in a native, newly hatched Fayoumi chicks which protected them up to 3 weeks-old from infection. Clinical signs, pathological lesions, pathogenesis and serological response after experimental infection with a highly pathogenic IBDV strains were described and discussed.

Amer et al., (1984) isolated 18 strains of IBDV out of 45 chicken flocks with symptoms and post mortem lesions suspected to be IBDV. The isolated strains caused embryonic mortalities ranging from 20-75% compared with 40% caused by the reference virus.

Mousa et al., (1984) described a severe form of IBD in 4-8 weeks old Fayoumi chicks at Nagi-Hamady. The mortality rate reached 40% within 1 week.

Amer et al., (1986) studied the prevalence of Gumboro disease virus among chicken flocks serologically using AGP test. Precipitating antibodies against vaccination to IBD usually disappear at early age (25 days post vaccination of maternally immune chicks at 10 days of age). So, detection of precipitating antibodies in old ages indicates recent infection.

Mousa et al., (1986) isolated IBDV from turkeys and identified them as non-serotype 1 IBDV.

Mousa et al., (1988a) developed and evaluated a vaccine from the T-73 virus isolate of IBDV recovered from a subclinical case in turkeys by experimental vaccination of commercial chicks possessing maternal antibodies against IBDV. T-73 as vaccine produced excellent protection when administrated at 4, 8 and 12 days of age. The strain was characterized as intermediate vaccinal strain producing moderate bursal lesions with no immunosuppressive effect against Newcastle disease vaccination.

Mousa et al., (1988b) characterized 4 commercial used IBDV vaccines in Egypt depending on the criteria of safety, efficacy and immunosuppressive effect and found that the vaccines were varied in their virulence and invasiveness to the bursa of Fibricus.

Amin, (1990) studied the effect of prepared live IBD vaccine on the immune response of chickens against live Newcastle disease vaccine. Also the author tried to isolate and identify a mild IBDV strain for live vaccine preparation and determining the suitable route for its inoculation in embryonated chicken eggs(ECE).

El-Batrawi and El-kady (1990) reported the interaction between maternal antibodies and the age of susceptibility to virulent IBDV.

Sultan, (1995) studied the virulence of recent local isolates of IBDV and their control by vaccination. The author indicated that the currently circulating IBDV field viruses are in the majority of the highly virulent pathotype, producing acute disease with sever clinical picture and high mortalities up to 70% also the results revealed that the intermediate vaccine (D78) provoke satisfactory seroconversion and protection percentage against mortality but could not completely prevent bursal damage as a result of experimental vvIBDV challenge. Also, the vaccine (D78) gave early vaccinal protection against mortality in the experimental challenge, ranging between 30-40% during the first 48 hours post vaccination, vaccination can be helpful when an outbreak occurs in one flock of multiple-housed farms where protection by vaccination of adjacent houses appears necessary.

Madbouly and Afifi (1995) reported that Dot-ELISA is faster, more economic and easily applicable in the detection of IBDV antigens than solid phase ELISA and AGPT. The indirect double antibody sandwich technique is more sensitive than the single antibody ELISA system.