Al-Qadisiya University

College of Veterinary Medicine

An overview of Certain Viral Diseases in Broiler Chickens that found in Al-Dewaniya

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By

Reem Numaan Talib

Supervised by

Dr. ALaa A. Jawad

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بسم الله الرحمن الرحيم

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زِدْنِـي عِلْـماً)

صدق الله العلى العظيم

Certificate of Instructor

We certify that **Reem Numaan talib** has completed the fulfillment of her graduation project entitled **An overview of certain viral diseases in Broiler Chickens that found in Al-Dewaniya** for the year 2015/2016 under our construction.

Instructor

Dr. Muthanna Hadi Hussain

21. 4. 2016

Head of Department of Internal and Preventive Medicine

Dr. Asaad J. Abed

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21. 4. 2016

Supervisor's Certificate

We certify that this study (**An overview of Certain Viral Diseases in Broiler Chickens that found in AL-Dewaniya**) was prepared under our supervision at the College of Veterinary Medicine / Al-Qadisiya University, as a partial requirement of the Bachelor degree of Veterinary medicine .

Signature

Dr. Alaa A. Jawad

College of Veterinary Medicine

Al-Qadisiya University

Date: / / 2016

Dedication

TO:

my family and all my friends

Acknowledgements

In the name of Allah most gracious most merciful, the first who deserves all thanks and appreciation for granting me with will, strength and help with which this study had been accomplished.

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Content

Introduction

Poultry industry has expanded rapidly over the last fourth decades and is playing a vital role in the economy of the country. However, the industry is confronted with a variety of problems, particularly the diseases of viral origin , poultry production is one of the best available sources for the production of high biological value animal protein in terms of meat and eggs, commercial hybrids, both broilers and layers, are being propagated for meat and eggs production throughout the world.
Viral infection are one of the important problem which can caused large economic losses represented in mortality and morbidity ratio especially, new castle disease virus, infectious bursal disease, infectious bronchitis disease virus and avian influenza virus . The data will be useful in further evolutionary studies and for a better understanding of the infection biology of the virus. Furthermore, the information will be helpful for the development of novel diagnostic assays and vaccine candidates.

Literature

1: Newcastle disease (ND)

1:1:Newcastle disease

Newcastle disease (ND) is one of the most important infectious diseases of poultry. It is distributed worldwide and has the potential to cause large economic losses in the poultry industry(Spradbrow, 1988) . Its causative agent is Newcastle disease virus (NDV), a virus that is able to infect over 240 species of birds and which spreads primarily through direct contact between infected and healthy birds(Kaleta and Baldauf , 1988). The first outbreaks of ND in Newcastle-upon-Tyne, England, were reported during the mid-1920s . Within a few years ND had spread throughout the

world and became endemic in many countries(Spradbrow, 1988) . NDV occurs in the field as a variety of strains which differ extensively in the organ systems that they affect and in the severity of the symptoms that they produce in infected birds. Based on the severity of the disease in chickens, NDV has been classified into three pathotypes:lentogenic, mesogenic and velogenic. Lentogenic NDV strains cause subclinical infection with mild respiratory or enteric disease and are considered low-virulent. Mesogenic NDV strains are of intermediate virulence causing respiratory infection with moderate mortality (< 10%), while velogenic NDV strains are highly virulent causing mortality rates up to 100%. Velogenic strainsare further classified into viscerotropic velogenic and neurotropic velogenic strains. Viscerotropic velogenic strains produce lethal haemorrhagic lesions in the viscera, whereas neurotropic velogenic strains cause neurological and respiratory disorders(Alexander,1997).

1:2:Newcastle disease virus NDV

NDV is a paramyxovirus and viruses from this family are enveloped, non-segmented, negative-sense RNA viruses, which - together with the Pneumovirinae – constitute the family of Paramyxoviridae . NDV, or avian paramyxovirus type 1 (APMV-1), is classified in the genus Avulavirus of the subfamily Paramyxovirinae(Mayo,2002). NDV viruses belong to one serotype and there are two classes. The genome of class I viruses consists of 15 198 nucleotides (nt) and the genome of class II viruses consists of 15 186 or 15 192 nt. (Czeglédi *etal.*, 2006). The genome contains six open reading frames (ORF) which encode the nucleoprotein (NP), the phosphoprotein (P), the matrix protein (M), the fusion protein (F), the haemagglutinin- neuraminidase (HN) and the large protein (L). At least one additional, non-structural protein (V) and

possibly a second one (W), are generated by RNAediting during P gene transcription (Steward *etal.*, 1993).

1: 3 Virus infection

The virulence of a virus is determined by multiplegenetic factors. These may involve its tissue or organ tropism, its ability to deal with the host's immune systemand/or its efficacy of replication. Virus infection is initiated by attachment of the virion to the surface of the target cell. Binding of the viral HN glycoprotein to sialic acid-containing cell surface proteins, which serve as receptors, triggers the F proteinpromoted fusion of the viral envelope with the plasma membrane of the host cell through a pH-independent mechanism, similar to other paramyxoviruses(Lamb *etal.*, 2007) . The viral nucleocapsid or ribonucleoprotein complex (RNP) contains the RNA genome encapsidated with NP and associated with the polymerase complex composed of the P and L proteins. After entry, the viral nucleocapsid dissociates from the M protein and is released into the cytoplasm. Subsequently, the polymerase complex transcribes the viral genomic RNA to produce the mRNAs that are required for the synthesis of the viral proteins. Binding of the polymerase complex to the nucleocapsid is mediated by the P protein, whereas the catalytic activities are functions of the L protein (Curran,1996). The switch from transcription to genome replication takes place when sufficient amounts of viral protein have accumulated. The polymerase complex is responsible for the synthesis of full-length plus-strand antigenomic RNA, which in turn serves as the template for synthesis of minus-strand genomic RNA. Viral nucleocapsidsare then assembled by association of NP with the newly formed genomic RNA and with the polymerase complex. All components of the virus particle are transported to the plasma membrane where they are assembled under the direction of the M

protein. Virionsare released from the cell by a process of budding (Harrison *etal.*, 2010). Finally, the neuraminidase activity of the HN protein facilitates the detachment of the virus from the cell and removes sialic acid residues from progenyvirus particles to prevent self-aggregation(Takimoto and Portner., 2004). The genome of negative-strand RNA viruses is exclusively present in viral particles in the form of the RNP; naked viral RNA is not infectious. However, the development of reverse genetics for negative-strand RNA viruses has allowed the production of infectious virus from cloned cDNAs and has made genetic modification possible (Conzelmann , 1996). Currently, reverse genetics systems for NDV are available for the lentogenic strains LaSota (Huang *etal.*, 2001). Hitchner B1 and AV324/96 (Nakaya *etal.*, 2001; Dortmans *etal.*, 2009) , the mesogenic strains Beaudette C and Anhinga (Krishnamurthy *etal.*, 2000; Estevez *etal.*, 2007) and the velogenic strains Herts/33 , ZJ1 and RecP05 (De Leeuw *etal.*, 2005; Liu *et al.*, 2007; Morales *etal.*, 2011) .

2: Infectious bursal disease virus (IBDV)

Infectious bursal disease virus (IBDV) is an acute, highly contagious pathogen, which causes intensive irreparable immunosuppression or often death in young chickens, often at an age of 3-6 weeks when the B lymphocytes are developing, and as a result imposes severe losses to the poultry industry annually worldwide. Younger chickens are passively protected by maternal antibodies transmitted via egg yolk and older ones are able to produce antibodies against the virus, therefore rarely develop clinical signs of the disease. Since the first outbreaks occurred in Gumboro, Delaware, "Gumboro disease" became a synonym for IBD (Macreadie *et al.*, 1990) .

2:1:Virus classification

IBDV belongs to the genus *Avibirnavirus* of the family *Birnaviridae*. The virion is composed of a non-enveloped icosahedral capsid with a bi-segmented double strand RNA genome. It has two distinct serotypes (I and II) but only serotype I is virulent for chickens. An intrinsic characteristic of RNA viruses is the high variability of their genome due to the low proofreading activity of their viral replicases. Accordingly, many strains have been generated. Based on the pathotype detectable IBDV in the field can be divided to classical, antigenic variant and very virulent strains. Classical strains cause inflammation and severe lymphoid necrosis, immunosuppression and up to 30% mortality. Variant strains cause bursal atrophy and immunosuppression, with very low mortality whereas very virulent IBDV (vvIBDV) may cause acute clinical disease with 60-100% mortality (Brown and Skinner, 1996). vvIBDV strains have emerged in Europe in 1987 and spread to Latin America, South-East Asia, Africa and the Middle East since the last three decades. One reason for the worldwide distribution of the virus is that it is extremely resistant in the environment. The Office of International des Epizooties (OIE) estimates that IBD is present in more than 95% of the 117 member countries. The acute clinical form of IBD caused by vvIBDV isolates has been observed in over 80% of these countries. It has been reported in Europe, Asia, Africa, South America and Central America.

2:2:IBDV structure

The IBDV genome has two segments of double-stranded RNA, named A and B, which are packed in a non-enveloped icosahedral (T=13) shell with 60 nm in diameter (Böttcher *et al.*, 1997). The smaller segment B (~2.8 kb) encodes the protein VP1 (97 kDa), which is an RNA-dependant

polymerase. The segment A (~3.3 kb) contains two partially overlapping open reading frames. The smaller ORF encodes the non-structural protein VP5, which is not essential for virus replication neither *in vitro* nor *in vivo* (Mundt *et al.*, 1997) but has a role in virus release (Gariiga *et al.*, 2006; Wu *et al.*, 2009). The larger ORF encodes a precursor 110 kDa polyprotein (VP2-VP4-VP3) that is post-translationally cleaved into three distinct proteins: pre-VP2 (54 kDa), VP4 (25 kDa) and VP3 (28 kDa). Native IBDV has an icosahedral symmetry with triangulation number (T) of 13 and 60 nm diameter made of 12 pentamers and 120 hexamers according to trimers decoration (Luque *et al.*, 2007). Structural studies indicated that the virus capsid is composed of 780 VP2 molecules in form of 260 structural trimer subunits , which stand on the outer face of the particle (Böttcher *et al.*, 1997). On the inner side, 600 (or ~ 450) (Luque *et al.*, 2007), copies of VP3 in Y-shaped trimers make a scaffold responsible for the stability of the particle. VP4 is located on 60 positions next to 5-fold axes of icosahedrons (Hu *et al.*, 1999). The RNA genome is in contact with positively charged C-terminus of VP3 inside the particles (Böttcher *et al.*, 1997).

2:3: Route of infection and pathology

The route of IBDV infection is usually oral (from contaminated food, water or excrements), but may be via the eye or respiratory tract, with an incubation period of 2-3 days. Following oral infection, the virus replicates in gut-associated macrophages as well as lymphoid cells (Mahgoub, 2012). The virus attacks the immature IgM-bearing B-lymphocytes in the BF and destructs the tissue resulting in the immunodeficiency of its host (Shaarma *et al.*, 2000). Depression, anorexia, ruffled feathers, unsteady gait, trembling, huddling under equipment, watery diarrhea and the most prominent lesion, hemorrhage

and necrosis of bursa of Fabricius, followed by bursal atrophy are some clinical symptoms of this disease (Chang *et al.*, 2001). If chickens are infected within 1-3 weeks of hatch, they show immunodeficiency without symptoms whereas infection within 3-6 weeks of hatch accompanying symptoms leading to death; but no influence on older chickens hasbeen observed. Immunodeficient chickens are susceptible to other disease and show no adequate response to vaccination.

2:4: Virus detection, classification and diagnostic tests

To distinguish between classical, antigenic variant, very virulent and non-virulent strains, it is important to identify the IBDV serotype in order to be able to choose the best strategy for vaccination. Since the distinction between different strains is not possible through serological techniques like agar-gel precipitation test (AGPT) or enzyme linked immunosorbent assay (ELISA), reverse transcription-polymerase chain reaction (RT-PCR) has been used as rapid, sensitive and specific method for detection of IBDV in clinical samples and for phylogenetic studies (Lee *et al.*, 1992; Li *et al.*, 2009).

3:Infectious bronchitis (IB)

3:1:Infectious bronchitis disease

Infectious bronchitis (IB) is a highly contagious disease of serious economic importance in the poultry industry worldwide. The first report of IB by Schalk and Hawn referred to a highly contagious disease in young chicks with respiratory symptoms in North Dakota, USA in 1931. Pathogenic alterations in the upper respiratory tract of the birds were prominent; hence the disease was named "infectious bronchitis of young chicks". Five years later, it was demonstrated that the causative agent of

this disease is a virus, which was named Infectious Bronchitis Virus (IBV). After the initial description of infectious bronchitis, many cases of the disease were reported in the United States (Hitchner, 2004). Thereafter and to date, a wide range of different IBV serotypes and genotypes have been detected around the world (de Wit *et al.*, 2011).

3:2:Infectious Bronchitis Virus

IBV belongs to the order *Nidovirales*, family *Coronaviridae*, genus *Gammacoronavirus* (Gonzalez *et al.*, 2003). The enveloped viral particles are round and pleomorphic in shape. The virions are approximately 120 nm in diameter and contain club-shaped surface projection called spikes, which are 20 nm in size (Cavanagh & Gelb, 2008). The positive sense RNA genome is approximately 27.6 kb in size and is encompassing 5′ and 3′ untranslated regions (UTRs) with a poly(A) tail (Mo *et al.*, 2012). A major part of the genome is organized as two overlapping open reading frames (ORFs), 1a and 1b, which are translated into large polyproteins 1a and 1ab through a ribosomal frame shift mechanism. The remaining part of the genome consists of regions coding for the main structural proteins spike (S), envelope (E), membrane (M) and nucleocapsid (N). Two accessory genes have been described, ORF3 and ORF5, that express accessory proteins 3a & 3b and 5a & 5b, respectively (Pasternak *et al.*, 2006).

3:3:Clinical features of infectious bronchitis

Clinical cases of IB are associated with respiratory, reproductive, digestive and renal infections in domestic poultry and in various other avian species (Cavanagh, 2005). The disease is clinically manifested by coughing, sneezing, tracheal coarse crackles, nasal discharge, decrease of feed intake and conversion, loss of body weight, swollen sinuses, increased water intake, wet droppings, depression, lethargy and poor

growth in broilers. In layers the disease, "false layer syndrome", affects egg quality (thin, rough, fragile, misshapen egg shells and thin watery egg) and causes decrease in egg production. In some cases, the virus infection may cause severe damage to the oviduct and result in decreased or permanent loss of egg production (Cavanagh & Gelb, 2008; Worthington *et al.*, 2008). IB infections may lead to mortality up to 20-30% or higher at five to six weeks of age in chicken flocks (Seifi *et al.*, 2010). The mortality can increase due to immunosuppression, mycoplasma and other secondary bacterial infections caused by various accompanying infectious agents, e.g. Escherichia coli, Ornithobacterium rhinotracheale and Bordetella avium. The mortality rate can be as low as 1% and chickens may recover rapidly, if the infections are produced by mildly virulent strains and are not associated with secondary bacterial infections (Cavanagh & Gelb, 2008).

3:4:Host specificity

IBV infects a wide range of avian species, especially those reared close to domesticated poultry, for example domestic fowl, partridge, geese, pigeon, guinea fowl, teal, duck and peafowl (Cavanagh, 2007). In different hosts, the virus exhibits considerable similarities in its genome. For example, a virus that was isolated from teal and peafowl shared 90-99% sequence related to IBV (Liu *et al.*, 2005). Evidence based on the nucleotide sequences of viruses isolated from samples of ducks, whooper swans, turkeys and pheasants have also shown high similarity to IBV (Hughes *et al.*, 2009).

4:Influenza

4:1:Influenza disease:

Influenza viruses A, B and C belong to the *Orthomyxoviridae* family, a group of enveloped viruses that possess segmented, negative-sense, single-stranded RNA genomes (Palese and Shaw, 2007). The name *Orthomyxoviridae* derives from the Greek *orthos*, meaning 'standard, correct' and *myxa*, meaning 'mucus' (Cheung and Poon, 2007). Influenza A has the broadest host range and infects a variety of animals that includes humans, pigs, birds, horses and sea mammals. Aquatic birds are the source of all influenza A viruses in other species (Taubenberger and Morens, 2010). Influenza B viruses naturally infect humans and occasionally seals, while influenza C viruses benignly infect humans, pigs and dogs (Noda and Kawaoka, 2010). Only influenza A viruses have been responsible for all influenza pandemics (Cheung and Poon, 2007). The influenza viruses are classified according to antigenic differences exhibited by two of the internal structural proteins, the nucleocapsid and the matrix proteins(Noda and Kawaoka, 2010). In addition, antigenic variations in the surface glycoproteins, Haemagglutinin (HA) and Neuraminidase (NA), are used to subtype the influenza A viruses (Bouvier and Palese, 2008). Antigenic subtypes have not been identified for influenza B and C (Palese and Shaw, 2007). With regards to influenza A, there are now 16 different haemagglutinin subtypes (H1-H16) (Fouchier *et al*, 2005) and 9 different neuraminidase subtypes known (N1-N9) (Bouvier and Palese, 2008; Taubenberger and Morens, 2010). Only three HA (H1, H2 and H3) and two NA (N1 and N2) subtypes have caused human epidemics, defined by sustained and widespread person-to-person transmission (Palese and Shaw, 2007). However, the avian influenza strains H5N1, H7N7 and H9N2, have also

been transmitted to humans (Wright *et al*, 2007; Taubenberger and Morens, 2010). Different influenza strains are named according to their genus, the species from which the virus was isolated (omitted in the case of humans), the location of the isolate, the number of the isolate, the year of isolation, and in the case of influenza A viruses, the haemagglutinin and the neuraminidase subtypes. For example, an influenza strain isolated by Shope was given the designation A/Swine/Iowa/15/30 (H1N1) virus, meaning that it was the 15th isolate of an H1N1 subtype virus isolated from pigs in Iowa in 1930 (Palese and Shaw, 2007; Bouvier and Palese, 2008).

4:2:Structure of influenza A virus

The influenza virus is an enveloped virus, surrounded by a lipid bilayer that is derived from the host's cell membrane during the viral budding process.The virions are pleomorphic, displaying shapes that range from spherical to filamentous (Noda and Kawaoka, 2010; Rossman and Lamb, 2011). Laboratory isolated strains of influenza are roughly spherical with a diameter of 80-120 nm (Bouvier and Palese, 2008). The filamentous form was first observed in 1949 and it has been determined that this shape occurs in newly isolated strains. Recently, electron microscopic analysis of autopsied lung tissue, acquired from a patient who died as a result of the Swine- Origin H1N1 2009 pandemic influenza strain, revealed filamentous viral particles (Nakajima *et al*, 2010; Rossman and Lamb, 2011). The influenza virion is coated with the surface glycoproteins HA and NA, which are anchored in the lipid bilayer, in a ratio of approximately four to one. Laver and Valentine (1969) were the first to demonstrate that the surface projections, visible on electron micrographs, were the HA and NA proteins . The envelope, which also contains the matrix 2 (M2) protein, overlays the matrix 1 (M1) proteins

that enclose the virion core. The genome of the influenza A virus contains eight vRNA segments, which can be observed as an ordered 7 + 1 configuration in budding virions (Noda and Kawaoka, 2010). In total, from eight vRNA segments, twelve proteins are produced (Bouvier and Palese, 2008, Wise *et al*, 2009).

4:3:Antigenic drift

Antigenic drift occurs because the influenza's RNA polymerase complexes have no proof reading ability; therefore, high mutation rates can lead to point mutations in the antigenic sites (Taubenberger and Morens, 2010). Mutations that include deletions, substitutions, and insertions can affect the antigenic binding sites and are among the most important mechanisms for producing antigenic variation in influenza viruses (Carrat and Flahault, 2007). As strains evolve to evade detection by host antibodies directed against the surface glycoproteins, frequent amino acid changes occur at the antigenic sites within HA1, NA or both (Wright *et al*, 2007; Bouvier and Palese, 2008).

4:4:Antigenic Shift

The segmented nature of the genome means that a reassortment event known as antigenic shift can occur, where the influenza A strain acquires the HA segment, and sometimes the NA segment, from another influenza A subtype. Reassortment can produce genetic diversity rapidly, thus providing an evolutionary advantage, and can occur in cells simultaneously infected with different strains of human and animal viruses . Mixtures of parental gene segments may be assembled into virions and the resulting virus may encode antigenic proteins for which the human population has no pre-existing immunity. The result can be a pandemic or worldwide epidemic (Carrat and Flahault, 2007; Hutchinson

et al, 2010). Antigenic shift triggered the 1957 and 1968 pandemic outbreaks as well as the most recent swine-origin H1N1 pandemic virus . And, in addition, it likely produced the extremely virulent influenza A (H1N1) virus that caused the 1918-1919 'Spanish flu', where the HA, NA and PB1 genes all contributed to the high pathogenicity in an immunologically naive global population (Pappas *et al*, 2008; Bouvier and Palese,2008).

Conclusion

1: More studies illustrate that virulence is a complex trait that is determined by multiple genetic factors.

2: NDV, the multi-basic amino acid cleavage motif in the F protein is an absolute prerequisite for virulence.

3: VP2 protein in IBDV as a major host protective antigen has been attracted the most attention in vaccine development.

4: The complete genome sequence data and the phylogenetic analysis have revealed that the genome of IBV is under a continuous process of evolution, due to mutations, strong selective pressure and recombination events.

5:The genetic shift of influenza virus play important role in developing new genotypes and therefore develop e new serotypes .

Recommendations :

Many questions still remain concerning the molecular mechanisms used by virus to regulate tropism, transcriptionand translation, interference with the host defence machinery, and how they affect virulence.

2: understanding of the biology of NDV and can serve as an important guide for future research on the molecular principles that determine its virulence.

3: Further molecular studies for genotypic and phylogenic characteristics of these viruses.

4:Isolation and characterization of these viruses and detection of local strains in order to produce efficient vaccines to control and eradicate these pathogens.

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