BURLEIGH DODDS SERIES IN AGRICULTURAL SCIENCE

Optimising poultry flock health

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Introduction

Diseases remain a significant burden to poultry production and its future. Whilst it is widely recognised that vaccines have a major role in inducing protection, they can only be considered as part of the solution to this growing problem. Recent research efforts have focused on developing effective preventative measures that reduce the risk of disease outbreaks in flocks.

This volume reviews the wealth of recent research on the mechanisms of transmission for infectious diseases and how our understanding of this transmission can be used to improve poultry flock health. The book is split into three parts: Part 1 chapters focus on understanding infectious diseases in poultry, drawing attention to viral and bacterial disease transmission and epidemiology as well as parasite infections. Chapters in Part 2 review the ways of preventing diseases, such as improving biosecurity, using nutritional strategies to boost immune response and developments in vaccines to protect against disease. Part 3 examines optimising health at differing stages in poultry production, including chapters that focus specifically on chick health, poultry broilers, layers and breeder birds.

Part 1 Understanding infectious diseases in poultry

The first chapter of Part 1 reviews understanding the molecular biology of avian viruses and their role in poultry health. Chapter 1 first examines the two main groups of viruses, naked (or non-enveloped) and enveloped viruses and how they can survive in poultry environments. It then moves on to discuss viral capsids and viral interaction within the host cell, focusing specifically on infectious bursal disease virus VP2 protein, avian orthoreovirus capsid protein σC and fowl aviadenovirus hexon and fiber proteins. A section on viral glycoproteins is also included, which is then followed by a review of how poultry viruses can mutate via recombination and reassortment.

Chapter 2 looks at advances in understanding bacterial diseases in poultry, focusing on challenges and perspectives. The chapter first examines how identification and various pheno- and genotyping tools can be used to control and prevent bacterial diseases. It also discusses how epidemiology can help to identify the source of infection, method of transmission and host susceptibility. The chapter moves on to review disease pathogenesis and disease prevention by vaccination, as well as how biosecurity and good farm management can help to prevent the transmission of pathogens. It concludes by highlighting potential areas for future research development.

The final chapter of Part 1 focuses on advances in understanding parasite infections of poultry. Chapter 3 reviews research on understanding and controlling three of the most widespread and economically-relevant parasites of poultry and the infections they cause: *Eimeria* species (coccidiosis), *Histomonas meleagridis* (histomonosis) and *Dermanyssus gallinae* (the poultry red mite (PRM)). In each case the chapter reviews advances in areas such as: incidence and economic significance, host-pathogen interactions, population dynamics, detection, control and prevention as well as ongoing challenges in parasite management.

Part 2 Preventing diseases in poultry

Part 2 opens with a chapter that reviews improving biosecurity in poultry flocks. Chapter 4 first highlights the various ways to reduce sources of contamination, drawing specific attention to cleaning and disinfection of poultry barns, management of equipment and vehicles and water and feed hygiene. It also examines how managing insect, mite and rodent pests, wild birds and pets to help reduce contamination which is then followed by a section on manure, litter and dead bird management as well. The chapter also looks at separating healthy birds from sources of contamination through using methods such as zoning production sites and improving hatchery and regional biosecurity. Sections on biosecurity compliance and the economics of biosecurity are also provided.

Chapter 5 considers nutritional strategies to boost immune response in poultry. The chapter specifically focuses on research work from the year 2000 to present, drawing attention to immune responsiveness and disease resistance. In order to assess immune enhancement, suppression and balance, independent sections on vitamins (i.e. carotenoids and vitamin E), minerals, such as selenium and zinc and amino acids, such as arginine and threonine are presented. The chapter also includes sections on other nutrients, specifically highlighting vitamin D, manganese and copper. A section on the ingredients on immune modulation (i.e. fats, oils, wheat, rye, insect meal, algae meal, and alternatively processed oilseeds) is also included. A section on in ovo manipulation of embryo and subsequent chick immunity is presented. Studies assessing few immune parameters versus those assessing overall immune balance in the presence of challenges are discussed.

The final chapter of Part 2 examines developments in vaccines to protect poultry against diseases. Chapter 6 first introduces the three different types of vaccines currently available: inactivated, live and recombinant vaccines. The chapter then goes on to review the viral vectors used for recombinant vaccines, such as herpesviruses, Marek's disease virus-1 and -2, infectious laryngotracheitis virus, avian poxviruses, adenovirus and Newcastle disease virus. Techniques used for generating recombinant vaccines are also highlighted, which is then

followed by a discussion on gene editing of herpesvirus vectors. The chapter concludes by emphasising the importance of continuing to develop vaccines for disease prevention in poultry production.

Part 3 Optimising health at differing stages in poultry production

The first chapter of Part 3 looks at incubation and chick health. The chapter begins by discussing what happens during the incubation stage and how the quality of this process can influence the health of the chick involved. The chapter then reviews day-old chick quality and the factors that can influence the chick's quality and status, focusing specifically on breeder influences such as diseases, nutritional factors, chemical intoxication and biological intoxication. It also reviews how egg quality can affect the chick's quality, drawing attention to factors such as laying house period, egg washing, egg disinfection, egg position and storage. Biological and physical risk factors during the incubation period are highlighted. A section on processing, storage and transport of the day-old chick is also included.

The next chapter focuses on optimising the health of broilers. Chapter 8 first presents the critical infectious disease challenges faced in the top five broiler-producing countries. It discusses the current and future strategies to control these diseases, strategies include biosecurity, vaccination, surveillance, diagnostics, environmental management, nutritional interventions and genetic selection. The optimisation of broiler health comes with a better understanding and monitoring of the whole production system in each location. The data presented in this chapter indicates that intestinal and respiratory diseases are the main aspects to improve worldwide.

Chapter 9 draws attention to optimising the health of poultry layers. The chapter begins by highlighting key disease issues faced by pullets, discussing the main vertically transmitted diseases, viral diseases and bacterial diseases affecting these hens. The chapter moves on to examine the key disease issues faced by laying hens, focusing specifically on the various viral and bacterial diseases that can affect them. A section on other issues affecting layers and pullets is also provided, drawing attention to the fungal infections that can occur, how feed and water sources need to be monitored closely to prevent deficiencies and excess consumption. External and internal parasites of layers are also highlighted. The chapter also reviews surveillance strategies in laying hens to ensure good bird health before concluding with an overview of why using different approaches is important to optimise layer health.

The final chapter of the book focuses on optimising the health of broiler breeds. Chapter 10 first introduces the diseases that can occur during the rearing period, drawing attention to early chick mortality and coccidiosis,

various leg problems and spinal abscesses. The chapter then moves on to review the diseases that can arise during the laying period, such as *E. coli* Peritonitis Syndrome (EPS), infectious bronchitis and other respiratory infections. It also discusses fowl pox, neoplastic diseases, worm infections, histomonosis (blackhead disease), ruptured tendons and amyloidosis. Vertical transmitted diseases are also discussed, focusing on diseases such as *Mycoplasma gallisepticum*, *Salmonella* spp., chicken infectious anemia, avian encephalomyelitis (AEV), avian reovirus (ARV) and adenovirus infections. A section on vaccination programs for broiler breeders and the role of maternal immunity is also provided, which is then followed by a review of the effects of various management practices on broiler breeder health. It concludes by highlighting the effects of diet on the health of breeders.

Chapter 1

Understanding the molecular biology of avian viruses and their role in poultry health

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1 Introduction

A mature, complete, and fully infectious virus is known as virion. The simplest virions consist of two basic components:

- the viral genome (single- or double-stranded RNA or DNA);
- a capsid, which is a protein coat that functions as a shell to protect the viral genome from nuclease degradation and, within some limits, from some environmental factors such as desiccation or extreme pH conditions.

Some viruses also contain an outer coat called a viral envelope. Viruses also have receptor-binding domains (RBDs) that will recognize and attach to specific receptors located on the membrane of the target cells where viruses 'deliver' their genomes. Enveloped viruses have viral glycoproteins anchored to their envelope which have critical roles in the initiation of viral infection as attachment proteins (Maclachlan et al., 2017).

Viruses are obligate intracellular parasites. Viruses lack mechanisms for energy production and protein synthesis since they do not possess cellular

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organelles (Maclachlan et al., 2017). Viruses have been described as 'cell hijackers' because they use host cell processes to synthesize viral proteins and replicate nucleic acids. Viruses have different delivery pathways to reach host cells. Some can disseminate efficiently through direct respiratory transmission like infectious bronchitis or Newcastle disease viruses (NDVs). Some viruses such as arboviruses or poxviruses use arthropods like mosquitoes or mites, while others can infect through the oral route such as enteric viruses. Some viruses like NDV and other paramyxoviruses contain enzymes such as RNA polymerases inside the mature viral particles' proteins that are essential for the initial stages of viral replication (Maclachlan et al., 2017). In a similar way, poxviruses (fowlpox) carry inside their particles enzymes involved in genome replication (Cann, 2016). Inside the cell, the viral genome replicates and alters protein synthesis to manufacture viral proteins through interactions between cellular and viral components (Flint, 2004). Infected cells react to the presence of viruses in various ways such as apoptosis. As a response to the viral infection, innate and adaptive immune systems elicit diverse mechanisms that play major roles in the pathogenesis of viral diseases. This chapter discusses key features of viruses and their role in understanding the characteristics of common viral diseases in poultry, as well as the ways viruses evolve.

2 Enveloped and non-enveloped viruses

There are two main groups of viruses:

- naked (or non-enveloped) viruses;
- enveloped viruses.

Non-enveloped or 'naked' viruses do not have a lipid covering, and their capsid proteins directly interact with the host cell receptors to initiate the infection. Naked viruses rupture infected cells, leading to release of viral progeny (Van Der Grein et al., 2018). Examples of non-enveloped virus families affecting poultry include: *Birnaviridae* (infectious bursal disease (IBD)), *Reoviridae* (viral arthritis), *Anelloviridae* (chicken infectious anemia), *Adenoviridae* (inclusion body hepatitis), and *Picornaviridae* (avian encephalomyelitis).

Enveloped viruses are generally large particles with an outer coat, a phospholipid bilayer that originates from the cell membrane and is transformed by the virus. Examples of enveloped viral families of significance in poultry include: *Herpesviridae* (Marek disease and infectious laryngotracheitis), *Orthomyxoviridae* (avian influenza), *Paramyxoviridae* (Avian Paramyxoviruses, including Newcastle disease), and *Coronaviridae* (infectious bronchitis).

Viral envelopes mainly consist of envelope proteins (E), membrane proteins (M), and spike proteins (S). Envelope lipids are derived from the

host cell, while envelope glycoproteins are encoded by the virus (Banerjee and Mukhopadhyay, 2016). The envelope acts as an anchor for different viral glycoproteins that form the 'spikes' or 'peplomeres' (Maclachlan et al., 2017). Enveloped virus particles are formed by budding through a host cell membrane, during which the particle becomes coated with a lipid bilayer derived from the cell membrane (Cann, 2016). This is important because the cell is not destroyed during the process and some of these viruses can thus induce more persistent infections.

The presence or absence of envelope will determine the stability of viruses in the environment and their resistance to chemical agents such as disinfectants. In general, enveloped viruses are more susceptible to chemical disinfectants than non-enveloped viruses. However, organic matter, especially feces or secretions, can protect these viruses and allow them to persist longer in the environment. With some minor differences, most chemical agents can be effective for infectious bronchitis, Newcastle disease, avian influenza, and infectious laryngotracheitis since these are relatively unstable in the environment. Physical factors such as heat, extreme pH, hypertonic conditions, and dryness can also inactivate them. Enveloped viruses with a low lipid content in their envelope (*Poxviridae* and *Hepadnaviridae*) are sensitive to disinfectants but more resistant than viruses with a high lipid content (*Herpesviridae*, *Flaviviridae*, *Togaviridae*, *Retroviridae*, *and Coronaviridae*), which are the most sensitive to chemical disinfectants (Tarka and Nitsch-Osuch, 2021).

On the other hand, naked viruses are very stable, and they can persist in poultry houses even when thorough cleaning and disinfection procedures were carried out. Infectious bursal disease virus (IBDV) is an example of a very hardy virus because it can survive in the environment for up to 122 days after removing infected birds from a poultry house (Benton et al., 1967). This virus can even resist cooking temperatures of over 70°C (Mandeville et al., 2000). Reoviruses can survive for at least 10 days on feathers, wood shavings, egg shells, and in feed but for at least 10 weeks in drinking water with little loss of infectivity (Savage and Jones, 2003). Non-enveloped viruses with strong hydrophilic properties (*Picornaviridae* and *Parvoviridae*) are the most resistant to chemical disinfectants in comparison with those with reduced hydrophilic properties (*Reoviridae*, *Rotaviridae*, *Adenoviridae*, *and Caliciviridae*) (Tarka and Nitsch-Osuch, 2021).

3 Viral capsids and viral interaction with the host cell

Viral capsids are formed as single or double protein shells and consist of only one or a few structural protein species with many copies per viral particle. These multiple protein copies must self-assemble to form a continuous three-dimensional capsid structure. These protein shells are tough and elastic. There

are two kinds of capsid architectures (Cann, 2016; Maclachlan et al., 2017; Mateu, 2013):

- helical;
- icosahedral.

To form a viral capsid with helical symmetry, the protein subunits self-assemble into a helical array surrounding the nucleic acid. Such nucleocapsids may form either rigid and highly elongated rods or flexible filaments in enveloped viruses (Cann, 2016). Examples of viruses with helical symmetry are infectious bronchitis virus (IBV), avian paramyxoviruses, including NDV, and avian influenza (AI) virus.

In the icosahedral capsid, the protein subunits are arranged in the form of a hollow quasi-spherical structure enclosing the genome within, consisting of 20 triangular faces and 12 vertices. Examples of viruses with icosahedral symmetry are adenoviruses (inclusion body hepatitis, hemorrhagic enteritis of turkeys, and egg drop syndrome), herpesviruses (Marek's disease, infectious laryngotracheitis, and duck enteritis virus).

Capsid proteins are extremely important for non-enveloped viruses because they are responsible for the first interaction with the host cell and delivery of the genome in a form in which it can interact with the cell. The interaction between virus and cell is carried out through the binding of a specific virus-attachment protein to a cellular receptor molecule.

3.1 Infectious bursal disease virus VP2 protein

An example of a capsid protein is the IBDV VP2 protein. IBD is an important immunosuppressive disease in young birds, producing severe atrophy with consequent immunosuppression, an increase in mortality, and impairment of growth performance (Eterradossi and Saif, 2020). The genome of this virus codes for five proteins (VP1–VP5). VP2 is the major structural protein that builds the viral capsid and contains the antigenic domains (Lee et al., 2006). There are remarkable variations among isolates or subtypes commonly known as the hypervariable region (between amino acids 204 and 344) in VP2. This region seems to be responsible for the interaction with cellular receptors (Van Loon et al., 2002).

The VP2 protein plays a critical role in the pathogenesis of IBD. VP2 and the nonstructural protein VP5 induce programmed cell death (Huang et al., 2021; Qin and Zheng, 2017; Rodriguez-Lecompte et al., 2005; Vasconcelos and Lam, 1994). Apoptosis contributes to the depletion of lymphocytes. In addition to the rapid loss of B cells in the bursa (Fig. 1), a high level of apoptosis is found in peripheral blood lymphocytes during IBDV infection. Some level of apoptosis

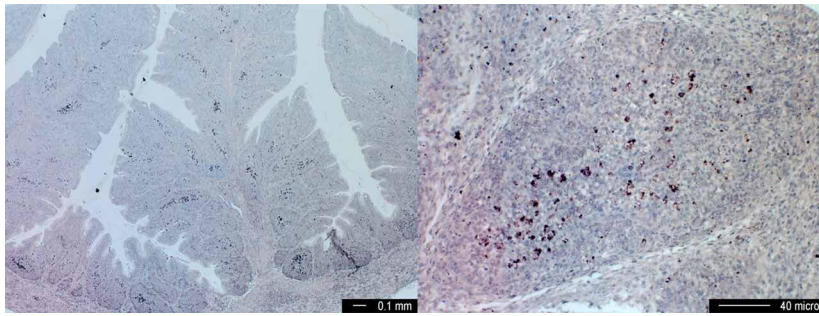

Figure 1 Bursa, commercial broilers inoculated with a Delaware E strain. Histological preparation with riboprobe in situ hybridization (ISH) targeting infectious bursal disease virus (IBDV) VP2 protein. (Left) Severe bursal atrophy with lymphoid depletion and interfollicular fibroplasia is appreciated. IBDV-infected lymphoid cells (ISH (+) in brown are located mainly in follicle centers (120× magnification). (Right) Detail of bursal follicle, immature IBDV-infected lymphoid cells, ISH (+) appear in brown in the center of the follicle.

has been observed in birds vaccinated with IBDV intermediate vaccine strains (Killian et al., 2017).

The protein VP2 has been seen as a suitable candidate for the development of recombinant vaccines. VP2 can be used to elicit a significant protective effect sufficiently comparable to that induced by an inactivated vaccine (Martinez-Torrecuadrada et al., 2003). Recombinant vaccine products using turkey herpesvirus (HVT) as a vector have been developed and have been very effective in controlling IBD even in the presence of very virulent IBDV strains (vvIBDV) (Gelb et al., 2016; Parker et al., 2014; Perozo et al., 2009; Sedeik et al., 2019). New research on vaccine development to control IBDV is based on subunit vaccines, virus-like particles, and DNA vaccines that also target VP2 to induce protection (Jackwood, 2017).

The study of IBDV VP2 has also helped in the development of efficient diagnostic tools. There are several types of IBDV, and classification or typing of strains by serological methods is difficult and lengthy and with limited discriminating power to detect minor antigenic differences between strains. Molecular methods such as reverse transcriptase-polymerase chain reaction (RT-PCR), and nucleotide sequence analysis assay have been used to identify or genotype IBDV strains. Most researchers have focused on the variable sequence region of the VP2 gene, known to encode one or more neutralizing epitopes of the virus (Jackwood, 2004; Wu et al., 2007).

3.2 Avian orthoreovirus capsid protein σC

Avian reoviruses are double-stranded, non-enveloped viruses with a segmented RNA genome. These viruses are the cause of viral arthritis/tenosynovitis in

chickens and turkeys. Clinical signs of this disease include swelling of the hock joints accompanied by lesions in the gastrocnemius and digital flexor tendons causing lameness, reluctance to move (Fig. 2), and other less frequent conditions such as hydropericardium, myocarditis, enteritis, encephalitis, and bursal atrophy. Reoviruses have been also associated with enteric and respiratory disease, malabsorption, and stunting syndromes (Jones, 2000). Although not all reoviruses are pathogenic, reoviral infections may be associated with poor weight gain, increased feed conversion ratios, and rejections at the processing plant, resulting in economic losses.

Attenuated vaccines including the strain S1133 or related strains were developed in the late 1970s and 1980s and had been used in breeders and young chicks to control clinical cases of viral arthritis and tenosynovitis. However, a dramatic increase in the number of clinical cases of tenosynovitis emerged in chickens and turkeys from 2012 in the United States, France, Israel, Canada, and some countries in South America. Molecular methods identified variant reoviruses belonging to different genotypes (Sellers, 2017). Current commercial vaccines do not provide adequate protection against these new variants, requiring vaccines with homologous viruses to confer protection (Lublin et al., 2011; Troxler et al., 2013). The U.S. poultry industry is now using custom-made inactivated vaccines produced from isolates from their farms known as autogenous vaccines (Sellers, 2017).

These disease variants showed wide variations in the gene coding for the capsid protein σC (Kant et al., 2003). Avian orthoreovirus minor outer σC is the viral cell attachment protein and is the most variable protein encoded

Figure 2 Viral arthritis in commercial broiler 20 days old with leg weakness, lateral recumbency, and moderate swelling of hock joints. The flock had history of lameness, leg weakness, birds with lateral recumbency, and concomitant bacterial infections. Avian reovirus was isolated from tendons after one passage in LHM cells.

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