Chapter

From Understanding the Immune Response against Coccidiosis to the Use of Coccidia Vaccines

Luis-Miguel Gomez-Osorio, Ben Dehaeck, Carlos Cuello, Jenny-Jovanna Chaparro-Gutierrez and Sara Lopez-Osorio

Abstract

Avian coccidiosis is the most costly global poultry parasitic disease, which represents a threat to food production and sustainability. Coccidiosis is still ubiquitous even in modern poultry production systems. Protective immunity against coccidia does develop but differs for each *Eimeria* species and depends on the method of immunization and the immune response (including both early innate immune response by several proteins and professional phagocytes as well as acquired immune response with specialized cells). In addition, GALT is a master tissue in the immune response against coccidiosis because of its crucial functions: acquired immunity in both the cellular and humoral immune responses. Here, we present an extensive review on the immune response against coccidiosis and the use of vaccines as an alternative for consideration in integrated sustained coccidiosis control programs.

Keywords: *Eimeria*, innate immune response, acquired immune response, cytokines, live vaccines, precocious vaccines

1. Introduction

Avian coccidiosis is by far the most costly parasitic disease in poultry [1], and it may represent a threat to guarantee the supply for sufficient, safe, and nutritious food. According to some projections, the global population in 2050 will be 10 billion which will increase the demand for food production by 70% and therefore achieving global food security is a staggering challenge [2].

Coccidiosis is an infectious disease caused by protozoa, genus *Eimeria*. The parasite is host-specific and has a direct life cycle [3]. Birds become infected by ingestion of sporulated oocysts omnipresent in poultry houses. Once ingested by the chicken, the parasite invades and multiplies in the gastrointestinal tract, destroying epithelial cells [4]. The severity of infection will depend upon the number of infective oocysts ingested as well as the pathogenicity of the wild strains. Intensive methods of production of poultry favor the reproduction of *Eimeria*. Therefore, coccidiosis is a continuing problem requiring constant attention and, in the case of broilers, a need for continuous coccidiosis control tools [5].

Even today, coccidiosis is still ubiquitous, and it is generally accepted that, under the current production systems, coccidiosis control remains necessary [4, 6]. Coccidiosis is also one of the main triggers for other gastrointestinal disorders including necrotic enteritis, dysbacteriosis, *Salmonella,* among others [7–9].

Birds suffering with clinical coccidiosis will show typical signs such as diarrhea, bloody droppings, increased mortality, decreased feed intake, and impaired performance. Inadequate coccidiosis control may also result in impaired growth and an increased feed conversion ratio, even in the absence of obvious clinical signs (referred to as subclinical coccidiosis).

In a recent study, the global prevalence of clinical coccidiosis was estimated at 5% and subclinical coccidiosis at 20% of global poultry production [10]. This supports that, under current production systems, coccidiosis is still a major health and welfare issue, which needs to be controlled.

Synthetic anticoccidials were the first to be introduced in the market. The first paper on prophylactic use of anticoccidials was published in 1948 by Leland Grumbles and describes the continuous use of Sulfaquinoxaline for the control of coccidiosis in poultry [11]. After their introduction, synthetics were found to be very efficacious and were very popular. Up until 1971, they were the only available option for coccidiosis control as ionophores were only introduced in the 1970s.

The introduction of the first ionophore coccidiostat (monensin) in the 1970s has proven to be critical for the development of modern poultry production [12]. The use of ionophores has significantly helped in the development of poultry production and has improved the health and welfare of broilers (Report from the Commission to the Council and the European Parliament on the use of coccidiostats and histomonostats as feed additives, 2008).

As expected, suboptimal control of coccidiosis will result in the increased use of antimicrobials, some of which are medically important for human medicine.

2. Methodology

Google Scholar (https://scholar.google.com) and PubMed (https://pubmed.ncbi.nlm.nih.gov) scientific databases were used to search for articles published between the years 2000 and 2022 containing the keywords, "immune response" AND "coccidiosis" in combination with "broiler chickens," "avian immunity," "intestinal immunity," "Coccidiosis Vaccines," "*Eimeria* Vaccines." Only manuscripts and book chapters in English or Spanish were included. Data from other animal species were also omitted except for the general overview of immune system. Data obtained in broiler chickens were grouped in tables including, the overview of avian immune response, peculiarities, intestinal immune response against coccidiosis and vaccines, type of *Eimeria* spp. infected, age at infection, among others.

3. A brief overview of the avian immune system

The immune system (IS) may be compared with a symphony orchestra in which a variety of molecules, cells, and tissues are finely organized to maintain the ideal state of homeostasis. In a nutshell, the IS may be defined as "A set of cells and molecules that defend the host against external (infections, trauma, among others) and internal aggressions (internal infections, autoimmunity, allergy as well as cancerous tumors)" [13].

The IS works as a passive system, meaning that it requires a threat to trigger an immune response (**Figure 1**). Once the IS is activated after the first contact with a foreign microorganism through the recognition of pathogen associated molecular patterns (PAMPs) and binding it with a variety of pattern recognition receptors (PRRs) the immune response is triggered. If innate immunity fails to eliminate the pathogen, adaptative immunity goes into action and activates more specific mechanisms to eliminate, obtain memory, and restore homeostasis [13].

Adaptative immunity comprises antigen presenting cells, lymphocytes (lym) including B and T cells as well as cytokines. There are fundamental properties of adaptative immune responses called cardinal features. Some include specificity, diversity, memory, nonreactivity to self (self-tolerance), and systemic localization (because of the ability of lym and other immune cells to circulate among tissues) [14]. There are two types of adaptative immunity: humoral and cell-mediated immunity which are mediated by different types of lym and work to kill different types of microbes [14]. Humoral immunity is conducted by molecules in the blood and mucosal secretions and is termed the secretory system [15].

T lym orchestrate cell-mediated immunity. Many pathogens can survive and replicate within the cells of the host. They are inaccessible to humoral response secretory molecules in these locations. As a result, cell-mediated immunity plays a role in the defense against this internal microorganism [14].

Protective immunity against a pathogen may be provided either by the host response (active immunity) or by transfer of secretory molecules that defend against the microbe. An important example of this form of immunity is the transfer of maternal antibodies by the bird to its offspring through the egg yolk, when the antibody is absorbed and enters the circulatory system, thus preventing or reducing clinical outcomes [16].

Among avian species, immune response in chickens is currently most studied followed by turkeys [17]. In theory, the avian immune response works similarly to the mammalian system. There are far more immunology studies conducted in mice compared with chickens. The use of pathogen infection models in mice has led to a greater advance of immunology understanding in mammals. Extrapolations from mammals to birds must be cautiously performed. A quote by the famous chicken evolutionist and immunologist Jim Kaufmann "chickens are not mice with feathers"

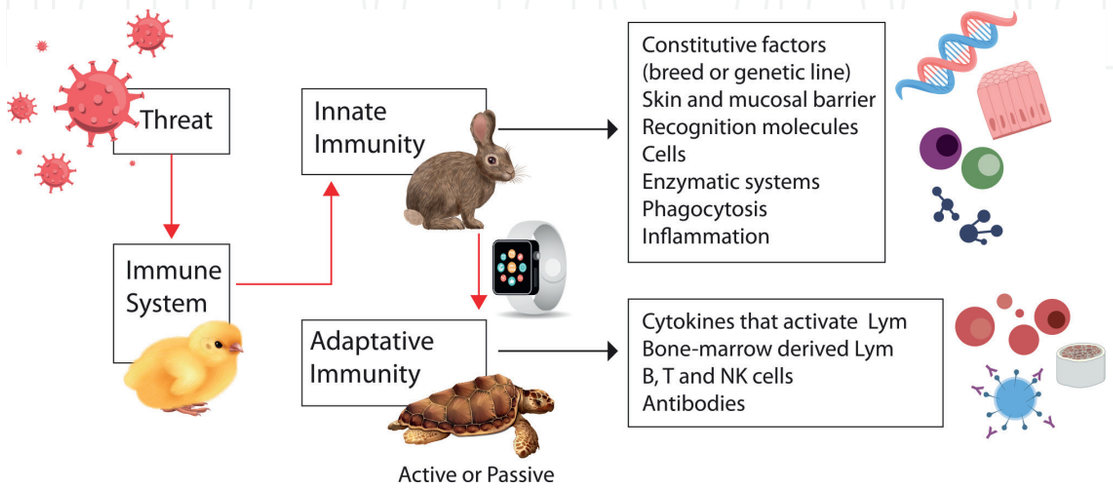

Figure 1.
Overview and the appropriate logic of the immune system. See the text for details.

supports that the study of the avian IS is worthwhile [18]. Avian IS seems to be simpler than mammals. Although both do the same actions, different pathways are sometimes used [19, 20].

The most known difference is that Avian B lym are developed in the Bursa of Fabricius (BF), a unique bird organ, and not in bone marrow as in mammals [21]. Other important differences include the major histocompatibility complex (MHC), tumor necrosis factor (TNF) and its receptor (TNFR) superfamilies, chemokines as well as the interleukin (IL) 1 superfamily, where the chicken repertoire is smaller. There are other cases with the opposite relationship such as the immunoglobulin-like receptor family where the chicken repertoire is greater than that of mammals [22]. The full descriptions and details about the avian immune system are found elsewhere and are beyond the scope of this review [17, 19, 23].

4. Intestinal immunity in birds

The gastrointestinal tract (GIT) is a complex environment because it is responsible for the digestion and absorption of nutrients, is constantly exposed to pathogens, and harbors beneficial microbiota of the host [24]. In addition, the GIT is the largest immune and nervous system, which is constantly challenged with immunogens from different sources including food, foodborne, and infectious pathogens as well as microbiota [25]. These actions may sound like a biological paradox which can be explained as follows: the poultry host must simultaneously maintain homeostasis (or the absence of disease) with nutrient absorption, intestinal integrity, exclusion of harmful microbes, tolerance of beneficial microbiota, and shaping mucosa immune response [26, 27].

The structure of the GIT varies throughout the length of the gut. In a nutshell, the intestine is a pipe with a tubular structure surrounded by a linear layer of epithelial cells embedded in a basement membrane (**Figure 2**). It is also composed of columnar absorptive cells (enterocytes), enteroendocrine, goblet cells, as well as immune intestinal cells. Tight junctions are an intercellular complex protein system that connects epithelial cells. These compartments are organized in protruding villus structures to increase the surface area of absorption. These structures are composed of an epithelial layer, a core of underlying lamina propria (containing the microvasculature), and a thin layer of smooth muscle (muscularis mucosae). In the intestine, each villus is an absorptive unit [28]. There are also structures, known as crypts, which are defined as the site of stem cells with proliferating abilities for self-renewal and differentiation, thus maintaining homeostasis in the intestinal epithelium [29]. These crypts are interspersed in indentations. The villus crypt blocks may vary in their maturation stage in distinct locations along the intestine. There is a zone known as “proliferative” within the crypt where stem cells are located and divide to form daughter cells that migrate from crypt to villus and survive between 48 to 96 hours, after which they are sloughed into the lumen and die by apoptosis in the tip [30]. The time depends on the length of the villus and age of the chicken. During this migration process, the enterocytes acquire differentiated functions in terms of digestion, absorption, and mucin secretion [31, 32]. The intestinal mucosa is covered by mucus, a complex hydrated gel that protects epithelial cells from chemical, enzymatic, microbial, and mechanical damage. The epithelium and its mucus layer permit the selective movement of ions, nutrients,and water, but restrict the translocation of microbes and toxins from the lumen [33].

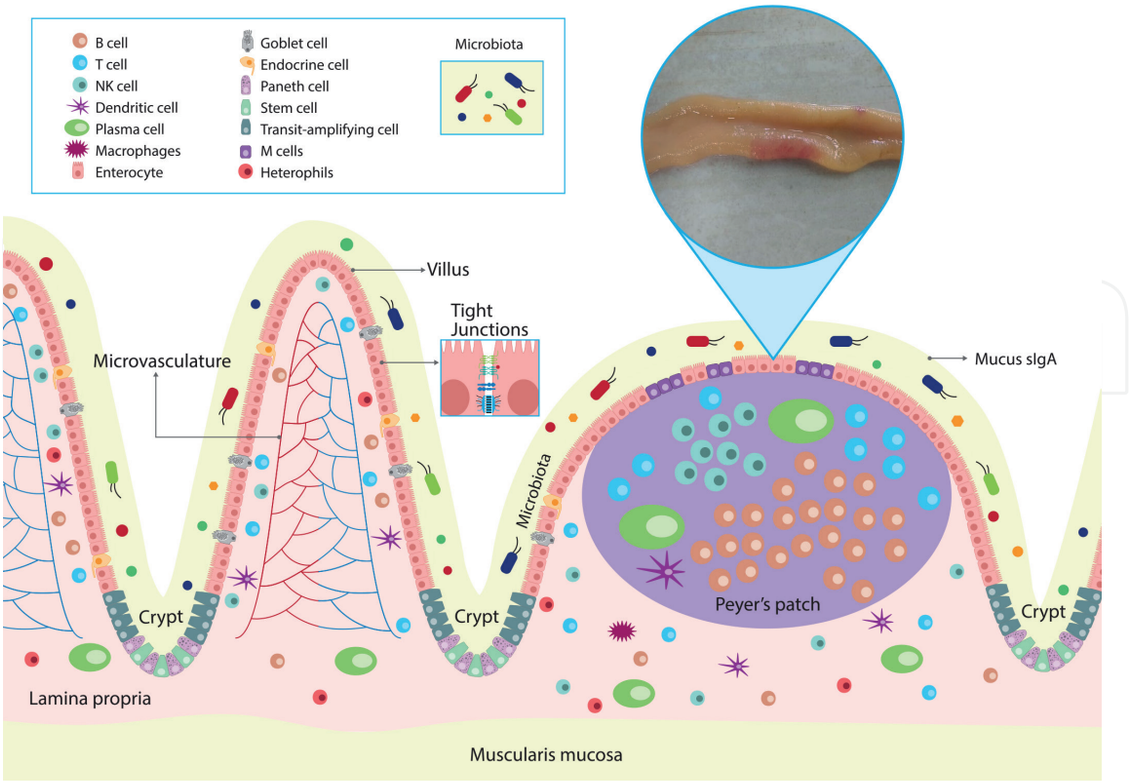

Figure 2.
Schematic diagram of the architecture of intestinal immune cells.

The structures between the small and large intestine of the birds are quite different. While villus/crypt units are present throughout the whole small intestine, the large intestine has villus-like outgrowth structures, with a ruffled structure, known as folds. Hyperactive crypts are found within each folded unit [29].

Gut mucosa is exposed to food immunogens as well as microbiota antigens that are required for the processing of nutrients and the education of the local immune system early after hatching. As a result, there are organized structures which function as key organized elements of cells and molecules to defend the host against intestinal threats. These structures are known as Gut Associated Lymphoid Tissue (GALT). GALT is the largest compartment of the immune system and is comprised of lymphoid cells residing in the epithelial lining and distributed in the underlining in the lamina propria. In addition, there are specialized lymphoid structures. GALT's main role is to limit progression of systemic infection by detecting and destroying infectious agents in their early stages. In poultry, GALT encompasses esophageal tonsils, pyloric tonsils, Meckel's diverticulum, Peyer's patches, and two caecal tonsils (this is the most GALT important organ) [34, 35]. GALT is comprised of more immune cells than any other host tissue including different cell subsets and including most major cell populations found at other sites. These include heterophils, macrophages, DC, natural killer (NK) cells, as well as B and T lym (although the proportions of each cell type differ according to locality, microbial status, and age) [29].

The entire GIT is covered by a protective mucus consisting of Mucins family proteins which are produced by Goblet cells. Lysozyme, native microbiota, gastric juices, bile salts, as well as cationic peptides and other substances which act as a nonspecific defense are also important participants in the process [36]. Thus, GALT detects not only harmful pathogens as a potential threat of the intestine but also normal gut

microbiota and self-antigens that can elicit autoimmune responses. Therefore, a comprehensive study of the avian GALT is crucial to develop oral vaccines which can be alternatives to replace antibiotic growth promoters and immunomodulatory molecules that maintain intestinal homeostasis with the best performance [36].

5. Intestinal immunity against coccidiosis

GALT is a master tissue in the immune response against coccidiosis because of three crucial functions: acquired immunity development in both cellular and humoral immune responses (including antigen processing and presentation), antibody production and cytokine production [37]. Cellular immunity seems to be the most important effector mechanism against coccidial infection [38]. It is orchestrated by subsets of lym bearing either αβ or γδ T cell receptor (TCR) [39]. Natural infections of epithelial cells such as *Eimeria* infections, for while TCR γδ cells are scarce in systemic circulation, they are commonly represented among IEL [40, 41]. Taking into account that *Eimeria* initiate the first contact with epithelial cells, it is tempting to speculate that IEL may be the first line of defense in response to *Eimeria* antigens which were processed and presented by major histocompatibility complex (MHC) expressed by epithelial cells [42]. Adaptative immune response against coccidiosis requires the involvement of these two pathways enabling proteins of MHC to be loaded with *Eimeria* epitopes. Only liganded expressed on the surface of antigen-presenting cells (APC) can activate T lym, which then execute effector functions, such as cytotoxicity, provision of help to B cells, and cytokine production [43]. In chickens, as in mammals, there are two subsets of lym classified by the system of cluster of differentiation (CD). These are CD4+ (known as T helper) and CD8+ (cytotoxic T cells). Adaptative immunity is highly dependent on T helper cells, and its activation is determined by MHC antigens [44]. Whereas CD8+ only recognizes peptides presented in the context of MHC-I molecules, T lym CD4+ recognizes peptides in the context of MHC-II molecules, and supports for co-stimulatory signals and other molecules. These molecular interactions underlying the regulation of the immune response between T lym and APC are known as immunological synapses [45].

This process is critical during the anticoccidial immune response in chickens. During the infection, the immune system inhibits parasitic development at three key stages in the *Eimeria* life cycle. The first is the sporozoite's search for binding sites in the epithelium cell, which allows it to penetrate the epithelium. While this is relevant, it is not particularly significant. Immune selection against life-cycle stages after the sporozoite stage may be more significant. Sporozoites are usually mentioned because immunity is so effective, but there are several studies that have studied later lifecycle stages and revealed that immunity can also inhibit multiple stages later in the life cycle.

The second stage is when sporozoites are placed within intraepithelial lymphocytes in the villus (IEL). Finally, sporozoite migrate from lamina propria to the crypt [46]. T cells are undoubtedly the protagonist in modulating anticoccidial immunity. Cytotoxic lym has been observed after a primary *Eimeria* challenge with the subsequent increase of interferon gamma (IFNγ) activating proinflammatory pathways to inhibit intracellular *Eimeria* parasite development in host cells [47]. Natural killer (NK) cells are also an important component of the intestinal immune response against coccidiosis [48]. Some subpopulations of NK mediate spontaneous cytotoxicity in chicken intestinal IEL underlying the statement that they are crucial for

intestinal immunity. NK cell activity depends on the infection stage, which decreases during early stages of infection, and recovers to normal levels 1 week after primary infection as well as in the early stages of secondary infection [49].

There are several cytokines and chemokines reported that play a predominant role during coccidiosis infection including IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IFNγ, transforming growth factor (TGF)-β1, and tumor necrosis factor, among others [38, 50]. Despite the high number of cytokines described in the pathogenesis of the disease, IFNγ and IL-10 are the key cytokines for host protection and susceptibility against parasitic infections, respectively [51, 52]. Detrimental effects on the parasite have been reported as a result of IFNγ release. This is because of the inhibition of parasite invasion and survival in the host cell as well as the promotion of local inflammation [53], free radical production [54, 55], activation of antibody-dependent cell-mediated cytotoxicity [56] and/or the promotion of the release of cytoplasmic granules containing perforin and proteases [57]. IL-10 has an inhibitory role in the intestinal immune response due to the interference with Th1 response and this decreases the ability of the host to eliminate the parasite [58]. Therefore, IL-10 is a proposed mechanism of host evasion by *Eimeria*. IL-10 have different functions such as the inhibition of nuclear factor kappa B and the suppression of proinflammatory cytokines enrolled in parasite cleaning from the intestinal cells [59]. In the end, the balance between Thl and Th2 responses is crucial to the outcome of the infection, and the cytokine network involved in the control of the immune response needs to be elucidated.

The role of humoral immunity against coccidiosis is still controversial, and there is more consideration paid to cellular immunity responses. Humoral immunity appears to play a minor role in resistance against infection. In one of the classical studies, in which the BF was removed, chickens were not affected after a secondary infection despite their ability to produce immunoglobulins [60]. During *Eimeria* infection, specific antibodies are produced, but they do not seem to be involved in controlling the infection [61] and immunoglobulin levels are not correlated with disease susceptibility [62]. IgA was also considered important as humoral protection against parasite invasion in earlier studies [63]. In a chicken kidney cell line model of *Eimeria* infection, caecal content from immunized chickens was co-cultured. Sporozoite invasion did reduce. However, there was no correlation in either antibody levels or the neutralization of sporozoites [64]. One of the major challenges has been to replicate *in vivo* results from the *in vitro* findings regarding the humoral immune response against Coccidiosis.

Immunoglobulins, therefore, do not appear to play an important role in protective immunity against Coccidiosis and cell immunity seems to be more crucial. Manuscripts underlying the key role of antibodies and humoral immunity as a protective mechanism against coccidiosis have been published, however [65]. It was determined that IgY antibodies injected systemically are capable of reaching the site of infection and effectively blocking parasite development in the intestine [66]. A positive association between antibody titers and protection [67] was also shown. In other studies, it was established that egg IgY from hens immunized with live infections of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* could be used as a feed additive to passively protect young chicks against all three species [68, 69]. The results described above support the concept that providing large amounts of protective antibodies to young chicks, through passive or maternal immunization, can interrupt the growth, development, and replication of *Eimeria*. Although antibody production is a mechanism to limit the propagation of several pathogens [65], T-cell

mediated response is the major criterion for the control of intracellular parasites such as *Eimeria* [39, 70].

6. Vaccines as a strategy to control coccidiosis

Vaccines provide an effective strategy for the control of coccidiosis in chickens and benefit the sustainability of the poultry industry worldwide [71]. The first vaccine against coccidia utilized a sporulated oocyst of a live *Eimeria tenella* wild type strain, and it was initially launched in 1950. This vaccine was based on the concept that low doses of oocysts over a number of days induced protective immunity against a homologous challenge [72, 73]. Current *Eimeria* vaccines are marketed and consist of live wild-type (virulent) parasites or live attenuated vaccines (precocious lines). Thus, up till now, there are more than 25 commercial anticoccidial vaccines utilized in poultry (reviewed in [73, 74]).

In breeders, vaccination programs based on live vaccines are tremendously useful and have been very successful. There are, however, some hurdles such as homogenous mass application to the flock. If the application is not done correctly, it may lead to suboptimal immunization and insufficient protection against the different *Eimeria* species. Even with homogenous mass application to the flock, there are additional hurdles which can lead to uneven application, triggering outbreaks [75].

A recent report showing the vaccine-induced immune response was published [76]. Briefly, three important findings were reported. First, *Eimeria* species can elicit an innate immune response by expressing TLR21 in macrophages through the recognition of pathogen-associated molecular patterns (PAMPs). Next, Coccidia vaccine induced a Th1 pattern characterized by proinflammatory cytokines and cell subsets in both systemic and local lymphoid organs. Second, *Eimeria tenella* induced the strongest activation of macrophages. Cellular analysis showed that vaccination led to an increase in macrophages and activated T cells (immunophenotypes CD8 + CD44+ and CD4 + CD44+). Other important effects were reported, including a decrease in fecal oocyst shedding as well as an improvement in body weight gain. However, this was not statistically different.

Precocious lines are defined as lines of *Eimeria* selected from a population that complete their endogenous life cycle in the host more quickly than wild-type parent strains. They are not only different because of an abbreviated life cycle but also by significant attenuation of virulence [77, 78]. Therefore, precocious lines are proposed as a successful strategy to control coccidiosis because they are less pathogenic than their parents, no adverse effects are observed in vaccinated birds and, despite their reduced multiplication within the intestine are able to stimulate protective immunity which is virtually as good as that induced by their pathogenic parent strains [75].

7. Conclusions

For more than 70 years, the main tools for the prevention and control of coccidia were performed using coccidiostats. As the number of available products is limited and no new molecules have been introduced in the last 30 years, it is a challenge to keep coccidiostats as effective as they were at their introduction to the poultry industry. Parallel to the advances in our knowledge of the avian immune system and the study of avian coccidiosis immune responses, strategies which can protect the birds

against different species of *Eimeria* are being considered to overcome health issues caused by coccidiosis.

The application of both types of vaccines (wild-type live strains and attenuated or precocious vaccines) are still a challenge due to mass application. Advantages and disadvantages of each vaccine exist. Therefore, it deserves continuous research and field work in different scenarios and facilities to identify effective control strategies for avian coccidiosis which will ultimately benefit the sustainability of the global poultry industry.

Acknowledgements

Thanks to Elizabeth Cruz Tapias for her outstanding commitment with the illustrations in this chapter. We would like thank Lydia-Jane Harrison for her English grammar correction.

Conflict of interest

L.M. Gomez-Osorio, C. Cuello, and B. Dehaeck are employees of Huvepharma N.V. which commercializes the vaccine Advent® against coccidiosis as well as anticoccidials. J.J. Chaparro-Gutierrez and S. Lopez-Osorio do not have any conflict of interest.

Author details

Luis-Miguel Gomez-Osorio[1,2*], Ben Dehaeck[3], Carlos Cuello[4], Jenny-Jovanna Chaparro-Gutierrez[1] and Sara Lopez-Osorio[1]

1 CIBAV Group, Universidad de Antioquia, UdeA, Medellín, Colombia

2 Country Manager Huvepharma, Colombia

3 Global Product Manager Anticoccidials, Belgium

4 Technical Manager LATAM Huvepharma, Ecuador

*Address all correspondence to: lgomezosorio@gmail.com

References

[1] Blake DP, Knox J, Dehaeck B, et al. Re-calculating the cost of coccidiosis in chickens. Veterinary Research. 2020;**51**:1-14

[2] Ehrlich PR, Harte J. To feed the world in 2050 will require a global revolution. Proceedings of the National Academy of Sciences of the United States of America. 2015;**112**:14743-14744

[3] Mesa-Pineda C, Navarro-Ruíz JL, López-Osorio S, et al. Chicken coccidiosis: From the parasite lifecycle to control of the disease. Frontiers in Veterinary Science. 2021;**8**:1-15

[4] Reid WM. History of avian medicine in the United States. X. Control of coccidiosis. Avian Diseases. 1990;**34**:509-525

[5] Long PL. Gordon memorial lecture coccidiosis control: Past, present and future1. British Poultry Science. 1984;**25**:3-18

[6] Chapman HD. Anticoccidial drugs and their effects upon the development of immunity to Eimeria infections in poultry. Avian Pathology. 1999;**28**:521-535

[7] Collier CT, Hofacre CL, Payne AM, et al. Coccidia-induced mucogenesis promotes the onset of necrotic enteritis by supporting Clostridium perfringens growth. Veterinary Immunology and Immunopathology. 2008;**122**:104-115

[8] Prescott JF, Parreira VR, Mehdizadeh Gohari I, et al. The pathogenesis of necrotic enteritis in chickens: What we know and what we need to know: A review. Avian Pathology. 2016;**45**:288-294

[9] Hofacre CL, Smith JA, Mathis GF. An optimist's view on limiting necrotic enteritis and maintaining broiler gut health and performance in today's marketing, food safety, and regulatory climate. Poultry Science. 2018;**97**:1929-1933

[10] Kadykalo S, Roberts T, Thompson M, et al. The value of anticoccidials for sustainable global poultry production. International Journal of Antimicrobial Agents. 2018;**51**:304-310

[11] Campbell WC. History of the discovery of sulfaquinoxaline as a coccidiostat. The Journal of Parasitology. 2008;**94**:934-945

[12] Chapman HD, Jeffers TK, Williams RB. Forty years of monensin for the control of coccidiosis in poultry. Poultry Science. 2010;**89**:1788-1801

[13] Rojas W, Aristizabal B, Cano L, et al. Generalidades y definiciones. In: Anaya J, Gomez-Osorio L, Aristizabal B, et al., editors. Inmunologia de Rojas. Fondo Editorial: CIB; 2023. pp. 3-16

[14] Abbas A, Lichtman A, Pillai S, editors. Properties and Overview of Immune Response. Amsterdam: Elsevier; 2021

[15] Gomez-Osorio L, Jiang Z, Zhang Q, et al. Secretory Defense Response in the Bird's Gastro-Intestinal Tract and Nutritional Strategies to Modulate It. In: Advances in Poultry Nutrition Research. London, UK: Intechopen. pp. 225-240

[16] Faulkner OB, Estevez C, Yu Q, et al. Passive antibody transfer in chickens to model maternal antibody after avian influenza vaccination. Veterinary Immunology and Immunopathology. 2013;**152**:341-347

[17] Sharma JM. Overview of the avian immune system. Veterinary Immunology and Immunopathology. 1991;**30**:13-17

[18] Tregaskes CA, Kaufman J. Chickens as a simple system for scientific discovery: The example of the MHC. Molecular Immunology. 2021;**135**:12-20

[19] Kaufman J. Innate immune genes of the chicken MHC and related regions. Immunogenetics. 2022;**74**:167-177

[20] Kaufman J. From chickens to humans: The importance of peptide repertoires for MHC class I alleles. Frontiers in Immunology. 2020;**11**:1-10

[21] Oláh I, Nagy N. Retrospection to discovery of bursal function and recognition of avian dendritic cells, past and present. Developmental Complement Immunology. 2013;**41**:310-315

[22] Kaiser P. The long view: A bright past, a brighter future? Forty years of chicken immunology pre- and post-genome. Avian Pathology. 2012;**41**:511-518

[23] Kaspers B, Schat K, Gobel T, et al. Avian Immunology. Third ed. Amsterdam: Elsevier; 2021

[24] Takahashi D, Kimura S, Hase K. Intestinal immunity: To be, or not to be, induced? That is the question. International Immunology. 2021;**33**:755-759

[25] Wickramasuriya SS, Park I, Lee K, et al. Role of physiology, immunity, microbiota, and infectious diseases in the gut health of poultry. Vaccines. 2022;**2022**:10. DOI: 10.3390/vaccines10020172

[26] Farré R, Fiorani M, Rahiman SA, et al. Intestinal permeability, inflammation and the role of nutrients. Nutrients. 2020;**12**:1-18

[27] Kogut MH. Role of diet-microbiota interactions in precision nutrition of the chicken: Facts, gaps, and new concepts. Poultry Science. 2022;**101**:101673

[28] Ensari A, Marsh MN. Exploring the villus. Gastroenterol Hepatology from Bed to Bench. 2018;**11**:181-190

[29] Smith A, Claire P, Richard B. The avian enteric immune system in health and disease. In: Kaspers B, Schat K, Gobel T, et al., editors. Avian Immunology. Third ed. Amsterdam: Elsevier; 2021. pp. 303-326

[30] Uni Z, Geyra A, Ben-Hur H, et al. Small intestinal development in the young chick: Crypt formation and enterocyte proliferation and migration. British Poultry Science. 2000;**41**:544-551

[31] Zhang H, Li D, Liu L, et al. Cellular composition and differentiation Signaling in chicken small intestinal epithelium. Animals. 2019;**2019**:1-12

[32] Shini S, Aland RC, Bryden WL. Avian intestinal ultrastructure changes provide insight into the pathogenesis of enteric diseases and probiotic mode of action. Scientific Reports. 2021;**11**:1-15

[33] Podolsky DK. Healing the epithelium : Solving the problem from two sides. Journal of Gastroenterology. 1997;**1997**:122-126

[34] Peralta MF, Danelli MGM, Vivas A. Rediscovering the importance of mucosal immune system (MIS) in poultry. Academy Journal of Biotechnology. 2016;**4**:91-095

[35] Peralta MF, Magnoli A, Alustiza F, et al. Gut-associated lymphoid tissue: A key tissue inside the mucosal

immune system of hens immunized with Escherichia coli F4. Frontiers in Immunology. 2017;**2017**:8. DOI: 10.3389/fimmu.2017.00568

[36] Lillehoj HS, Lillehoj EP. Avian coccidiosis. A review of acquired intestinal immunity and vaccination strategies. Avian Diseases. 2000;**44**:408-425

[37] Tellez G, Shivaramaiah S, Barta J, et al. Coccidiosis: Recent advancements in the immunobiology of Eimeria species, preventive measures, and the importance of vaccination as a control tool against these apicomplexan parasites. Veterinary Medical Research Reports. 2014;**2014**:23

[38] Kim WH, Chaudhari AA, Lillehoj HS. Involvement of T cell immunity in avian coccidiosis. Frontiers in Immunology. 2019;**10**:1-8

[39] Min W, Kim WH, Lillehoj EP, et al. Recent progress in host immunity to avian coccidiosis: IL-17 family cytokines as sentinels of the intestinal mucosa. Developmental and Comparative Immunology. 2013;**41**:418-428

[40] Vervelde L, Jeurissen SHM. Postnatal development of intra-epithelial leukocytes in the chicken digestive tract: Phenotypical characterization in situ. Cell and Tissue Research. 1993;**274**:295-301

[41] Lillehoj HS, Chung KS. Postnatal development of T-lymphocyte subpopulations in the intestinal intraepithelium and lamina propria in chickens. Veterinary Immunology and Immunopathology. 1992;**31**:347-360

[42] Smith AL, Hayday AC. An αβ T-cell-independent immunoprotective response towards gut coccidia is supported by γδ cells. Immunology. 2000;**101**:325-332

[43] Jensen PE. Recent advances in antigen processing and presentation. Nature Immunology. 2007;**8**:1041-1048

[44] Pishesha N, Harmand TJ, Ploegh HL. A guide to antigen processing and presentation. Nature Reviews. Immunology. 2022;**22**:751-764

[45] Capitani N, Baldari CT. The immunological synapse: An emerging target for immune evasion by bacterial pathogens. Frontiers in Immunology. 2022;**13**:1-11

[46] Jeurissen SHM, Janse EM, Vermeiden AN, et al. Eimeria tenella infections in chickens: Aspects of host-parasite: Interaction. Veterinary Immunology and Immunopathology. 1996;**54**:231-238

[47] Shah MAA, Song X, Xu L, et al. The DNA-induced protective immunity with chicken interferon gamma against poultry coccidiosis. Parasitology Research. 2010;**107**:747-750

[48] Lillehoj HS. Role of T lymphocytes and cytokines in coccidiosis. International Journal for Parasitology. 1998;**28**:1071-1081

[49] Lillehoj HS. Intestinal intraepithelial and splenic natural killer cell responses to eimerian infections in inbred chickens. Infection and Immunity. 1989;**57**:1879-1884

[50] Hong YH, Lillehoj HS, Lee SH, et al. Analysis of chicken cytokine and chemokine gene expression following Eimeria acervulina and Eimeria tenella infections. Veterinary Immunology and Immunopathology. 2006;**114**:209-223

[51] Yun CH, Lillehoj HS, Choi KD. Eimeria tenella infection induces local gamma interferon production and intestinal lymphocyte subpopulation

changes. Infection and Immunity. 2000;**68**:1282-1288

[52] Rasheed MSA, Tiwari UP, Jespersen JC, et al. Effects of methylsulfonylmethane and neutralizing anti–IL-10 antibody supplementation during a mild Eimeria challenge infection in broiler chickens. Poultry Science. 2020;**99**:6559-6568

[53] Lillehoj HS, Choi KD. Recombinant chicken interferon-gamma-mediated inhibition of Eimeria tenella development in vitro and reduction of oocyst production and body weight loss following Eimeria acervulina challenge infection. Avian Diseases. 1998;**42**:307-314

[54] Ovington KS, Smith NC. Cytokines, free radicals and resistance to Eimeria. Parasitology Today. 1992;**8**:422-426

[55] Hong YH, Lillehoj HS, Lillehoj EP, et al. Changes in immune-related gene expression and intestinal lymphocyte subpopulations following Eimeria maxima infection of chickens. Veterinary Immunology and Immunopathology. 2006;**114**:259-272

[56] Fleischer B. Effector cells in avian spontaneous and antibody-dependent cell-mediated cytotoxicity. Journal of Immunology. 1980;**125**:1161-1166

[57] Schroder K, Hertzog PJ, Ravasi T, et al. Interferon-gamma: An overview of signals mechanisms and functions. Journal of Leukocyte Biology. 2004;**75**:163-189

[58] Shanmugasundaram R, Sifri M, Selvaraj RK. Effect of yeast cell product (CitriStim) supplementation on broiler performance and intestinal immune cell parameters during an experimental coccidial infection. Poultry Science. 2013;**92**:358-363

[59] Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. Nature Reviews. Immunology. 2010;**10**:170-181

[60] Pierce AE, Long P. Studies on acquired immunity to coccidiosis in bursaless and thymectomized fowls. Immunology. 1965;**9**:427-439

[61] Rose ME. Some aspects of immunity to Eimeria infections. Annals of the New York Academy of Sciences. 1963;**113**:383-399

[62] Lillehoj H, Ruff M. Comparison of disease susceptibility and subclassspecific antibody response in SC and FP chickens experimentally inoculated with Eimeria tenella, E. acervulina, or E maxima. Avian Diseases. 1987;**31**:112-119

[63] Davis P, Parry S, Porter P. The role of secretory IgA in anti-coccidial immunity in the chicken. Immunology. 1978;**34**:879-888

[64] Davis P, Porter P. A mechanism for secretory IgA-mediated inhibition of the cell penetration and intracellular development of Eimeria tenella. Immunology. 1979;**36**:471-477

[65] Wallach M. Role of antibody in immunity and control of chicken coccidiosis. Trends in Parasitology. 2010;**26**:382-387

[66] Rose ME. Protective antibodies in infections with Eimeria maxima: The reduction of pathogenic effects in vivo and a comparison between oral and subcutaneous administration of antiserum. Parasitology. 1974;**68**:285-292

[67] Smith NC, Wallach M, Miller CMD, et al. Maternal transmission of immunity to Eimeria maxima: Enzyme-linked immunosorbent assay analysis of

protective antibodies induced by infection. Infection and Immunity. 1994;**62**:1348-1357

[68] Lee SH, Lillehoj HS, Park DW, et al. Induction of passive immunity in broiler chickens against Eimeria acervulina by hyperimmune egg yolk immunoglobulin Y. Poultry Science. 2009;**88**:562-566

[69] Lee SH, Lillehoj HS, Park DW, et al. Protective effect of hyperimmune egg yolk IgY antibodies against Eimeria tenella and Eimeria maxima infections. Veterinary Parasitology. 2009;**163**:123-126

[70] Lillehoj HS, Min W, Dalloul RA. Recent progress on the cytokine regulation of intestinal immune responses to Eimeria. Poultry Science. 2004;**83**:611-623

[71] Chapman HD, Cherry TE, Danforth HD, et al. Sustainable coccidiosis control in poultry production: The role of live vaccines. International Journal for Parasitology. 2002;**32**:617-629

[72] Williams RB. Fifty years of anticoccidial vaccines for poultry (1952-2002). Avian Diseases. 2002;**46**:775-802

[73] Soutter F, Werling D, Tomley FM, et al. Poultry coccidiosis: Design and interpretation of vaccine studies. Frontier in Veterinary Science. 2020;**7**:1-12

[74] Ahmad TA, El-Sayed BA, El-Sayed LH. Development of immunization trials against Eimeria spp. Trials Vaccinology. 2016;**5**:38-47

[75] Shirley MW, Bedrník P. Live attenuated vaccines against avian coccidiosis: Success with precocious and egg-adapted lines of Eimeria. Parasitology Today. 1997;**13**:481-484

[76] Gaghan C, Adams D, Mohammed J, et al. Characterization of vaccine-induced immune responses against coccidiosis in broiler chickens. Vaccine. 2022;**40**:3893-3902

[77] Jeffers TK. Attenuation of Eimeria tenella through selection for precociousness. Parasitology. 1975;**61**:1083-1090

[78] McDonald V, Shirley MW. Past and future: Vaccination against Eimeria. Parasitology. 2009;**136**:1477-1489