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Immunity to Avian Coccidiosis: A Review

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Review Article

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Abstract

This paper review the exposure of birds to coccidiosis and the role of innate/acquired immunity (adaptive immunity), probiotics, prebiotics as well as vaccines developed and their applications in inducing immunity or resistance in birds.

Introduction

Coocidiosis causes annual losses of US $ 2.4 billion to the poultry industry worldwide in both the layer and broiler industries [1]. Conventional disease control strategies depend on vaccination or immunization. *Eimeria* infection or its developmental stages promotes antibody and cell-mediated immune responses [2]. However cellular immunity is mediated by various cell populations, including lymphocytes (cytokines and CD_4^+ cells count, Kaze, et al.), natural killer (NK) cells and macrophages plays a major role in disease resistance [2,3]. There is increase evidence of CD_4^+ and intraepithelial lymphocyte (IEL) involvement during a primary infection, while T-cell receptor α -and β -chain-positive CD_8^+ IEL play a key role in secondary infection [3]. The low level of homology between chicken genes and their mammalian counterparts has made it difficult to discover immunologically relevant chicken genes. However, there have been increasing numbers of chicken gene sequences appearing in the data bases due to the emergence of chicken genome projects. Among the cytokines cloned, one can find gene coding for interleukins (interleukin -1 β (IL-1 β), IL-2 and interferons (alpha/beta interferon [IFN- α / β] and IFN-γ and also for a macrophage growth and three isofornes of transforming growth factor β (TGF- β) [4-9]. In addition, several members of the chemokine family, have recently been cloned: C chemokines cc chemokines (macrophage inflammatory protein 1^{β} (MIP-1^{β}) and C X C chemokines (k60 and IL-8 [10-12]. A number of receptors have also been identified including the IL-1 receptor (IL-IR) and a putative chemokine receptor (chem.-R) [13,14]. However, studies by reverse transcription – polymerase chain reaction (RT-PCR) of the expression of an available panel of genes may provide initial clues about the development of immunity to *Eimeria* infection. In this paper, we intend to review immunity to avian coccidiosis response found in poultry farms.

Natural (innate) Immunity

The surface layer involved with the digestive, respiratory and reproductive tracts is referred to as epithelium and the underlying tissue is the lamina propria. The combination of these two tissues forms the mucosa. Mucosal membrane is considered as the largest organ system in vertebrates. To protect the body from infection within the mucosal immune systems of the gut, respiratory and reproductive tracts have highly

developed lymphoid tissues such as the gut associated lymphoid tissue (GALT) and bronchial-associated lymphoid tissues (BALT). In addition, there are well-developed immunological activities that provide essential protection in the different parts of these systems. Within the gut, there are different immunological requirements in different locations, because of the nature of the different local conditions and the specialized functions within different regions. In mammalian species, the GALT contain more lymphocytes than secondary lymphoid tissues, such as the spleen and lymph nodes. It is likely that this is also the case in avian species.

The mucosal surfaces have a number of common features. Since each forms a major barrier between the external environment and internal milieu, they provide an important portal of entry for pathogens. This is especially the case with the gut and respiratory tract where the continuous movement of external substances, nutrients and air, respectively, and the need to transport or exchange essential molecules across the mucosal surface for organs to function properly and for the animal to remain healthy. Some organisms (mainly bacteria) may reside and have a beneficial effect on digestive processes, while pathogenic organisms can replicate in the mucosal epithelial cells or cross the mucosal surface to enter the body proper and cause disease [15]. A small-scale, low-density production system can allow a low level of exposure to Coccidia, which permits the chicks to develop immunity without triggering the disease.

However, birds may not pick up enough parasites to cause immunity. In addition, immunity is only species-specific; exposure to one type of Coccidia will not protect a chicken from the other species that can infect it [16]. Oral inoculation of *E. tenella* led to parasite invasion of the intestinal caeca and caecal tonsils, protective immunity to *E. tenella* infection produce intestinal lymphocytes and gamma interferon [17]. Previous applications used vaccination to protect broilers via maternal antibodies, protein complex extracted from gamytocytes stage of *E. maxima* elicited maternal protection and enabled young chicks to exposed *Eimeria* spp. without usual sings and consequences of coccidiosis, protection was heterologous against *E. tenella* and *E.acervulina* as well as against the homologous [18].

Acquired Immunity

Acquired immunity to *Eimeria* is even more stronger than innate resistance to primary infections. It has been acknowledged that immunity to reinfection with *Eimeria* is conspicuously effective and is T cell-dependent and that B cells (antibodies) are not involved in acquired immunity since bursectomized birds and mice lacking B cells are perfectly capable of developing immunity to reinfection [19,20]. It has been proven almost impossible to correlate any immune parameter with immunity to reinfection because the expression of that immunity in experimental settings, at least, is so rapid and efficient.

However, studies using gene knock-out mice have proved extremely useful in determining which factors may play a role. Thus, as for primary infection, CD4 T cells are crucial for immunity to reinfection with *E. vermiformis* [20]. However, in contrast to primary infection, IFN-g plays no role in this acquired immunity [20]. On the contrary, some studies demonstrate that CD8 T cells can be used to transfer immunity (e.g. to *E. falciformis*; Pogonka *et al*) or that depletion of CD8þ T cells can increase, very slightly, susceptibility to *E. vermiformis*. Evidence from poultry experiments is more difficult to interpret because experiments showing an increase in oocyst excretion in secondary infection of birds depleted of CD8þ T cells did not include a concomitant primary infection control, making it hard to assess how significant the increased oocyst production really was [21]. More, and more sophisticated, analyses of acquired immunity to *Eimeria* are required to resolve the mechanism(s) that are operating.

Maternal Immunity

The immune system of young animals is 'uneducated' rendering them more susceptible to infectious disease. Protection against infection during this vulnerable period is provided via transfer of antibodies from mother to young. In chickens, this occurs via the egg yolk; indeed, the ability of hens to transfer remarkable quantities of IgY (IgG) antibodies to their hatchlings has long been appreciated, including in regard to the transfer of antibodies that protect chicks from infection with *E. tenella* [22]. In many of the progeny from hens deliberately infected with high doses of *E. maxima*, this maternal immunity can be absolute (i.e. result in the complete absence of oocysts in the faeces of chicks), at least during the first week post-hatching. Maternal antibody levels (in egg yolk or chicks) are correlated with protection. Moreover, maternal immunity induced by *E. maxima* confers partial protection against *E. tenella*, possibly via cross-recognition of conserved proteins (or, at least, epitopes) in different *Eimeria* species an idea lent further credibility by the ability of maternal immunization with conserved macrogametocyte proteins to protect hatchlings against multiple species of *Eimeria* [23].

The effectiveness of maternal, antibody-mediated immunity to *Eimeria* appears contradictory to the body of evidence, reviewed above, indicating that antibodies play only a minor role in resistance to *Eimeria*. The protection conferred by antibodies was later demonstrated to be correlated tightly with levels of parasite-specific IgG [23]. Immune sera can even partially protect highly susceptible T cell-deficient animals [19]. Thus, antibodies certainly can protect against *Eimeria* but the effect must be described as variable – from absolute to negligible even if similar immunization regimens are used [23,24]. Maternal immunization, however, does appear to be a phenomenon that can be harnessed to control poultry coccidiosis [23].

Development of Immunity during Coccidial Infection

Following the ingestion of *Eimeria* sporulated oocysts, the chicken response in various ways, the non-specific portion of the immune system is antagonistic in the form of low pH, enzymes, and inflammatory reactions. This will limit the number of potent sporozoites that reach the location of infection. When the infection is manifested, the specific immunity system will become strong in the form of specific antibodies and specific cellular immunity [24,25]. Brandtzaeg *et al* defined three general functions of the specific immune response GALT in the host defence against pathogenic infections, including coccidiosis [26]:

a. Processing and presentation of antigens.
b. Production of intestinal antibodies.
c. Activation of cell-mediating immunity.

The function of the specific antibodies in immunity against coccidial infection is limited, but they are present in the circulation and mucosal secretions. The circulating IgY and the biliary IgA that are specific for coccidial parasites have been detected one week after the infection and reach peak values within 8-14 days and persist for two months [27]. Lillehoj reported that bursectomised chickens could show full protection against coccidiosis in the absence of antibodies, illustrating that the role of antibodies is minor in the process of immunity against coccidiosis [28].

In vitro studies showed that immune sera increased the phagocytosis of sporozoites and merozoites [29,30]. It is possible that antibodies reduce the invasion of some, but not all *Eimeria* species, or enhance the intraluminal destruction of the sporozoites if they come into close contact with local antibodies before they enter the host cells [31]. On the other hand, T cells have been reported to play an important role in the immune responses to coccidiosis [32,33]. Trout and Lillehoj studied the role of $CD4^+$ and the cytokines produced in coccidiosis infection, and found that depletion of $CD4^+$ cells have no effect on *E. acervulina* infection, but results in a significant increase in oocyst production following *E. tenella* primary infection [34]. The authors suggested that this difference could be related to the changes that occur during these infections or that the immune mechanisms may vary from one gut location to another. In contrast, depletion of $CD8^+$ results in a substantial increase in oocyst production following a challenge with *E. acervulina* infection in chickens. The direct role of the $CD8^+$ T cells in resistance to coccidiosis has not been proven yet. However, increased numbers of these cells were seen, and in direct contact with parasite-infected epithelial cells, in a tissue section of the gut following secondary infection, suggesting that infected epithelial cells may be the target of the cytotoxic T cells [31,35].

Early Vaccines Trials against *Eimeria* Species

(a) Probiotics: Metchnikoff (1908 as cited by Hussain, 2010) proposed that the consumption of live microorganisms (mainly lactic acid bacteria) could improve intestinal health and well-being of the host. Probiotics was also defined as "a live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance" [36]. Probiotic preparations may consist of a single strain *Lactobacilli* or *Streptococci* or may contain any number up to eight strains [36,37]. The use of probiotics aims to fasten the development of a stable and beneficial intestinal microflora, which will lead to improvement of intestinal health and modulate the immune system, enhancing host resistance to enteric pathogens [38-40].

Tortuero demonstrated the antagonism between *Lactobacilli* and enterobacteria and showed that lactobacilli reduced the severity of clinical signs in *E. tenella* infection [41]. Dalloul and Lillehoj reported that a *Lactobacillus* containing diet fed to broilers infected with *E. acervulina* resulted in an immunoregulatory effect on the local immune system and improved the broilers' resistance to *E. acervulina* infection [42]. Furthermore, it has been reported that *lactobacillus* species inhibit the invasion of *E. tenella in vitro* [43]. Recently, Lee *et al* reported that *Pediococcus acidilactici* effectively enhanced the resistance of birds and partially protected against the negative growth effects associated with coccidiosis [44].

(b) Prebiotics: Gibson and Roberfroid defined a prebiotic as "a non-digestible food ingredient that beneficially

affects the host by selectively stimulating the growth and/or activity of one or limited number of bacteria in the colon that can improve the host health" [45]. Prebiotics have the advantage, when compared with probiotics, that they are targeting the bacteria already present and hence those that are adapted to the gastrointestinal tract environment. Many studies have proved that the non-digestible polysaccharides inulin, oligofructose, and oligomannose, enhance the growth of the beneficial bacteria (*Bifidobacteria and Lactobacillus)* and reduce that of the pathogenic bacteria (*E. coli* and *Salmonella*) and also stimulate the immune system of the host [46-48].

Mannanoligosaccharides (MOS), derived from the cell wall of the yeast, can be considered as prebiotics. MOS is non-digestible and is utilized by lactic acid producing bacteria. MOS also competes with mannose-specific binding of type-1 *fimbriae* of pathogenic, gram-negative bacteria such as *E. coli* and *Salmonella*, resulting in a reduction of their colonization reported an increase in faecal *Bifidobacteria* and a reduction in susceptibility to *Salmonella enteritidis* colonization in young chickens fed a diet supplemented with MOS [49]. Addition of MOS to the diet of broilers reduced the severity of the infection due to either *E. tenella* alone or a mixture of *E. acervulina, E. maxima* and *E. tenella* [50].

Vaccines for *Eimeria* Species

Vaccines are the most valuable public health tools that have been developed by man. The development of resistance to coccidia and anticoccidial drugs, the concern about drug residues in poultry products, the pressure imposed by consumers to avoid chemotherapeutics and the recent announcement by the EU to ban several anticoccidial drugs used in broilers, have led to interest in the vaccination of poultry against coccidiosis. In addition to the vaccines currently available, many others are presently under development [51-55]. Jeurissen and Veldman listed factors making coccidiosis as a disease that can be controlled by vaccines [25]. These factors are:

1. Immunity to avian coccidiosis is strongly species-specific.
2. Coccidiosis infection induces a quick and strong protective immunity.
3. Lack of antigenic variation in *Eimeria* species.

However, as described above, *Eimeria* exhibit a complex life cycle comprising stages both inside and outside of the host. During the in-host stage, there are both intracellular and extracellular stages and both asexual and sexual reproduction. This complexity provides the immune system with only three moments to inhibit *Eimeria* development. The first is when the sporozoites search for a site of penetration and actually bind with the epithelium. The second is when the sporozoites are in the villus epithelium, inside and between intraepithelial leucocytes. The third moment of possible attack by the immune system is during the passage of the lamina propria into the crypt epithelium [25].

There are four major brands of vaccines commercially available, and they are based on the use of wild type (Coccivac® D/B and Immucox®) and attenuated (Paracox® and livacox®) *Eimeria* species [55,56]. The non-attenuated vaccines contain a mixture of oocysts of wild-type-strain *Eimeria* that will not produce pathogenic effect, but induce immunity. The methods of administration of vaccines have been reviewed by Williams [56]. In the past, vaccines were applied via drinking water or feed when the chickens are about one-week of age, but recently the method of vaccination is a single dose at day one with Coccivac D®, Immuncox® Coccivac B® [57]. Administration of vaccines as a single dose at day-one of age is important in initiating immunity as early as possible in broilers as they are reared only to about 6 weeks of age. However, some studies indicated that vaccination on day one could not evoke a strong immunity since the immune system in young chicks is immature [58]. In contrast, other studies have shown that chicks infected at day one of age indeed are capable of building an effective immunity [3,58]. Many scientists have reported that even embryos have a functional immune system [59,60].

There are various methods of administration of coccidial vaccines, including intra-ocularly (Coccivac®), by hatchery spray (Coccivac® and Nobils®), by edible-gel (Immucox®), or by spraying on feed (Coccivac®, and Paracox®) [55,56]. Immunological protection against *Eimeria* is strongly species specific, a number of species have been incorporated in vaccines, varying from two species (*E. acervulina* and *E. tenella* as in LivacoxD®) to eight species (*E. acervulina, E. brunetti, E. maxima, E. mitis, E. mivati, E. necatrix, E. praecx,* and *E. tenella* as in CoccivacD®) [61,62]. However, Williams recommended the inclusion of *E. acervulina, E. maxima,* and *E. tenella* and the exclusion of *E. brunetti,* and *E. necatrix* in vaccines as the latter two species rarely infect younger chickens.

Following vaccination, immunity is initially stimulated by the vaccine oocysts and is subsequently boosted and maintained by multiple re-infections initiated by the viable oocysts in the litter either originating from the

vaccine or from local wild-type strains [51,55]. This synchrony of infection development is called "trickle" infection and has been shown to be crucial in stimulating solid protective immunity [63].

Types of Vaccines

Coccidial vaccines licensed in the US include Coccivac, Immucox and Advent vaccine. These vaccines can actually cause some lesions and occurrence of coccidiosis in birds because they are not attenuated or weakened in some way. It is a controlled occurrence, but it may be necessary to treat for secondary gut disease, using antibiotics or alternatives such as probiotics. Coccidiosis vaccines used in Europe are attenuated. They are altered because the coccidia used in the vaccine are designed to mature quickly and have a short life cycle and low fertility. They are not pathogenic disease causing and are more costly to produce than the non attenuated vaccines. They include Paracox, Livacox, and Viracox, which are marketed in other countries but not currently in the US.

More types of vaccines are likely to be developed, because the government approval process is much cheaper for vaccines than for anticoccidial drugs. Anticoccidial vaccines include mixtures of species of *Eimeria* that affect chickens. It is especially important to include the three types that cause the most damage in chickens; *E. acervulina*, *E. maxima*, and *E. tenella* [64].

Methods of Vaccine Applications

Spray cabinets; these are used at hatcheries on day-old chicks, resulting in 90 to 95 percent of chicks exposed to the vaccine. Edible gel; gel pucks are placed in transport crates or on the floor of the house when the chicks arrive. Feed spray: vaccines are mixed with water in a garden pressure-sprayer and sprayed on a 24-hour supply of feed [64]. The chicks should be slightly water-starved to encourage them to drink. Since oocysts are heavy and fall to the bottoms of drinkers, they are mixed with a suspension agent to keep them evenly distributed. This method can be used for older chicks. Vaccines cannot be given through proportioners or nipple drinkers. It is important to apply vaccines uniformly to ensure the birds get equal exposure. If birds receive too much of a non-attenuated vaccine, the parasites can cause lesions. If attenuated vaccines are not given in adequate doses, the birds will be susceptible to field strains of the coccidia. The environment must allow the oocysts to sporulate, since the goal of vaccination is to introduce the parasite in small numbers. Litter should be damp but not wet after vaccination; birds excrete fresh oocysts onto the litter. Birds then eat these (second cycle) oocysts. Two cycles of replication are needed for good protection.

Vaccination against Coccidiosis

The application of attenuated vaccines for the prevention of chicken coccidiosis has increased exponentially in recent years. In *Eimeria* spp. infections, protective immunity is thought to rely on a strong cell-mediated response with antibodies supposedly playing a minor role. However, under certain conditions antibodies seem to be significant in protection. Furthermore, antibodies could be useful for monitoring natural exposure of flocks to *Eimeria* spp. and for monitoring the infectivity of live vaccines [65]. Western blotting analysis of parasite antigens prepared from the lining of caeca infected with the attenuated strain of *E. tenella* revealed two dominant antigens apparently associated with trophozoites and merozoites that were present at high concentrations between 84 and 132 hours post-infection. When cryosections of caeca infected with *E. tenella* were probed with IgY purified from immune birds the most intense reaction was observed with the asexual stages. Western blotting analysis of proteins of purified sporozoites and third generation merozoites and absorption of stage-specific antibodies from sera suggested that a large proportion of antigens are shared by the two stages. The time-courses of the antibody response to sporozoite and merozoite antigens were similar but varied depending on the inoculation regime and the degree of oocyst recirculation [65].

In the past, most broiler producers have controlled coccidiosis by providing anticoccidial drugs in poultry feed; this approach is becoming less desirable in light of growing public concern about food safety. Vaccination consists of infecting young poultry with a known dose of live coccidian parasites. This vaccination will immunize poultry against the disease [66]. Avian coccidian and their developmental stages are highly immunogenic and primary infections can stimulate solid immunity to homologous challenges [2]. Therefore, it would seem obvious that vaccines could offer excellent alternatives to drugs as a means controlling coccidiosis.

Live vaccine for coccidiosis control have been used to a limited degree by the poultry industry. For about 60 years, their effectiveness hinges on the recycling of initially very low doses of oocyst and the gradual build up of solid immunity. They have been used primarily to protect breeder and layer flocks. However, their use, particularly in broiler flocks, is increasing. Live vaccine contains attenuated or not coccidial strains. Advantage of

attenuated vaccines is that they have low reproductive potentials. This present crowding in the specific mucosal areas of infection. Thereby resulting in the development of optimal immunity with minimal tissue damage. It is believed that the drug-sensitive, attenuated strains and wild, native strains interbred, reducing both virulence and drug resistance in local population. Thus, the useful period of anticoccidial drugs could be extended by rotating their application with live vaccine [66]. A low-molecular-weight immunogenic antigen with a single immunodominant epitope was reported to be present in all endogenous stages of *E. tenella.* Metabolic antigens from developing sporozoites, merozoite antigens and gamete antigen all elicit various degree of protective immunity.

A delivery mechanism for coccidial vaccines that produces optimum resistance to challenge infection is not yet determined. Immunogenic *Eimeria* antigens have been administered as isolated proteins with adjuvants as recombinant antigens in live vectors such as non pathogenic strains of *Escherichia coli, Salmonella enteric, serovar and typhimurium*, poxviruses, fowlpox virus and turkey herpesvirus and by direct plasmid DNA injection with various degree of success [66]. A species-specific immunity develops after natural infection [67]. The degree of which largely depends on the extent of infection and the number of reinfections. Protective immunity is primarily a T-cell response.

Commercial vaccines consist of low doses of live, sporulated oocysts of the various coccidial species administered at low doses in day-old chicks. Because the vaccine serves only to introduce infection, the vaccine strains of coccidia may or may not be attenuated. The self-limiting nature of coccidiosis is used as a form of attenuation for some vaccines, rather than biological attenuation, Layers and breeders that are maintained on floor litter must have protective immunity. Often, they are given a suboptimal dosage of an Anticoccidial drug during early growth, with the expectation that immunity will continue to develop from repeated exposure to wild types of Coccidia. This method has never been particularly successful because of the difficulty in controlling all of these factors [68]. Anticoccidial vaccines may not induce complete immunity in chickens with lowered immunocompetence due to stress, including certain viral diseases.

In Ovo Vaccination

Developed by the Poultry Health Division of Pfizer Animal Health, is delivered via *in ovo* administration and will provide a new tool for the broiler industry to help control one of the global poultry industry's most prevalent and costly diseases. *In*-ovocox is administered *in ovo* to 18 or 19 day-old incubated broiler chick eggs via an in ovo injection system. The *in ovo* administration of Inovocox helps ensure that every bird receives a uniform dose for effective protection. This technology is based on more than a decade of research, involving millions of birds to evaluate Inovocox for efficacy and safety.

The Inovocox vaccine contains highly immunogenic, anticoccidial-sensitive, sporulated oocysts of *E. acervulina, E. tenella* and two strains of *E. maxima*. These originated from field isolates, which were screened and selected for their ability to help protect against challenge when administered *in ovo*, and for their sensitivity to anticoccidial drugs. Pre-hatch exposure to coccidial organisms will allow birds to develop early immunity to the disease [69]. Early and uniform flock immunity to coccidiosis helps provide control of clinical and subclinical coccidiosis and may result in more uniform growth and development of the flock throughout the grow-out. Inovocox has no significant effect on hatch rate. Performance trials show Inovocox-vaccinated flocks will help provide attractive weight gain, feed conversion and settlement costs.

In addition, Inovocox vaccine may be used as a year-round coccidiosis control program, or as part of an annual rotation program. One dose of Inovocox helps provide broiler birds with life-long immunity against coccidiosis, the new vaccine is a useful addition to the use of *in ovo* injection systems, which already is utilised on a large scale in the broiler industry. It seems that in ovocox will be a new convenient, efficient and precise method of coccidiosis protection [69-75].

Conclusion

Epidemiological studies have established the economic importance of coccidiosis as a major parasitic disease of poultry (Chapman, 2008). Knowledge of the immune response to the different stages of *E. tenella* will give an insight on the possibility of control to the disease through vaccine production, which will ultimately lead to increase in productivity. Consequently, the use of vaccines has become more desirable than ever before.

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