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Immunity Against Coccidiosis in Poultry- A Review

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Abstract: Coccidiosis is the most important parasitic infection in poultry worldwide. Control is largely limited to good husbandry and prophylactic chemotherapy using a range of drugs against which resistance is rapidly acquired. Attempts at vaccination using conventional vaccines have been disappointing and there is now a need for new approach. These include biology of parasite to identify the life cycle stages that are vulnerable to immune attack; antigenic characterization, heterogenicity; variability; and effector mechanisms responsibility for immunity. The purpose of this review is to update/summarize the recent advances in the development of vaccines against the avian coccidiosis.

Key words: Immunity, coccidiosis, poultry

Coccidiosis, caused by various species of genus *Eimeria*, is one of the major menace for poultry industry (Shirley, 1986). Seven species of *Eimeria* are generally accepted to be the causative agents of avian coccidiosis, namely *Eimeria (E.) acervulina*, *E. burnetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox* and *E. tenella* (Shirley, 1986). *E. tenella* is found to be the most prevalent and pathogenic species followed by *E. maxima* and *E. acervulina* through out the world (Ayaz *et al*., 2003; Shirley and Bedrink, 1997). These parasites have been assigned species status on the basis of characteristic differences in their biology such as site of development, morphological appearance of life cycle stages, prepatent and patent times, and immunological specificity, together with, in most cases, knowledge of reproductive isolation (Shirley and Bedrink, 1997). *E. tenella*, *E. acervulina* and *E. maxima* are considered to be of most important to the poultry industry from consideration of their ubiquity in broiler flocks, innate pathogenicity and/or immunological features (Shirley and Bedrink, 1997). It is a disease of economic importance causes heavy economic losses through out the world (Allen and Fetterer, 2002). Since every flock is at risk from coccidiosis, all the 30 billion chickens reared worldwide annually must be protected by prophylactic chemotherapy with specific drugs (most common) or by vaccination (increasing importance) (Shirley and Johnson, 2001). But the development of anticoccidial resistance has threatened the economic stability of the poultry industry (Calnek, 1990). Although there has been little effort by the pharmaceutical industry to develop new anticoccidials, the mounting problem of drug resistance of *Eimeria* species has prompted major research efforts to seek alternative means of control through vaccination. Studies revealed three phenomena responsible for immunity against *Eimeria* infections. First, the actual passage and presence of parasites in the lamina propria to induce immunity. Second, the sporozoite seems to be the most important parasite stage for immunity, and third, cytotoxic T cells are necessary to inhibit parasites (Jeurissen *et al*., 1986).

It has long been known that infection with any of the species of *Eimeria* can induce a potent protective immune response in the host that is exquisitely specific to each species of parasite (Beach and Corl, 1925; Edger, 1958). It was observed that if fowl immunized by *E. tenella* when given an additional infection with *E. necatrix*, produced precipitates in the serum that reacted with antigens of both the species (Rose and Long, 1962). Solid immunity to any species occurred after infections but some species like *E. tenella* and *E. maxima* are so highly immunogenic that an intake of only few oocysts can induce almost complete immunity to homologous challenge (Devies *et al*., 1963). It is also documented that some indigenous breeds of chicken could produce immunity earlier than the other breeds (Rehman, 1971).

At that time, virtually nothing was known about the specific stage of the parasite and nature of the antigen that may elicit potent and unique immune responses; and have fundamental role in induction of protection hindered by multi-stage complexity of coccidial life cycle (Allen and Fetterer, 2002). Jeffers and Long (1985) observed that intracellular sporozoites induced little protective immunity against the intestinal lesions but substantial protection against changes in body weight. It was also observed that early events after invasion of *E. tenella* sporozoites elicited the protective immune responses (Jenkins *et al*., 1991; 1991a)

Eimeria infection in chickens primarily confined to the intestinal tract and the gut associated lymphoid tissue (GALT) which serves as the first line immune defense against colonization by this organism (Lillehoj *et al*., 2000). GALT serves three functions in host defense

against *Eimeria* viz processing and presentation of antigens; production of intestinal antibodies (primarily secretary IgA); and activation of cell-mediated immunity (Ganguly and Waldman, 1980; Brandtzaeg *et al*., 1989; Neutra *et al*., 1996. Yun *et al*., 2000). IgA secreting splenic cells in chickens immunized with egg adopted gametocytes (*E. tenella*) vaccine gave protection against heavy doses of challenge with mixed species of genus *Eimeria* (Akhtar *et al*., 2002; Ayaz, 2003). The importance of cell mediated immunity in acquired resistance to coccidiosis has been documented in the literature (Lillehoj, 1998; Yun *et al*., 2000), and convincing evidence has been obtained by cellular depletion studies (Lillehoj, 1987; Isobe and Lillehoj, 1993) including selective deletion of T-cell subpopulations by treatment with monoclinal antibodies (Trout and Lillehoj, 1996). Another way of investigating the importance of cell mediated immunity in coccidiosis relies on the influence of host genetic factors (Lillehoj and Bacon, 1991; Lillehoj, 1994; Vervelde and Jeurissen, 1995; Zhang *et al*., 1995; Bessay *et al*., 1996; Choi *et al*., 1999). Parasite reactive serum IgY (IgG) and biliary sIgA antibodies usually detected within one week after oral infection and reached to maximum levels on days 14 (Lillehoj and Ruff, 1987; Guzman *et al*., 2003). Humoral immunity against coccidiosis inhibit the development of *Eimeria* (Rose and Hesketh, 1987). The direct role of these antibodies in protective immunity against coccidiosis is minimal, if any, because immunity to reinfection is not diminished in agammaglobulinemia chickens produced by hormonal and chemical bursectomy (Lillehoj, 1987) and because of the lack of correlation between antibody levels and oocyst output (Talebi and Mulcahy, 1995). Rather, parasite specific antibodies may serve as an indirect role in immunity by reducing infectivity as a consequence of parasite agglutination, neutralization, stearic hindrance, reduced mortality, induction of conformational changes in the parasite's host receptor molecule(s), and/or inhibition of intracellular parasite development (Sasai *et al*., 1996; Sasai *et al*., 1998; Lillehoj *et al*., 2000).

Hein (1976) showed that growth performance was not affected and oocyst production was negligible after reinfection of chickens immunized with live oocysts of *E. acervulina*. It was also demonstrated that the dose of oocysts was critical for the development of complete immunity whereas only partial resistance to reinfection was achieved by immunization with two doses of *E. acervulina*, more than three low doses of oocysts were necessary to induce complete and long lasting active immunity against high dose challenge with *E. acervulina*, *E. brunetti* or *E. necatrix* pathological effects of the live coccidial occysts prevented higher doses from being tested. It was also important to maintain a minimum of 14 days between primary and secondary infections to avoid interference during the second infection due to tissue damage caused by the initial infection.

Chickens immunized with live *E. acervulina*, either by the trickle procedure or in a single dose, demonstrated both resistance to reinfection, as evidenced by reduced fecal oocyst output, and reversal of growth reduction compared with non-immunized controls (Galmes *et al*., 1991). Prolonged exposure of chickens to *E. tenella* was shown to induce protective immunity against challenge by the homologous parasite (Nakai *et al*., 1992). The ability of *Eimeria* given repeatedly to protect against heterologous challenge was investigated using a foreign host (Augustine *et al*., 1991). These studies demonstrated only partial success in chickens inoculated recurrently with oocysts of the turkey coccidian *E. adenoids* and challenged with the chicken coccidian *E. tenella*. Watkins *et al*. (1995) speculated that parasite introduced *in ovo* might complete their life cycle within developing chicks and thereby induce protective immunity. But they failed to demonstrate such protection after *in ovo* administration of adult chickens with viable *Eimeria* parasites in the presence of drugs that inhibit parasite development (Long and Jeffer, 1982) or recombinant bovine somatotropin (Allen *et al*., 1997) has also produced inconsistent results.

Early foundations for the work were laid by Rose (1971), who examined the protective capacity of sera collected from chickens at different times after oral inoculation with *E. maxima*. Sera taken at 14 days post infection were able to provide up to 97 per cent protection in passively immunized recipients that were later challenged. Certain regions on the cell surface of the coccidial parasite have been shown to possess discrete immunogenic properties (Danforth *et al*., 1989). Due to the logistical difficulties inherent in the isolation of native *Eimeria* cell surface proteins in sufficient quantities to permit characterization and testing for vaccine efficacy, workers have utilized the biotechnological isolation of the gene (s) coding for these antigenic proteins to produce mass quantities of recombinant antigenic protein in host bacterial or yeast cells. Recombinant coccidial protein gave partial protection against coccidial infection by a particular *Eimeria* species (Danforth, *et al*., 1989). Cloned surface antigen (p250) of *E. acervulina* merozoites also gave partial protection upon challenge. The plasmid carrying the cloned antigen gene survived in the intestinal flora, even after the *E. coli* which initially harbored the plasmid were no longer present (Kim *et al*., 1989). Miller *et al*. (1989) discloses a cloned protein from *E. tenella* which was identified using an antibody raised against *E. acervulina* sporozoites. Live recombinant *E. coli* harboring the gene for the cloned protein provided a degree of partial protection. Substantial number of DNA sequences was identified, coding for antigens of *E. tenella*, by direct screening of genomic libraries with immune serum. No protective

effects were seen for any of these antigens (Clare and Danforth, 1989).

In an attempt to construct a DNA vaccine against chicken coccidiosis, the TA4 gene of *E. tenella* strain BJ was ligated to the mammalian expression vector pcDNA3.1/Zeo(+) to give pcDNA3.1-TA4 (pcDT). Then, Et1A (*E. tenella* refractile body gene) was ligated to it, upstream, aiming to be expressed in fusion with TA4, giving pcDNA3.1-Et1A-TA4 (pcDET). The constructed DNA vaccines were given to broiler chicks. Chickens were challenged with sporulated oocysts of *E. tenella* BJ seven days after the second injection. Results indicated that both pcDT and pcDET could induce protective immunity against coccidial challenge (Wu *et al.*, 2004).

In another study, A cDNA library was constructed with *E. necatrix* merozoite mRNA and immunologically screened by chicken sera against this parasite. One of the positive clones containing an insert of 879 nucleotides, pNP19, showed similarity to part of a published gene expressed in *E. tenella* merozoite by the homology search system. The inserted DNA was subcloned into baculovirus, and a 35-kD protein was expressed, purified, and used for the antigen in enzyme-linked immunosorbent assay. Antibodies from the chickens vaccinated with the *E. necatrix* attenuated strain, Nn-P125, were detected from 14 days after vaccination. The mean absorbance increased rapidly to a peak around 21 days after vaccination; thereafter, it began to decline. Even though some of the vaccinated chickens showed very low levels of antibody response to the recombinant protein 56 days after vaccination, they were protected against challenge with virulent strain of *E. necatrix* (Tajima *et al.*, 2003)

Several reports provide evidence that sporolated oocysts of *Eimeria* species gave protection against heavy doses of challenges; further, the vaccinated birds revealed a significant cellular and humoral responses (Akhtar *et al.*, 1998; 1999; 2000; 2001; 2001a; 2001b; 2003; Ayaz, 1999; Khan, 1999; Ayaz *et al.*, 2002; 2002a). *In ovo* vaccination with infective stages of coccidia and recombinant 3-1E *Eimeria* protein induced protective intestinal immunity against coccidiosis which could be enhanced by co-administration of genes encoding immunity-related cytokines enhanced (Weber and Evans, 2003; Weber *et al.*, 2004; Ding *et al.*, 2004).

In a series of parallel and complementary studies, the immunizing ability of sexual stages (when injected as a sub-unit vaccine or arising from natural infection) have been examined and their work has led to field trials of vaccine derived from purified gametocytes. Wallach *et al.* (1989) tested a similar range of sera by Western blotting techniques (with gametocyte extract as antigen) and identified two major gametocyte protection antigens; the appearance of which correlated well with the results of the protection studies by Rose (1971). Wallach *et al.* (1989) thus postulated that the antigens might play a role in protective immunity. Pugatsch *et al.* (1989) subsequently demonstrated the immunogenicity of purified gametocytes of *E. maxima* in mice, rabbits and chickens, and Wallach *et al.* (1990) showed partial protection to challenge in chickens that had been inoculated with antibodies directed against preparation enriched for the two immunodominant antigens. Wallach *et al.* (1992, 1995) subsequently showed that immunization of hens with affinity purified gametocyte antigen's conferred significant protection against challenge and, more interestingly as the finding may have major practical implications. Wallach (1997) observed that maternal immunization with gametocyte antigens from *E. maxima* protected against challenge with other *Eimeria* species, probably because gametocyte antigens are well conserved within the genus.

Not surprisingly, the ability of a gametocyte vaccine to protect the young offspring against challenge with *Eimeria* spp, has stimulated the conduct of large scale floor pen and field trials. In Israel, Several thousand breeder hens and their offspring are being vaccinated in order to access the practical and commercial feasibility of the material vaccination approach for the control of coccidiosis (Wallach, 1997a). Parallel reports by Smith *et al.* (1994) showed that infection of broiler breeder hens with 20000 sporulated *E. maxima* oocysts led to the production of protective immunoglobulin G antibodies, which were passed into the egg yolk and subsequently to hatchlings. Protection was around 90 per cent following challenge of 3-day old chicks hatched from eggs 3 weeks after infection of the hens, but dropped to between 47 and 68 per cent in chicks that were hatched from eggs collected 7 or 8 weeks after infection of the hens. However, it was possible to prolong the period for which protective antibodies could be transferred to hatchlings, and intramuscular injection with an emulsifying agent before immunization of the laying hens gave protection values of more than 60 per cent, even from eggs collected 19 weeks after infection of the hens. In another study, egg adapted gametocytes (*E. tenella)* vaccine protected the broiler chicks against heavy doses (60,000-70,000) of challenge with mixed species of genus *Eimeria* (Ayaz *et al.*, 2002; Ayaz *et al.*, 2004; Ayaz, 2003a). In another study, it was found that immunity produced due to egg adapted vaccine transferred to the progeny and protected them against challenge with homologus species (Hafeez, 2004).

Conclusion: This review has considered some of the research work carried out on different aspects of immunity against coccidiosis in poultry. Emphasis has been given on the different types of the parasite antigen(s) that had been used/are being used or needed to be explore to prepare an effective vaccine against avian coccidiosis.

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