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Review Paper

OVERVIEW OF CURRENT DEVELOPMENT IN IMMUNOMODULATION STRATEGIES AGAINST AVIAN COCCIDIOSIS

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ABSTRACT: Coccidiosis is one of the major diseases striking the poultry flocks and leading to huge losses and, thus, has direct impact on the economics of this ever growing poultry industry. During the past 70 years, researches have gone through many ways to control this threatening parasitic disease and, surely, the success of this industry today is largely due to the efforts made by them. Among the two effective controls for this problem are anticoccidial drugs and vaccines. Now researchers are also working on different plants and phytogenic products to use them against coccidiosis through feed. As the use of therapeutic drugs is being condemned all over the world, there are two options left to overcome the disease *i.e.* the use of vaccines and anticoccidial plant products. A comprehensive comparison and application potential of the reviewed strategies are presented.

Key words: Poultry, coccidiosis, immunomodulation vaccines, phytogenic products.

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INTRODUCTION

Poultry chicken birds are raised for the provision of inexpensive and quality meat and protein to the masses. About 50 billion chickens are raised each year for this purpose all over the world (Muthamilselvan *et al.* 2016). The success of this industry depends on saving the birds from diseases. Coccidiosis has a huge role in this regard. The diseases causes immense losses to the industry that are up to 3 billion US dollars a year. In China and United States of America these losses are nearly 30 to 60 million dollars (Wang *et al*. 2017) and 127 million dollars (Masood *et al.* 2011) each year respectively, and these losses are none less than millions of dollars in Pakistan. Rashid *et al*., (2018) conducted a study in and around Multan city to check the prevalence of coccidiosis in that area and also assessed the losses caused by the parasite to the broiler, layer and golden industries. They reported a financial loss of US dollars 45,405 due to treatment and prophylaxes measures while the production losses in form of lower meat and eggs production as US dollars 2,750,779 for broiler, US dollars 13,974.98 for layer and US dollars 50,228.76 for golden for young birds while for old birds these losses were estimated as US dollars 104.74 and 203.77 for layer and golden respectively. The prevalence of the parasite reported by them was 14.16% in broiler birds, 11.01% in layer birds and 19.57% in golden birds. These losses include death loss, poor FCR, the expenses on medication and immunization. As far the prevalence of coccidiosis is concerned, it varies according to different areas of Pakistan as 71.8% in Rawalpindi, 65% in Muzaffargarh (Bachaya *et al*.2015), 44% in Dera Ismail Khan (Jamil *et al* 2016), 43.89% in Faisalabad (Awais *et al.* 2011), and 42.85% in Lahore (Sultana *et al*. 2009). The disease called "coccidiosis" is an infectious one and is caused by protozoa that comes from the genus Eimeria that belongs to phylum Apicomplexa (Reid *et al.*, 2014). The disease is mostly found in warm and humid environments and it hits the species being farmed intensively including cattle, sheep, rabbits, pigs and poultry (Clarke *et al.* 2014). Focusing upon the poultry, the Eimeria species that are economically important include *Eimeria tenella, Eimeria acervullina and Eimeria maxima* (Kadykalo *et al.* 2017), although 9 species of *Eimeria* have been reported in the chicken. The parasite spreads in flocks when the hygienic conditions are not proper, overcrowding is there and the infected birds are not separated from the non-infected birds (Clarke *et al.* 2014). The *Eimeria* parasite has different sexual and asexual stages occurring in the host and in its surroundings (Kadykalo *et al.* 2017). The infection starts when a bird ingests oocyst of parasite shed with the feces of the already diseased birds. The oocyst goes to intestinal cells and releases the sporozoites there. These sporozoites go through multiple cycles and merozoites are formed that change into sexual stage called the gametocyte. Male gametes fertilize the female gametes and again the oocytes are formed that can infect other birds when ingested by them (Clarke *et al.* 2014). These sporulated oocysts can survive for long periods, usually months, in the environment. The prepatent period is about 4 to 7 days (Kadykalo *et al.* 2017). The signs of the disease include lesions on the intestine, diarrhea, decreased growth, poor feed conversion ratio (Clarke *et al.* 2014), and decreased water and feed intake, decreased production of eggs and death. As far as the prevalence of coccidiosis is concerned, clinical cases are up to 5% and subclinical cases are up to 20% (Kadykalo *et al.* 2017).

To overcome this problem therapeutics have been used on a large scale. For example, 30 or more different sulphonamides have been used that act by interfering with synthesis of cofactor, functions of microbes and function of cell membrane of *Eimeria* parasites (Ahmad *et al.* 2016). The invasion of the parasite to epithelial cells was much reduced in presence of an effective compound (Madsen et al. 2001 & Silpa *et al*. 2015). Coccidiocidal drugs destroy the parasite while it is developing and the coccidiostatic drugs retard the growth of parasite (Hansen *et al.* 2008). Synthetic coccidiocidals have been prepared that destroy the intracellular stages of parasite after its entry to the intestinal cells. Ionophores make complexes with many ions that include sodium, calcium and potassium. The ionophores move them into membranes and also across the membranes. For this movement, ionophores may act as carriers of ions or they may form channels to move the ions. This general mode of action enables them to effectively control a wide variety of parasites and they can act against the intracellular as well as extra cellular stages of the coccidial parasite (Clarke *et al.* 2014). Ionophores can also act against sporozoites in the lumen of intestine but they do not destroy the sporozoites completely. As a result, a few sporozoites survive in the host and result in the development of some immunity in it (Kadykalo *et al.* 2017). The anticoccidial drugs are often used as feed additives. Up to 80% of the anticoccidial drugs administered to chicken may be excreted by them. As their waste material is used as a fertilizer the drugs are spreading in the environment (Hansen *et al*. 2008). Now all these drugs are being banned to use in poultry because of development of resistance in parasites against them and their residues in the poultry products (Lee *et al*., 2001, Hashemi and Davoodi, 2011).

Vaccination has a great effect in minimizing these losses, therefor, vaccination is done to save the birds from this lethal parasite. Along with vaccination, in recent years, the use of phytogenic plants and their products is becoming a good alternative source to avoid the problems. Here, in this article, we will discuss about the efficacy of vaccination and use of plant products against the *Eimeria* species.

Vaccination against *Eimeria* species: Vaccination is an excellent source for immunizing the poultry birds against various diseases in order to avoid the development of resistance in parasites and other microbes against the anti-parasitic and anti-microbial drugs used in this industry. Vaccines are comparatively expensive than the drugs and the cross protection against different strains of a specie is always questioned. An Eimerian parasite passes through different stages during its life cycle that are both sexual and asexual and each of them invades a particular host and a particular tissue in the host, so, the immunity in the host against the invaded parasite varies (Dalloul and Hyun 2005). The first vaccine against coccidiosis was developed around 1950 after the work of Edgar who assured that the administration of oocytes of *E. tenella* could help the chicken birds resist the diseases (Brown, 2007). The vaccines currently available against coccidiosis in the market have oocysts of either live attenuated or live non-attenuated *Eimeria* strains. The methods of their application are different as they may be used via drinking water or through feed sprayed with the vaccine. Now in the hatcheries spray cabinets are used (Chapman and Jeffers 2014). The first developed vaccines had live, non attenuated wild type strains of the parasite. These vaccines induce a healthy immune action in the bird but it is necessary that the exposure with the oocytes occur again through the litter to keep the immune status at the same level that saves the birds (Sharman 2010). Towards the generation of immunity, T-cells play a crucial role especially those involved in expression of αβ type of T-cell receptors and IFN-gamma. B cells and natural killer cells do play a little role in immunity against coccidiosis (Shirley *et al.*, 2006). The inoculation of live vaccines caused problems in birds as higher dose resulted in poor feed conversion ratio and even clinical symptoms while smaller dose rendered the birds unable to develop the immunity (Blake and Fiona 2013). Another problem is that the immunity produced is species specific i.e. the bird secured against one specie is still liable to the others that's why the vaccine should have oocysts of all *Eimeria* species. This can result into the exposure of birds to a parasite that had no contact with the birds ever before (Lillehoj and Erik, 2000). The vaccines consisted of dead parasites used in different ways were found to be unable to immune the birds. Therefor the focus was largely on the live vaccines, the proper working of which was dependent on administration of small doses of Eimeria oocysts that develop the desired responses by evoking both the humoral and cellular mechanisms to control different developmental stages of the parasite (Ahmad *et al*. 2016).

The immune system of chicken controls the parasite where they interact in the epithelial cells of the intestine of host, in vicinity of intraepithelial lymphocytes inside the epithelium and when the parasite moves from lamina propria to the crypts of epithelium (Lillehoj and Erik, 2000). The lymphoid tissue of the gut (GALT) performs three jobs to save the host from invaders (Brisbin *et al*., 2008). They process and present the antigen, produce antibodies and then stimulate the cell mediated immunity. Immunoglobulin A, IgM and IgY are produced, not the IgE and IgD, in the chicken whenever an *Eimeria* specie strikes. While the cytokines produced in chicken include gama-interferon, transforming growth factor (TGF), tumor necrosis factor, IL-1, IL-2, IL-6, IL-8, IL-15 and some others as well (McKay and Baird, 1999; Dalloul and Hyun, 2005; Hong *et al.* 2006).

The vaccines having live attenuated antigens are safer to use as these antigens have lower pathogenicity and that's why their effectiveness is not sufficient during heavy infection (Ahmad *et al.* 2016). Different methods that have been used for attenuation of parasite. Initially, heat treatment and radiation were used both of which failed to produce effective products (Sharman *et al*. 2010). Then other methods like vaccines of de-routed sporozoites and inoculation of merozoites in high concentrations intra-rectally were also used. But only the development of precocious lines of *Eimeria* and development of egg adopted lines of *Eimeria* were used at commercial levels (Ahmad *et al.,* 2016). Precocious lines are the *Eimeria* that complete their life cycle in the host rapidly than their parents and they were first described by Jeffers (Shirley and Bedmik, 1997). The ability of these lines to cause disease is lost due to smaller size and lesser invasiveness of schizonts of second generation8 (McDonald and Shirley, 2009; Sharman *et al.,* 2010).

Undoubtedly, the active immunization achieved through the live attenuated vaccines makes the chicken birds immune against coccidiosis but still this is also a source of transient infection for the birds resulting in poor performance (Rasheed, 2016, Chapman *et al.,* 2002). Furthermore, the problems with live attenuated vaccines include the dose of vaccine. The lower first dose of antigen can cause mild infection in the birds (Velkers *et al*., 2010; Smith *et al*. 2011). The drugs that depress the immune system and affect T-cells (dexamethasone, betamethasone), make the host more prone to the parasite by lowering or fully diminishing the immunity against it (Lillehoj and Erik, 2000). Furthermore, the use of any drug or feed additive that effects the development of coccidial parasite is prohibited (Vermeulen *et al*., 2001). Another effort was made to immune the chickens by using ionophores at early age and vaccinating with the *Eimeria* strains resistant to the ionophore used. This approach can save the birds at early age when the immunity is underdeveloped (Vermeulen *et al*., 2001). But there was a danger that the oocytes of vaccine may evolve into a more lethal form and problem of their resistance against ionophore made them less useful (Ahmad *et al*., 2016).

Besides the extensive use of the active immunization, passive immunization is an important way to save the poultry birds from coccidiosis (Wallach 2010; Dalloul and Lillehoj 2006). Passive immunization was done for the first time in 1988 using monoclonal antibody against a surface antigen (1073.10) of *Eimeria tenella* (Ahmad *et al.,* 2016). The birds that survive the disease have large amounts of IgM, IgG, IgA antibodies in their body fluids (serum, intestinal secretions, and bile). The sera having peak levels of IgG were found to provide the naïve chicks with great passive immunity. This was also found that maternal IgG transferred through eggs saves the chicks too against same species of *Eimeria* (Wallach, 2010). In spite of excellent effectiveness of passive immunity, still it has lesser role in immunization against *Eimeria* possibly because the active immunization can cope with the challenge of disease and also due to the focus on developing a subunit vaccine (Wallach, 2010).

Many attempts were made, during 1980s and 1990s, to recognize the antigens and the genes that encoded them in the parasite. These antigens and their coding genes were set as the targets of a new vaccinal product, the subunit vaccine (Blake and Fiona 2013). These anticoccidial vaccines are made up of antigens that include micronemes, refractile bodies, rhoptries, gametocytes or merozoites of *Eimeria* (Ahmad *et al*., 2016). The methods to develop the vaccine include testing the recombinant protein, DNA, exosome derived from dendritic cells and vectored subunit vaccine and these all depended on the recognition of antigen (Blake and Fiona, 2013). Recognition of molecules that evoke immune response and those which are involved in a protective immune response is critical issue to identify vaccine candidates. DNA vaccines generate immunity by the mechanism in which cross-priming and direct transfection of APCs play their part. APCs process the exogenous proteins intracellular for presentation by MHC class 1 and priming of CTLs responses takes place. Myocytes are mainly infected when DNA vaccines are manipulated intramuscularly or via gene gun and antigen presenting cells (APCs) derived from bone marrow act to induce response of cytotoxic T-cells (Donnelly *et al.,* 2000). They have limited use as it is not an easy job to identify and produce an antigen and also due to their less reliability (Ahmad *et al*., 2016). These vaccines are expensive and need the production of new antigens or use of more than one antigens at a time to get desired response. These vaccines also need much labor for administration therefor not acceptable for poultry production (Blake & Fiona, 2013).

Phytogenic feed additives in poultry: Phytogenic products are the chemicals naturally found in plant kingdom synthesized in them to save themselves from parasites and even from the animals (Hashemi and Davoodi, 2011). The medicinal benefits of many of these products is known for centuries and these plant products have been used to treat many diseases like malaria in Pakistan, Africa, China, India and many other parts of the world (Wang *et al.,* 2017). In the current era, the increasing development of drug resistance in pathogens have made these herbal products of worth consideration as an important alternative source to overcome the problems like coccidiosis and the other microbes (Hashemi and Davoodi, 2011).

Different types of phytogenic products used: Windicsh and Kroismayr in 2006 described the groups of phytogenic plants as herbs; the products found from the flowering and non-woody plants along with the non-persistent plants, botanicals; that are the whole plants or parts of plants that are processed e.g. roots, leaves. Essential oils; volatile plant products obtained from hydro distillation and oleoresins; non-aqueous solvent extracts (Hashemi and Davoodi, 2010). The medicinal plant products have also been grouped as phenolic, polyphenols, terpenoids, alkaloids, essential oils, lectins and polypeptides (Hashemi and Davoodi, 2011).

Mechanism of action: The plant products have different ways to apply their anti-microbial actions. Tannins work by preventing iron, hydrogen bonding and reacting with proteins like the enzymes. They also act by decreasing the growth of bacteria in G.I.T (Smith *et al.* 2005), e.g. *Bacteroides fragilis, E.coli* etc. Alkaloids inhibit topoisomerase activity thereby inhibiting DNA synthesis (Fang *et al.* 1993). Saponins form compounds with sterols found in membranes of microbes and damage it so the cell is collapsed. The mechanism of action by which essential oils perform their anti-microbial action is not fully known but it is assumed that they act by lipophilic characters and chemical structure. They also act owing to their characters like the functional groups and aromatic structures (Hyldgaard *et al.,* 2012). Terpenoids and phenylpropanoids have lipophilic character due to which they enter the microbial cell and find access to the inner parts (Hashemi and Davoodi, 2011).

Phytogenic plants and products used against coccidiosis: Many plants and plant products have been used worldwide to control the *Eimeria* species. Muthamilselvan *et al*., (2016) described 68 plants and plant products that have been used against coccidiosis. The mechanism by which they act is known for some of them (32 products) and not for the others. Here we'll discuss about a few plants and medicinal plant products used by researchers in recent years just in order to have a glimpse of their usefulness against coccidial parasites.

Ahad *et al*., (2017) used *Artemisia vestita* to study its beneficial effects in comparison to amprolium against *Eimeria tenella* in broiler chicken. The Artemisia vestita showed great efficacy against the parasite as indicated by the results. The results showed decreased oocyst shedding (71.5 ± 12.2), the weight gained by the birds (1406.4 ± 12.2), and the FCR (1.58 ± 0.06). The study also confirmed that the plant has active ingredients of alcoholic nature. The plant extracts were also safe to be used even at a level of 2000 mg/Kg. In a study, Wang *et al*., (2017) proved that the extracts of areca nuts have equally good results against *Eimeria tenella* as does the diclazuril. Both the areca nut extracts and diclazuril lowered the intestinal damage done by the parasite. The areca nut extracts also decreased the cecal lesion scores as compared to the negatively controlled group ($P<0.05$). The ANE also improved the level of IL-2 as compared to negatively controlled group. Abbas *et al.,* (2017) studied the anti-coccidial effects of sugar beet. Results of their study showed that sugar beet, when used as feed additive, improved FCR, decreased lesion scores and oocysts per gram of feces. It also had no adverse effects on serum profile.

Jitviriyanon *et al.,* (2016) working with essential oils obtained from the plants *Boesenbergia pandurata* and *Ocimum basilicum* showed that these oils reduced the sporulation and degenerated the oocysts. The active compounds of *B. pendurata* and *O. basilicum* include methyl cinnamate and camphor, and methyl chavicol respectively. They have cytotoxic activity against coccidian as well. Malik *et al*., (2014) studied the anticoccidial effects of Berberine in comparison to amprolium. The results of their study proved that there is synergism between both compounds. The Berberine reduced number of oocyst shed per gram of feces, improved feed intake and better weight gain as did the amprolium. In a study, Orengo *et al*., (2011) used *Echinacea purpurea* plant extracts and cinnamaldehyde to test the anti-coccidial activity against *Eimeria acervulena*. The results of their study showed the reduction in lesion scores ($P<0.05$). Lee *et al*., (2011) evaluated a couple of phytonutrient mixtures for their effects on the chicken birds. Components of one mixture included Carvacrol, Cinnamaldehyde, and Capsicum oleoresin (VAC), and that of second included Capsicum oleoresin and turmeric oleoresin (MC). The birds fed the mixtures had higher body weights, better serum antibodies levels against parasite and more proliferation of lymphocytes. The chickens vaccinated for *E. tenella* fed MC added feed had more CD4+, CD8+, TCR1+ and T cells as compared to the birds fed on no supplement added feed. The birds fed on VAC supplemented feed had more level of just K1+ macrophages. In another study, Chandrakesan *et al.,* (2009) used a herbal complex comprised of Solanum nigram (35%), Aloe vera (15%), Moringa indica (35%) and Mentha arvensis (15%) in order to test them against Eimeria tenella. They compared this herbal complex with salinomycin. The herbal complex showed improved weight gains during the period of 4^{th} and 5^{th} week (344.34 g), better FCR (1.77) and no mortality.

Conclusion: Coccidiosis can be controlled in chicken bird flocks by the use of anti-coccidial vaccines. Phytogenic products also have efficacy against the coccidiosis but further studies should also be conducted to check the efficacy of herbal products on farm levels.

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