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Chapter 7

Avian Coccidiosis, New Strategies of Treatment

Rosa Estela Quiroz-Castañeda

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Abstract

The control of avian coccidiosis since the 1940s has been associated with the use of ionophores and chemical drugs. Recently, a significant interest in natural sources has developed due to the pressure to poultry industry to produce drug-free birds. Consequently, the search of products derived from plants and other natural sources has increased in the last years. Today, many commercial products containing essential oils, extracts, and other compounds are available. The use of these compounds of natural origin is related to an increased immune response, a body weight gain, destruction of oocyst, among other benefits. The main inconvenience of these products is the act on some species of *Eimeria*, but not all. This genetic variability found in the parasite makes the use of products difficult to control and treat coccidiosis. In this chapter, several proposals of treatment are presented based on the use of natural products, considering the new strategies of treatment with minimal consequences to birds.

Keywords: *Eimeria*, coccidiosis, natural treatments, vaccines, herbal extracts

1. Introduction

Chickens’ production is positioned as an important source of meat around the world. About 60 billion chickens are produced worldwide every year. However, *Eimeria* protozoan parasites of Phylum Apicomplexa are considered the main risk to avian production since they are the causative agent of avian coccidiosis [1]. For Avian coccidiosis is caused by seven species of *Eimeria*, which parasite chickens intestine resulting in economic losses around $2.4 billion per year worldwide [2].

The life cycle of the *Eimeria* is complex. They have developmental cycles with an exogenous phase in the environment and an endogenous phase in the intestine of chickens [3]. The efforts to prevent this disease have been focused on developing vaccines and drugs with...
coccidiostatic and coccidicidal activity [4]. So far, genomic studies have revealed the wide antigenic variability of species such as *Eimeria tenella*, one of the most pathogenic parasites to chickens, which leads to develop a vaccine resistance rapidly [1].

The main problem of coccidiosis treatment is that the resistance to anticoccidial drugs can evolve rapidly leading to a continuing need to develop novel and effective therapies [5].

In this chapter, avian coccidiosis is briefly reviewed and a special focus on novel strategies of treatment is presented. The strategies include the use of herbal compounds to genomic analysis of *Eimeria* species.

2. Avian coccidiosis

Poultry industry raises approximately 40 billion chickens annually and more than 100 tonnes of chicken meat. Today, there exist a growing demand of this meat not only because it is cheaper than other types of meat, but also due to the increasing number of inhabitants around the world [6]. In spite of the high production of poultry industry which still exists, some factors are affecting the productivity such as handling, housing, and rearing of birds in addition to disease control (nutritional, metabolic, and parasitic diseases) [7].

2.1. Causative agents of coccidiosis

Avian coccidiosis is a parasitic disease caused by protozoa belonging to the phylum Apicomplexa, genus *Eimeria*. It affects virtually all domestic and wild species, causing signs such as paleness, diarrhea with or without blood, high feed conversion, weight loss, and in severe cases even death. It is the most important parasitic disease of the poultry industry worldwide, causing serious economic losses both for the prevention and the control of subclinical or clinical disease [8]. The disease transmission is fecal-oral by consuming oocysts eliminated by infected birds. Those oocysts present in bed sporulate when conditions are favorable. Currently, the problem tends to worsen because conditions of intensive poultry production as the amount of oocysts per m<sup>2</sup> in bed are higher and therefore, the challenge for the birds [9].

More than 1200 species of *Eimeria* are described, most of them parasitizing the intestinal epithelium of vertebrates, and as a consequence, infected birds reduce feed intake, have bloody diarrhea, and have hampered weight gains [10].

In domestic fowl (*Gallus gallus domesticus*), seven species of *Eimeria* are reported. Those causing hemorrhagic disease are *E. brunetti*, *E. necatrix*, and *E. tenella*. Considered as mildly pathogenic and causing malabsorptive disease *E. acervulina*, *E. mitis*, *E. maxima*, and *E. praecox* [5]. Each species has a specific site of development in the small intestine (upper, middle, lower, rectum, and caeca) Table 1.

2.2. Life cycle

*Eimeria* life cycle is complex and comprises of three stages, one occurs on litter under the conditions of humidity, temperature, and oxygen supply (sporogony), and two stages occurs in
the cells of intestinal epithelium [merogony or schizogony (asexual reproduction) and gametogony (sexual reproduction)].

During the sporogony, which is considered a noninfective stage, the oocyst is excreted in chicken feces and undergoes sporulation in the presence of humidity, warmth, and oxygen and thus becoming a sporulated oocyst, now infective. Merogony or schizogony occurs in the intestine and comprises of several rounds of asexual multiple division (from two to four times), followed by gametogony that involves the formation of male and female gametes, fertilization and formation of a zygote (oocyst) that will be excreted in feces [11]. Infection starts when the host ingest sporulated oocysts (Figure 1).

<table>
<thead>
<tr>
<th>Species</th>
<th>Site of development</th>
<th>Pathogenicity</th>
<th>Disease type</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. necatrix</td>
<td>Jejunum, ileum, caeca</td>
<td>++++</td>
<td>Hemorrhagic</td>
</tr>
<tr>
<td>E. brunetti</td>
<td>Caeca and rectum</td>
<td>+++</td>
<td>Hemorrhagic</td>
</tr>
<tr>
<td>E. tenella</td>
<td>Caeca</td>
<td>+++</td>
<td>Hemorrhagic</td>
</tr>
<tr>
<td>E. maxima</td>
<td>Jejunum, ileum</td>
<td>++</td>
<td>Malabsorptive</td>
</tr>
<tr>
<td>E. mitis</td>
<td>Ileum</td>
<td>+</td>
<td>Malabsorptive</td>
</tr>
<tr>
<td>E. acervulina</td>
<td>Duodenum, ileum</td>
<td>++</td>
<td>Malabsorptive</td>
</tr>
<tr>
<td>E. praecox</td>
<td>Duodenum, jejunum</td>
<td>+</td>
<td>Malabsorptive</td>
</tr>
</tbody>
</table>

Table 1. Location and pathogenicity of *Eimeria* species [5, 7].

Figure 1. Infection process of *Eimeria* spp. in chickens. First, *Eimeria* oocysts are ingested by chickens, once inside, *Eimeria* sporozoites colonize and infect different regions of intestine epithelium. Oocysts are formed again and released to litter to begin another cycle.
Due to the chemical and mechanical proventriculus and gizzard action, and the presence of CO\textsubscript{2} in the lumen, the oocyst releases the sporozoites, which gets the intestinal lumen and attached to enterocytes using this anchoring and penetration proteins present in the apical complex (rhoptries and micronemes) entering the cell in order to continue the second phase: esquizogony or merogony, producing a schizont with thousands of merozoites inside to be released back into the lumen to infect new intestinal cells. Thus, after several stages of merogonies, some of the merozoites inside the intestinal cell forms macrogamonts with a macrogametocyte (inmobile cell considered the female gamete) and some forms microgamonts with several microgametocytes inside (mobile cells considered male gametes). These microgametocytes come out of the cells that originated to locate and fertilize to macrogametocyte producing a zygote to be excreted in the feces again (not sporulated oocyst) to begin another cycle [12] Figure 2.

Once the oocyst is formed, it is considered the most persistent structure of \textit{Eimeria} life cycle. It has a significant resistance to mechanical, chemical, and proteolytic damage, due to the composition of the two walls that confer the oocyst and outstanding resistance [13, 14] Figure 3.

The unsporulated oocyst is considered as the noninfective stage while the sporulated oocyst is the infective stage, in \textit{Eimeria}, the oocyst has four sporocysts, each with two sporozoites Figure 4.

Environmental factors such as humidity (40–80%), temperature (24–28°C), and oxygen supply (aeration) makes the sporulation occurs, at least in \textit{E. acervulina} the most important environmental

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure2.png}
\caption{Life cycle of \textit{Eimeria}. (A) Sexual and asexual stages reproduction occurs in epithelium cells and oocyst formation occurs outside the birds under specific environmental conditions. (B) Structure of \textit{Eimeria} spp. sporozoite. Figure taken from https://doi.org/10.1016/j.trivac.2016.02.001.}
\end{figure}
factor to onset sporulation is temperature [15, 16]. Nevertheless, the temperature is an environmental factor that is hard to control due to the ideal temperatures that are easily reached in the poultry litter.

3. Strategies of treatment

During the last years, research has focused on development of anticoccidial drugs, with interest focused on the sexual and asexual stages of the parasites (stages occurring within the host). However, exists a tendency to ban the use of drugs in animals for human consumption, so the development of new drugs to control avian coccidiosis demands another way to control the disease.

The control of avian coccidiosis is a challenge of veterinary parasitology. So far, any treatment, including, anticoccidial drugs, vaccines, or natural alternatives control avian coccidiosis by itself. It proposed the use of combination of different strategies to achieve an effective control (Figure 5).
3.1. Chemotherapy

This treatment comprises of ionophorous compounds (ionophores) and synthetic drugs (chemicals). Ionophores usually cause the death of parasite and are produced by the fermentation and chemicals inhibit several biochemical pathways of the parasite and are produced by chemical synthesis [17, 18]. In Table 2, the most important ionophores and chemicals are shown.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Examples</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionophores</td>
<td>Lasalocid, Monensin, Narasinm, Salinomycin, and Semduramicin</td>
<td>Disruption of ion gradient across the parasite cell membrane</td>
</tr>
<tr>
<td>Chemicals</td>
<td>Quinolone drugs (Decoquinate and nequinatem buquinolate).</td>
<td>Inhibition of parasite mitochondrial respiration</td>
</tr>
<tr>
<td></td>
<td>Pyridones (Meticlorpindol)</td>
<td>Inhibition of the folic acid pathway</td>
</tr>
<tr>
<td></td>
<td>Sulphonamides</td>
<td>Competitive inhibition of thiamine uptake</td>
</tr>
<tr>
<td></td>
<td>Amprolium</td>
<td>Mode of action unknown</td>
</tr>
<tr>
<td></td>
<td>Diclazuril, Halofuginone, and Robenidine</td>
<td>Inhibition of the development of the first and second generations of the schizont stage</td>
</tr>
<tr>
<td></td>
<td>Nicarbazin</td>
<td>of the parasites</td>
</tr>
</tbody>
</table>

Table 2. Most commonly used ionophores and chemical in coccidiosis treatment.
However, after prolonged uses of a drug treatment, several drug-resistant strains may emerge, which represents a severe problem [19]. To combat resistance, shuttle, mix, and rotation systems of drugs are employed.

3.2. Vaccines

Passive or active immune responses induce immunity in animals. This immunity can reduce the pathogenic effects of coccidiosis such as less macroscopically visible lesions, decreasing of oocyst production, and increasing performance of birds [16].

The first commercial live coccidiosis vaccine was CocciVac® registered in the USA in 1952 [20]. Currently, two types of vaccines are used with the aim of controlling coccidiosis in a chemical-free way: nonattenuated and attenuated vaccines.

The main risk of using live nonattenuated vaccines (Coccivac, Advent, Immucox, and Inovocox) is the live parasites that can develop a severe reaction in birds. Many times their use is accompanied by chemical treatments to control the inherent pathogenicity of the parasites [21].

On the contrary, the success of live attenuated vaccines (Paracox and HatchPak CocciIII) relies on the low risk of disease occurring because of the reduction in the proliferation of parasites and consequently a less damage in birds’ tissue [22].

Nonattenuated and attenuated vaccines may have different routes of administration (oral, eyes drops, in ovo) in birds and several Eimeria species as target [23].

Subunit vaccines consist of purified antigenic determinants obtained from Eimeria parasite. These vaccines are obtained from DNA recombinant technology and may consist of native antigens or recombinant proteins of various stages (sporozoites, merozoites, and gametes) of Eimeria [16]. Distinct protective antigens used are micronemes, rhoptries, refractile bodies, merozoites, or gametocytes of Eimeria parasite [24].

These kinds of vaccines involve native or recombinant subunit second generation extract or DNA vaccines. The only commercial subunit vaccine was CoxAbic®, based on purified native protein isolated from gametocytes of Eimeria and inhibits the development of oocysts but with only 53% of protection against challenge with Eimeria infections, resulting in a very limiting vaccine [22, 25].

Proteomic analysis of E. tenella life cycle stages (unsporulated oocyst, sporulated oocyst, sporozoite, and second generation merozoite) revealed specific proteins in each stage and many other proteins are shared in all stages. During parasite invasion, proteins RON2 and RON5 are expressed, these proteins have been previously identified in Toxoplasma gondii, and also were found in E. tenella, where may have a role in host adhesion during process of invasion [11].

A collection of epitope mapping of T-cell mediated antigenic determinants was applied in an in silico analysis to investigate promising epitopes from the sporozoite and merozoite stages. Several epitopes showed a significant predicted efficacy [26].
So far, the use of recombinant vaccines is limited mainly due to the low protection of antigens with the potential to induce potent protective immune response against *Eimeria*. Certainly, genomic and proteomic analyses of *E. tenella* genome will allow the design and development of potential immunogens that could be used as vaccine in future [5, 27, 28].

### 3.3. Natural compounds, alternative treatments

The search of alternatives to anticoccidial drugs and vaccines against avian coccidiosis has led to discover in fungal extracts, plant extracts, and probiotics a source of new compounds with anticoccidial activity. Many of them with the oocyst as target being that if the dispersion of oocysts is controlled then the possibilities of infection reduce.

The role of fats, essential oils and herbal and medicinal plants has been explored to control avian coccidiosis.

#### 3.3.1. Fats

Fatty acids from fish or flax seeds reduce the severity of *E. tenella* infections in young broiler chicks. Diets supplemented with docosahexaenoic acid, linoleic acid, and eicosapentaenoic acid allowed a maintained weight gain in birds, which strongly suggests their use as part of birds nutrition. Cecal lesions were reduced as well as the parasite invasion rate and development of parasite. Unfortunately, this benefits only were observed in birds infected with *E. tenella* and not with other species, thus, limiting the use of fatty acids to control coccidiosis [29, 30].

#### 3.3.2. Essential oils

The use of essential oils as a therapy to control *Eimeria* oocysts is widely reported. Although the mechanism of oils is still unclear, they destroy the most resistant structure of *Eimeria*, the oocyst, thus reducing dispersion and the risk of infection [7].

Functional oils comprise those oils that have an action beyond nutritional value [31]. Recently, a variety of essential have been used at different stages of life cycle of *Eimeria* with good results Figure 6.

In vitro assays are used to test potential oocysticidal activity of essential oils. The use of oils from Artemisia, thyme, tea tree, and clove showed a clear destruction of oocyst after 3 hours of treatment and a LC$_{50}$ < 1 mg/ml of oocyst [33]. Commercial oils carvacol, carvone, isopulegol, thymol, and eugenol were used to destroy a mixture of *Eimeria* species oocysts, a release of oocyst internal substances was observed after treatment suggesting the potential of these oils to control oocysts of *E. tenella, E. maxima, E. acervulina, E. necatrix, and E. mitis* [34].

A commercial essential oils product called Essential (Oligo Basics Agroind. Ltda, www.oligobasics.com.br) containing ricinoleic acid and alkylphenolic oil of the shell of the cashew nut (*Anacardium occidentale*) showed an improvement in the energy utilization and the livability and decreased lesion caused by coccidiosis (*E. acervulina, E.maxima, and E. tenella*) in birds treated with Essential [31].
3.3.3. Herbal derivatives


The usage of these plants varies from organic extracts (ethanol, petroleum ether and acetone extracts), ground powder, essential oils, and decoction. The parameters measured to evaluate efficiency of the anticoccidial compounds are body weight gain, oocyst count, feed consumption, lesion scores, bloody diarrhea, and mortality [7, 32]. Recently, plant *Bidens pilosa* was used in diet of birds, which significantly elevated body weight gain and lowered feed conversion ratio. Also, *B. pilosa* reduced cecal damage, villus destruction and decreased villus-to-crypt ratio in chicken ceca [35, 36].
Table 3 shows some compounds related to different action to control avian coccidiosis. Information based on [7, 32, 37].

The use of these compounds is not limited to laboratory conditions, many products that contain natural compounds are commercially available for prevention and treatment of coccidiosis. This highlights their potential use in poultry industry. In Table 4, commercial natural products are show.

<table>
<thead>
<tr>
<th>Action</th>
<th>Compound (Plant/fungi)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of <em>Eimeria</em> life cycle</td>
<td>Artemisin (<em>Artemisia annua</em>)</td>
<td>Induce reactive oxygen species (ROS) that inhibit oocyst wall formation and sporulation</td>
</tr>
<tr>
<td></td>
<td>Tannins, Pine (<em>Pinus radiata</em>)</td>
<td>Inhibition of life cycle and decreased sporulation of the oocyst</td>
</tr>
<tr>
<td></td>
<td>Allicin and sulfur compounds, Garlic (<em>Allium sativum</em>)</td>
<td>Antimicrobial activity and inhibition of sporulation of <em>E. tenella</em></td>
</tr>
<tr>
<td></td>
<td>Selenium, Phenolics and Green tea (<em>Camellia sinensis</em>)</td>
<td>Inhibition of sporulation of coccidian oocysts.</td>
</tr>
<tr>
<td></td>
<td>Papain (<em>Carica papaya</em>)</td>
<td>Inhibition of coccidiosis probably by proteolytic degradation of <em>Eimeria</em></td>
</tr>
<tr>
<td></td>
<td>Saponins (<em>Cyanopsis tetragonoloba</em>)</td>
<td>Suppression of coccidiosis</td>
</tr>
<tr>
<td></td>
<td>Essential oils from thyme, tea tree and clove</td>
<td>Destruction of <em>Eimeria</em> oocysts</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate extract (<em>Meyerozyma guilliermondii</em>)</td>
<td>Destruction of <em>Eimeria</em> spp. oocysts</td>
</tr>
<tr>
<td>Immune response modulators</td>
<td>Probiotics (<em>Pediococcus acidilactici</em> and <em>Saccharomyces boulardii</em>)</td>
<td>Enhanced humoral immunity, changes in body weight gain and fecal oocyst shedding rates.</td>
</tr>
<tr>
<td></td>
<td>Arabinoxylans (<em>Triticum aestivum</em>)</td>
<td>Immunostimulatory and protective effects against coccidiosis in broiler chickens</td>
</tr>
<tr>
<td></td>
<td>Sugar cane (<em>Saccharum officinarum</em>)</td>
<td>Enhancement of anticoccidial antibodies and antigen-specific cell proliferation in splenocytes via cellular and humoral immunity to <em>E. tenella</em></td>
</tr>
<tr>
<td></td>
<td>Polysaccharides (<em>Astragalus membranaceus</em> Radix, <em>Carthamus tinctorius</em>, <em>Lentinula edodes</em>, <em>Tremella fuciformis</em>)</td>
<td>Protection against <em>E. tenella</em> infection. Increase in NK cells, macrophages, CD4+ T cells, CD8 + T cells and cytokines IFN γ and IL-6.</td>
</tr>
<tr>
<td></td>
<td>Phytonutrients mixtures: VAC (carvacrol, cinnamaldehyde, <em>Capsicum oleoresin</em>).</td>
<td>Protection against <em>E. tenella</em> infection.</td>
</tr>
<tr>
<td></td>
<td>MC (<em>Capsicum oleoresin</em> and turmeric oleoresin)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lectins (<em>Fomitella fraxinea</em>)</td>
<td>Enhancement of both cellular and humoral immune response</td>
</tr>
</tbody>
</table>

Table 3. Natural compounds identified with potential to inhibit *Eimeria* life cycle and acting as immune system modulators.
4. Future vision of avian coccidiosis

The development of new treatments against avian coccidiosis is a challenge to many researchers. However, information of parasites still can be revealed using new strategies such as omics approaches. Recently, a transcriptional profile analysis of virulent and precocious strains of *E. tenella* revealed that some genes involved in carbohydrate metabolism, proteases, transporters, cell attachment proteins, and mitochondrial proteins express are upregulated in the virulent strain [38].

The vaccine development against *Eimeria* spp. also requires new approaches. Marugan-Hernández et al., [39] reported the expression of viral proteins in parasite *E. tenella*. They found that the chicken immune system recognizes the expressed viral proteins, which is a significant precedent to develop a vaccine in future, due to the chance to express antigens that allow recognition of the parasite. A study about the population structure or *E. tenella* as well as their genotype distribution and the presence of the Apical Membrane Antigen 1 (AMA1) showed that this antigen outweighs immune evasion [1].

The recent studies of *Eimeria* spp. focus on genomic, transcriptomic, and proteomic analysis, besides genetic diversity and molecular phylogenetics, which strengthens the need to explore other fields of interest [5, 40–42].

<table>
<thead>
<tr>
<th>Commercial name</th>
<th>Ingredients</th>
<th>Producer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential</td>
<td>Ricinoleic acid and alkylphenolic oil of the shell of the cashew nut (<em>Anacardium occidentale</em>)</td>
<td>Oligo Basics Agroind. Ltda,</td>
</tr>
<tr>
<td>Avihicox</td>
<td>Clove and <em>Bocconia cordata</em> extract</td>
<td>Centaur</td>
</tr>
<tr>
<td>Nutrimin</td>
<td>Apple cider vinegar</td>
<td>Chicken Lickin</td>
</tr>
<tr>
<td>Kochi free</td>
<td>Olive leaf, mustard seed, black seed, cloves, grapefruit seed extract</td>
<td>Amber Technology</td>
</tr>
<tr>
<td>Coccinon</td>
<td>Blend of plant extracts and natural compounds</td>
<td>Natural farm health</td>
</tr>
<tr>
<td>Oil of oregano</td>
<td>80% Carvacrol</td>
<td>Natural factors</td>
</tr>
<tr>
<td>Garlic granules</td>
<td>Garlic</td>
<td>Flyte so fancy</td>
</tr>
<tr>
<td>Poultry Provita</td>
<td>Probiotics and prebiotic inulin</td>
<td>Vets plus</td>
</tr>
<tr>
<td>Eimericox</td>
<td>Blend of essential oils</td>
<td>Phytosynthese/Trouw nutrition</td>
</tr>
<tr>
<td>Enteroguard</td>
<td>Garlic and cinnamon</td>
<td>Orffa</td>
</tr>
<tr>
<td>Coxynil</td>
<td><em>Allium sativum</em> Linn 15%, <em>Cinnamomum camphora</em> Nees &amp; Eberum 15%, <em>Elephantopus scaber</em> Linn 15%, <em>Valeriana wallichii</em> DC 15%, Sulfur dioxide 25% and NaCl 15%</td>
<td>Growell India</td>
</tr>
</tbody>
</table>

Table 4. Commercially available compounds of natural origin: plants and herbal extracts, fatty acids, probiotics and others).
5. Conclusions

The development of drugs to control and treat avian coccidiosis since the 1940s until now has increased significantly leading to a wide variety of products. The use of these drugs interferes with cofactor synthesis, mitochondrial functions, and cell membrane function of *Eimeria* spp. Although their use was the first alternative, today, most governments and health policies preferred meal free of antibiotics and drugs, as in most European countries.

This view guided research efforts to search for new compounds with a natural origin. Thus, plants, fungi, and bacteria were considered sources of metabolites and molecules with potential anticoccidial activity. In this chapter, we present recent information related to compounds that can be used to prevent, control, and treat avian coccidiosis. Many of them were with good results when an immune response is involved.

This disease may not be controlled or treated with the use of only one compound, on the contrary, it requires the combination of immunostimulators that induce a good response in bird and herbal extracts, essential oils and other natural compounds that can destroy *Eimeria* spp. oocysts or interfere with life cycle. In summary, avian coccidiosis control demands many shared efforts that with the advancement of omics technologies will certainly open new lines of investigation.

Author details

Rosa Estela Quiroz-Castañeda

Address all correspondence to: quiroz.rosa@inifap.gob.mx

Anaplasmosis Unit, National Center for Disciplinary Research in Veterinary Parasitology, CENID-PAVET, INIFAP, Jiutepec, Morelos, México

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