Anticoccidial Resistance In Poultry: A Review

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Abstract: Cocidiosis is an important and a major parasitic disease of poultry caused by *Eimeria* Species. The disease has a great economic impact in poultry productions partly due to resistance of the organisms to anticoccidial drugs. Cross and multiple resistance of anticocidial drugs occurred when these drugs were tested on various *Eimeria* organisms. Hence, the need to understand the status of past and present state of resistance of coccidiosis. Here in this paper review, the various attempt in the past and present to combat coccidiosis in poultry have been reported. The history of the emergence of resistance to drugs and vaccines and how detected were also discussed. Adoption of some alternatives to anticoccidial agents and economic burden of the disease were also highlighted. In conclusion, this paper have shown that there is need to further explore the possibility of developing a more viable anticocidial drugs which will be effective against all the *Eimeria* Species in order to reduce the economic impact of this disease on poultry production. The use of herbs and herbal extracts seems to be promising in the control of coccidiosis although this needs to undergo more experimental investigations to ascertain their effectiveness.
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1. Introduction

Coccidiosis is a major parasitic disease of poultry, it is an intestinal infection caused by the Apicomplexan protozoan belonging to the subclass Coccidia, family Eimeriidae and genus *Eimeria* (Finlay et al., 1993; Lillehoj & Lillehoj, 2000). There are various species of the intracellular protozoan parasites causing avian coccidiosis, these are; *Eimeria tenella*, *Eimeria necatrix*, *Eimeria brunetti*, *Eimeria praecox*, *Eimeria acervulina*, *Eimeria mitis*, and *Eimeria maxima* (Shirley, 1986). Each Specie has a particular predilection site in the chicken digestive tract. For example, *Eimeria acervulina* affects the upper part of the small intestine, *Eimeria maxima* affect the entire small intestine, and *Eimeria tenella* attacks the caecum (Fanatico, 2006).

The most common and pathogenic species that affects the poultry industry is *E. tenella* (Ayaz et al., 2003), resulting in 100% morbidity and a high mortality due to extensive damage of the digestive tract (Cook, 1988). The incidence of coccidiosis in commercial poultry can range from 5 to 70% (Du and Hu, 2004). Penetration of the intestinal villous and crypt epithelial cells by asexual (schizogony or merogony) and/or sexual (gametogony) stadia may result in clinical disease (Renaux et al., 2001).

The disease is manifested clinically by intestinal haemorrhage, malabsorption, diarrhoea, reduction of body weight gain due to inefficient feed utilization, impaired growth rate in broilers and reduced egg production in layers (Lillehoj & Lillehoj, 2000, Lillehoj et al., 2004).

2. BRIEF HISTORY OF ANTICOCCIDIALS

The economic impact of coccidiosis in poultry production cannot be over emphasied hence, this review is carried out to estimate the current status of the disease, management and control and advancement in vaccine production, also to look at the way forward in handling the disease. Sulphanilamide was discovered by Levine in 1939 (Levine, 1939) when he cured coccidiosis in chickens using the drug. This has been in use until 1948 when producers began to use preventive approach with the continuous use of sulfaquinoxaline in feed. Nitrofurazone and 3-notroroxarsone were introduced in the 1950s; early 1960s amprolium and nicarbazine came to the market. Due to emergence of resistance to the above chemicals, the ionophore-based drugs was developed and introduced in the early 1970s (Matsuda et al., 1989).

3. ECONOMIC BURDEN OF COCIDIOSIS

Coccidiosis is recognized as the parasitic disease with the greatest economic impact on poultry industries worldwide (Allen and Fetterer, 2002) due to production losses and costs for treatment or prevention (Shirley et al., 2005). The control of coccidiosis in replacement birds, which includes broiler breeders and egg producing stock, is a continuing problem. Most anticoccidial drugs cannot be given to birds in egg production yet most adult birds live in an infected environment (Long et al., 1979).

Coccidiosis is responsible for 6–10% of all broiler mortalities, and the global economic losses occur as a result of reduction in growth rate and feed conversion efficiency, (Weber, 1997; Banfield et al., 1999). The annual cost of anticoccidial drugs worldwide is estimated at about US $800 million (Williams, 1998).

4. THE EMERGENCE OF RESISTANCE

Coccidiosis is mainly controlled using chemical coccidiostats administered in feed (Shirley et al., 2005), an underdose of anticoccidial used in feeds could lead to resistance (Daugschies et al., 1998). The continuous use and misuse of anticoccidial drugs have led to the emergence of drug-resistant strains (Long, 1982; Ruff and Danforth, 1996), this limits their use, and is a problem faced with most of the anticoccidial drugs, chemicals and ionophore compounds alike (Chapman, 1986, 1997; Rotibi *et al*. 1989; Bafundo and Jeffers 1990]. During 1980s and early 1990s sulphaquinoxaline, nitrofurans and amprolium were the drugs which were commonly used for the control of poultry coccidiosis, with time there was increasing inability of these drugs to control coccidiosis with an increasing number of resistant *Eimeria* field isolates in various countries, Brazil (Kawazoe and Difabio 1994), China [Li et al, 2004], India [Panda et al., 1973; Gill and Bajwa, 1979, Yadav and Gupta, 2001), and Pakistan (Abbas et al, 2008). Isolates from different farm or geographical locations showed varying degree of resistance (Kawazoe and Difabio 1994), thus, reports available on the development of resistance in one country cannot be used as such to minimize the development of resistance and to plan for effective coccidiosis control strategies in other countries because of strain variations of coccidian species in different geographical locations and different schedules of using anticoccidials (Abbas et al., 2008). For example, Yadav and Gupta, (2001) reported absence of resistance of the used ionophores against all the field isolates of *E. tenella* of Gurgaon (north India) he suggested that this might be due to the facts that these compounds are not in use for long period in India. Also, in another work done by Kawazoe and Difabio (1994), they showed that diclazuril was effective against strains that have never been exposed to the drug, while resistance to the drug in field isolates of *Eimeria (E. acervulina, E. maxima* and *E. tenella*) following use of the drug was possible. Guo et al., (2007) reported the excellent performance of decoquinate on field and laboratory strain of *Eimeria* and its value as a broiler anticoccidial in China. Isolate of *E. tenella* resistant to nicarbazin and monensin was obviously fully sensitive to meticlorpindol plus methylbenzoquate in the battery and field trials. *E. acervulina* was insensitive to MET (Raether, 1988). Peak and Landman (2003) used Diclazuril (Clinacox†), Halofuginone (Stenorol†), Lasalocid (Avatec†), Maduramicin (Cygro†), Meticlorpindol/Methylbenzoquate (Lerbek†), Monensin (Elancoban†), Narasin (Monteban†), Nicarbazin (Nicarb†) and Salinomycin (Sacox†) in 1996, 1999 and 2001 study and reported resistance of various strain to the drugs, eventhough *Eimera acervulina* from field isolates showed no resistance against salinomycin and maduramicin. Abbas et al., (2008) reported that none of the *E. tenella* field isolates showed complete sensitivity or complete resistance to the anti-coccidials used (salinomycin, maduramicin, and clopidol). There was cross-resistance between salinomycin and maduramicin (polyether ionophore antibiotics) but not with clopidol. Resistance had been reported with clopidol but no cross resistance between clopidol and quinolone anticoccidials was reported (McLoughlin and Chute. 1973; Abbas et al., 2008). However, multiple resistance among salinomycin, maduramicin, and clopidol were shown (Abbas et al., 2008). Also there was multiple resistance among monensis, halofuginone, nicarbazin, robenidine, diclazuril and toltrazuril (Stephan et al., 1997). Previous works have also demonstrated the sensitivity of *Eimeria* isolates to maduramicin (Schenkel et al., 1984; Bedrnik et al., 1989; Yadav and Gupta, 2001; Peak and Landman 2003; Abbas et al., 2008). Yadav and Gupta, (2001), also demonstrated that Salinomycin had varying degree of efficacy ranging from good to partial resistance, indicating that its use should either be restricted or if possible be discontinued because in the near future development of resistance against this anticoccidial will be seen especially in their area. Robenidine prevents excretion of oocytes (Stephan, et al., 1997) while Monensin at concentrations of 40-50 ppm, was found to be a suitable drug to use in the rearing of replacement birds (Long, 1979).

5. DETECTION OF *EIMERIA SPP*

Different formulae have been used by different researchers to find out the sensitivity or resistance to anticoccidial drugs. The older formulae or indices used for determining sensitivity or resistance were the performance index (Morehouse and baron, 1970) and the anticoccidial index (Jeffers, 1974; Ramadan et al., 1997). In them, no importance was given to feed conversion rate (FCR), which is considered to be an important parameter in determining resistance or sensitivity to any anticoccidial drug because feed costs constitute some 70% of the cost of producing broiler chickens [Jordan and Pattison, 1998]. Stephen *et al*., (1997), developed the global index (GI), calculated on the basis of weight gain (%), feed conversion (g/g), lesion scores, oocyst index, and mortality (%), by using the formula $GI = \%WG_{NNC} - [(FM - F_{NNC}) \times 10] - (OI_M - OI_{INC}) - [(LS_M - LS_{INC}) \times 2] -$

(%mortality/2), where GI is the global index, WG is weight gain, F is the FCR, OI is the oocyst index, LS is the lesion score, M is the medicated group, NNC is the noninfected, nonmedicated control group, and INC is the infected, nonmedicated control group. In this formula, all 5 parameters—weight gain, FCR, lesion scores, oocyst scores, and mortality—have been given their due importance. Weight loss cannot be used as indices because ionophores like toltrazuril, nicarbazine and monensin observed high intestinal lesion indices side by side with excellent weight gains, thus, the degree of resistance may actually be underestimated by the GI% value for this group of anticoccidials. *Eimeria* species exhibit variable levels of pathogenicity, Species can be distinguished by oocyst morphology, pre-patent period, site of infection or minimum sporulation time, but all of these methods are labour intensive, time consuming and can be very difficult and unreliable with a mixed sample (Vrba *et al*., 2010) which prompt the development of DNA-based molecular methods (Haug et al., 2007; Vrba et al., 2010).

6. PLANTS AS AN ALTERNATIVE TO ANTICOCIDIAL AGENTS

A number of natural feed additives have shown anticoccidial activity. Among them fish oils, flaxseed oil, and whole flaxseed containing high concentrations of n-3 fatty acids (docosahexaenoic acid, eicosapentaenoic acid and linolenic acid) (Allen et al., 1996, 1998). Artemisinin isolated from the Chinese herb Artimisia annua (Allen et al., 1997). Extracts from Sophora flavescens Aiton, Pulstilla koreana, Sinomenium acutum, Ulmus macrocarpa and Quisqualis indica (Young & Noh, 2001) had anticocidial activity. Feed supplements such as g-tocopherol, the spice turmeric and curcumin (Allen et al., 1996) also have anticoccidial properties. Wahba, (2003) and Dkhil et al., (2011), reported that Treatment of mice with garlic extract significantly decreased the oocysts expulsion by about 44%; this indicates that garlic impairs the development of parasites in the host before the relatively inert oocysts are formed and finally released. The fact that garlic possesses anticoccidial activity has been also reported in rabbit coccidiosis (Toula and AL-Rawi, 2007).

Eimeria papillata infections caused a moderate inflammatory response of the liver treatment of mice with garlic extract largely prevented the E. papillata-induced histopathological changes in the liver. Anticoccidial properties was also found in the fruits of *Gleditsia japonica* Miquel var. *koraiensis* Nakai, *Melia azedarach* Linne var. *japonica* Makino and *Torilis japonica* Decandolle, full herbs of *Artemisia annua* Linne and *P. aviculare* Linne, leaves and stalks of *Artemisia asiatica* Nakai, nuts of *Quisqualis indica* Linne, the roots of *Bupleurum chinese* DC, *Inula helenium* Linne, *P. koreana* Nakai, *Sophora flavescens* Aiton and *S. japonica* Miquel, seeds of *Torreya nucifera* Siebold and Zuccarini, seeds and barks of *Ulmus macrocarpa* Hance, and trunks and roots of *Sinomenium acutum* (Youn and Noh 2001). There was milder bloody diarrhoea in the groups treated with *S. flavescens* and *S. acutum*, survival rate in the groups treated with *U. macrocarpa*, *P. koreana*, *T. japonica*, *A. asiatica* and *S. flavescens* were 20–30% more improved than those of the infected control group (Youn and Noh 2001). Also, there was decreased lesion scores, body weight gain, when birds were treated with various herbs as compared to the infected control groups (Youn and Noh, 2001). *Curcuma longa* L. (Zingiberaceae), commonly known as turmeric was reported to suppress the development of coccidiosis in chickens in diets at different concentrations (Allen et al., 1998). Abbas et al, 2010, reported milder bloody diarrhoea with an appreciable body weight gain and feed consumption rate in groups treated with ration supplemented with salinomycin sodium and 3% turmeric powder. An increased in weight gain was found when broilers were treated with coumestans from *Eclipta alba* at 120 ppm, with 80% reduction in excreted oocysts, none of the chickens showed signs of toxicity (hepatic or muscular lesions) but at 180ppm signs of toxicity were seen (Michels, et al., 2011).

The crude aqueous extracts of the stem barks of *Khaya senegalensis*, *Anona senegalensis* and *Butyrospermum paradoxum* produced anticoccidial effects mediated through substantial reduction or complete termination of oocyst production and sporulation as well as appreciation of the PCV and weight gain of *Eimeria* infected birds treated with the extracts. These effects were highest with *A. senegalensis* and *K. senegalensis*. The toxicity of these plants was reported, *Khaya senegalensis* exhibited a highest margin of safety, produced only mild clinical signs and lesions without mortality even at very high concentrations in chickens (Nwosuh et al., 2011) and in rats (Onyeyili *et al.* (2000). while *B. paradoxum* was the most toxic by both routes of administration (orally and intraperitoneally) which precludes its possible usefulness as an anticoccidial agent (Nwosuh et al., 2011).

7. VACCINES

The invasion of the *Eimeria* sporozoites to the intestinal epithelium results in inflammation, leading to initiation of the immune response and resulting in massive infiltration of macrophages, granulocytes, and lymphocytes into the lamina propria. The macrophages modulate the severity of the infection and the lymphocytes, in particular the CD^{+4} T cells, and act as inducer of an effective immune response (Lillehoj, 1994; Trout and Lillehoj, 1995; 1996; Breed et al., 1997; Jeurissen and Veldman, 2002). Various types of *E. tenella* derived antigens viz. sporulated oocyst, sporozoite, merozoite antigens among others have been tried as vaccinal candidates with various degrees of success (Rose, 1982; Karkhanis et al., 1991; Rhalem et al., 1993) and even with recombinant antigens (Danforth et al., 1989; Crane et al., 1991).

Due to difficulty and expenses involved in developing new drugs, a search for new approaches to controlling coccidiosis involving immunological, biotechnological and genetical methods were employed, of these; immunological approach is assuming more importance (Garg et al., 1999). Birds immunized with the precocious line (PEN E-281/20) were protected against challenges with homologous (E-281) or heterologous (W-79) field strains (McDonald et al., 1986; Montes et al., 1998). In rabbits it was also seen that precocious line of *Eimeria magna* was effective in conferring protection (Drouet-Viard et al., 1997). Garg et al., (1999) was able to show that chickens immunized with the antigen emulsified in Feunds Complete Adjuvant showed best protection against homologous oocyst challenge as evidenced by a reduced number of oocyst production (57.4% reduction in oocyst count), no mortality and lower mean lesion scores (+1.9). Increasing resistance to the available coccidiostats and consumer concern about residues in meat and eggs have resulted in increasing use of live vaccines containing attenuated parasite strains to control the disease (Chapman et al., 2002; Shirley et al., 2005).

The problems of controlling pathogenicity, coupled with limited shelf life of live, virulent and avirulent vaccines has prompted work towards the development of subunit vaccines against poultry coccidiosis (Chapman, 1988). The first subunit vaccine, CoxAbic®, is based on transfer of protective antibodies from immunized hens to embryos (Belli et al., 2004), one of the most intriguing features of the vaccine is its ability to cross-protect against every species of *Eimeria* tested (Wallach et al., 2008). Long, et al., 1979 reported the use of Dinitolmide at 60 ppm in the food for 20 weeks allowed some immunity to develop. Advantage of attenuated vaccines is that they have low reproductive potentials, thus avoiding crowding in the specific mucosal areas of infection and resulting in the development of optimal immunity with minimal tissue damage. It is believed that the drug-sensitive, attenuated strains and wild, native strains interbreed, reducing both virulence and drug resistance in local populations. Thus, the useful period of anticoccidial drugs could be extended by rotating their applications with live vaccines.

Immune responses induced by DNA vaccination can be enhanced by co-injection with recombinant cytokines or plasmids encoding cytokines or by co-expression of antigens with cytokines (Song et al., 2009 and Xu et al., 2008). The results showed that the DNA vaccines encoding MZ5-7 or co-expressing MZ5-7 and chicken IL-17 could induce significant higher body weight gain lower lesion scores and oocyst output. This indicated that MZ5-7 antigen could be an effective candidate to develop vaccine against *E. tenella* infection. Cytokines are major modulators of immune responses to infection, and therefore they represent natural sources of immunostimulation that could be used as adjuvants in vaccines. In the application of cytokines as adjuvants, their rapid degradations and clearances might decrease their activities (Lowenthal et al., 1999). Administering them in DNA form or in a viral or bacterial vector could overcome this problem and provide a more practical method for treating the large trail flock (Allen and Fetterer, 2002). Considering that countless coccides in different phases of its life cycle colonize the host at the same time, they cause a reaction characterized by the presence of different cellular types (macrophage, heterophyles, linphocytes) covering areas with acute and chronicle inflammatory reaction. The severe inflammatory reaction in the intestinal mucosa and the mobilization of local defense cells are important immunological reactions to *Eimeria* infection (Michels et al., 2011). The use of vaccination was associated with an increased sensitivity of *Eimeria* spp. field isolates to anticoccidial drugs (Peek, 2010).

8. MANAGEMENT AND CONTROL

Management has always been important to coccidiosis control, especially before drugs were available. Management focuses on reducing the number of coccidia to keep infection at a minimum until immunity is established. Good brooding practices involve giving birds adequate floor space and feeder/waterer space to prevent overcrowding. Disinfectants are not effective against coccidia, so sanitation focuses on good hygiene and removing infected droppings. Keep the litter dry to reduce sporulation of oocysts, remove any wet or crusted litter (Chapman, 2003; Fanatico, 2006).

9. CONCLUSION

- The issue of cross and multiple drug resistance in the treatment of coccidiosis still posses challenges, hence, the need to develop a better anticoccidial drug which all the *Eimeria* Species will be sensitive to. Also, the issue of over usage and under dosing of anticoccidials in feeds and treatment needs to be addressed in order to reduce the development of drug resistance.
- Robenidine prevents excretion of oocytes which is advantageous, indicating it to limit the spread of the disease in the flock, whereas Monensin was found to be suitable in rearing replacement birds.
- The use of herbs proved to be effective in the control of coccidiosis due to its anticoccidial properties. Also, some of the

herbs are said to have a wide margin of safety. Herbal extracts seems to have a promising future in the control of coccidiosis. Efforts should be geared towards the use of herbs and herbal extracts in the control and prevention of coccidiosis.

- Subunit vaccine has the ability to crossprotect against every specie of *Eimeria*.The combination of vaccination and anticoccidial drugs seems to be very effective in the control of coccidiosis.

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