# Coccidiosis in poultry: review on diagnosis, control, prevention and interaction with overall gut health

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Coccidiosis in poultry is still considered as one of the main diseases affecting performance of poultry reared under intensive production systems. Although a lot of research efforts have been allocated towards molecular techniques, and a lot of progress has been noted in this field, practical use of these techniques are not available today, except in the field of diagnostics, where several polymerase chain reaction (PCR) tests for chicken *Eimeria* spp. are available today albeit not yet commonly used. On the other hand, with currently available diagnostic methods such as oocyst counts and lesion scoring, an interpretation of the impact of (subclinical) coccidiosis is not easy. Another problem difficult to address with currently available tools, is the interpretation of the efficacy of an anticoccidial program. Anticoccidial sensitivity testing is the only reproducible method available today, but interpretation is far from easy. The result of all this is that, although coccidiosis is not, by some, perceived as a major problem in poultry production, economical impact of coccidiosis is most probably underestimated and optimisation of anticoccidial programmes might be advantageous to the broiler industry. In addition to this, a link between subclinical coccidiosis and bacterial enteritis complicates choosing the right tools and strategy for poultry producers. Implementing sound shuttle and rotation programs can be part of the answer in order to not only control clinical, but also subclinical coccidiosis.

**Keywords**: *Eimeria,* diagnosis, prevention, anticoccidials, vaccination, resistance, chicken

## Introduction

Coccidiosis is a disease that is caused by protozoan parasites of the genus *Eimeria,* developing within the intestine of most domestic and wild animals and birds. Seven species of *Eimeria* (*E. acervulina, E. brunetti, E. maxima, E. mitis, E. necatrix, E. praecox* and *E. tenella*) are recognized as infecting chickens. Although coccidiosis is a disease known for many years, it is still considered as the most economical important parasitic condition affecting poultry production worldwide. Based on a compartmentalized model (Williams, 1999), cost of coccidiosis in poultry in Sweden was estimated to be € 0.023 per kg live weight (Waldenstedt, 2004). Extrapolated to assess the worldwide impact of coccidiosis, and assuming 50 billion broilers of 2 kg live weight annually produced (Sørensen et al., 2006) this cost is probably more than 2.3 billion €. Noteworthy in this aspect is that the Swedish poultry production is at high standards and therefore coccidiosis is, within the Swedish poultry industry, not considered to be an important issue. Very important is the finding that almost 70 percent of this estimated cost is due to subclinical coccidiosis, by impact on weight gain and feed conversion rate. One of the reasons for these remarkable findings is probably the difficult diagnosis of subclinical coccidiosis, which prevents the industry to evaluate the best possible strategies for control of coccidiosis.

For the control of coccidiosis in chickens and turkeys, a number of preventive medications have been approved for use world-wide, but reduced sensitivity and resistance are increasingly important as no new anticoccidial compounds are known to be under development. Also live attenuated and non-attenuated vaccines are available, but next to cost reasons, the fact that live vaccines need host cells to replicate and to instigate an active immunity, cause them to result in subclinical coccidiosis and this is a disadvantage. This is associated with a diminution of performance and, in the absence of growth promoters, even attenuated vaccines are considered by many poultry producers to be associated with a higher incidence of bacterial enteritis. In spite of this, live vaccines are significant and important tools in the anticoccidial arsenal, as will be explained in this paper. New approaches such as vaccination of breeders with the goal of confer of protective maternal antibodies and recombinant vaccines are not popular in practice or not yet available. Molecular techniques such as polymerase chain reaction (PCR) methods are becoming increasingly important, but no quantitative method is available on a cost effective and large scale basis. Fundamental research on coccidiosis today is mainly focused on improving molecular techniques that might result in improved diagnostics or the development of recombinant vaccines; but hitherto molecular techniques have not solved many practical questions on what kind of prevention is adequate for a certain poultry production unit. So, although significant and promising steps have been made in describing the biology, diagnosis, epidemiology and prevention of coccidiosis, a number of issues important to the industry are not sufficiently addressed today.

The purpose of this review is to provide a brief overview and interpretation of the, for the poultry industry, most practically relevant insights on diagnosis, control, prevention and the impact of coccidiosis on the overall gut health of chicken.

## Diagnosis and drug sensitivity testing

As indicated, next to the fact *Eimeria* are very effective parasites, one of the main reasons coccidiosis is still a major problem, is the difficult diagnosis. The classical parasitological methods of diagnosis are labor intensive and therefore costly. <u>Oocyst per gram</u> (OPG) counts in faeces or litter have a poor relation with the impact of the parasite on the performance of a flock. Identification of different species based on morphology of oocysts is very challenging and requires expertise. <u>Lesion scoring</u> is an interpretation based on macroscopic visible lesions caused by *Eimeria*, usually following a scoring system from zero to four (Johnson and Reid, 1970). The individual scores for all the species are usually compiled for a certain number of birds (e.g. six) per flock resulting in a Total Mean Lesion Score (TMLS). The method is extremely labor intensive, sometimes subjective and only reliable when performed by skilled people. The correlation between lesion scores and performance is believed to be stronger than with OPG but still there is a difficult appreciation of the level of lesions towards impact on performance, especially at subclinical levels. A limitation is for instance the fact that *E. mitis*, although quite pathogenic, does not cause typical lesions and is mostly disregarded when using this method. Lesion scoring still remains the most frequently applied diagnostic method today. The seven species of *Eimeria* infecting chickens are considered not equally important. Generally, it is agreed upon that from the species recognized in broiler chickens, the most pathogenic are *E. acervulina*, *E. maxima* and *E. tenella*. The latter is, amongst broiler farmers, the best known. It infects the caeca and because of its deep development in the mucosa and subsequent wide-spread damage with distinct gross lesions and loss of blood in the faeces, it is easily recognized also by farmers. On the other hand, when performing field necropsies on a larger scale, *E. tenella* appears to be the least prevalent of the three species mentioned. Also, the damage is being limited to the caeca, relative less important parts of the gut with regard to digestion and absorption, thus effects on growth and feed conversion rate. Diagnosis of clinical disease caused by *E. tenella* is quite easy and action (therapy on the short term, change of preventive means on the long term) can be swift. These facts make its impact on the productivity of the broiler industry is relatively limited compared to the other species, although many broiler farmers associate coccidiosis only with caecal coccidiosis. This is a good example of perception not being in accordance with the facts. E. *acervulina* and *E. maxima*, both much more prevalent, are less perceived to be related with clinical coccidiosis in the field. *E. acervulina* is causing white lesions in duodenum and in heavier infections also more caudal, interfering even with the ability for *E. maxima* to develop (Mathis, 2005). *E. maxima* causes petechiae in the midgut. To assess the level of damage caused by these two species, lesion scoring can be performed. An important debate is still ongoing on what levels are to be considered clinical (and requiring treatment) and what levels are subclinical. Some consider lesions higher than 1.5 per species as indicative for clinical disease, and levels below as subclinical, not requiring treatment. *E. praecox* and *E. mitis* are not scored for and are completely disregarded using the lesion scoring method, although both species are shown to be able to cause losses through an increased feed conversion rate and in the latter case even morbidity (Gore and Long, 1982; Fitz-Coy and Edgar, 1992; Williams, 1998). Moreover, it has been demonstrated there can be a poor relation between macroscopic and microscopic lesions, emphasizing using macroscopic lesion scoring alone is not suitable to detect all economical relevant coccidiosis infections (Idris et al, 1997). It is frequently disregarded that all macroscopic, but also microscopic lesions, in fact any infection of coccidia, requires an invasion and thus destruction of host cells. This is both true when the parasitical life cycle can complete, but even so when an intervention of the immune system occurs. In the latter case not only host cells are destroyed, but also the activation of the immune system requires use of nutrients that cannot be addressed to the conversion of nutrients into meat, the ultimate goal of broiler production. As a consequence it is important to understand that any level of coccidiosis is causing a real, but difficult to quantify, loss in performance. As coccidiosis is a disease that cannot currently be eradicated, the objective of coccidiosis prevention is finding the economical optimal balance between costs of diagnosis, prevention, treatment and development of host immunity while trying to keep the subclinical loss as low as possible. It is clear that producers achieving a better balance will have a competitive advantage over other producers.

Necropsy sessions are performed in cooperation with the pharmaceutical industry in a number of countries. Basically, such systems consist of a planned, organized and benchmarked assessment of the lesion scores and gut health on poultry complex (group of farms on the same anticoccidial program) basis. A number of times per year and always at the same laboratory, preferably the same, well-trained specialists assess a significant number of poultry houses, thus improving the reproducibility compared to a field lesion scoring session. This methodology is suitable for assessing the overall efficacy of the anticoccidial program, including reduced sensitivity and resistance of drugs in use. In order to make firmer conclusions, session data are compared with historical data.

A nice overview of advances in diagnosis of coccidiosis and analysis in genetic variation in *Eimeria* is given by Morris and Gasser (2006). This review covers both biochemical and molecular methods such as multilocus enzyme electrophoresis, southern blot analysis, pulsed-field gel electrophoresis and several PCR techniques. These techniques are a major addition for scientific research and more practical applications such as establishing vaccine quality control, but unfortunately, the lack of a rapid, low-cost and especially quantitative test is preventing their broad scale use. The main application of these techniques for field diagnosticians today is the possibility of defining presence of species currently disregarded such as *E. praecox* and *E. mitis*. Still, the lack of the quantitative aspect of the techniques is preventing an accurate appreciation of different coccidial species certainly with the widespread use of ionophores that also allow some multiplication of sensitive parasites.

A very innovative technique can be found on a website (Gruber et al., 2007) and is called Coccimorph. This is a computational approach for parasite diagnosis, in this case *Eimeria* spp. from chicken and rabbit. Images from sporulated oocysts from a confirmed species were assessed on different features: curvature characterization, size and symmetry and internal structure characterization. Users can upload their digital images from unidentified oocysts and have the program identify the species concerned. This is very accessible and the low cost is a major advantage. A disadvantage is only sporulated oocysts can be identified, which limits the use of this technique to litter sample identification only.

Anticoccidial sensitivity testing (AST) is a well-known technique to try to assess resistance of a certain coccidial isolate to different anticoccidial drugs (McDougald, 1987; Chapman, 1998; Naciri et al., 2003; Peek and Landman, 2003). Although a valid method for a certain isolate, this technique is not routinely used. The main reasons are the long duration and very high cost associated with the complicated, *in vivo* character of the test. The short period of testing (usually about six days) without allowing the initially naive birds to recover from an artificially high infective dose makes interpretation of the results not easy. One way to decrease the cost is using strains originating from different houses in one AST. In this way, a worst case result for the different strains may give good information on what anticoccidials could be effectively used on a big portion of farms part of a broiler complex. By meta-analysing AST results from strains with a known drug history, a better knowledge can be obtained on how fast resistance is induced, how long it remains established in a certain coccidial population and on whether there is cross-resistance amongst drugs.

## Prevention and control of coccidiosis

There are basically two means of prevention of coccidiosis: chemoprophylaxis and vaccination. Chemoprophylaxis using so-called anticoccidial products (ACP) or anticoccidials in the ration is by far the most popular: it is estimated that 95% of the broilers produced (Chapman, 2005) receive anticoccidials. Sometimes the term 'coccidiostats' are used with regard to ACP but in reality most of the ACP currently on the market are coccidiocidal and not just static.

Generally two groups of anticoccidials are considered, ionophorous antibiotics or 'ionophores' and synthetically produced drugs, also denominated as 'chemicals'. Chemicals were the first type of drugs being used in treatment and later on in prevention of coccidiosis. In 1948, sulphaquinoxaline was the first drug administered in the feed continuously and at lower doses (Chapman, 2003, McDougald, 2003). Other chemicals followed in the years after, allowing the poultry industry to expand and upscale production. Most of the initially marketed chemicals have disappeared from the market. The main reason for this is the rapid selection for resistance in coccidia when these chemicals were used, requiring their judicious use, switching to another drug before resistance has built up. This limits the commercial potential which, in combination with increasingly high costs associated with registration of anticoccidials, explains the short life-cycles of some chemicals. There are a couple of chemicals that are marketed today, such as amprolium, nicarbazin, robenidin, diclazuril, zoalene, decoquinate, halofuginone. The fact that they are still being marketed is a demonstration of their value to the poultry industry and thus an indication of the more limited potential for resistance build-up compared to the ones which disappeared. The resistance status of chemicals can be assessed using ASTs (McDougald et al., 1987; Peek and Landman, 2003; Naciri et al., 2004). If coccidiocidal, chemicals can and are in practice often used in order to reduce the infection pressure of coccidiosis (De Gussem, 2005), in a so-called clean-up program. Clean-up programs and consequent reduced (subclinical) infection pressure is expected to have a positive impact on performance. To achieve this, chemicals are preferably used during a complete grow-out, a so-called full program. Some producers do not, in order to limit risk for resistance, use chemicals in full program, but switch from one chemical to another in the same grow-out, in a so called shuttle program. Switching after a certain grow-out from one anticoccidial to another or to a shuttle program is called rotation (Chapman, 2005). However, most popular ACPs are carboxylic true ionophores. The main reason for their popularity is the relatively limited risk for complete resistance to these products, at least compared to the risk for resistance towards chemicals. Indeed, after introduction of the first ionophore on the market, monensin, in the 1970's it is remarkable to see that these drugs are still predominant in the prevention of coccidiosis. An explanation for this slow acquisition of resistance to ionophores is the fact that they allow for some leakage of sensitive oocysts. This leads to a less stringent resistance selection than with chemicals. The mode of action of the different ionophores is similar: they facilitate cation transport across the parasitic cell membrane. This causes ionic gradient and content modifications (Gumila et al., 1996) with parasite cell death as a final consequence. Based on their cation selectivity, transport rate capacitity and structure, three classes of ionophores can be discriminated (Presmann, 1976; Westley, 1982), monovalent, monovalent glycoside and divalent ionophores. The ones registered and marketed worldwide are the monovalent ionophores monensin, salinomycin, narasin, the monovalent glycosides maduramicin and semduramicin and the divalent ionophore lasalocid. One of the main debates still ongoing amongst coccidiologists is the ability for acquiring resistance to one drug by the use of another drug, the so-called cross resistance (Chapman, 2007). Evidence of incomplete cross resistance within a certain ionophore class is illustrated by the fact that, after years of use of the monovalent ionophore monensin, resistance to narasin in United States was encountered before the product was commercially launched (Weppelman et al., 1977). Several papers indicate this cross resistance is less obvious between products of different classes, for instance between maduramicin and monovalent ionophores or between lasalocid and monovalent ionophores (McDougald, 1987; Bedrnik et al., 1989; Marien et al, 2007). The debate is of particular importance when defining rotation programs: stricto sensu rotating between one monovalent drug to another can be considered rotation, but taken into account the above described incomplete cross resistance within a class of ionophores, the relevance of this type of rotation could be questioned. Therefore, relevant or true rotation for anticoccidials could be suggested to be between classes of ionophores or chemicals. Some producers do not use rotation programs, although a majority of producers has accepted this principle as valuable in order to maintain and safeguard the efficacy of anticoccidials. Chapman (2005) pointed out that one of the reasons producers can afford limited rotation, thus working with not fully effective drugs, is the importance of immunity towards coccidiosis. This might be true when drugs are used to prevent clinical coccidiosis, but to control subclinical coccidiosis this is probably an inadequate strategy. As solid flock immunity is achieved, in built up litter conditions, only at 6 to 7 weeks of age (Chapman, 1999), subclinical levels will cause economical damage. Logically deducting, a more efficient anticoccidial will cause lower levels of subclinical coccidiosis, thus less economical damage. Subclinical damage is therefore considered by some coccidiologists today to be the most important reason for rotation programs.

Live vaccination, as indicated higher, is today less applied in broiler production. Two types of vaccines are discriminated, attenuated and virulent (Chapman et al., 2002). Attenuated vaccines lack a part of the life cycle (less asexual reproductive cycles) of the original strain they were derived from, and as a consequence have a lower reproductive and pathogenic potential. This is a major advantage towards performance of virulent coccidial vaccines, but because of the lower reproductive potential of attenuated vaccines, production costs are significantly higher. Another discrimination to be made are vaccines consisting of anticoccidial-sensitive strains and others made of more or less resistant strains. The main advantage of the live ACP sensitive vaccines is their ability to alter the level of resistance in a certain coccidial population. There are several reports on this very interesting feature of vaccines (Mathis, 2003; Chapman and McFarland, 2004; Mathis and Broussard, 2006; Peek and Landman, 2006), still many questions remain on how many consecutive grow-outs should be applied to overcome or prevent resistance to the different anticoccidials marketed. Also the stability of this resensitized populations are not well known. Still, the approach of live vaccination to optimize the efficacy of anticoccidials is very important and next to simple resting (Chapman and McFarland, 2003) of anticoccidials the only method known to help reducing the portion of resistant parasites in a given coccidial population.

For the design of anticoccidial programs, above aspects of resistance and restoration of sensitivity may be used to optimize rotation and shuttle programs. A first consideration is on the definition of shuttle and rotation programs. Strictly spoken, changing from one drug to another is enough to talk about shuttle or rotation, but in view of the cross resistance described, a more narrow definition would suggest rotation and shuttle to be more valid if switching from one class of drug to another. Indeed, no proof exists that a shuttle between two monovalent ionophores will slow down resistance development; therefore no indication exists to perform this type of shuttles. Another consideration is on giving a simple rest to anticoccidials: as proven by Chapman and McFarland (2003), resting monovalent ionophores is advantageous to the efficacy of a coccidial population towards the ionophore previously used, but cross resistance might invalidate this rest. Therefore, in order to substantially control coccidiosis, and also subclinical losses, prudent use of anticoccidials might include consolidation of ionophores from the same class in the same shuttle or to simply use full programs, and after this use of a class, rotating away, ideally to chemicals or vaccines. For practical reasons, also another class of ionophores can be considered for a next phase in a rotation program. Still a lot of research is needed to better validate these seemingly obvious ideas.

## Interaction of coccidiosis with microbial intestinal flora

Over the last years, interesting research models have been developed to study impaired gut health in the absence of growth promoters. Indeed, one of the main concerns for poultry integrations is the vast majority of flocks suffering from several degrees of gut disorders (Van Immerseel, 2004). These disorders are poorly defined, variable in etiology, severity and appearance. Nomenclature is very diverse but some popular terms to describe this condition of impaired gut health are dysbacteriosis, bacterial enteritis (BE), small intestinal bacterial overgrowth, clostridiosis and wet litter.

Signs during necropsy associated with the conditions described are

- ✓ thin, fragile, often translucent intestinal walls,
- ✓ ballooning of the gut,
- ✓ hyperaemia of the mesenteric blood vessels and blood vessels on the serosal side of the intestine,
- ✓ flaccid gut edges after incision, lack of tonus
- ✓ watery or foamy contents,
- ✓ poorly digested feed particles at the end of the gastro-intestinal tract (GIT)
- ✓ multi-coloured oily aspect of the gut contents in contact with the mucosa

During a visit in a typical affected poultry house, following are frequently encountered signs:

- ✓ wet litter, initially in patches under drinking or feeding lines where condensation is typical, in more severe cases wet litter is generalized
- ✓ greasy aspect of the wet litter
- ✓ droppings with greasy and poorly digested feed particles are common
- ✓ Initially and typically feed consumption is stalling, while water consumption shows daily increase following standards for the breed concerned, causing an increased water:feed ratio (WFR). During a typical episode of bacterial enteritis the WFR is exceeding 2 in normal ambient conditions. In a later phase, also water consumption is stalling.
- ✓ Because of wet litter, birds have dirty feathers
- ✓ Feeding and drinking activity is reduced

Because in affected animals, *Clostridium perfringens* (*Cp*) has been isolated in much larger numbers and more proximal in the GIT than in healthy birds, BE is often associated with NE, a condition also associated with *Cp*. Three predisposing factors are associated with BE: feed factors known to cause BE, (subclinical) coccidiosis and management. Most of the research models combine two of these predisposing factors namely (i) feed known to instigate BE and (ii) subclinical coccidiosis. Models usually combine the instigating feed components with a consequent coccidial challenge sometimes with an additional *Cp* challenge to exacerbate BE. Some researchers claim models to be as efficient without the additional *Cp* challenge. *Clostridium perfringens* is a very common and very abundant bacterial species in the caeca, even in normal conditions and it is assumed that *Cp* is able to relocate to more proximal locations in the gut whenever the conditions are appropriate: availability of nutrients for *Cp*. It is however not clear yet whether *Cp* is the cause or rather a consequence or indicator of BE: a lack of knowledge of the exact pathogenesis of the condition exists, or even, a lack of knowledge of the several possible pathogeneses that can lead, to a similar outcome described as BE. The well-known impairment of digestive function caused by coccidia is therefore probably a main factor in conditioning the guts for *Cp* to grow. In several models, attenuated *Eimeria* strains are used, indicating that subclinical coccidiosis is sufficient as a predisposing factor. Still, other factors impairing digestion and absorption of nutrients, such as enzymatic dysfunction, viral infections or mycotoxins are likely to be equally effective as a predisposing factor, although in practical conditions and with current knowledge, subclinical coccidiosis is believed by many to be the most important one. As a consequence, the last commonly recognized predisposing factor, poor management, is probably not so important in inducing the disease but more important in defining the degree of severity of BE and the subsequent impact on the zootechnical performance of a flock.

A very important debate is ongoing in the role that ACP have in prevention of BE. A publication on reduction of *Cp* counts in the intestinal tract (Elwinger et al., 1998)of birds medicated with narasin, a monovalent ionophore, strongly suggest the positive impact of ionophore compounds on reducing the impact of BE. This reduction of *Cp* counts is a consequence of the well known antibacterial activity of ionophorous compounds. Minimal inhibitory concentrations (MIC) for the different ionophores are to be found in several publications. However, as it is not proven that *Cp* is the main etiologic agent of BE or rather an opportunistic bacterium, no conclusions can be made whether ionophores have a direct preventive effect on BE. Maybe they simply reduce the effects of one of the consequences of BE, *Cp* proliferation. This discussion might seem not so relevant at first sight, but in practice one of the main drivers on the choice of ACP in anticoccidial programs is this presumed effect on BE. A few questions remain unanswered in rightfully assessing the role of ionophores in prevention of BE:

1. All over the world, the vast majority of anticoccidial programs consist of ionophores. As indicated by the large number of antibiotic treatments through drinking water, BE is still considered one of the main problems in poultry production. What would be the number of antibiotic treatments if ACP were used that have anticoccidial activity, equivalent to ionophore efficacy, but not the antibacterial activity? Maybe the number of treatments would be higher in absence of the antibacterial activity exerted by the ionophores, but yet there is neither clear evidence nor numbers.
2. As (subclinical) coccidial infections are known to be a predisposing factor of BE, what would be the number of treatments if an ACP existed that had no antibacterial activity but was able to very effectively suppress coccidial infections? Some of the chemicals would apply to this category of ACP.
3. Even if assuming *Cp* is a cause and not a consequence of BE, are the differences in *Cp* MIC for the different ionophores relevant? As all ionophores are used at concentrations in the gut that approach or largely exceed *Cp* MIC, there is no inference or even an indication that this is a valid hypothesis.
4. Is, when using the same ionophores because of the (maybe perceived) important role in prevention of BE, there a risk of installing a vicious circle through overuse of ionophores, reduced anticoccidial sensitivity (not resistance), higher coccidial challenge thus a more prominent role of coccidiosis as predisposing factor for BE?

## Conclusion

Although coccidiosis has been the subject of a lot of research over the last decades, a number of very significant questions remain unanswered. As poultry production is subject to continuous changes, also the problems related to coccidiosis change over the years. A lot is to be expected from recent progress made with molecular techniques, but practical applications of these techniques are scarce today. The industry has to rely on established techniques in order to diagnose coccidiosis, and these techniques still bring added value if used in a correct way. The assessment of efficacy of different ACP is important in order to optimize anticoccidial programs, not only to limit cases of clinical coccidiosis, but mainly to reduce the impact of subclinical coccidiosis, estimated to be a lot more costly to the poultry industry. An important debate is ongoing on how rotation programs can be made as efficient as possible. Live vaccines could have an interesting role to boost the efficacy of ACP. As bacterial enteritis is today one of the most important problems affecting the performance of the poultry industry, the role of coccidiosis in this multi-factorial condition is to be carefully assessed. Anticoccidials could have a beneficial effects by reducing the impact of BE, but if these effects are due to direct antibacterial, or indirect by the effects on coccidia, remains to be further investigated.

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