Coccidiosis in Poultry: An Overview

Introduction

Coccidiosis is one of the most important diseases of poultry caused by protozoa, *Eimeria* and is generally characterized by bloody diarrhea, the primary symptom and high mortality. The bloody diarrhea is mainly due to intestinal epithelium dying off since a large number of oocysts and merozoites burst out of the cells. The disease spreads from one bird to another by contact with infected feces or ingestion of infected tissue. Thus it is a major problem in intensively reared poultry. Despite recent advances in control and treatment, the disease remains one of the most expensive and common disease of poultry worldwide. In India, coccidiosis is a serious problem and is one of the biggest causes of economic losses in poultry. It inflicts heavy mortality each year mainly in broilers and also in grower raised on deep litter by impairing the growth and feed utilization of infected birds. The comparison of economic traits revealed the maximum loss due to reduced body weight gain, followed by increased FCR and chemoprophylaxis. Control of coccidiosis by the poultry industry is dominated by prophylactic chemotherapy but drug resistance is a serious problem. Because of the complexity of the host immunity and the parasite life cycle, a comprehensive understanding of host-parasite interactions and protective immune mechanisms becomes necessary for successful prevention and control practices.

Etiology and Predisposing Factors

Coccidian is cosmopolitan and ubiquitous in distribution. Seven species of *Eimeria viz, E. tenella, E. necatrix, E. brunetti, E. maxima, E. acervulina, E. mitis* and *E. praecox,* infect chickens in order of their pathogenicity. *Eimeria acervulina, E. Brunetti, E. maxima, E. necatrix* and *E. tenalla* produce moderate to severe intestinal or caecal lesions, whereas *E. praecox* and *E. mitis* do not. *Eimeria acervulina* infects primarily the duodenum and upper jejunum. *Eimeria brunetti* moves down the intestine during infection with the most damage being caused to the lower ileum, colon and proximal areas of the caeca. *Eimeria maxima* and *E. necatrix* infect the mid-gut, from the lower duodenum to the mid-ileum; however, *E. necatrix* oocysts develop in the caeca and *E. tenella* infects primarily the caeca. Prepatent periods range from 4 days for *E. acervulina* to 5.5 days for *E. necatrix* with the other species falling in between. Patent periods generally range from 4 to 6 days. Subclinical infection is thought to result in heavy economic loss by impact on weight gain and FCR.

The predisposing factors includes-

- Birds affected with immunosuppressive diseases like chicken infectious anaemia (CIA), Marek's disease (MD) and infectious bursal disease (IBD).
- Dampness and bad quality of litter.
- Defective feeders and drinkers, which allows birds to enter into the utensils and defecate.
- Overcrowding of birds.
- Intermittent lighting, which increases the risk as compared with continuous lighting.
- Infrequent change and turning of litter increase the concentration of coccidial oocysts.
- Anticoccidial drugs if used over a long time may lead to emergence of drug resistant population of coccidian.

The Disease

Chickens of all ages and breeds are susceptible to coccidiosis. But it is not largely a disease of young birds because immunity quickly develops after exposure and gives protection against later outbreaks. It usually affects chicks below 10 weeks of age with maximum incidence in 3-6 weeks old chicks. It is rarely seen in birds less than 3 weeks of age unless they are brooded on contaminated litter. Oocysts are not infective until they sporulate under optimum condition of warmth (21-32°C) with adequate moisture and oxygen, and this requires 1-2 days, which they gets on the ground, soil or litter. Sporulated oocysts (infectious form of coccidian) may survive for long periods, depending on the environmental factors and are protected by the thick oocyst wall, and are therefore, able to survive for months or even years. Due to the short prepatent period and high biotic potential, the number of

oocysts in the litter rises rapidly on arrival of susceptible new flock of birds. Coccidia are almost invariably present in poultry rearing operation but clinical disease occurs only after ingestion of relatively large numbers of sporulated oocysts by susceptible birds. Both clinically infected and recovered birds shed oocysts in their droppings, which contaminate feed, water, litter and soil. Threat of coccidiosis is greater in cooler wet weather (rainy and winter seasons) and is lesser during hot dry weather (summer).

The disease is transmitted through horizontal course. Transmission of oocysts may takes place by shoes, feed, trucks, crates, pets, rodents, and moving equipments. Consumption of as few as 10,000 sporulated oocysts will produce coccidiosis. Birds pick up the infection through ingestion of oocyst contaminated feeds and drinking water. Oocysts remain viable in litter for many months. In this way they can contaminate a farm from year to year. These are able to tolerate most common disinfectants. Oocysts are killed by freezing, extreme dryness and high temperature above 56°C and desiccation (drying) can kill them.

Shortly following infection, sporozoites can be seen invading the cells of intestinal (or caecal) surface epithelium and development of some species occurs here *(E. brunetti* and *E. praecox).* Other species develop in the epithelium of the crypts *(E. acervulina, E. maxima, E. necatrix, E. tenella). Eimeria* primarily infect the intestine of the susceptible host and cause damage, resulting in dehydration, blood loss and increased susceptibility to other infectious agents. Microscopic examinations of lesion scrapings show numerous gametocytes and oocysts. Diarrhoea, severe weight loss, poor feed conversion and loss of skin pigmentation are the common signs. During severe infection of *E. maxima*, numerous petechiae appear on the serosal surface of the intestine and the walls get thicken; ballooning of the intestine may occur.

The disease appears in two forms i.e. caecal and intestinal form. Caecal form is generally observed in young chicks whereas adult birds usually suffer from intestinal form of coccidiosis. Clinical signs in young chicks include depression and droopiness, anorexia, huddling together, having ruffled feathers, watery diarrhea and by the fourth day blood begins to appear in the droppings. The greatest amount of blood appears by day five or six and by the eighth or ninth day the bird is either dead or on the way to recovery. Severe haemorrhagic diarrhoea may produce anemia. Comb and wattles become pale. Mortality is highest between the fourth and sixth days. Birds that recover may develop a chronic illness as a result of a persistent caecal core. However, the core usually detaches itself by eight to ten days and is shed in the droppings. Clinical signs in adult birds include gradual loss of appetite, ruffled feathers, progressive emaciation and birds disincline to move, comb and wattles become pale. Chocolate colored droppings are characteristic.

Immunity

Eimeria species have a good immunizing power. This immunity is species specific. There is no cross immunity (cross protection) between *Eimeria* species and later outbreaks may be the result of different species. For example, immunity against *E. tenella* does not give complete protection against *E. maxima* and so on. It is easier to produce chickens resistant to the effects of the disease than to produce chickens absolutely resistant to infection. The duration of immunity in the complete absence of reinfection is relatively short, depending upon species of *Eimeria* and it is probably in the order of 6-7 weeks. Immunity is not important in broiler chickens, which are kept only for 6-8 weeks before marketing, as in layers, which are kept much longer. Day-old chicks usually do not get protective maternal antibodies from the hen. Therefore, birds of any age are susceptible to coccidiosis. Most chicks get infected in the first few weeks of life, which produces a good immunity, and it may persist for life due to frequent low-grade re-exposure to infections. However, in the absence of re-infection, immunity may decrease gradually. Immunity is best produced by repeated exposure to low numbers of oocysts (Trickle infection). Immunity leads to a reduction in tissue damage and marked decrease in oocyst output. The mechanisms of immunity are not fully understood, but cell-mediated immune response appears to be extremely important whereas humoral immunity plays a very minor role in resistance against infection. By contrast, some studies have pointed towards the ability of antibody to block parasite invasion, development and transmission and to provide passive and maternal immunity against challenge infection. Secretory IgA may also contribute to protective immunity, but circulating antibodies (IgM, IgG) play only a minor role.

Diagnosis

The diagnosis is not very difficult and is usually depends on clinical signs, gross lesions and microscopic examination. Faecal or intestinal/caecal scrapping should be made using microscopic slides so as to demonstrate oocyst. Sometimes at farm no mortality due to coccidiosis is seen for 2-3 days but weight loss is apparent, in those cases, faecal samples should be examined for oocyst by microscopy. The presence of faeces with blood, dysentery and diarrhea suggests coccidiosis. However, with less harmful coccidian, the only symptoms are reduced growth and poor feed conversion. The earliest symptoms with the most harmful species may be a sudden increase in daily mortality. For confirmatory diagnosis, postmortem examination is necessary. The type and location of lesions in the gut indicates the species of *Eimeria*. *E. acervulina* affects the upper part of the small intestine. Affected parts of the intestine are grossly distended and small red spots and white bands may be noted on it. *E. maxima* affect the entire small intestine. The intestines look watery, and in later stages have blood and mucus. The intestine may look thickened and ballooned with red pinpoint lesions. *E. tenella* affects the blind sacs (ceca) of the gut. Haemorrhagic lesions may be noted in the wall of the caecum. Caecum may be filled with blood and pus and turn into a solid core. Recently, Polymerase Chain Reaction (PCR) based assay for the detection, identification and differentiation of pathogenic species of *Eimeria* in poultry is found effective. An attractive genomic DNA target for PCR based detection of *Eimeria* is the Internal Transcribed Spacer 1 (ITS 1) gene of ribosomal DNA (rDNA). Real-time PCR assay has also been developed for diagnosis of different species of coccidia. This real-time PCR mainly targets one genetic marker called ITS-2 and can be multiplexed in pairs such that the seven species of *Eimeria* can be screened at a time using species-specific probes.

Treatment

There are basically two means of prevention of coccidiosis; chemoprophylaxis and vaccination. Using anticoccidials or coccidiostats in the ration is the most popular. It is estimated that 95% of the broilers produced recieve anticoccidials. These anticoccidials can be classified as (1) chemicals which have specific modes of action against parasite metabolism such as amprolium, clopidol decoquinate, halofuginone, diclazuril, toltazuril or (ii) polyerther ionophores such as monensin, losalocid, salinomycin, narasin, and maduramycin, which act through altering ion transport and disrupting osmotic balance. The use of toltrazuril as the sole anticoccidial for two consecutive days in the drinking water between 10 and 14 days of age would be the best method for coccidiosis control. Combinations of anticoccidial such as salinomysin and roxarsone with a digestive enhancer such as bacitracin are widely used in broilers. Acute infection of coccidia is generally treated with anticoccidials, but the economic losses are mainly because of subacute infection. Prophylactic use is preferred because most of the damage occurs before signs become apparent, and delayed treatment may not benefit the entire flock. Only a few of the prophylactic drugs are also effective therapeutically. Usually, coccidiosis is controlled by prophylactic in-feed medication of an anticoccidial drug on continuous basis in broilers and upto 16-18 weeks in layers. Water medication is generally preferred over feed medication for treatment. Antibiotics and increased levels of vitamins A and K are sometimes used in the ration to improve rate of recovery and prevent secondary infections. Continuous use of anticoccidial drugs may result in selection for and survival of drug-resistant strains of coccidia. Drug resistance is a genetic phenomenon, and once established in a line of coccidian remains for many years. To overcome this problem, shuttle programme and frequent rotation of drugs may be practised.

The use of one anticoccidial in the starter and another in the grower ration is called a shuttle programme which is meant to improve coccidiosis control, for example, intensive use of the ionophore drugs for many years has produced strains of coccidia that have reduced sensitivity for the ionophores. It is a common practice to use another drug such as nicarbazin or halofuginone in either the starter or grower ration to strengthen the programme of control against coccidiosis. The use of shuttle programme has been found to reduce drug resistance. Rotation of drugs may improve productivity. This is because the new drug will be effective against the build up of coccidia that had reduced sensitivity against the previous product that had been used for a long time. Anticoccidial drugs cause growth depression in the absence of *Eimeria* infection. Moreover, when the

anticipated drug effect is not observed the user tends to increase the dosage, which invariable leads to toxicity and is reflected by reduced feed intake, growth depression, reduced egg production, and leg weakness. Ionophores are not recommended in turkeys as these are highly susceptible to ionophores toxicity.

Prevention and Control

Coccidiosis can be controlled and treated well if adequate measures are taken timely. Coccidial vaccines have been developed with an objective to solve the problems faced by the use of drugs. The immunological control is recognized as the major practical alternative to chemotherapy for the control of coccidiosis. Advantage of vaccine is, it can repopulate drug sensitive *Eimeria* spp. in a particular locality, and vaccines leave no residues in the meat. The live vaccines have been found to be very effective which administered at day old age, as here booster is not required. The first commercial anticoccidial vaccine, CocciVac (Alabama, USA), was introduced to the US market in 1952 and was a live unattenuated vaccine comprizing several wild-type strains of *E. tenella* oocysts. Since it was including only one species of *Eimeria*, it would not protect flocks from other species. Therefore, the vaccine went through a number of reformulations over the past 50 years. Thus variants of the original product – Coccivac-B, Coccivac-D and Immucox (Ontario, Canada) – are still in use today. However, the use of these live unattenuated vaccines is limited somewhat by the pathogenicity of the parasites used in making of these vaccines. Thus attenuated strains are now, arguably, the preferred products.

Unattenuated vaccines comprise mixtures of wild type strains of *Eimeria*. The number of oocysts in these vaccines are calculated and at correct dose, pathogenic effects should not been observed. Attenuated vaccines comprise mixtures of strains that have been selected so that they have reduced or no pathogenicity. There is general agreement that the potentially pathogenic species *E. acervulina*, *E. maxima* and *E. tenella* should be present. It is therefore possible to provide protection to all, or a selection of the species that might be encountered in the field. How many species should be included in a vaccine depends upon an assessment of their importance and the risk of exposure to them. Because, breeders have long life span than broilers, it is likely that they will be exposed to more species of *Eimeria*. Therefore, vaccines for breeding stock should contain all the *Eimeria* species while fewer may be necessary in broilers.

A subunit vaccine comprising a purified protein isolated from gametocytes of *Eimeria* is apparently near to launch in Israel. It is the first developed subunit vaccine and comprises of affinity purified gametocyte antigen (AGPA). Trials results indicate that there is transfer of maternal immunity and cross protection. Using antigens of interest of various stages of *Eimeria* life cycle, sub unit vaccines are made which may be administered as such or through a vector. Advantages with subunit vaccine includes, can be produced on mass scale, and don't produce clinical illness and cross species immunity. A variety of recombinant antigens of potential use as vaccine like GX3262 of *E. tenella* and GM28 of *E. acervulina* expressed in *E. coli* used to vaccinate laying hens, broiler breeders, just prior to the start of their laying period, produce protection through maternal antibodies in their progeny chicks for 6-7 weeks and against other species like *E. tenella* and *E. acervulina* too. These antibodies have been reported to interfere with oocyst wall formation in the parasite.

As the immunity to coccidian is species specific, it is recommended to include in a vaccine all the *Eimeria* species for which immunity is desired, depend on the geographical area. Moreover, due to the possible strain variation, within the *Eimeria* species (*E. maxima*, *E. acervulina*) a vaccine developed for one geographical region should be tested for protection against the local isolates in another area. All the seven *Eimeria* species have been reported from different geographic regions of India, from time to time in commercial broilers and layers. Methods of control that rely upon immunity are likely to be more important in future. Certain vaccines have been used mainly in parent breeder flock but not in commercial broiler layer flocks due to its cost factor.

Vaccination methods include administration of vaccine through drinking water, in feed, edible gel ingestion, intra ocular, eye spray (by spray cabinet or individually by eye spray) and *in ovo*. *In ovo*-administration of *E. tenella* oocysts into broiler chick embryos after 18 days incubation have been reported to provoke good immunity. In eye Spray cabinet technique, a coloured dye is added to the

vaccine in a spray cabinet, and a predetermined volume of vaccine is automatically sprayed onto the birds and, they get infected by pecking at the coloured dye on the feathers. Other preventive measures comprises-

- Nutrition, in which diets rich in protein and essential amino acids along with additional vitamin E and zinc may help in maintaining a strong immune response.
- Use good quality coccidiostats judiciously.
- Meticulous usage of anticoccidial drugs.
- Adopting better management and husbandry practices.
- Good sanitation and hygiene.
- Prevention from immunosuppressive agents (MD, IBD, CIA).
- Avoid indiscriminate use of live hot IBD vaccines.
- Strict biosecurity measures.

Sanitation and hygiene

It is necessary to maintain good hygiene and sanitation in the farm as disinfectants are not effective against coccidia. So, some points should be considered to maintain good hygiene:

- Litter should be replaced and poultry houses disinfected on a regular basis.
- Caked material beneath drinkers should be removed and a top dressing of new litter applied.
- Drinkers should be cleaned and sanitized.
- Put waterers and feeders at a height level with the backs of the birds, so they cannot defecate or scratch litter into them.
- Clean the pens and remove droppings regularly.
- Prevent the access of infected droppings to the non-infected birds.
- Keep older birds away from chicks, since old birds are carriers.
- Avoid moisture and humidity in litters.
- Keep the litter dry by frequent turning of litter to reduce the sporulation of the oocysts.
- Avoid over-crowding in the house.

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 scare 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. Anticoccidial vaccines, especially live attenuated vaccines, appear to be a viable alternative for drugs in control of coccidiosis in breeder flocks and commercial egg layers. Investigations should be carried out for interactions between anticoccidial drugs and vaccines and to develop the suitable means of control of coccidiosis. Due to acquired resistance many drugs are less effective than when they were first introduced. Methods of control that rely upon immunity are therefore, likely to be more important in the future. Of course, biosecurity should never be breached for profitable poultry farming. In future, coccidiosis should be controlled by adopting an integrate approach where drugs and vaccines are both used to prevent this disease. Induction of protective immunity by active immunization with antigens not involved in natural infection may also be possible as indicated by recent immunization studies using recombinant antigens. Immunity is an important consideration in broiler breeders and commercial egg laying flocks that are reared on the ground. Outbreaks of coccidiosis have occasionally been reported among laying hens on wire floors or cages, suggesting that immunity is desired in these birds as well. Antigenic variation between strains of a given species has been reported (*E. maxima*). Thus it is suggested that the vaccines be reformulated to include such strains. So before, using a vaccine developed from 'foreign' strains it is clearly desirable to carry out cross protection tests to demonstrate efficacy against parasite of local origin. The use of live vaccines (particularly those based on non-attenuated strains) could result in the introduction into the environment of species not already

present in a poultry farm. Use of vaccines with a limited number of species is an option if the introduction of species is of concern.