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COCCIDIOSIS IN POULTRY INDUSTRY*

Lilić S., Ilić Tamara, Dimitrijević Sanda

A b s t r a c t: Coccidiosis is a permanent health problem in poultry industry especially in intensive production systems. It is the most important poultry disease as far as economy is concerned since yearly costs of prophylaxis, as well as of therapy exceed 2 billion Euros, at the global level. In Serbia the disease has the highest prevalence in chicken, less in turkeys, gees, ducks and pheasants. The causes of the infection are protozoa belonging to the Eimeridae family, spore oocists being the infective form. The source of the infection are already infected birds, whereas the disease can spread in the sussceptible bird population by direct and indirect contact such as dust, objects on the farm, people, rodents, wild birds, as well as insects. The incidence of the disease depends on the lack of space on the farm, high temperature and high relative humidity, improper feeding, other diseases and all factors that can compromise bird immunity and general resistance to infectious diseases. Coccidiosis is the disease of the spring and fall, i.e. humid seasons with plenty of rain. The parasite development takes place in epithelial cells of the intestine of all bird species. The parasite can develop also in epithelial cells of the kidney glomerully in gees whereas merozoits and shizonts (as a developing form of the parasite) cause severe lesions and desquamation of the mucus. Local symptoms are accompanied with general health disturbance and typical diarrhea which is the characteristic symptom. Diagnosis is on the basis of the general symptoms, gross and microscopic findings as well as feces sample testing. To control coccidiosis in poultry, there is a vaccine or the disease is controlled by anticoccidials in the feed. Coccidiosis is possible to treat with anticoccidials (coccidiostatics and coccidiocides). Economical consequences of the coccidiosis in poultry are decreased feed conversion, smaller weight gain, inadequate feed conversion, smaller body weight at the end of the fattening period, prolonged fattening period, as well as therapy costs.

Body weight gain is reduced, as well as accumulation of abdominal fat. The disease has a negative impact on chemical and sensory meat appearance. One of the problems as far as coccidiosis is concerned is drug resistance. Today, coccidiosis control strategies are the „shuttle" and „switch" program of the prophylactic medication, good manufacturing praxis and proper sanitation.

***Key words**: coccidiosis, poultry, economical impact.*

Kokcidioza u proizvodnji živine

S a d r ž a j: Kokcidioza je oboljenje koje predstavlja stalan zdravstveni problem, naročito u uslovima intenzivnog uzgoja živine. Najznačajnija je bolest živine u ekonomskom pogledu, jer godišnji troškovi za profilaksu i terapiju kokcidioze prevazilaze dve milijarde eura na globalnom nivou. U našoj zemlji najzastupljenija je kod kokošaka, a ređe se javlja kod ćuraka, gusaka, pataka i fazana. Uzročnici oboljenja su protozoe iz familije Eimeridae, a infektivni oblik predstavljaju sporulisane oociste. Izvor infekcije su inficirane jedinke, a bolest se prenosi direktnim i indirektnim kontaktom – pribor, oprema, prašina, ljudi, glodari, divlje ptice i insekti. Na rasprostranjenost bolesti utiču: nedovoljan prostor, visoka temperatura i relativna vlažnost vazduha, neadekvatna ishrana, pojava drugih oboljenja i svi faktori koji smanjuju otpornost organizma. Najčešće se javlja u proleće i jesen, odnosno u kišnim periodima. Razvoj parazita odigrava se u epitelnim ćelijama creva svih ptica, odnosno epitelu bubrežnih kanalića gusaka, a razvojni oblici (merozoiti i šizonti) dovode do teških oštećenja i deskvamacije sluznice, praćenih promenom opšteg stanja i karakterističnim prolivom. Dijagnoza oboljenja postavlja se na osnovu kliničke slike, koprološkog, patomorfološkog i patohistološkog nalaza. Profilaksa oboljenja sprovodi se vakcinacijom ili primenom antikokcidijala u smešama za ishranu, dok se terapija sprovodi antikokcidijalima, koji mogu biti kokcidiostatici i kokcidiocidi. Ekonomski gubici ogledaju se u povećanom utrošku hrane, smanjenom prirastu, nižoj konverziji hrane, manjoj prosečnoj telesnoj masi na kraju tova, produženom trajanju tova i troškovima lečenja.

Prinos trupova i deponovanje abdominalnog masnog tkiva su manji, a oboljenje negativno utiče i na hemijske i senzorne parametre kvaliteta mesa. Problem u suzbijanju oboljenja je brz razvoj rezistencije na lekove, a kontrolne strategije u suzbijanju kokcidioze su „shuttle" i „switch" program profilaktičke medikacije, dobra proizvođačka praksa i sanitacija.

***Ključne reči:** kokcidioza, živina, ekonomski aspekti*

Poultry coccidiosis

Coccidiosis is a parasitic disease that is a constant health problem, especially in intensive poultry industry. It is the most important infectious poultry disease, as far as economy is concerned. Coccidiosis is a global disease and costs on yearly basis, for prophilaxis, as well as therapy exceed two billion Euros (*Dallouil and Lillehoj*, 2006).

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AUTHORS: Slobodan Lilić, slobo@inmesbgd.com, Institute of Meat Hygiene and Technology, Belgrade, Serbia; Tamara Ilić, Sanda Dimitrijević, Faculty of Veterinary Medicine, Belgrade, Serbia
AUTORI: Slobodan Lilić, slobo@inmesbgd.com, Institut za higijenu i tehnologiju mesa, Beograd, Srbija; Tamara Ilić, Sanda Dimitrijević, Fakultet veterinarske medicine, Beograd, Srbija.

Several domestic species are susceptible, however concerning the incidence, as well as economic consequences, coccidiosis is most important in poultry, rabbits, ruminants, carnivores and less in swine. In Serbia, coccidiosis is most important in the chicken industry, less in turkey, gees, ducks and pheasants.

Causative agent of the disease belong to phylum *Apicomplexa*, class *Sporozoa*, subclass *Coccidia*, ordo *Eucoccidia*, suborder *Eimerinae*, and family *Eimeridae* that has two ordo: *Eimeria* and *Tyzzeria*. Depending on the localization, disease in poultry has two forms: coccidiosis of the caecum that is caused by *Eimeria tenella* and intestinal coccidiosis that is caused by a number of parasites: *E. necatrix, E. acervulina, E. maxima, E. brunetti, E. mitis, E. mivati, E. praecox* and *E. hagani*. Coccidiosis of turkeys is caused by: *E. adenoides, E. meleagrimitis, E. gallopavonis, E. dispersa, E. inocua, E. meleagridis* and *E. subrotunda*. In gees the disease can be in the form of renal infection *E. truncata* and intestinal coccidiosis: *E. anseris*, *E. nocens*, *E. parvula* and *E. stigmosa*. Duck coccidiosis is caused by *Tyzzeria perniciosa* however, *E. anatis* and *E. danailovi* can also cause the disease. In pheasants, coccidiosis is caused by *E. dispersa, E. phasiani, E. langeroni, E. pacifica, E. megalostomata, E. gennaeuscus, E. duodenalis, E. colchici, E. picta* and *E. tetartooimia*.

Epidemiology

The source of the infection varies and depends on the technology in the poultry industry. In the case of extensive poultry farming the source of infection is one bird. In case of intensive production the source of infection is the old bird population (*Hammond and Long***,** 1973). In flock, disease is spreading by direct, as well as indirect contact (*Williams*, 2002). Oocysts that are infectious could be distributed by equipment, dust, people, rodents, wild birds as well as insects (*Dimitrijević and Ilić*, 2003). *Coleoptera* spp, which are usually present in the broiler population, can serve as mechanical vectors (*Calnek,* 1997).

Distribution and prevalence is influenced by several factors: high animal density cramped ona small space, high air temperature, high relative humidity, different (especially different age) categories of birds at same place, feed change, quality of feed, as well as all other factors that compromise resistance to the disease and general health status of the birds (*Calnek,* 1997). Onset of the disease depends on the age of the bird at the time of the first infection and number of passages of the infect (for one passage to be completed it is required 10 days), as well as on ability of the bird to develop proper specific immune response (*Hofstad,* 1984; *Ilić et al.,* 2003).

The highest incidence of coccidiosis is during spring and fall, especially when weather is cold and humid (rain). The incidence is significantly smaller during hot and dry weather conditions (*Maungyai et al,* 1990; *Calnek,* 1997; *Razmi and Kalideri*, 2000). The intensity of the infection depends on the number of oocists that are ingested and the immune status of the bird (*Hofstad*, 1984). In case of release of the chicken on to the floor that was used for the previous flock. *Šibalić and Cvetković* (1996), reported the acute form of the coecal coccidiosis and mortality as soon as in eight days old chicken.

Infection of young chicken can not be avoided in intensive production systems, whatever prophylactic measures have been taken. So, infection takes place in the first weeks of life. Intensive poultry production systems, high density of totally susceptible birds and many passages of the causative agent in the new bird generation, pose almost ideal circumstances for infection to persist and spread within the flock (*Jordan,* 1990). To heavy load of infectious oocists on the floor is one of the most important prerequisite conditions for infection to persist in the flock (*Hofstad*, 1984).

Clinical disease can be prevented by continuous adding of the anticoccidials in feed. However, persistence of the sub clinical disease is always a possibility. According to some authors (*Braunis,* 1980; *Razmi and Kalideri*, 2000), sub clinical forms of the disease depend on the size of the flock. Prevalence of the sub acute form of the disease is significantly higher in flocks with more than 40,000 birds in comparison to flocks with less of 10,000 birds. The subclinical form of the disease is most frequent in six weeks old chicken and infection occurs in nearly all flocks (*Jordan and Pattison*, 1996). *Voeten* (1987) showed that sub clinical coccidiosis is most prominent from four to six week old chicken in the case if anticoccidials are not added to the feed.

Pathogenesis

The nfectious form of the causative agent are oocists in the form of spores. Infection is by oral route, with contaminated feed and/or water. After ingestion, infectious oocysts excist, liberating the infective form: the sporozoit. Sporozoit infect epithelial cells of the intestine and kidney epithelial cells. Transfer of the sporozoits up to the locus of the primary lesion is with the help of intraepithelial lymphocytes (*Lawn and Rose*, 1982; *Daszak,* 1999). The pathogenic process starts during shizogonic phase of the parasite development. The pathogenic

process during the first generation of shizonts is negligible. However, the most pathological stadium is during the second generation of shizonts. Their development, deep in the cells of Lüberkinii glands, results in inflammation, mucus desquamation, capillary rupture and haemorrhagiae. This stadium of the disease is accompanied with severe clinical symptoms. In this stadium, possible outcome could be death of the bird. Death is a consequence of haemorrhagiae (bird can loose 60 to 80 percent of the blood volume), toxemia or as a consequence of gangrene or rupture of the intestinal wall.

During coccidiosis, there can be other infections such as reovirus infection, Marek disease, New Castle virus infection and infectious bronchitis virus infection. In such a case, symptoms are mixed depending on causative agents (*Ruff*, 1991). Especially in Nordic countries, there are mixed infections with *Eimeria* spp, *Cl. perfringens* or *E. coli*. This is because the use of antibiotics is banned (*Van Der StroomandVan der Sluis,* 1999).

Endogenous development of renal coccidiosis in gees takes place in tubules of the kidney. As a result, there is desquamation of the epithelia, obstruction and dilatation of the tubuli by mature gamonts. Kidneys are enlarged, there are urate salts deposits in the urinary tract, as well as kidney failure.

Developmental cycle of the parasite

All coccidia develop in same way. There are two phases: endogenous and exogenous. *Vide infra* is the infectious cycle of the *E. tenella* which is highly pathogenic and the most prevalent in our region.

The endogenous phase is in the animal (bird) and there are two sub-phases: shizogonia (nonsexual sub phase) and gametogonia. Shizogonia is characterized by producing one after another generations of shizonts that carry merozoits as the infectious form of the parasite (*Soulsby and Rose*, 1972). During the sexual sub-phase (gametogonia), oocysts form that are responsible for further infection spreading. Exogenic phase take place out of the bird. During this phase, oocysts sporulate (sporogonia).

One to two hours (*Lawn and Rose,* 1982), after ingestion of the oocysts they excyst as the wall of the oocysts ruptures and releases sporocysts. From oocysts, by further degradation, release of the sporozoits occurs. Sporozoits attack the surface of the caecal epithelium (*Patillo,* 1959; *Davies et al*., 1963), penetrate the basal membrane and enter the *lamina propria mucosae* whether free or inside the macrophages. Finally, they attack epithelial cells that cover the bottom of the Lüberkinii cripts (*Lillehoj and Trout*, 1993).

In most cases, from the second generation of merozoits microgametocyte develop, as well as makrogametocites. Sexual phase of the parasite development, takes place in the cells of the mucus and sub mucus. That phase starts from 6th day of the infection (*Pellerdi,* 1974). Microgametocytes (12,4 x 8,7 μm) (*Tyzzer,* 1929), enlarge and undergo through a number of divisions resulting in microgamete development (*Davies et al,* 1963)**.** Microgamets are mobile, fusiform in the shape approximately 5 μm long with three active flagella evenly distributed on one end of the cell (*Joyner and Kendall*, 1963).

Macrogametes are as big as oocysts. During growth they transform into the macrogamete. Macrogamete have granular cytoplasm and centrally placed nucleus (*Pellerdy*, 1974). When micro and macrogametes join they form zygote. After the fertilization phase, the macrogamete mucoproteinaceous granule that is placed on the periphery of the cell, form the outer membrane of the zygote. From that form, nonporous oocyst develops.

Once the cyst wall is formed completely the oocyst leaves the host through feces. Prepatent period is the time from the start of the infection up to the moment when first oocyst could be found in feces. This period of time is unique for the species and in case of *E. tenella,* it is up to 6 to 7 days *(Pellerdy,* 1974). The maximal number of oocysts in feces is at 10th day after infection. After that time, number of the oocysts in feces sharply decline (*Hammond and Long*, 1973).

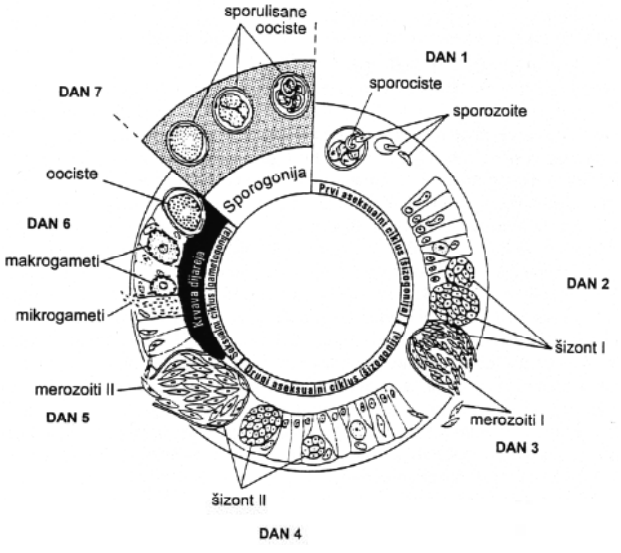

Figure 1. Scheme of the developing cycle of *Eimeria tenella*
Slika 1. Shematski prikaz razvojnog ciklusa *Eimeria tenella*

Diagnosis

Diagnosis of the coecal coccidiosis is made on the basis of clinical signs, coprology, patomophological and patohistological analysis. Clinical diagnosis is not reliable. One of the basic symptoms that could lead to diagnosis is bloody diarrhea, as well as changes in feces appearance (*Dimitrijević,* 1999).

Disease signs (clinical)

The first and most frequent symptom is at the beginning yellow diarrhea. As the disease progresses, because of the blood in feces, feces are red or resemble the color of chocolate (*Jordan,* 1990). The feathers around the cloacae are covered with bloody deposits. Feces are stained with blood. Birds that survive first few days of the infection, can survive the next 10 to 15 days. During that time, birds are thirsty and rapidly loose weight (*Calnek*, 1997). Symptoms of the disease start to appear at the time when the second generation of shizonts starts rapidly to replicate, grow, mature and release the second generation of merozoits. Second generation of merozoits cause inflammation of the sub epithelial mucus, desquamation of the epithelia and capillary rupture in the caecum wall. As a consequence, bloody diarrhea occurs (*Jordan,* 1990).

Other signs of the disease are anorexia, thirst (*Pellerdy,* 1974), somnolentia, goose pimples, dropped wings, closed eyes, leg paralysis, anemia of the crest and outer mucous membranes, enterorrhagiae, skin depigmentation and in spite of the loss of appetite the gizzard is filled with feed (*Ruff,* 1991). Death usually occurs 5. and 6. day after infection (*Hammond and Long*, 1973).

It is postulated that death is the result of blood loss, as well as infection. However, the precise cause of death is not jet clear (*Calnek*, 1997). Death of the bird can be the result of gangrene or rupture of the caecal sac (*Hofstad*, 1984).

In gees with renal coccidiosis somnolence, leg weakness, birds are reluctant to move, eyes are closed, inapetencia, thirst, whitish diarrhea, dropped wings, nervous signs, neck twisting, weight loose, and death are present.

Coprology

Coprology is performed on native samples by flotation, using concentrated solutions of NaCl. The most reliable method is to find oocysts and count them by using the McMaster method. However, it is not enough to confirm the causative agent or cause of death since death can occur before onset of oocysts in the feces (*Dimitrijević,* 1999; *Dimitrijević and Ilić*, 2003). Positive results only show that there is infection that is at least seven days old (*Hofstad,* 1984). In the case of renal coccidiosis of gees, oocysts can be found in feces however this finding is not enough for diagnosis, since there are difficulties to differentiate them and oocysts of the intestinal coccidias.

Patomorphological leasons

In cases of intestinal coccidiosis, the first and second day after infection, on the microscopic level (patohistology) there are focal lesions of the intestinal epithelium and small necrotic foci in the subepitelial connective tissue. Those changes are the result of first generation shizont maturation. On the third day, caecums are enlarged in diameter and there are regions with petechiae in the mucosa. The most prominent macroscopic lesions are from the fourth and fifth day after infection. It is obvious since in that period the second generation of the shizonts completely matures and on the fifth day after infection there is transformation into the second generation merozoits. Entrance of the second generation of merozoits into the healthy epithelial cells, mark the moment when haemorrhagiae of the caecum start. Such findings accompanied with heterofil infiltration of the *lamina propriae* and submucosis, as well (*Calnek*, 1997).

The intestine is shortened and the intestinal wall is thickened. The lumen is enlarged two to three times. The color is dark blue with sub serous petechiae. Mucosa is thickened; surface of the epithelium, as well as the epithelium of the Lüberkini crypts is desquamated with haemorrhagic patches. The intestinal content is watery, bright red in colour with desquamed cells, erythrocytes and plenty of coccidia in different stages of development. Later on, the content becomes thick and thecolour is changed to dark red. Gradually, fibrinous tissue encirculates the content of the intestinum, resembling gray-yellow hard cork (*Nešić,* 1999).

During the sixth and seventh day of infection the content of the intestinum hardens and becames dry. Epithel regeneration is fast and can be accomplished in 10 days after infection. However, as a consequence of intensive local lesions, it is possible that the epithel never returns to the previous condition (*Calnek,* 1997). Recovery starts with the appearance of fibroblasts and angioblasts (*Pellerdy*, 1952).

Microscopic examination of the intestinal wall, reveals plenty of parasites in different stages of maturation and development. Native sample-slide is especially useful since it shows oocysts and macrogamets (*Jordan,* 1990). The pathognomonic finding is the presence of shizonts in the material (*Calnek,* 1997).

Diagnosis is made on the basis of gross lesions in the intestinum, as well as microscopically by using the content of the intestinum as a sample (*Calnek,* 1997). Intensity of the infection can also be estimated especially if there is a doubt whether coccidias are the only cause of the fatal outcome of the disease. Intensity of infection is in proportion with the number of oocysts that were ingested and is in positive corelation to other parameters such as loss of body weight and changes in feces appearance (*Hofstad,* 1984).

Postmortem examination of gees that succumbed to renal coccidiosis are cahectic and gross lesions and are localized only in kidney. Kidneys are enlarged, circular in shape, smooth and bright at the surface, grey-white or grey-yellow in color. Sometimes the color changes to gray-red and red-brown. The surface of the kidneys have plenty of softened foci that are white or yellow, circular and 0.5 to 1 mm in diameter. These foci are not clearly separated from rest of the kidney tissue. It is possible to find whitish stripes and petechiae (*Dimitrijević and Ilić*, 2003).

Histology

Using standard pathohistology staining procedures (hematoxillyn-eosin) different stages of parasite development can be seen (*Hofstad*, 1984). In order to differentiate and identify them, it is better to use Shiff's reagent. Polysaccharides accompained with refractary granula, as well as aggregates that form the macrogamete wall, stain bright-red (*Calnek,* 1997; *Nešić*, 1999).

Appart of the abovementioned standard technique, there are other more specialized diagnostic methods that use monoclonal antibodies conjugated with fluorescent markers (*Calnek,* 1997).

Microscopical examination of the sample reveals that second shizont generation migrate deep into the lamina propria. Around them, there is a strong inflammatory cell reaction with eosinophils, plasma cells and in some cases giant cells (*Hofstad,* 1984). Oocysts can be found in tissue sections, and the finding depends on the stage of the infection when the sample was taken. Oocysts can be seen in giant cells next to the muscular lamina of the intestinal wall (*Pellerdy*, 1974). The first shizont generation, that matures two to three days after infection, can be seen microscopically scattered as a wide belt. Small focal hemorhagiae and necrosis can be seen in the vicinity of blood wessels in the stratum circulare internum of the intestinal wall muscular lamina (*Jordan,* 1990).

Kidney tubuli from infected gees are dilated and filled with epithelial cells and oocysts. Ureters are dilated and filled with mucous yellow-brown mass. At some places, epithel of the renal tubuli totally dissapeared and as a consequence, there are cists filled with parasites in different stages of development and cell detritus. Around most of the tubuli, there is fibrinous tissue proliferation with a number of inflamatory cells (*Dimitrijević and Ilić,* 2003).

Prophilaxis and therapy of coccidiosis

Coccidiosis can be treated with anticoccidials. They can act either as coccidiostatics, that inhibit growth and development of the intracellular parasite form or coccidiocides. Coccidiocides destroy the parasites during their developmental stages. Most of the anticoccidials are coccidiocides or they are at the beggining of the action coccidiostatics and in later stage, coccidiocides (*Long and Jeffers*, 1986). In order to prevent coccidiosis, it is possible to add some of the above mentioned substances in the feed for birds. In case therapy is needed, the drug is given diluted in drinking water.

Basically, anticoccidials are devided in 12 groups: benzenacethonitril derivatives (clazuril and diclazuril), benzyl-purin (arprinocid) derivatives, xarbanilid derivatives (nicarbazine), gvanidine derivatives (robenidin), dinitrobenzamide derivatives (dinitolmid), ionofors-polyether antibiotics (monensin, lasalocid, narasin, salinomicin, maduramicin, alboriksin), piridins (klopidol), quinazolines (halofuginon), hinolons (dekokvinat, metilbenzakvat), sulphonamides (sulphakvinoksalin), symmetric triazinons (toltrazuril) and tiamine antagonists (amprolium).

With the exeption of ionophors, there is a possibility that coccidias develop resistance (*Jordan*, 1990; *Dimitrijević et al.*, 1992; 1998). It is required only that several sporozoits survive and start the asexual cycle. That leads to production of several thousands of parasites that are resistant to a particular drug. In order to avoid resistance, it is better to use coccidiocides that act onthe late stages of shizogony (*Jezdimirović*, 1997).

In order to minimize the possibility for resistance to develop, it is possible to use "shuttle" and "dual" program. The basis of such program is to change drugs during flock raising. Another program is the "switch" program i.e. changing the drug for the next flock. Whatever drug is in use, it is essential to change drugs according to the mode of action of the active substance. Only in that case there is a real chance to avoid development of resistance within the parasite population (*Calnek*, 1997; *Dimitrijević and Ilić,* 2003).

After treatment, whether prophylactic or in therapy, there is need to take care of drug withdrawal period. Nowadays, in order to prevent the disease, most often iodophors are in use. Drugs are omitted in the feed for the final fattening period. Nevertheless, even witho ionophores there is a possibility for the parasite to develop resistance (*Chapman,* 1997).

Immunity

Broilers that survived coecal coccidiosis, immunity is life lasting and that is normal in natural infeciton (*Pellerdy,* 1974). Chicken, acquire immunity from their mothers only if hens are actively immunized against coccidiosis (*Hammong and Long,* 1973).

Immunity against parasitic diseases develops in the same way as protection against all other infectious diseases. It is dependent on the age (*Ruff,* 1991) and genetic background (*Jeffers and Shirley*, 1982). At the same time, it depends on the number of oocysts that are innoculated. Immunity against coccidiosis is highly specific and cross protection has not been documented. That means that different species of the parasite can cause disease in susceptible birds (*Hofstad*, 1984; *Ilić et al.,* 2003a).

Early informations on immunity against coccidiosis show that in order to stimulate the immune reaction, it is required to have, as immunigen, shizonts of the second generation. However, it has been shown that the immune reaction develops as early as 72 (*Kendall and McCullogh*, 1952) hours after ingestion or after intracutaneous injection (*Pellerdy*, 1974), of the infective oocysts at the time when there are not second generation of the shizonts developed jet.

Good protection in the case of coccidiosis means that there is no development of the parasites and onset of oocysts during reinfection. That is achieved after several natural infections. Better protection is achieved with every day infection of chickens with a small number of infective oocysts in comparison with one single dose (*Joyner and Notrhon*, 1973). In practice, simulation of multiple dose immunization is during floor husbandry when continous reinfection keep the immune system in contact with the immunogen (*Šibalić and Cvetković*, 1996; *Jordan,* 1990).

The immune response to coccidia is complex. Animals infected with Eimeria spp. develop parasite-specific immunoglobulins that are present in the circulation, as well as on the mucous membranes, in secretions. However, it has been shown that specific antibodies play a minor role in the protection against coccidiosis. Nowdays there is evidence that cell imunity plays a major role in the protection against infection (*Challey and Burns*, 1959; *Pattillo,* 1959; *Daviesandsar.*, 1963; *Soulsby*, 1972; *Lillehoj and Trout*, 1996; *Ilića et al.,* 2003a, 2003b).

Early investigations show that the basis for protection against coccidiosis are of the humoral type (*McDermot and Stauber,* 1954; *Itagaki and Tsubokura*, 1955). However, today it has been shown that the protection is of the cellular type (*Long and Pierce*, 1963). Details of the protective mechanisms that are activated during infection are not clarified jet however, it is clear that cellular immunity plays the most important role in bird protection (*Lillehoj and Bacon***,** 1991).

As a result of infection, T lymphocites produce cytokines. At the same time, T lymphocites are cytotoxic to infected cells (*Lillehoj and Trout*, 1996). However, detailed mechanisms of that protection are still obscure. One of the theory is that the major mechanism of protection is the presence of intestinal immune system of chickens, that means that the intestinal lymphoid tissue poses as the first specialized line of defence of the mucous surfaces. That system encirculates not only immunoregulatory, but effector cells, as well.

Vaccination

Because of the resistance against anticoccidials that often develops, vaccination is the most appropriate method for desease control (*Augustine et al.,* 2001). Vaccination is the simpliest and cheapest way to achieve immunoprophilaxis. In that way, the immune system is activated so natural infection causes a secondary immune reaction which is faster and better in comparison to the primary immune reaction (*Naglić and Hajsig*, 1993; *Dimitrijevi and Ilić,* 2003a).

An ideal vaccine will stimulates long lasting immunity against all epitopes in the coccidia structure. That immunity has to be not only specific for the basic pathogenic coccidia species, but also against strains that develop during epizootia (*Dimitrijević,* 1993). The vaccine also has to be harmless for birds that are vaccinated. At the same time the vaccine must not contaminate the natural habitat with potentially pathogenic coccidia. Vaccines that are in use, can have atenuated (alive), recombinant or antiidiotypic immunogens. As immunogens, atenuated vaccine can have non-virulent coccidia strains or can be produced on the basis of virulent coccidia strains **(***Lillehoj and Trou*t, 1993; *Liand et al.,* 2005).

Live vaccines that posess virulent coccidia strains comprise of a mixture of all species of virulent coccidia. Such a vaccines can be used in drinking

water (*Jordan*, 1990)**.** They elict the most potent immune reaction since immunogenic characteristics match with the ability of the parasite to replicate and with the level of pathogenicity (*Naglić and Hajsig*, 1993). They are the best vaccines however, such vaccines have to be used in small doses in order pathogenic changes not to occur (*Orlić et al.,* 1996*; Dimitrijević,* 1997). For maximal effect, birds have to be revaccinated several times (*Orlić et al.*, 1996; *Dimitrijević,* 1997).

Recently, as the immunogen in vaccines, there are alive *Eimeria* species that are tollerant to iodophores. Advantage of such vaccines is that in vaccinated flock iodophores can be used in the first 3-4 weeks of bird life, at the time when immunity is not jet fully developed (*Danforth*, 2000). Vaccinated birds, for not jet clear reasons, have a smaller mortality in comparison untreated ones (*Williams*, 2002). Live, virulent immunogens (vaccines) are not quite appropriate for broilers since there is a possibility of accumulation of parasites in the floor (*Lillehoj and Trout*, 1993).

Live attenuated vaccines can be divided into two groups. The first group comprises of vaccines that are made of natural strains that are of low virulence. The second group of such vaccines, have laboratory produced low virulence strains as immunogens (*Shirley,* 1989).

Special advantages of live vaccines is that vaccine strains compete with natural, highly virulent strains that are resistant to drugs (*Hofstad*, 1984). In that case, vaccine strains overgrow natural strains in the vaccinated bird population. By attenuation of infectious oocysts, live cycle of coccidia can be shortened in order to enable required number of immunizing stages and still not possessing an infectious potential (*Dimitrijević,* 1993).

Advantages of attenuated vaccines, in comparison to virulent vaccines are that in the production of a great number of oocysts, there is minimal danger of infection to occur. The disadvantage is that there is only a partial protection against natural „field" coccidia strains (*Shirley*, 1989; *Augustine et al.,* 1993).

Vaccines based on recombinant techniques consist of immunogens that were produced in bacterial vectors. In that way, large quantities of immunogen can be produced (*Dimitrijević,* 1997). They are a kind of cocktail consisting of different antigens originated from several coccidia species. At the same time, such vaccines consist of different antigens from the same coccidia species. To produce them, it is required to use complex technology and their production is still a matter of future in vaccinology. Disadvantages of such vaccines are low immunogenicity and possible selection of mutant coccidias that do not possess the cloned gene. So, such mutant parasite can freely replicate in the vaccinated bird population. At the beggining, in few parasite generations, mutants represent a small population however, during epizootia, they became dominant. That means that in such a case, there is a need to produce new recombinant immunogens frequently (*Lillehoj and Trout***,** 1993; *Dimitrijević and Ilić,* 2005).

Anti-idiotypic vaccines are a special variety of vaccines that use anti-idiotypic immunoglobulins (*Lillehoj and Trout*, 1993). The mode of action of such antibodies is based on idiotypic-antiidiotypic network. Anti-idiotypic vaccines open new possibilities in coccidiosis immunoprophilaxis however, they are very expensive. At the same time they lack immunogenicity (*Naglić and Hajsig*, 1993). In future, such immunization could be used for overcoming certain genetical limitations that are still causing problems in vaccination against some other diseases (*Lillehoj and Trout*, 1993).

Coccidiosis – economic impact

In the last few years the poultry industry and as a consequence chicken meat represents 80 percent of the whole production of meat originating from birds. Still, production is the fastest growing in the meat industry. According to analysis, production, as well as consumption of chicken meat, will rise because of: good feed conversion in comparison to other animal species, there is not religious aspect of poultry meat consumption, poultry meat is healthy (low fat and high protein content), has good sensory quilities, low price and fast production which mean a short generative time. Poultry, during coccidiosis and after therapy, have poor productive results. Daily feed quantity and feed conversion rise. Chicken daily growth weight is reduced, as well as body mass at the end of the fattening period (*Jordan*, 1990; *Vermeulen et al.,* 2001). As a result the fattening period should be prolonged. At the same time, care should be taken for the withdrowal period for the drug wich further rises costs of production (*Jordan,* 1990; *Williams,* 2002).

Because of coccidiosis, carcass yield is smaller, as well as the proportion of more valuable parts of the body. Also, fat deposits are smaller in the abdominal fat tissue. In broilers' meat, there is higher water content and less proteins. Relative proportion of proteins of the fibrinous tissue in the total protein mass is higher. Sensory characteristics of the broilers' meat are bad in comparison to the population where coccidiosis was absent (*Lilić,* 2007). In liver of infected borilers, content of iron

and copper is smaller. Meat of infected broilers have a decreased iron manganese and phosporous content (*Koinarski et al.,* 1998).

A great economic problem is resistency to anticoccidial drugs. Such drugs are not easy to use. Also, development of new drug generations, that are for prophilaxis and therapy, is expensive. As an alternative, there are investigations whose target is to use immunological, biotechnical and genetical methods for prevention and control of coccidiosis (*Grag et al*, 1999). Of all coccidias that cause the disease, *Eimeria tenella* is widely distributed and serves as a gold standard in order to sequence the genetical material of the causative agent. At the same time, *E. tenella* is the first candidate for eradication (*Augustine et al.,* 2001).

Poultry meat consumption, at a global level is constantly rising. So, there is a need to intensify broiler production. In such a production system, the possibility for coccidiosis is higher inspite of using anticoccidials in feed. At contrary, world trends in food production are to produce organic meat, with no drugs added to the feed. This means that the risk of coccidiosis is higher. Nevertheless, strategies to control coccidiosis are still based on prophilactic medication through feed and vaccination (*Vermeulen et al,* 2001), not to exclude good production praxis and good hygiene and sanitation.

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