Review Article
Progress in Control Measures for Chicken Coccidiosis

A. KITANDU, R. JURANOVÁ

Avian and Exotic Animal Clinic, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

Abstract

Kitandu A., R. Juranová: Progress in Control Measures for Chicken Coccidiosis. Acta Vet. Brno 2006, 75: 265-276.

For many years, prophylactic use of anticoccidial drugs has been the primary means of controlling chicken coccidiosis in broiler industry and has played a major role in the growth of this industry. Also the use of live vaccines is well established in the control of the disease. Three groups of live vaccines can be distinguished based on the characteristics of the *Eimeria* species included in the product: vaccines based on live virulent strains, vaccines based on live attenuated strains, vaccines based on live strains that are relatively tolerant to the ionophores, and non-live subunit vaccines. The mounting problem of drug resitance of *Eimeria* species has prompted major research efforts to seek alternative means of control through increased knowledge of understanding the immunomodulation, natural-product feed additives, advances in live and recombinant vaccines. This article reviews the above mentioned methods in control of chicken coccidiosis.

Chickens, anticoccidial drugs, vaccines, immunomodulators, Eimeria, immunity

Chicken coccidiosis is an intestinal infection caused by the intracellular protozoan parasite of the genus *Eimeria*. Seven species have been recognised to infect chickens: *Eimeria tenella, Eimeria necatrix, Eimeria acervulina, Eimeria maxima, Eimeria brunetti, Eimeria mitis,* and *Eimeria praecox*. Each species has its own characteristics with respect to preferred site of infection, pathogenicity and immunogenicity. It is the major parasite disease of poultry, with substantial economic burden estimated to cost the industry more than $ 800 million in annual losses worldwide (Williams 1998). These estimates include the costs of prophylactic in-feed medication for broilers and broiler breeders, alternative treatments if medication fails, and losses due to mortality, morbidity, impaired growth rate, temporary reduction of egg production in layers and poor feed conversion of chickens that survive outbreaks.

Modern intensive poultry production is largely dependent upon chemoprophylaxis for the control of coccidiosis (Chapman 1999; Allen and Fetterer 2002), although there is a rising problem of drug resistant strains of *Eimeria*. In addition the use of live vaccines for control of coccidiosis is also well established (Williams 2002).

The life cycle of *Eimeria* comprises intracellular, extracellular, asexual and sexual stages, so it is not surprising that host immunity is also complex and involves many facets of non-specific and specific immunity (cellular and humoral immune mechanism) (Lillehoj 1998), and their pathogenicity varies in birds of different genetic background. Therefore, in the natural host, the immunity is species specific (e.g. chickens immune to one species of *Eimeria* are susceptible to others). Additionally, *Eimeria* species exhibit different tissue and organ specificity in the infected host, so, understanding the interplay between the host and the parasites in the intestine is crucial for the design of novel control approaches agaist coccidiosis (Dalloul and Lillehoj 2005). The introduction of alternative prevention

---

Address for correspondence:
Dr. Amos Kitandu
P.O. Box 7238
Dar-Es-Salaam
Tanzania

E-mail: drkitandu4@yahoo.com
http://www.vfu.cz/acta-vet/actavet.htm

measures such as non-chemical feed supplements that effectively enhance productivity and non-specific immunity may help to limit the use of anticoccidials in control of chicken coccidiosis.

Control using anticoccidial drugs

The effective use of anticoccidial drugs over the past 50 years has played a major role in the growth of poultry industry and has allowed the increased availability of high quality, affordable poultry products to the consumer. Numerous products were introduced, many of which are available and used today. However, there is increasing concern about rising levels of drug resistance (Chapman 1997). The anticoccidial drugs can be classified as 1) Synthetic drugs (chemicals), 2) Polyether ionophores. Synthetic drugs have specific modes of action against parasite metabolism, sulphamides and related drugs compete for the incorporation of paraminobenzoic acid (PABA) and metabolic of folic acid, amprolium compete for absorption of thiamine by the parasite. Quinoxaline and clopidol inhibit energy metabolism in the cytochrome system of coccidia. The quinolones and ionophores arrest or kill the sporozoite or early trophozoite, nicarbazin, robenidine and zoalene destroy the first or second generation schizonts and the sulphonamides act on the developing schizonts and also on the sexual stages. The ionophores kill coccidia by interfering with the balance of important ions such as sodium and potassium. The host cells are able to manage these ions in the presence of ionophores, but the parasites cannot.

1. Synthetic drugs: These drugs were introduced first, then the ionophores followed and are now an important component of coccidiosis control. The recent anticoccidial drugs in control of coccidiosis are diclazuril and toltrazuril (Chapman 1999). The use of toltrazuril as the sole anticoccidial for two consecutive days in the drinking water between days 10 and 14 would be the best time for good coccidiosis control (Mathis et al. 2004). Mehlhorn et al. (1988) reported that toltrazuril is effective against all species of *Eimeria* infecting chickens. Mehlhorn et al. (1984) also reported that toltrazuril is active against all intracellular developmental stages including those of schizogony and gametogony. It has been reported that despite high efficacy toltrazuril does not intrefere with the development of natural immunity but can even enhance it (Greif 2000). Mathis et al. (2003) demonstrated that toltrazuril was an effective aid to certain anticoccidial program as well as effective as a stand alone anticoccidial. Vanparijs et al. (1989ab, 1990), McDougald et al. (1900ab) reported that diclazuril has a potent, broad-spectrum anticoccidial activity against *Eimeria* species and it was noted to be highly effective against the six major pathogenic species of *Eimeria (Eimeria acervulina, E. maxima, E. tenella E. brunetti, E. necatrix and E. mitis)*. Chapman et al. (2004) reported that roxarsone has important anticoccidial activity particularly against *E. tenella* and works very well in combination with ionophores. Also combinations of anticoccidials such as salinomycin and roxarsone with a digestive enhancer such as bacitracin are widely used in the starter and grower feeds of broilers for control of coccidiosis and improvement of growth in broilers (Chapman and Johnson 2002). It is quite clear that some degree of resistance to all anticoccidial drugs, including ionophores, has developed (Chapman 1997). To minimize the effects of resistance, poultry producers rotate the use of various anticoccidials with successive flocks, where drugs from different classes are used sequentlly on a single crop of birds, one class might be used in starter feed, another in growers, returning to the first for the finisher diet, followed by a withdrawal diet (Sangster 2001).

2. Polyether ionophores: Since 1971 the preferred drugs for coccodiosis prevention have been ionophore antibiotics. These drugs still achieve sufficient control despite resistance being common; for example, salinomycin, narasin, monensin, lasalocid, maduramicin and semduramicin remain useful agents except in situation of heavy parasite challenge

(Chapman 1997). In addition it has been demonstrated that some ionophores can be used in combination with live virulent vaccines, therefore the use of ionophores-tolerant resistant strains would probably have a wider application of the development of anticoccidial vaccines for suitable control of coccidiosis (Danforth 2000; Vermeulen et al. 2000a). The advantage of such ionophores is that they prevent infection during the first 3-4 weeks of age when immunity is not developed, such use limits the increase of infection pressure due to the expanding field strains during the development of immunity, which further reduces the overall risk of contracting coccidiosis (Vermeulen et al. 2000a; 2001). It is known that coccidiosis is aggravated by microflora, for example *Clostridium perfringens* interacting with intestinal mucosa damage as well as the developmental stages of *Eimeria* parasites. This problem can be reduced by the use of antibacterial properties of the ionophores. The use of ionophores such as monensin, narasin and salinomycin in combination with live virulent vaccines (Nobilis COX ATM) can protect birds against coccidiosis as well as necrotic enteritis. Advantages claimed for this method are protection by the ionophore against coccidiosis (due to wide-type strains) in the period before effective immunity has developed, protection against species not included in the vaccine and effectiveness against bacterial diseases such as necrotic enteritis due to the additional properties of the ionophores (Chapman et al. 2002; Vermeulen et al. 2000ab).

Common anticoccidial drugs

Class	Names	Acts on life cycle stage
Ionophores	Monensin, lasalocid, narasin, Maduramicin and semduramicin	Trophozoite/sporozoite
Sulphonamides	Sulphaquinoxaline (+DHFR inhibitors)	Second and later schizont
Quinolones	Decoquinate	Sporozoite
Pyridones	Clopidol	Sporozoite
Thiamine mimics	Amprolium	First generation schizont
	Halofuginone	Asexual stages
Guanidine	Robenidine, nicarbazin, Diclazuril and toltrazuril	Multiple stages

Vaccines against *Eimeria* parasites

It is known that when chickens are infected with low number of *Eimeria* parasites, protective immunity is induced after two or three consecutive infections (Joyner and Norton 1973; Long et al. 1986). Therefore, it would seem obvious that vaccines could offer excellent alternatives to drugs as a means of controlling coccidiosis. Thereby live vaccines have been used mostly in breeder stocks and, to a lesser extent, in commercial broilers and replacement hens. This strategy is based on the well-documented protective immunity that develops in chickens after a primary coccidial infection (Williams 2002).

Three groups of live vaccines are used in control of disease:
1) Live, virulent strains
These vaccines comprise a varible number of wild type strains depending on their formulation and field of application (Lee 1987). For broiler-breeders up to eight *Eimeria* species are included in these products (Coccivac® D, Immuncox® C2). For use in the broiler industry this number is restricted to up to four species (Coccivac® B, Immucox® C1).
The advantage of live virulent vaccines is that they can provide equal or superior performance compared with prophylactic medication when given in gel form on day 1 of

age because this method ensures the synchronous exposure of all birds to small uniform number of oocysts (Danforth et al. 1997a). The disadvantage of virulent vaccines is a risk of introducing unwanted *Eimeria* species in the environment.

2) Live, attenuated strains

This type of vaccines can be obtained by repeated selection for early maturation (precociousness) or serial passage through embryonated eggs (TA lines) (Long 1972; Jeffers 1975). This has led to the development of two attenuated vaccines, Paracox® (precocious strains, Shirley and Millard 1986; Shirley 1989; Williams 1992) and Livacox® (precocious and TA strains, Bedrník 1989).

The advantage of live attenuated vaccines is that they have low productive potentials, thus avoiding crowding in the specific mucosal areas of infection and resulting in the development of optimal immunity with minimal tissue damage (Williams 1994) and there is no risk of introducing *Eimeria* species into the environment. Furthermore, a major advantage of these vaccines is that they cannot induce coccidial drug resistance (Williams 1992).

The virulent or attenuated vaccines are given to chickens in the first week of age, which results in early low-level infection followed by cycling of the vaccine parasites through the litter and induction of immunity against a heavy field challenge. Any use of therapeutics or feed additives that interfere with *Eimeria* development cannot be used during the period of development of immunity. Hence, when vaccination is used to control coccidiosis the risk of contracting coccidiosis is higher at the early age (weeks 1-3), and decreases as immunity has developed from weeks 3 to 4 onwards (Vermeulen et al. 2001).

3) Live, tolerant to ionophores

There is a new development that made live *Eimeria* strains relatively tolerant to ionophores. An experimental vaccine comprising a strain of *E. acervulina* and a strain from *E. maxima*, both of which were partially tolerant to salinomycin, has been used in USA (Danforth 2000). A live vaccine that can be used with different ionophores has been also introduced (Nobilis® COX ATM) to the market (Schetters et al. 1999). Vermeulen et al. (2000ab) reported that the combination of Nobilis COX ATM and narasin gave good results in protection of chickens against challenge infection. The vaccine comprises strains of three different species (*E. acervulina, E. maxima* and *E. tenella*) which are relatively tolerant to ionophores. The advantage of these vaccines is that they allow the use of ionophores during the first 3-4 weeks when immunity is not complete. Such use limits the increase of infection pressure due to expanding field strains during the period of development of immunity, which further reduces the overall risk of contracting coccidiosis (Vermeulen et al. 2001). Also the vaccine is claimed to protect chickens against coccidiosis as well as necrotic enteritis (Chapman et al. 2002).

Recently a new subunit vaccine (CoxAbic vaccine) against coccidiosis has been registered. This vaccine consists of affinity purified sexual stages (gametocyte) antigens (APGA) isolated from *Eimeria maxima*. It is a novel vaccine that unlike the currently available vaccines is based on the concept of transmission blocking immunity. Immunization of breeding hens prior to the start of lay, gives rise to a protective immunuglobulin G (IgG) response, which is transferred to the developing embryo providing protection to their offspring broiler chickens (Wallach 2002). In a multinational field trial involving four countries, it was observed that chickens vaccinted with CoxAbic showed reduced oocyst shedding of the three major species of *Eimeria (Eimeria tenella, E. acervulina* and *E. maxima*) that cause coccidiosis in chickens, and they performed at least as well as the coccidiostat fed broiler controls (Michael 2002).

Recombinant vaccines

Due to emergence of drug-resistant *Eimeria* strains and high cost in manufacturing live vaccines much research has focused on recombinant vaccination strategies as potential alternative methods of disease control. The conception of genetic vaccines emerged from the observation that injection of naked plasmid DNA resulted in transfection of murine muscle cells and production of the plasmid-encoded protein β-galactosidase (Wolff et al. 1990). Later, analyzing the mechanism of operation made it clear that DNA not only is simply a vehicle to ensure protein production in transfected cells but it also has intrinsic adjuvant properties because of the presence of immunostimulatory CpG dinucleotide in the backbone of the bacterial DNA (Krieg 2002).

Identification of antigens specific to parasite life cycle stages conveying protective immunity is a pivotal step in subunit vaccine development. In *Eimeria* spp. recombinant forms of both parasite surface antigens and internal antigens have been examined as vaccine candidates (Min et al. 2004; Schaap et al. 2004). Belli et al. (2004) cloned and expressed two recombinant proteins of the genes gam56, and gam82, encoding the immunodominant components of a commercial subunit vaccine called CoxAbic® derived from *E. maxima* gametocytes. This vaccine has been shown to provide partial protection against *E. acervulina, E. maxima* and *E. tenella* (Wallach 2002), but its production is both laborious and costly (Belli et al. 2004).

After multiple immunization with recombinant proteins, alone or in combination, breeding hens elicited a dose-dependent antibody response indicative of similar antigenic and immunogenic properties to the native protein vaccine. These proteins can be potentially used in developing recombinant vaccines at lower costs than with CoxAbic®. Ding et al. (2004) used a purified *E. acervulina* recombinant protein (3-1E) to vaccinate chickens in ovo against both alone and with expression plasmid encoding the IL-1, IL-2, IL-6, IL-8, IL-15, IL-16, IL-17, IL-18, or IFN-γ genes. They revealed that in ovo vaccination with 3-IL protein enhanced protective immunity against *E. acervulina* infection as measured by reduced fecal oocyst shedding and increased body weight gain compared with non-vaccinated controls.

Also, covaccination with 3-IL plus the IL-2, IL-15, IL-17, IL-18, or IFN-γ genes further reduced the oocyst output beyond that induced by 3-IE protein alone. A second potential recombinant protein was evaluated as a coccidiosis vaccine, its gene (EtMIC2) was cloned, the encoded protein expressed and purified, and the efficacy of in ovo immunization to protect against *Eimeria* infections was determined (Lillehoj et al. 2005). They demonstrated that in ovo vaccination with recombinant EtMIC2 protein induced significantly higher antibody responses, lower fecal shedding, and increased weight gains after *E. tenell*a infection compared with negative controls. Furthemore, combined embryo immunization with the EtMIC2 protein plus cytokine or chemokine genes (IL-8, IL-16, TGF-β4, and lymphotactin) demonstrated enhanced protection compared with vaccination with EtMIC2 alone (Lillehoj et al. 2005). Taken together, these results provide the first evidence that in ovo vaccination with the recombinant 3-IE and EtMIC2 *Eimeria* species (spp.) proteins induced protective intestinal immunity against coccidiosis. Furthermore, their protective effects were enhanced by coadministration of genes encoding immune related cytokines, paving the way for a potentially effective method to control coccidiosis.

On the other hand, DNA vaccines use genes encoding immunogenic proteins of pathogens rather than the proteins themselves. They are administered directly in conjuction with appropriate regulatory (e.g., promotors, enhancers) permitting the encoded protein to be expressed in its native form and thereby to be recognized by the host's immune system in a manner that stimulates natural infection. Kopko et al. (2000) were able to ligate SO7', a reflactile body encoding gene derived from *E. tenella* sporozoites, to the mammalian expression vector pcDNA3. After intramuscular injection of the pcDNA3-SO7' construct

and subsequent *E. tenella* challenge, significant protection against cecal lesions and weight loss was achieved. Recently, Wu et al. (2004) constructed two DNA vaccines based on antigens present on *E. tenella* sporozoites. After DNA immunization and *E. tenella* challenge, there was reduced oocyst shedding as well as decreased weight loss. Lillehoj et al. (2000) observed immune protection manifested by significantly reduced fecal oocyst shedding in chickens vaccinated subcutaneously with a cDNA encoding *E. acervulina* 3-IE protein. Further protection was obtained when the 3-IEcDNA was administered in conjuction with cDNAs encoding chicken IFN-γ or IL-2. Later, Min et al. (2001) examined the effect of injecting a plasmid encoding the 3-IE gene in combination with a plasmid encoding IL-Iβ, IL-2, IL-8, IL-15, IFN-α, IFN-γ, TGF-β4, or lymphotactin and delivered twice subcutaneously to chickens, followed by challenge 1 week later. Body weight loss was significatly reduced in chickens given the DNA vaccine with the IFN-α, or the lympotactin-encoding plasmid, whereas parasite replication was reduced in chickens injected with the IL-8, lymphotactin, IFN-γ, IL-15, TGF-β4or IL-1β-encoding plasmids compared with chickens vaccinated with the 3-IE DNA vaccine alone.

Furthermore, the groups of chickens that were given the IL-8 or IL-15 genes had significantly increased number of CD3^{+} T cells compared with the other groups. More recently, Lillehoj et al. (2005) used a similar scheme to inject the 3-IE and cytokine encoding plasmid in ovo and assess its protection against *E. acervulina* infection. In ovo vaccination with the 3-IE gene generated an antibody response against the expressed parasite protein that was enhanced by covaccination with the IL-1, IL-2, IL-15, or IFN-γ genes. In ovo vaccination with 3-1E demonstrated protective immuinty against *E. acervulina* infection as measured by reduced oocyst shedding and improved body weight gain compared with nonvaccinated controls. The data also showed that covaccination of 3-1E with the IL-2, IL-15, or IFN-γ genes further curtailed oocyst output and exceeded weight gain beyond that induced by 3-E alone.

Alternative controls including nutritional and probiotics (immunomodulators) or natural-feed additives

Natural medicinal products as feed supplements have been widely used as growth and health promotors in farm animals in China (Li 1998). A current estimation of the number of immunoactive natural medicinal products ranges between 200 and 300 and most products originate from plants and fungi (Li 2000). The immunoactive components of these plants and fungi include polysaccharides, glycosides, alkaloids, volatile oils, and organic acids, of which polysaccharides are considered to be the most important (Xue and Meng 1996; Li 2000). Polysaccharides may act as immune enhancers or immunomodulators, and these components may display antibacterial activity (Xia and Cheng 1988) and could affect both innate and adaptive immunity including cellular and humoral responses (Lien and Gao 1990). Also some mushrooms and herb polysaccharides which were used as feed supplements or vaccines adjuvants showed antibacterial (Yuan et al. 1993) antiviral (Yu and Zhu 2000), or antiparasitic (Pang et al. 2000) effects.

Mushroom and herb polysaccharides

The polysaccharide extracts from 2 mushrooms, *Lentinus edodes* (LenE) and *Tremella fuciformis* (TreE), and a herb, *Astragalus membranaceus* (AstE) when given as supplement feed to chickens resulted in a significant impact on the inductive responses against *E. tenella* infection in chickens by enhancing both cellular and humoral immunity (Guo et al. 2004).

Sources of fats

Sources of fats containing high concentrations of n-3 fatty acids (n-3 FA) (docosahexaenoic acid, eicosapentaenoic acid, and linolenic acid), such as fish oils, flaxseed oil, and whole flaxseed, when added to starter rations and fed to chicks from 1 day of age, effectively reduced lesions resulting from challenge infections with *E. tenella* (Allen et al. 1996, 1997) but not *E. maxima* (Allen et al. 1997). The fish oil and flaxseed oil diets significantly reduced the degree of parasitization by and development of *E. tenella* (Allen et al. 1996), and caused ultrastructural degradation of both asexual and sexual stages, characterized by cytoplasmic vacuolization, chromatin condensation within the nucleus, and lack of parasitophorous vacuole delineation (Danforth et al. 1997b). These results are consistent with reports of the effects of high n-3 FA diets on other parasites (Allen et al. 1998) and suggest that these diets induce a state of oxidative stress (due to the high concentration of easily oxidized double bonds) that is detrimental to parasite development.

Herbs

Artemisinin is a Chinese herb isolated from *Artemisia annua*; it is a naturally occurring endoperoxide with antimalarial properties. It has been found effective in reducing oocyst output from both *E. acervulina* and *E. tenella* infections when fed at levels of 8.5 and 17 ppm in starter diets (Allen et al. 1997). The mode of action is also thought to involve oxidative stress.

Most recently, extracts from 15 Asian herbs were tested for anticoccidial activity against *E. tenella*. Of the species tested, extracts from *Sophora flavescens* Aiton was the most effective in reducing lesion scores, maintaining body weight gain, and reducing oocyst production (Youn and Noh 2001).

Feed supplementation with antioxidants such as γ-tocopherol (8 ppm), found plentifully in seed oils such as wheat, corn, and soybean, and the spice tumeric (1%), as well as its main medicinal component, curcumin (0.05%), appear effective in reducing upper- and mid-small-intestinal infections caused by *E. acervulina* and *E. maxima* (Allen et al. 1998).

Products that can generate a state of oxidative stress, such as n-3 FA and artemisinin, are particularly effective against the cecal parasite *E. tenella*. Products that have antioxidant properties, such as γ-tocopherol and curcumin, seem to be more effective against the mid- and upper-small intestinal species *E. maxima* and *E. acervulina*. The osmoprotectant betaine appears to be most active against *E. acervulina*. Practical applications of these findings, such as the use of the products in starter rations or combinations of them with current anticoccidials or vaccines, appear possible.

Vitamin A

It is known that nutrition plays a significant role in the development of the chicken immune system (Dalloul and Lillehoj 2005). Essential nutrients such as vitamins may affect both humoral and cell-mediated immune responses. Vitamin A, known for its role in the differentiation of epithelial cells, is essential for maintaining the integrity of mucosal surfaces (Chew and Park 2004). Deficiency of vitamin A increases host susceptibility to enteric diseases like coccidiosis (Chew 1995; Dalloul et al. 2002). Indeed, vitamin A deficiency impaired the local immune defenses within the gut lymphoid tissues of broiler chickens (Dalloul et al. 2002). This effect was best characterized by a reduction in intraepithelial lymphocyte (IEL) subpopulations, mainly CD4$^+$ T cells. Alteration in the IEL subpopulations caused by lack of vitamin A lowered the ability of broilers to resist *E. acervulina* infection, resulting in greater oocyst shedding. Furthermore, vitamin A deficiency affected the systemic immune system by reducing the ability of splenic

T lymphocytes to respond to *in vitro* mitogen stimulation and also resulted in lower IFN-γ secretion (Dalloul et al. 2002). In fact dietary vitamin A levels can affect gut immunity in broiler chickens, and its deficiency can cause immunosuppression at those sites and result in increased susceptibilty to coccidiosis.

Betaine

Betaine is another dietary supplement, a naturally occurring amino acid derivative, that has been investigated as potential enhancing agent against coccidiosis. Klasing et al. (2002) reported an increase in duodenal IELs of *E. acervulina*-infected chickens as well as an improved functionality of phagocytes. Other studies have shown differential effect on the rate of body weight gain in chickens infected with different *Eimeria* species, where it was effective only during *E. maxima* infection but not during *E. acervulina* or *E. tenella* (Fetterer et al. 2003) infections. When given to salinomycin-treated chickens, betaine significantly reduced invasion by *E. acervulina* and *E. tenella* as compared with invasion in chickens on salinomycin or betaine alone (Allen et al. 1998).

Probiotics enhance gut defensive mechanisms

It is known that the gut microflora constitutes an important component of these first lines of defence in both humans and animals. Probiotic supplementation of the intestinal microflora has been shown to enhance gut defensive mechanisms in poultry (La Ragione et al. 2004). The development and use of probiotics for animals, including poultry, is based on the knowledge that the gut flora is involved in resistance to enteric infections where it has been shown to be involved in protection against a variety of pathogens, including *Escherichia coli* (Chateau et al. 1993; La Ragione et al. 2004), *Salmonella* spp. (La Ragione et al. 2004; Stern et al. 2001), *Campylobacter jejuni* (Hakkinen and Schneitz 1999; Stern et al. 2001), and, more recently, *Eimeria* spp. (Dalloul et al. 2003ab). Therefore, feeding probiotics to animals to maintain a good balance of intestinal microflora could prove effective in the prevention and therapy of such enteric infections by possible modulation of the mucosal immune system and enhancing the host's resistance to enteric pathogens.

Several studies have demonstrated that a *Lactobacillus*-based probiotic stimulated the local immune system of the broiler chickens and improved resistance to *E. acervulina* (Dalloul et al. 2003ab; Lillehoj et al. 2003). The studies involved supplementing broiler chicken diet with a commercial probiotic followed by *E. acervulina* infection. Both local (intestinal) and systemic (serum) immune responses were then assessed by measuring cytokines (namely, IFN-γ and IL-2), antibodies, weight gain, and oocyst shedding (Dalloul et al. 2003b). Upon examinig the effects of feeding the probitic on the IEL subpopulations and protection against coccidiosis, a significant increase in IEL T lymphocyte subpopulations expressing the surface markers CD3, CD4, CD8, and α/β-T cell receptor was observed in probiotic-fed birds. Upon cytokine and antibody levels in sera and intestinal secretions, the probiotic-fed chickens showed a significantly higher IFN-γ and IL-2 at 3 days post infection, which was much earlier than shown by control birds (Dalloul and Lillehoj 2005). These authors reported that probiotic-fed chickens shed fewer oocysts than did chickens without probiotics, even in vitamin A deficient birds, thus confirming improved resistance to coccidiosis in chickens fed probiotic supplement.

CpG oligodeoxynucleotides (ODNs)

The short ODNs containig unmethylated CpG motifs have been shown to be effective immunoprotective agents in mammalian models by inducing both innate and adaptive

immune responses (Krieg 2002). Recently, CpG ODNs were reported to have both *in vitro* and *in vivo* immunostimulatory effects in domestic animals, including chickens (Dalloul et al. 2004; Gomis et al. 2003; He et al. 2003; Mutwiri et al. 2003; Vleugels et al. 2002). In mammalian systems, bacterial DNA displays impressive immunomodulatory action that influences DNA vaccination (Gurunathan et al. 2000; Klinman et al. 2004).

Since its initial discovery (Krieg et al. 1995), ODNs have shown to play a role in host defense, both by stimulating T cells and by inducing cytokines or enhancing innate immunity (Klinman 2004). Dalloul et al. (2004), Xie et al. (2003) recently identified CpG sequences that activate chicken innate immunity and enhance protective immune response against *Salmonella* spp. and coccidia. One of the ODNs, CpG 2006, had strong stimulatory effects on chicken macrophages as demonstrated by increased proinflammatory cytokine IL-6 secretion, enhanced nitric oxide release, upregulated cell surface marker expression, and increased intracellular bacterial killing (Xie et al. 2003). *In vivo* trials were demonstrated to investigate the immunomodulatory effects of CpG ODNs on disease susceptibility in *E. acervulina*-infected chickens, SC and TK, two genetic chicken lines with different immune responses to *Eimeria* infection: Tk is more susceptible than SC. The results showed a CpG effect on body weight gain in both SC and TK chickens but an oocyst shedding effect in TK chickens (Dalloul et al. 2004). Only CpG ODN with a phosphorothioate backbone (S-CpG ODN) reduced the number of oocysts shed by TK chickens but not in SC chickens. In previous work, reduced oocyst shedding in TK birds was observed with intravenous CpG ODN injection. However no clear correlation was found between weight gains and oocyst shedding. Enzyme linked immunosorbent assay results showed higher antibody response in SC chickens injected with the S-CpG ODN. In contrast, no such effect was found in TK birds despite the reduced shedding of oocysts.

Mechanismy kontroly kokcidiózy drůbeže a jejich perspektivy

Dlouhodobě byla prevence kokcidiózy ve výkrmu brojlerů založena převážně na používání antikokcidik a významně ovlivnila rozvoj drůbežnictví. Také vakcinace drůbeže živými vakcínami přispěla ke kontrole kokcidiózy. Podle vlastností použitého druhu kokcidií rodu *Eimeria* rozlišujeme tři základní skupiny živých vakcín: vakcíny obsahující virulentní kmeny kokcidií, vakcíny s atenuovanými kmeny kokcidií, vakcíny obsahující živé kmeny s relativní tolerancí k ionoforovým antikokcidikům a subjednotkové vakcíny. Vzrůstající problém rezistence kokcidií rodu *Eimeria* na používaná antikokcidika vedl k rozvoji výzkumu alternativních způsobů kontroly kokcidiózy na základě nových poznatků v imunomodulaci, využití léčiv rostlinného původu a nových živých a rekombinantních vakcín. Tento článek podává přehled o výše uvedených metodách kontroly kokcidiózy drůbeže.

References

ALLEN PC, DANFORTH HD, AUGUSTINE PC 1998: Dietary modulation of avian coccidiosis. Int J Parasitol **28**: 1131-1140

ALLEN PC, DANFORTH HD, LEVANDER OA 1996: Diets high in n-3 fatty acids reduces cecal lesion scores in broiler chickens infected with Eimeria tenella. Poult Sci **75**: 179-185

ALLEN PC, DANFORTH HD, LEVANDER OA 1997: Interaction of dietary flaxseed with coccidia infections in chickens. Poult Sci **76**: 822-827

ALLEN PC, FETTER RH 2002: Recent advances in biology and immunobiology of Eimeria species and in diagnosis and control of infection with these coccidian parasites of poultry. Clin Microbiol Rev **15**: 58-65

AVERY S, ROTHWELL L, DEGEN WGJ, SCHIJNS VET, YOUNG JR, KAUFMAN J, KAISER P 2004: Characterization of the first nonmammalian T2 cytokine gene for cluster: the cluster contains functional single copy genes for IL-3, IL-4, IL-13 and GM-CSF, a gene for IL-5 that appears to be a pseudogene, and a gene encoding another cytokine-like transcript, KK34. J Interferon Cytokine Res **24**: 600-610

BEDRNÍK P 1989: The role of different Eimeria species in a protective coccidiosis vaccine. In: P. Yvoré, P.(Ed.), Coccidia and Intestinal Coccidiomorphs (pp. 666-670). Paris: INRA.

BELLI SI, MAI K, SKENE CD, GLEESON MT, WITCOMBE DM, KATRIB M, FINGER SI, WALLACH MG, SMITH NC 2004: Characterization of the antigenic and immunogenic properties of bacterially expressed, sexual stage antigens of the coccidia parasite Eimeria maxima. Vaccine **22**: 4316-4325

DALLOUL RA, LILLEHOJ HS SHELLEM TA, DOERR JA 2003a: Enhanced mucosal immunity against Eimeria acervulina in broiler fed a Lactobacillus based probiotic. Poult Sci **82**: 62-66

DALLOUL RA, LILLEHOJ HS, OKAMURA M, XIE H, MIN W, DING X, HECKERT RA 2004: In vivo effects of CpG oligodeoxynucleotide on Eimeria infection in chickens Avian Dis **48**: 783-790

DALLOUL RA, LILLEHOJ HS, SHELLEM TA, DOERR JA 2002: Effect of vitamin A deficiency on host intestinal immune response to Eimeria acervulina in broiler chickens. Poult Sci **81**: 1509-1515

DALLOUL RA, LILLEHOJ HS, SHELLEM TA, DOERR JA 2003b: Intestinal immunomodulation by vitamin A deficiency and Lactobacillus based probiotic in Eimeria acervulina-infected broiler chickens. Avian Dis **47**: 1313-1320

DALLOUL RA, LILLEHOJ HS 2005: Recent advances in immunomodulation and vaccination strategies against coccidiosis. Avian Dis **49**: 1-8

DANFORTH HD 2000: Increase in anticoccidial sensitivity seen after field trial studies with five oocyst vaccination of particularly drug-resistant strains od avian Eimeria species (abstract). In: Proceeding of the 75th Annual meeting of the American Society of Parasitologists and the 53rd annual meeting of Protozoologists, p. 90

DANFORTH HD, ALLEN PC, LEVANDER OA 1997b: The effect of high n.3 fatty acid diets on the ultrastructural development of E. tenella. Parasitol Res **83**: 440-444

DARNFORTH HD, LEE EH, MARTIN A, DEKICH M 1997a: Evaluation of a gel-immunisation technique used with two different Immucox vaccine formulations in battery and floor-pen trials with broiler chickens. Parasitol Res **83**: 445-451

DING X, LILLEHOJ HS, QUIROZ MA, BEVENSEE E, LILLEHOJ EP 2004: Protective immunity against Eimeria acervulina following in ovo immunization with a recombinant subunit vaccine and cytokine genes. Infect Immun **72**: 6939-6944

FETTERER RH, AUGUSTINE PC, ALLEN PC, BARFIELD RC 2003: The effects of dietary betaine on intestinal and plasma levels of betaine in uninfected and coccidia-infected broiler chicks. Parasitol Res **90**: 343-348

GOMIS S, BABIUK L, GODSON DL, ALLAN B, THRUSH T, TOWNSEND H, WILLSON P, WATERS E, HECKER R, POTTER A 2003: Protection of chickens against Escherichia coli infection by DNA containing CpG motifs. Infect Immun **71**: 857-863

GREIF G 2000: Immunity to coccidiosis after treatment with toltrazuril. Parasitol Res **86**: 787-790

GUO FC, KWAKKEL RP, WILLIAMS BA, PARMENTIER HK, LI WK, YANG ZQ, VERSTEGEN MWA 2004: Effects of mushroom and herb polysaccharides on cellular and humoral immune responses of Eimeria tenella-infected chickens. Poult Sci **83**: 1124-1132

GURANATHAN S, KILNMAN DM, SEDER RA 2000: DNA vaccines: immunology, application, and optimization. Ann Rev Immunol **18**: 927-974

HAIKKINEN M, SCHNEITZ C 1999: Efficacy of a commercial competitive exclusion product against Campylobacter jejuni. Br Poult Sci **40**: 619-621

HE H, CRIPPEN TL, FARRELL MB, KOGUT MH 2003: Identification of CpG oligodeoxynucleotide motifs that stimulate nitric oxide and cytokine production in avian macrophage and peripheral blood nuclear cells. Rev Comp Immunol **27**: 621-627

CHAPMAN HD 1997: Biochemical, genetic and applied aspects of drug resistance in Eimeria parasites of the fowl. Avian Pathol **26**: 221-244

CHAPMAN HD 1999: Anticoccidial drugs and their effects upon the development of immunity to Eimeria infections in poultry. Avian Pathol **28**: 521-535

CHAPMAN HD, CHERRY TE, DANFORTH HD, RICHARD G, SHIRLEY MW, WILLIAMS RB 2002: Sustainable coccidiosis control in poultry production: the role of live vaccines. Int J Parasitol **32**: 617-629

CHAPMAN HD, JOHNSON ZB 2002: Use of antibiotics and roxarsone in broiler chickens in the USA: analysis for the years 1995-2000. Poult Sci **81**: 356-364

CHAPMAN HD, MARSLER P, La VORGNA MW 2004: The effects of salinomycin and roxarsone on the performance of broilers when included in the feed for four, five or six weeks and infected with Eimeria species during the starter or grower phase of production. Poult Sci **83**: 761-764

CHATEAU N, CASTELLNOS I, DESCHAPS AM 1993: Distribution of pathogen inhibition in the Lactobacillus isolates of a commercial probiotic consortium. J Appl Bacteriol **74**: 36-40

CHEW BP, 1995: Antioxidant vitamins affect food animal immunity and health. J Nutr 125: 18045S-18085S

CHEW BP, PARK JS 2004: Carotenoid action on the immune response. J Nutr **134**: 257S-261S

JEFFERS TK 1975: Attenuation of Eimeria tenella through selection for precociousness. J Parasitol **61**: 1083-1090

JOYNER LP, NORTON CC 1973: The immunity arising from continuous low-level infection with Eimeria tenella. Parasitology **67**: 907-913

KLASING KC, ADLER KL, REMUS JC, CALVERT CC 2002: Dietary betaine increases intraepithelial lymphocytes in the duodenum of coccidia infected chicks and increases functional properties of phagocytes. J Nutr **132**: 2274-2282

KLINMAN DM 2004: Immunotherapeutic uses of CpG oligodeoxynucleotides. Nat Rev Immunol **4**: 249-259

KLINMAN DM, CURRIE D, GURSEL I, VERTHELYI D 2004: Use of CpG oligodeoxynucleotides as immune adjuvants. Immunol Rev **199**: 201-216

KOPKO SH, MARTIN DS, BARTA JR 2000: Responses of chickens to a recombinant refractile body antigen of *Eimeria tenella* administered using various immunizing strategies. Poult Sci **79**: 336-342

KRIEG AM 2002: CpG motifs in bacterial DNA and their immune effects.Ann Rev Immunol **20**: 709-760

KRIEG AM, YI AK, MATSON S, WADSCHMIDT TJ, BISHOP GA, TEADALE R, KORETZKY GA, KLINMAN DM 1995: CpG motifs in bacterial DNA triger direct B-cell activation. Nature **374**: 546-549

La RAGIONATE RM, NARBAD A, GASSON MJ, WOODWARD MJ 2004: In ovo characterization of *Lactobacillus johnsonii* F19785 for use as a define competitive exclusion agent against bacterial pathogens in poultry. Litt Appl Microbiol **38**: 197-205

LEE EH 1987: Vaccination against coccidiosis in commercial roaster chickens. Can Vet J **28**: 434-436

LI CM 1998: Chinese herb medicime feed additives (Chinese). 2nd rev. ed. Chinese Agricultural University Press, Beijing.

LI XY 2000: Immuno-modulation components from Chinese medicines. Pharm Bio **38** (Suppl.): 33-40

LIEN EJ, GAO H 1990: Higher plant polysaccharides and their pharmacological activities. Int J Orient Med **15**: 123

LILLEHOJ HS 1998: Role of T lymphocytes and cytokines in coccidiosis. Int J Parasitol **28**: 1071-1081

LILLEHOJ HS, DALLOUL RA, MIN W 2003: Enhancing intestinal immunity to coccidiosis. World Poult **19** (Coccidiosis 4): 18-21

LILLEHOJ HS, DING X, QUIROZ MA, BEVENSEE E, LILLEHOJ EP 2005: Resistance to intestinal coccidiosis following DNA immunization with the cloned 3-1E *Eimeria* gene plus IL-2, IL-15, and IFN-γ. Avian Dis **49**: 112-117

LILLEHOJ HS, CHOI KD, JENKINS MC, VAKHARIA VN, SONG KD, HAN JY, LILLEHOJ EP 2000: A recombinant *Eimeria* protein inducing interferon-gamma production: comparison of different gene expression systems and immunization strategies for vaccination against coccidiosis. Avian Dis **44**: 379-389

LONG PL 1972: *Eimeria tenella*: reproduction, pathogenicity and immunogenicity of a strain maintained in chick embryos by serial passage. J Comp Pathol **82**: 429-437

LONG PL, JOHNSON J, McKENZIE ME, PERRY E, CRANE MstJ, MURRAY PK 1986: Immunization of young broiler chickens with low level infections of *Eimeria tenella, E. acervulina* or *E. maxima*. Avian Pathol **15**: 271-278

MATHIS GF, FROYMAN R, IRION T, KENNEDY T 2003: Coccidiosis control with toltrazuril in conjunction with anticoccidial medicated or nonmedicated feed. Avian Dis **47**: 463-469

MATHIS GF, FROYMAN R, KENNEDY T 2004: Coccidiosis control by administering toltrazuril in the drinking water for a 2-day period. Vet Parasitol **121**: 1-9

McDOUGALD LF, MATHIS GF, SEIBERT BP 1990a: Anticoccidial efficacy of diclazuril against recent field isolates of Eimeria from commercial poultry farm. Avian Dis **34**: 911-915

McDOUGALD LF, SEIBERT BP, MATHIS GF, QUARLES CL 1990b: Anticoccidial efficacy of diclazuril in broilers under stimulated natural conditions in floor-pens. Avian Dis. **34**: 905-910

MEHLHORN H, ORTMAN-FALKENSTEIN A, HABERKORN A, MAXIMA E 1984: The effects of symmetrical triazinetrione on developmental stages of *Eimeria tenella*, and *E. acervulina*: a light and electron microscopical study. Z Parasitenkd **70**: 173-182

MEHLHORN H, SCHMAHL G, HABERKORN A 1988: Toltrazuril effective against a broad spectrum of protozoan parasites. Parasitol Res **75**: 6

MICHAEL A 2002: The practical use of a maternal vaccine against coccidiosis. World Poult **18**: 2-4

MIN W, DALLOUL RA, LILLEHOJ HS 2004: Application of biotechnology tools for coccidia vaccines development. J Vet Sci **5**: 279-288

MIN W, LILLEHOJ HS, BURNSIDE J, WEINING KC, STAEHELI P, ZHU JJ 2001: Adjuvant effects of IL-1 beta , IL-2, IL-8, IL-15, IFN-alpha, IFN-gamma, TGF-beta 4 and lymphotactin on DNA vaccination against Eimeria acervulina. Vaccine **20**: 267-274

MUTWIRI G, PONTAROLLO R, BABIUK S, GRIEBEL P, van DRUNEN LITTLE-van den HURK S, MENA A, TSANG C, ALCON A, NICHANI A, IOANNOU X 2003: Biological activity of immunostimulatory CpG DNA motifs in domestic animals. Vet Immunol Immunopathol **91**: 89-103

PANG FH, XIE MQ, LING HH 2000: The investigation of immunodulators tested for the results on the control of a coccidial infection. Chin J Vet parasitol **8**: 1-3

SANGSTER NC 2001: Managing parasiticide resistance. Vet Parasitol **98**: 89-109

SHIRLEY MW 1989: Development of a live attenuated vaccine against coccidiosis of poultry. Parasitol Immunol **11**: 117-124

SHIRLEY MW, MILLARD BJ 1986: Studies on the immunogenicity of seven attenuated lines of Eimeria given as a mixture to chickens. Avian Pathol **15**: 629-638

SCHAAP D, ARTS G, KROEZE J, NIESSEN R, ROOSMALEN-Vos SPREEUWENBERG K, KUIPER CM, BEEK VERHOEVEN NV, KOK JJ, KNEGTEL RM, VERMEULEN AN 2004: An *Eimeria* vaccine candidate appears to be lactate dehydrogenase, characterization and comparative analysis. Parasitology **128**: 603-616

SCHETTERS TPM, JANSEN HAJM, VERMEULEN AN 1999: A new vaccination concept against coccidiosis in poultry. In: van der Sluis, W. (Ed), World poultry. Elsevier, Amsterdam, pp. 23-24

STERN NJ, COX NA, BAILEY JS, BERRANG ME, MUSGROVE MT 2001: Comparison of mucosal competitive exclusion and competitive exclusion in treatment to reduce *Salmonella* and *Campylobacter* spp. colonization in broiler chickens. Poult Sci **80**: 156-160.

VANPARIJS O, MARSBOOM R, DESPLENTER L 1989a: Diclazuril, a new broad spectrum anticoccidial in chickens. 1. Dose titration studies and pilot-pen trials. Poult Sci **68**: 489-495

VANPARIJS O, MARSBOOM R, HERMANS L, Van der FLAES L 1989b: Diclazuril, a new broad spectrum anticoccidial in chickens. 2 Battery trials. Poult Sci **68**: 496-500

VANPARIJS O, MARSBOOM R, HERMANS L, Van der Flaes L 1990: Diclazuril, a new broad-spectrum anticoccidial for chickens 3. floor-pen trials. Poult Sci 69: 60-64

VERMEULEN AN, SCHAAP DC, SCHETTERS Th PM 2001: Control of coccidiosis in chicken by vaccination. Vet Pathol **100**: 13-20

VERMEULEN AN, SCHETTER TPM, SCHAAP DC 2000a: Control of coccidiosis by vaccination. Paper no S1. 10.03 in CD-ron version of proceedings XXI world's poultry congress, Montreal. Montreal, Canada

VERMEULEN AN, SCHETTERS TPM, JANSEN HAJM, HANRNBERG EROM, DONKERS AMJ 2000b: A new vaccination/control concept against coccidiosis in poultry: combining a vaccine with ionophore treatment. In proceedings of the Cost 820 animal workshorp, immunity to coccidial parasites: from natural infections to molecular vaccination, Dublin (p.53). Dublin. Eire.

VERMEULEN AN, SCHAAP DC, SCHETTERS ThPM 2001: Control of coccidiosis in chickens by vaccination. Vet Parasitol **100**: 13-20

VLEUGELS B, VERVERKEN C, GODDEERRIS BM 2002: Stimulatory effect of CpG sequences on humoral response in chickens. Poult Sci **81**: 1317-1321

WALLACH M 2002: The development of CoxAbic® a novel vaccine against coccidiosis. World Poult **18**: 2-4

WILLIAMS RB 1992: The development, efficacy and epidemiological aspects of Paracox, a new coccidiosis vaccine for chickens. Harefield, UK: Pitman/Moore Europe

WILLIAMS RB 1994: Safety of the attenuated anticoccidial vaccine Paracox in broiler chickens isolated from extraneuos coccidial infection. Vet Res Commun **18**: 189-198

WILLIAMS RB 1998: Epidemiological aspects of the use of live anticoccidial vaccines for chickens. Int J Parasitol **28**: 1089-1098

WILLIAMS RB 2002: Anticoccidial vaccines for broiler chickens: pathways to success. Avian Pathol 31: 317-353

WOLFF J, MALONE RW, WILLIAMS P, CHONG W, ACSADI G, JANI A, FELGNER PL 1990: Direct gene transfer into mouse muscle in vivo. Science **247**: 1465-1468

WU SQ, WANG M, LIN Q, ZHU YJ, SUO X, JIANG JS 2004: Construction of DNA vaccine and their induced protective immunity against experimental *Eimeria tenella* infection. Parasitol Res **94**: 332-336

XIA EN, CHENG QH 1988: Isolation, analysis and bioactivities of Tremella fuciformis fruit body polysaccharides. Acta Mycol Sin **7**: 166-174

XIE H, RAYBOURNE RB, BABU US, LILLEHOJ HS, HECKERT RA 2003: CpG- induced immunomodulation and intracellular bacterial killing in a chicken macrophage cell line. Dev Comp Immunol **27**: 823-834

XUE M, MENG XS 1996: Review on research progress and prosperous of immune activities of bio-active polysaccharides. J Tradit Chin Vet Med **3**: 15-18

YOUN HJ, NOH JW 2001: Screening of the anticoccidial effects of herb extracts against *Eimeria tenella*. Vet Parasitol **96**: 257-263

YU JG, ZHU LY 2000: The use of Astragalus Radix polysaccharides against Infectious Disease in chickens. J Tradit Chin Vet Med **6**: 3-4

YUAN YJ, FAN BT, ZHANG YX 1993: Efficacy of 8301 polysaccharides as an adjuvant against avian Pasteurellosis in chickens. J Tradit Chin Vet Med **3**: 6-10

Abbreviations: DNA = deoxyribonucleic acid, IFN = interferon, IL = interleukin, TFG = transforming growth factor, MHC = major histocompatibility, TCR = T- cell receptor, ODN = oligodeoxynucleotide