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DNA vaccines as sustainable Coccidiosis control strategies in chickens

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Abstract

Coccidiosis, still one of the most widely reported diseases of the world within the poultry industry from economic point of view. The control is highly dependent on use of coccidiostates and some extent on live vaccines. But their negative impacts like drug residues, drug resistance, extreme production cost, poor results, high mortality and morbidity have restricted their application. DNA vaccines with advantages of their stability, costly effectiveness and the non-requirement of cold chain have proved their worth. Immunity against *Eimeria* depends mainly on the cellular arm of the immune response including CD4+ and CD8+ T cells which is sufficiently provided by DNA vaccines. In the review, different aspects of DNA vaccines against Coccidiosis in chickens are summarized.

Key words: Chicken, Coccidiosis, DNA, vaccine.

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Introduction

Coccidiosis, still one of the most widely reported diseases of the world within the poultry industry from an economic point of view [1]. The coccidia are present normally in poultry raising operations almost throughout the world; however, disease occurs following the presence of significant number of oocysts inside the intestines. Signs and symptoms of Coccidia infection include severe weight loss, bloody diarrhea, decreased egg production and sometimes mortality. The infection caused severe gross lesions in the intestines of infected chickens (Fig. 1).

There are totally seven species of Eimeria as the causative agent of Chicken Coccidiosis named; *E. tenella*, *E. maxima*, *E. acervulina*, *E. brunetti*, *E. necatrix*, *E. praecox* and *E. mitis*. These species are highly specific and resident in different part of intestine of host (Table 1).

Table 1: Habitant place of *Eimeria* species different parts of chicken intestine.

Species name	Intestine parts
E. tenella	Caeca
E. acervulina	Duodenal loop and upper small intestine
E. necatrix	Upper small intestine
E. maxima	Small intestine
E. mitis	Duodenal loop and upper small intestine
E. mivati	Small intestine
E. brunetti	Small intestine and caeca

Today, control of *Eimerian* Coccidiosis is dependent on the use of coccidiostats and some extent on the live vaccines. But there are still negative impacts persisting like drug residues presence, drug resistance, extreme production cost, poor results, high mortality and morbidity. For example, we have provided drug withdrawal periods of some of commonly used drugs for Coccidiosis (Table 2). Therefore, there is great need for alternate and specific techniques, which can give a better solution for these shortcomings as well as provide good quality levels of protection.

Table 2: Withdrawal periods of commonly used drugs against Coccidiosis.

Name	Withdrawal period (days)
Amprolium	0
Halofuginone hydrobromide	4–7
Lasalocid sodium	3
Maduramicin ammonium	5
Clopidol or meticlorpindol	0
Amprolium + ethopabate	0
Amprolium+Sulfaquinoxaline	5

DNA immunization has been widely studied as a novel strategy to elicit protection against Coccidiosis for its ability to stimulate cellular immune response [2-15]. These recent immunological research of vaccines is directed towards this goal. Cytokines the natural modulators of the immune system and are being used as vaccine adjuvant to enhance host immune responses induced by DNA vaccines in Coccidiosis [16]. It was stated that interferon gamma (chIFN gamma) is a strong adjuvant candidate for both the general enhancement immune response induced by vaccine antigen and modulation of the immune response generated. It induces a microbicidal state, which is involved in the resolution of the infection by

intracellular parasites, thus enhancing vaccine efficacy [17].

DNA vaccination, the underlying principle

DNA vaccines, generated using plasmids, include a gene encoding target antigen under the transcriptional control of an effective viral/eukaryotic promoter, along with a polyadenylation signal sequence (poly-A) and a bacterial origin of replication. The poly-A provides stability and effective translation; and the antibiotic resistance gene facilitates selection of bacteria. For complete optimization of DNA vaccine, the plasmid should have a Kozak sequence (GCCA/GCC) upstream of initiator codon and an enhancer, downstream of the poly-A signal [14]. But, to elicit an effective immune response, the protein should undergo post-translational modification and retain their tertiary structure.

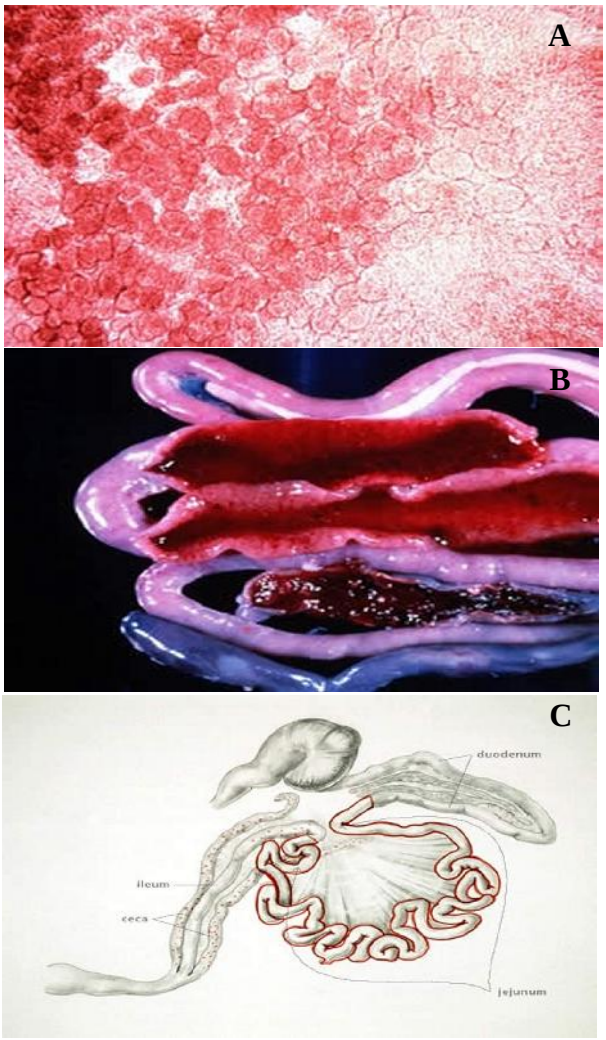

Fig. 1: *Eimeria necatrix*. (A) Developmental stages (B) Gross lesions (C) oocysts in small intestine. Courtesy by Merck Veterinary Manual

After *in vivo* generation, the antigenic peptides are processed and presented by professional antigen presenting cells (APCs), like dendritic cells, by getting primed through direct or by obtaining proteins from myocytes (Fig. 2).

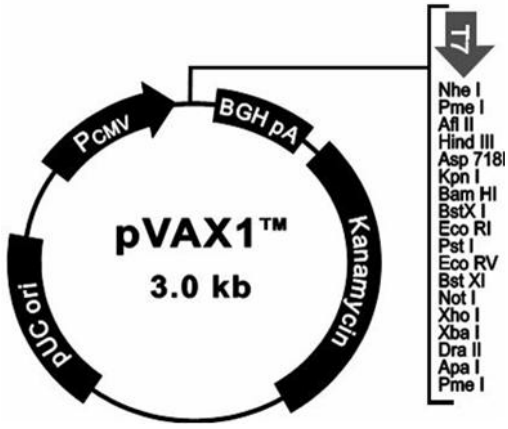

Fig. 2: A schematic presentation of mammalian expression vector pVAX1 (Invitrogen).

DNA Vaccination with reference to *Eimeria*

Immunity against *Eimeria* depends on the cellular arm of the immune response including CD4+ and CD8+ T cells with antibodies playing only a minor role in protection [18]. Thereby, DNA immunization has been widely studied as a novel strategy to elicit protection against Coccidiosis for the ability to stimulate cellular immune response [2-10].

The administration of a simple plasmid can induce a broad spectrum of immune responses against *Eimeria* parasites. Vaccination of Coccidiosis with DNA is one of the most promising novel immunization techniques against *Eimeria* pathogens. A lot of effective efforts have been put forward to identify methods of enhancing the immune response of plasmid DNA, to enable its practical implementation. The most importance has been given to develop vaccines to elicit both Humoral and cellular immune responses. The use of different types of immune modulators, cytokines and co-stimulatory molecules, in this DNA Coccidiosis vaccination reflect the positive approach to enhancing the immunity level against *Eimeria*. If the potency is improved, plasmid DNA vaccines, having numerous

advantages, can be useful for the active immunization against Coccidiosis.

Considering the present status and many other possibilities, efforts should be targeted towards improving their delivery or to increase their immunogenic potential. Poor cellular uptake and rapid *in vivo* degradation of plasmid DNA has to be taken into account and novel delivery systems has to be developed along with the optimization of the plasmid vector. Some of the recently studied *Eimeria* DNA vaccines are tabulated in Table 3.

Table 3: Recently studied DNA vaccines.

DNA vaccine	Species studied	Reference
pVAX1-cSZ-2-IL-2	*Eimeria tenella* *Eimeria necatrix* *Eimeria maxima* *Eimeria acervulina*	[2-5]
pcDNA-TA4-IL-2	*Eimeria tenella* *Eimeria necatrix* *Eimeria maxima* *Eimeria acervulina*	[8,9]
pcDNA3.1-Et1A-TA4	*Eimeria tenella*	[11]
3-1E cDNA-pBK-CMV	*Eimeria acervulina*	[12]
pcDNA3.1-3-1E-ChIL-15	*Eimeria acervulina*	[24]

Factors affecting success of DNA vaccination

A different types of factors determine the success of DNA immunization, such as the structure of the plasmid backbone, quantity of plasmid delivered, route and times of immunization, age of animals and so on [19]. Different routes for DNA vaccine delivery have been used intramuscular, intradermal, intravenous, intranasal and epidermal [13]. Studies in mammalian systems clearly documented that route and dose of DNA immunization influenced the types of immunity elicited. Generally, intramuscular and intradermal inoculations induced T helper (Th1) responses. In contrast, epidermal injection elicited T helper 2 (Th2) responses [20, 21].

Song et al. [9] studied some of factors are which are highly influencing efficacy of immunization, for instance, the routes, doses, and timing. A number of the factors such as immunization dose, immunization route, primary immunization age, immunization time and so on, affected the magnitude and type of immune response induced by plasmid DNA. Among these factors, under certain delivery methods, immunization dose was the most important. It was reported that chickens of 3 days old immunized with 50 µg of pcDNA3.1-Et1A-TA4 intramuscularly showed the highest ACI in different doses [11]. Lillehoj et al. [22] immunized chickens with *E. acervulina* gene 3-1E. The anti-3-1E antibody titers were enhanced in a dose dependent manner and decreased the number of oocysts and increased the weight gain at relatively high-dose. More plasmid correlated with stronger response, but not necessarily linearly. Among chickens immunized intramuscularly with various doses of pMP13 expression vector ranging from 5 µg, to 100 µg, both 5 µg and 50 µg DNA group presented more effective in reducing the oocyst production after challenge with *E. acervulina*. However, the highest dose group of 100 µg was the worst one. Immunization route plays an important role in the induction of protective immune responses to DNA immunization. Intramuscular, intravenous and mucosal administration of DNA provided some protective immunity against a lethal challenge with avian influenza virus [13]. There is report that intramuscular injection provided higher levels of serum antibody subcutaneous injection in chickens immunized with the 3-1E gene of *E. acervulina*. Primary immunization age and immunization times are very important to DNA immunization. Young primary immunization age has obvious effect on the efficacy of intramuscular injection, because the immature muscle cells of young animals are beneficial to be transformed.

Future prospects

Coccidiosis is caused by many species of *Eimeria*, and the commercial vaccines are often a mixture of many *Eimeria* species accompanied with loads of drawbacks; therefore, there is need of a vaccine which can provide protection to more than one species. The administration of cytokine genes combined with antigen genes possibly helps the host immune system to elicit a correct type of response. It is documented that DNA vaccination of chickens in concert with cytokines like IL-2, IL-15, and IFN-gamma enhanced protective intestinal immunity against Coccidiosis [22]. Hilton et al. [23] stated that recombinant chIFN-gamma prolonged secondary antibody response that persisted at higher levels and for longer periods as compared with live antigen. Weight gain can be regarded as an important factor for evaluating the efficacy of anti-coccidial drugs. The homologous challenge with *E. acervulina* the respective ACI for chicken vaccinated with pVAX1-cSZ-2, pVAX1-chIFN-gamma and pVAX1-cSZ-2- chIFN-gamma were 173, 164, and 171, respectively [4].

The major challenge in the future will be the improvement of the transfection efficiency of the DNA Coccidiosis vaccines. Gene gun and electroporation have increased the transfection and improved immune responses to a great deal, but these technologies are accompanied with their drawbacks especially the economic issues. Therefore, these technologies have not yet practiced for routine.

Another promising approach is the development of microparticles/nanoparticles as delivery systems or the non-invasive plasmid DNA immunization. Although the potency of the immune response has been weak while using oral administration methods and novel adjuvant may significantly improve them.

Further, the properties of DNA Coccidiosis vaccines have to be modulated via using cationic liposomes for promoting mucosal and systemic immunity simultaneously. The current scenario of incorporating such novel methodologies unveils much promise regarding the development of effective, safe and economically viable DNA Coccidiosis vaccines

Conclusion

DNA *Emeria* vaccination is a promising technology to prevent coccidioisis in the Poultry farming industry. However, there is a great need to increase the potency of DNA *Emeria* vaccine against all species. This may be carried out by a multivalent/multiepitopes vaccine against different species or by improving delivery devices, good formulated adjuvant base; which might enhance the immunity to its anticipated level. The cost of the vaccine must also be taken into consideration.

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