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Advancements in Research Related to Vaccination Against Avian Coccidiosis

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Abstract

Coccidiosis is a fatal disease of poultry, caused by *Eimeria* species and considered the most prominent and destructive disease within all protozoal diseases. Chemotherapy is considered the main preventive strategy against coccidiosis. Chemotherapy is partially successful and has many drawbacks as it is practiced after the outbreak of disease, until then coccidiosis causes huge production and health losses. As an alternative, prophylactic measures like vaccination and management betterment are of eminent importance and can help to overcome this disease. Vaccination can induce antibody-mediated immunity, cell-mediated immunity or both. However, ordinary vaccines do not offer enough potential to overcome *Eimeria* species at its full extent. Different research trials and studies have shown that DNA vaccines can induce both antibody and cell-mediated immunity. Several DNA vaccines have been tested in the last few years with marvelous results but still no vaccine is available commercially. Here we will discuss different types of vaccines against *Eimeria*, especially in poultry. A comparative analysis along with the recent advancements will reveal the types of vaccines that bear sufficient potential to overcome *Eimeria* species to its full extent. This review will also describe the fruitful and advanced steps towards the synthesis of molecular vaccines in the future.

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Introduction

Coccidiosis is one of the most prominent and disastrous protozoal diseases of poultry, cause serious threat towards overall health and production. It is caused by species of *Eimeria* and *Isospora*, belongs to phylum *Apicomlexa*, which mostly affects the intestines of birds [1]. It leads towards severe losses as poultry meat and eggs are the cheapest sources of protein, hence contributing a lot to maintain good health and nutritional requirements. This fatal disease affects the overall health and production of birds by disturbing the gut integrity and efficiency of intestines. This condition is followed by poor absorption of feed, low feed conversion ratio, less production of birds and compromised immune system [2]. The decrease in egg production, feed conversion ratio, meat production, and high mortality in poultry sector leads to huge economic losses [3]. There are seven recognized species of genus *Eimeria* responsible for coccidiosis in poultry and the most important pathogenic species is *E. tenella*. It affects caecum in birds causing caecal coccidiosis and is responsible for enormous losses to the poultry industry [4].

A lot of methods to confront coccidiosis like chemotherapy and prophylactic measures are in regular practice. Chemotherapy is followed by resistance, which reduces the effects of drugs. Drug residues are also a matter of concern for human consumption [5]. Prophylactic measures have a solid effect on coccidiosis. There are many ways relating to prophylactic measures, which induce immunity against coccidiosis, *i.e*., vaccination and management betterment. Vaccination is one of the most liable of all techniques to overcome coccidiosis [2]. Live and dead vaccines have a strong impact on immunity. Nowadays, many other new strategies are also contributing to minimizing *Eimeria* effects. These techniques include subunit vaccines, DNA vaccination and roles of different adjuvants. In this review, we will discuss and analyze all the strategies of vaccination against *Eimeria* in poultry along with the recent advancements.

Life cycle of *Eimeria*

Oocyst of *Eimeria* remains at different places for many years. Once it sporulates, it becomes active and after ingestion by the specific host, it goes to intestine and exocyst there. Sporozoites released from sporulated oocysts, penetrate the intestinal cells (enterocytes) of the host, encapsulate in a vacuole and replicate [2]. This replication is known as merogony that results in the formation of merozoites. These merozoites are released from the vacuole in tremendous numbers; rupture the host's enterocytes along with invasion into new cells. These merogonic divisions are genetically programmed, depends upon different *Eimeria* species. The more it reinvades the enterocytes, the more damage it causes, results in severe intestinal lesions [5, 6]. In the end, merozoites invade the intestinal cells as directed by specie specific merogony and differentiate into gametocytes (male and female gametes). Fertilization occurs within the host's cell; oocytes ruptures the enterocytes and expose to the environment along with feces (Fig 1).

Preventive and prophylactic measures against coccidiosis

Drugs always possess a central role to alleviate clinical signs of any disease and disease control programs. However, resistance is the main issue towards drug therapy, especially against parasitic infestation in humans and livestock. Every anthelmintic class is followed by resistance, especially in livestock hosts as mentioned by Kaplan [7]. Prophylactic measures have a potent role to escape from parasites. These strategies are followed by their own drawbacks. There should be some novel methods and techniques to eradicate, more or less, to control threats of parasitic infestations. Strategies related to prevention and prophylaxis against coccidiosis are discussed below.

Anticoccidial drugs

Starting from anticoccidial compounds, the first drug used against coccidiosis was sulphaquinoxaline in 1948 and was approved along with nitrofurazone by American fund and drug administration (AFDA) [8]. Similarly, a lot of drugs were used onwards against coccidiosis without registration and approval with better effects. Later, some drugs were refused by farmers due to resistance and some drugs have their own drawbacks, *i.e*., safety issues, residues and efficacy [9]. Anticoccidial drugs have different categories like synthetic, polyethylene, ionophores and mixed. Polyethylene or ionophores are further classified into monovalent ionophores, monovalent glycosidic ionophores and divalent ionophores (Table 1).

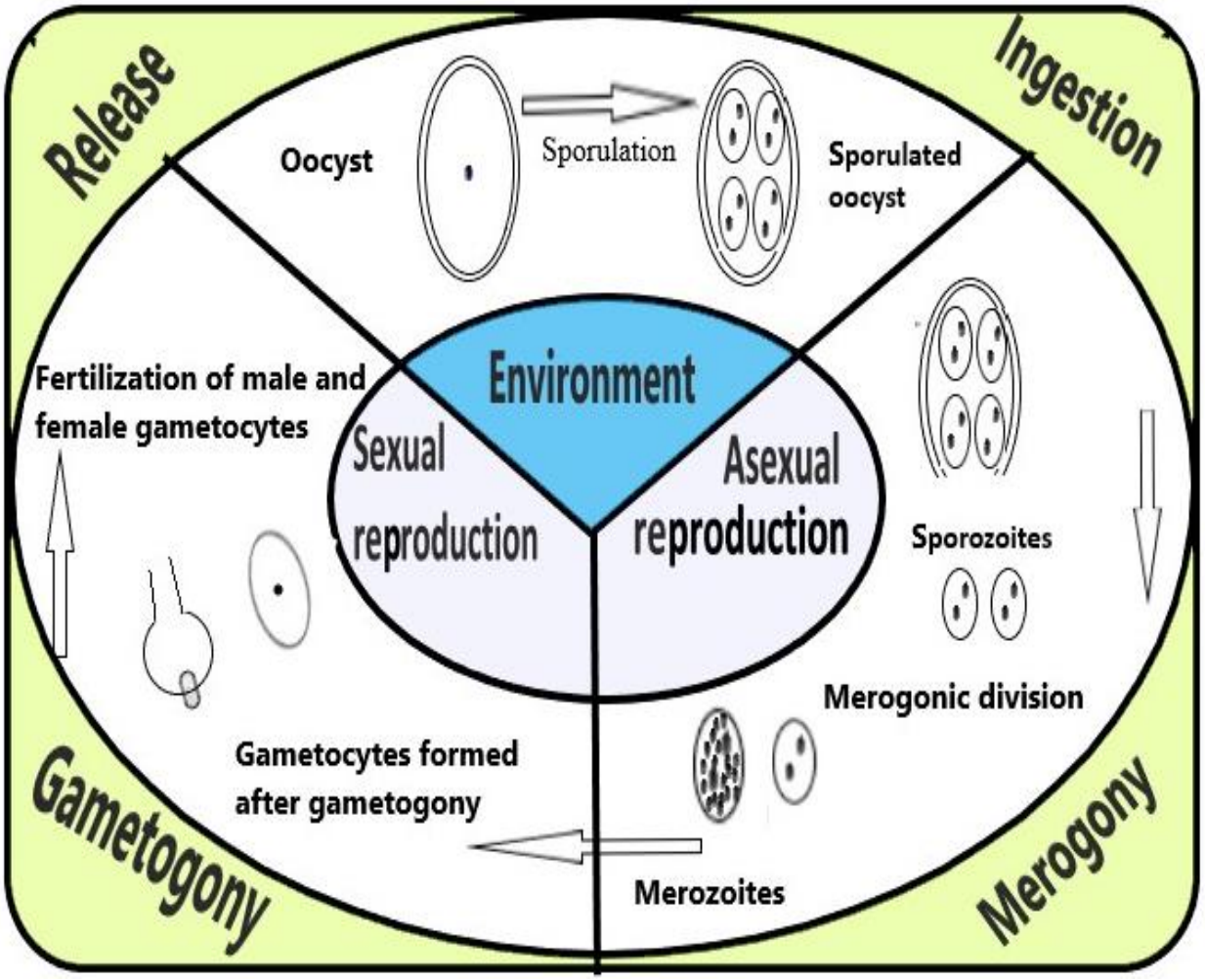

Fig. 1 Different stages of the life cycle of *Eimeria tenella.*

Resistance

According to World`s Health Organization, resistance against any drug is defined as "The ability of any microorganism/parasite to survive and reproduce itself, instead of having a proper dose of the drug within the tolerance range of host" [10]. Generally, intensive usage of anticoccidial drugs leads to resistance within hosts. Very quick resistance is observed in the case of quinolones and pyridines [11]. Long term exposure to anticoccidial drugs, especially belongs to a single group, leads towards resistance. Even many developed countries like United States (USA), Europe and China bear continuous threats of resistance [12]. A lot of natural herbs and their extracts were also practiced against coccidiosis like *Trachyspermum ammi* (Ajwain) by Abbas et al. [13] but they have no significance as compared to chemotherapy. These compounds can act a little against some *Eimeria* species but not all due to huge genetic variability, which degrades their effect [14, 15].

Vaccination

Some necessary prophylactic measures are adopted to confront coccidiosis in which the most important is vaccination. A vaccine is necessary to induce immunity against coccidiosis. Immunity achieved may be active or passive, according to the immune status of the host. Vaccination against *Eimeria* was first studied by Beach and Corl in 1925 [16], which lead to the foundation of modern vaccines against coccidiosis. Following those strategies, the first commercial vaccine was formulated in 1952 and was registered as coccivac in the USA [17]. After this achievement, a lot of vaccines were formulated by using this technique. Different vaccines were practiced against coccidiosis at different times, which are categorized into different types. A

detailed study of types of vaccines along with advancement in the vaccination process against *Eimeria* is as follows:

Live vaccines

Live vaccines are thoroughly composed of sporulated oocysts, either inactivated (non-attenuated) or inactivated (attenuated) form. Non-attenuated live vaccines consist of wild type strains of *Eimeria* species. Protective immunity against coccidiosis may be achieved either by applying a single high dose or by trickle infections (applying multiple low doses) [18, 19]. Non-attenuated vaccines contain *Eimeria* parasites, which are directly obtained from the field area (field strains) without modification and attenuation. Coccivac, immunocox, inovocox and advent are examples of non-attenuated vaccines. It is necessary to provide an equal amount of dose/bird while administering non-attenuated vaccines to avoid the risk of disease occurrence. It is essential to obey proper vaccination protocol in case of live non-attenuated vaccines against *Eimeria* [20]. Attenuated or inactivated vaccines are those which contain field strains after manipulation or lowering of virulence. There are many ways relating to attenuation like serial passages of parasites within chicken embryo and selection of precocious lines. In Livacox, *E. tenella* is present after treatment with serial passages while other *Eimeria* species are present by using precocious lines of attenuation process. Paracox contains all *Eimeria* lines following the precocious lines method of attenuation [21, 22]. Drawbacks of live vaccines are also of considerable importance. The most important is the reduction of effectiveness with the passage of time [23]. Dosage errors also result in non-satisfactory immune response or disease outbreaks. Feed formulations in poultry also contain anticoccidial drugs, which may act as lethal compounds for sensitive strains of *Eimeria*. Administration of live vaccines to immunocompromised birds may cause an outbreak. Sometimes reversing of virulence related to live, active or attenuated strains of *Eimeria* may become serious risk for farmers [9].

Subunit vaccines

This type of vaccine contains antigens that are purified after isolation from virulent and pathogenic *Eimeria* species. Subunit vaccines may contain antigens or proteins expressed from the DNA of different developmental *Eimeria* stages, like sporozoites, gametocytes or merozoites. Coxabic is the only registered subunit vaccine, which has been marketed since its manufacturing [24]. The drawback of subunit vaccine is that still, no antigen is able to provoke solid and potent protective immunity. It is necessary to understand the full genomic study of *Eimeria*, especially of *E. tenella* to sort out the actual mechanism of the immune response [25].

Exosomes derived from dendritic cells (DC) and antigen-presenting cells (APC) as a vaccine

Dendritic cells (DCs) were observed as an authentic pathway to induce immunity against coccidiosis. Different chemical signals are activated by DCs, which paved their role to activate T cells. DCs are also known as 'nature adjuvants' and the exosomes derived from these DCs possessing parasitic antigens might become an alternative to produce immunity against coccidiosis [26]. A trial in which the protective immunity was observed against coccidiosis (*E. tenella*) by using antigen loading DCs and DC-derived exosomes [27]. The study showed that antigen containing cells were present in the lymphoid tissues along with a higher concentration in germinal cells of cecal tonsils and spleen. A higher number of IgG and IgA antibodies were observed within cecal tonsils and spleen cells, reactive with *E. tenella* antigens along with the increased number of IL-2, IL-16, and interferon producing cells as compared to non-vaccinated groups. Similarly, in a research trial, DCs were isolated *in vitro* from sporozoites of *E. tenella*, *E. acervulina* and *E. maxima* [28]. These DCs were subjected to chickens for immunity induction tests. Results showed that spleen, payer's patches and cecal tonsils possessed a greater amount of IL-2, IL-16 and gamma interferon along with antigen-stimulated proliferative response and antigen reactive IgA and IgG producing cells in immunized birds. APC derived exosomes were also studied to check the immune inducing potential. A study determined the immunogenic capabilities of serum exosomes containing parasite antigens against avian coccidiosis [29]. Results revealed that a subset of protein AGS was found, especially in spleen and intestinal areas and an increase in the number of interleukins, especially IL-2, IL-4 and IL-6 along with interferon-gamma secreting cells were also observed in these areas.

DNA vaccines

It's a novel and magnificent approach towards prophylactic immunity against avian coccidiosis.

Table 1 Anticoccidial drugs, their categories and mechanism of action.

Sr. No.	Anticoccidial drug	Category	Mechanism of action
1	Amprolium	Synthetic	Competes with parasite for absorption of thiamine (Vit B1)
2	Monensin, Narasin and Salinomycin	Monovalent ionophores	Interfering balance of ions within parasite, which is very important for life, like Na, K, etc.
3	Maduramycin and Semduramycin	Monovalent glycosidic ionophore	Same as above (Ionophore)
4	Laslocid	Divalent ionophores	Same as above (Ionophore)
5	Maxban (Nicarbazin + narasin)	Mixed	Dual effect (synthetic + ionophores)
6	Pyrimethamine	Synthetic	Effects folate pathways of coccidia
7	Lerbek	Synthetic	Competes with parasite for absorption of thiamine (Vit B1)
8	Amprolium + Ethophobate	Mixed	Amprolium interferes thiamine, while Ethophobate blocks para-aminobenzoic acid (PABA)

DNA vaccine is also known as a third-generation vaccine, which is, in fact, a turning point towards preventive measures against coccidiosis [30] as it provokes cell-mediated immunity, which possesses a magnificent role in immunity [31]. DNA immunization or recombinant antigen can raise both humoral and cellular immune responses and continuous delivery of cytokines and chemokines as potential adjuvants could augment the impending for DNA or recombinant vaccines to induce extreme humoral and cellular immunity [32]. In this technique, immunogenic proteins are first isolated, identified and then inserted into a plasmid (vector); which is most probably a eukaryote, possesses the extreme capability to replicate. After purification of plasmid, the gene is directly inserted into the animal's body. These genes converted into respective proteins after administration, have ability to provoke the host immune system [33]. The selection of genes encoding proteins should be of optimal importance. They must possess any key role in the overall life cycle of *Eimeria*, *i.e*., *E. tenella* microneme protein genes are implicated in parasite recognition, motility, migration, and invasion of host cells [34]. DNA vaccines are more beneficial than all other types of vaccines as they can be generated with more ease and do not need a cold chain for storage. They possess long-lasting stability and they are less dangerous than traditional live vaccines with highly virulent live antigens [2].

Advancements regarding DNA vaccination against *Eimeria*

A lot of experimental trials related to DNA vaccines have been performed during the last two decades. In a trial, the respective changes in cytokines and IgG antibodies in serum of chickens were checked after administering a DNA vaccine encoding *E. acervulina* lactate dehydrogenase [31]. The results obtained satisfactorily suggested that this vaccine could raise the IgG level along with the induction of cytokines expressions. A DNA vaccine was checked against *E. tenella* and induced immunity [35]. In this study, the TA4 gene of *E. tenella* was ligated with a vector pcDNA3.1/Zeo and administered to experimental chickens. Results indicated that this vaccine-induced sufficient protective immunity against *Eimeria* challenge. Similarly, a DNA vaccine was prepared using *E. tenella* MZ5-7 gene and checked for the subsequent results against *E. tenella* challenge [36]. The efficacy of a DNA vaccine having *E. maxima* Gam56 as an antigen was determined against chicken coccidiosis [37]. This study indicated that the experimental vaccine could provide partial immunity after challenge with *E. maxima*. In this study, protection and practical application of DNA vaccine were investigated but the mechanisms involved related to the production of immune responses were left for further studies.

Role of different cytokines in DNA vaccine against *Eimeria*

There are several factors that mediate innate immunity in the avian immune system. These immune cells recognize the specific pathogen-associated molecular pattern (PAMPS) with the help of their specific pattern recognition receptors. The studies on these immune cells related to coccidiosis and *Eimeria* species emphasized the extraordinary qualities of these cells, results in the discovery of new uncharacterized genes [38]. A

vaccine consisting of *E. tenella* MZ5-7 along with chicken IL-17 was formulated and was evaluated against *E. tenella* challenge [36]. It was obvious that the vaccine along with cytokine produced more significant immunity then simple vaccines. It was observed that a new class of T-helper cells Th-17 was associated with interleukin IL-17 production. IL-17 works as a potential cytokine stimulator in avian immunity. In a similar study, IL-17 contribution was checked by using experimental *E. tenella* infection and the results showed that chicken IL-17 might help *E. tenella* induced immunopathology during infection [39]. In another study, *E. tenella* infected chickens treated with IL-17A antibodies suggested that IL-17A stops the migration of parasitized epithelial cells. Furthermore, a significant reduction of ROS and MM-9 along with a marked reduction in cecal lesions was found, which denoted IL-17A as a potential therapeutic agent against coccidiosis [40]. The role of many other cytokines like IL-2, IL-4 and IFN gamma, along with DNA vaccine was also studied. Here it is a special point that must be kept in mind while studying the role of cytokines that 2 different immune responses are governed by 2 different interleukins producing cells. IL-2 and IFN gamma are produced by Th1 cells while IL-4 and IL-1 are produced by Th2 cells [41]. All of these cytokines were studied separately and co-expression with different genes. DNA vaccines encoding *E. tenella* gene 541 along with IL-2 and IFN gamma (*IFN-γ*) were studied [42]. The results revealed that antigen 541 possesses good immunogenic qualities along with the confirmation of the role of cytokines that they can boost specific immunity by improving the efficacies of DNA vaccines against avian coccidiosis.

A DNA vaccine was tested in which immunity posed by DNA of *E. acervulina* cSZ-2 and IL-2 was seen against *E. tenella* challenge [43]. Chickens of one group were vaccinated with pVAX1-cSZ2, pVAX1-IL-2 and 2nd were vaccinated with pVAX1-cSZ2-IL-2. The results showed that the birds vaccinated with a co-expression of genes and IL2 cytokine depict more tolerance towards *E. tenella* challenge [43]. A similar trial was exercised, in which a gene encoding protein known as SO7 was tested for immunogenic analysis along with IL-2 [44]. The results expressed that IL-2 worked as a potential stimulator regarding immunity along with genes as compared to a vaccine containing SO7 gene alone. The role of IL-2 was also tested [45-47] in which a gene TA4 of *E. tenella* and pEtK2 gene were used along with IL-2. Results of IL-2 group were significantly protective regarding immunity as compared to other groups [48]. In a recent study, IL-10 was cloned and characterized. IL-10 possesses 45% identity with human IL-10, while 42% identical to murine IL-10 [49]. The chIL-10 gene structure contains 4 introns and 5 exons. The data and information gathered in this study revealed a genuine role of chIL-10 in changing Th bias during a protozoan infection [50]. These results showed that IL-10 possesses a solid role to reduce symptoms of *Eimeria* pathogenesis. One of the most popular works regarding cytokine role in DNA vaccine against *Eimeria* was done using 8 different cytokines (IL-1β, IL-2, IL-8, IL-15, IFN-α, IFN-γ, TGF-β4, lymphotactin) as adjuvants along with 3-1E gene of *E. acervulina* [51]. Flow cytometry revealed that chickens belong to groups vaccinated with IL-8 and IL-15 adjuvants showed increased numbers of CD3+ within intraepithelial lymphocytes of the duodenum. Similarly, all other adjuvants also contributed as immune boosters compared to pcDNA-3-1E alone. A chimeric DNA vaccine was examined in which IL-15 was used as an adjuvant along with 3-1E gene. The study proved that antigen 3-1E linker-mCh IL-15 is more efficient immunologically as compared to antigen 3-1E alone [52].

Available vaccines, now and then

A lot of formulated available vaccines are there. A brief discussion about these vaccines is given below.

Live non-attenuated anticoccidial vaccines

Coccivac-B

Coccivac-B is used for heavy broilers and is currently approved in Canada, United States and other countries. It contains *E. tenella*, *E. Acervulina*, *E. maxima* and *E. mivati* as nonattenuated antigens. Coccivac-B may be administered via different routes like hatchery spray, feed spray, ocular route and drinking water. The age of birds should be 1 day to 2 weeks [53].

Coccivac-D

Coccivac-D is used for heavy breeders and layers and is currently approved in Canada, United States and other countries. It contains *E. tenella*, *E. acervulina*, *E. maxima*, *E. mivati*, *E. hagani*, *E. brunetti*, *E. praecox* and *E. necatrix* as nonattenuated antigens. It may be administered via different routes like hatchery spray, feed spray,

ocular route and drinking water at the age of 1 day to 2 weeks [54].

Immucox C1

Immucox C1 is used for roasters and broilers mostly containing non attenuated antigens of *E. tenella, E. acervulina, E. maxima* and *E. mivati*. It is currently approved for Canada, United States and other countries. It can be administered through different routes like oral gel and drinking water. The age of birds should be 1 day to 2 weeks [53].

Immucox C2

Immucox C2 is used for breeders and layers. It contains *E. tenella, E. acervulina, E. maxima, E. mivati, E. Necatrix* and *E. brunetti* in nonattenuated form. It is currently approved for United States, Canada and other countries. Mostly administer through different routes like drinking water. The age of birds should be 1 day to 14 days [54].

Inovocox

Inovocox is used in ova as a single dose in broilers mostly. It contains *E. tenella, E. acervulina* and two strains of *E. maxima* in nonattenuated form. It is currently approved for use in United States [53].

Inovocox EM1

Inovocox EM1 is used as a single dose in ova of broilers mostly. It contains one strain of *E. maxima, E. tenella* and *E. acervulina* in nonattenuated form. It is currently approved for use in United States [53].

Advent

Advent is used in broilers mostly. It contains *E. tenella, E. acervulina* and *E. maxima* in nonattenuated form. It is administered as a single dose as hatchery spray mostly on the day of hatch or first feed as a feed spray. It is currently approved for use in United States [53].

Live attenuated anticoccidial vaccines

Livacox D

Livacox D is used in caged chickens mostly. It contains *E. tenella* and *E. acervulina* in attenuated form. It is currently approved for use outside of United States and Canada. It is mostly administered in drinking water as a single dose at the age of 1–10 days of birds [54].

Livacox T

It is used in breeders and broilers. It contains *E. tenella, E. maxima* and *E. acervulina* in attenuated form. It is mostly administered in drinking water and ocular form as a single dose at the age of 1-10 days. It is currently approved for use outside of United States and Canada [53].

Livacox Q

Livacox Q is used in broilers. It contains *E. tenella, E. maxima, E. brunetti* and *E. acervulina* in attenuated form. It is currently approved for use outside of United States and Canada. It is mostly administered in drinking water and ocular form as a single dose at the age of 1–10 days [53]

Paracox

Paracox is used in breeders, layers and broilers. It contains a great combination of *E. tenella* and two different antigenic strains of *E. maxima, E. mivati, E. praecox, E. brunetti* and *E. acervulina* in attenuated form. It is currently approved for use outside of United States and Canada. It is mostly administered in feed spray and drinking water as a single dose at the age of 1-9 days [53].

Paracox 5

It is used in broilers. It contains a combination of *E. tenella* and two different antigenic strains *of E. maxima, E. mivati, E. praecox,* and *E. acervulina* in attenuated form. It is mostly administered as hatchery spray, feed spray and drinking water as a single dose at the age of 1st or 3rd day. It is currently approved for use outside of United States and Canada. [53]

Nobilis COX ATM

Nobilis COX ATM is used in broilers mostly. It contains a combination of *E. tenella* and two different antigenic strains *of E. maxima* and *E. acervulina* in non-attenuated form. It is mostly administered as hatchery spray and drinking water as a single dose. For hatchery spray, age should be 1-5 days while 4-5 days of age is suitable in drinking water. It is currently approved for use outside of United States and Canada [53]

Subunit vaccines

CoxAbic

CoxAbic is the only subunit vaccine that has been marketed until now. Subunit vaccines mainly contain a purified antigen isolated from pathogenic organisms. Subunit vaccines are obtained by different technologies either contain recombinant proteins, antigens or DNA of different developmental stages of *Eimeria* species. CoxAbic provokes maternally derived antibodies [55].

Conclusion

Eimeria species are a constant threat to poultry production. While chemotherapy leads to many drawbacks, prophylactic measures are of eminent importance but ordinary vaccination can induce antibody-mediated immunity only, which does not offer enough potential to overcome *Eimeria* species at its full extent. Different research trials and studies have shown that DNA vaccines can induce both antibody and cell-mediated immunity. Several DNA vaccines have been tested in the last few years with marvelous results but still no vaccine is available commercially. It is necessary to take fruitful and advance steps towards the synthesis of molecular vaccines in the future.

Conflict of Interest

The author declares no conflict of interest in this study.

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