Vaccines against chicken coccidiosis with particular reference to previous decade: progress, challenges, and opportunities

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Abstract
Chicken coccidiosis is an economically significant disease of commercial chicken industry accounting for losses of more than £10.4 billion (according to 2016 prices). Additionally, the costs incurred in prophylaxis and therapeutics against chicken coccidiosis in developing countries (for instance Pakistan according to 2018 prices) reached US $45,000.00 while production losses for various categories of chicken ranges 104.74 to US $2,750,779.00. The infection has been reported from all types of commercial chickens (broiler, layer, breeder) having a range of reported prevalence of 7–90%. The concern of resistance towards major anticoccidials has provided a way forward to vaccine research and development. For prophylaxis of chicken coccidiosis, live virulent, attenuated, ionophore tolerant strains and recombinant vaccines have been extensively trialed and commercialized. Eimeria antigens and novel vaccine adjuvants have elicited the protective efficacy against coccidial challenge. The cost of production and achieving robust immune responses in birds are major challenges for commercial vaccine production. In the future, research should be focused on the development of multivalent anticoccidial vaccines for commercial poultry. Efforts should also be made on the discovery of novel antigens for incorporation into vaccine designs which might be more effective against multiple Eimeria species. This review presents a recap to the overall progress against chicken Eimeria with particular reference to previous decade. The article presents critical analysis of potential areas for future research in chicken Eimeria vaccine development.

Keywords Eimeria · Vaccine · Poultry · Antigens · Control

Introduction
The chicken industry is a rapidly developing enterprise around the globe. One of the foremost concerns of poultry industry is meeting the ever-rising competitive edge (Bosila et al. 2021). The overall flock health and performance is a critical parameter leading to higher economic returns (Abu et al. 2022). It is important to look up for and counter both clinical and sub-clinical forms of diseases in chicken (Davou et al. 2018; Iraqi et al. 2021; Tahir et al. 2021). Chicken coccidiosis is a disease of commercial poultry holding high economic value (Shahid et al. 2020; Lee et al. 2022). Coccidiosis in chicken causes sub-clinical infections as well as mortalities in persistently infected flocks. The disease can lead to morbidity reflected as increased feed conversion ratios, reduced weight gains, drop in production, lower reproductive performance, continuous Eimeria oocyst shedding, and increased susceptibility towards secondary bacterial infections (Gerhold 2016; Kadykalo et al. 2018; Venkatas and Adeleke 2019). Chicken coccidiosis accounts for more than £10.4 billion (according to 2016 prices) losses annually and has a variable prevalence ranging 7–90%, around the globe (Dalloul and Lillehoj 2006; Guven et al. 2013; Gyorko et al. 2013; Rashid et al. 2019; Blake et al. 2020a). Most recently, the overall economic loss owing to chicken coccidiosis was calculated to be UK £99.2 million (2016 prices) for vaccine, treatment, and other preventive
measures’ cost (Blake et al. 2020a). Moreover, it was estimated that there is a range of losses in different categories of chicken ranging US $104.74 to US $2,750,779.00 (Rashid et al. 2019). The economic burden due to production losses indicates the prime value of timely and effective Eimeria vaccines in sparing the economic burden thus created.

It is imperative to recognize at least 7 chicken Eimeria species that have, so far, been recognized as responsible for intestinal disease. These include Eimeria (E.) praecox, E. acervulina, E. mitis, E. brunetti, E. tenella, E. maxima, and E. necatrix (Clark et al. 2017; Idris et al. 2017; Abbas et al. 2019). Among all species, E. maxima, E. tenella, E. acervulina, and E. necatrix have reportedly highest clinical and sub-clinical disease burden, making them economically significant among Eimeria species of poultry (Gerhold 2016; Hinsu et al. 2018). The control options for coccidiosis in chicken have been a mainstay in commercial poultry farming, since almost 100 years (Blake et al. 2017). Prevention and control options like the use of anticoccidials, coccidiostats, anticoccidial vaccines, herbal extracts, plant-derived immunomodulatory agents, essential oils, nano-particles, feed additives, probiotics, and many bioactive compounds, coupled with an extensive list of good farm hygiene and biosecurity practices have been established so far (Dkhil and Al-Quraishy 2016; Awais et al. 2018; Craig et al. 2020; Moryani et al. 2021).

Over many decades, Eimeria vaccines have gained a considerable importance, by virtue of effectively preventing the disease without developing resistance in field strains as in anticoccidial chemotherapeutics (Sander et al. 2019; Liu et al. 2020; Zhao et al. 2020). Rather, the sensitivity towards previously resistant chemical anticoccidials has been enhanced by the use of Eimeria vaccines (Jenkins et al. 2010). Examples of some commercial Eimeria vaccines for chicken include Endrex®, Hipracox®, CoxAbic® (vaccine capable for transmitting maternally derived antibodies from breeders), Hatchpakcocci III® (vaccine available at USA having precocious strains of 3 most important broiler Eimeria), Cocciva®, Livacox®, Paraco® etc.

Many vaccine candidates for Eimeria have been validated in both lab and animal trials (Ma et al. 2011; Gerhold 2016; Venkatad and Adeleke 2019; Khater et al. 2020). “Paraco® 8” (MSD Animal Health, Madison, NJ, USA) vaccine had been in practice since 1991 to 2015 as the only vaccine in most of the European Union countries. The vaccine which consisted of 8 precocious strains of Eimeria maxima (CP and MFP strain), E. acervulina, E. tenella, E. mitis, E. necatrix, E. praecox, and E. brunetti (MSD Animal Health, 2016) was used both by breeder and by layer producers. Later, two new vaccines were added to prevent disease by Hipra company (Amer, Girona, Spain) and Huvepharma company (Huvepharma, Sofia, Bulgaria). The former company prepared “Hipra Evalon® (E)” containing 5 precocious strains, viz, E. maxima, E. tenella, E. acervulina, E. necatrix, and E. brunetti (Hipra, 2016). The latter company launched “Huveguard® Start” (now named Mmat) containing four precocious strains viz a viz E. acervulina, E. mitis, E. maxima, and E. tenella for its administration today old chick (Huvepharma 2016a). A separate product named “Huveguard® Plus” (now named as NB) was launched with E. necatrix and E. brunetti strains and tended to be given at the 14th day of age (Huvepharma 2016b). These vaccines provided considerable protection to both breeder and layer replacement. The third type of vaccines is non-attenuated vaccines consisting of parasites Eimeria which were not processed with a reduced pathogenicity in the laboratory. Salient examples of such vaccines are Advent™, Inovocox™, Immucox®, and Cocciva® (Chapman et al. 2002). Cocciva® consists of E. acervulina, E. maxima, E. mitis, E. praecox, and E. tenella; Advent™ comprised of E. acervulina, E. maxima, and E. tenella; Inovocox™ was prepared from E. acervulina, E. maxima, E. mitis, and E. tenella; and Immucox® is made of E. acervulina, E. maxima, and E. tenella (Peek and Landman 2011; Price 2012).

The Eimeria vaccines have been developed keeping in view the commercial demands for the control of Eimeria. The potential areas for addressing chicken coccidiosis in commercial flocks need to be focused on terms of cost-effective vaccine production and commercialization on practical basis. The academia and industry linkages should be strengthened to bring forward the commercialization of better vaccinal strains (based on indigenous Eimeria) and integrated strategies against chicken coccidiosis. This paper presents an updated review on development of Eimeria vaccines and future opportunities in the sector.

The antigenic diversity of chicken Eimeria

The Eimeria parasites are obligate, intracellular, apicomplexan parasites belonging to Eimeridae family and genus Eimeria (Brown-Jordan et al. 2018). The epizootiology of chicken Eimeria has shown a wide range of variability of 11–92% (Gyorke et al. 2013; Rashid et al. 2019) and a great antigenic diversity, exhibiting host specificity (Kadykalo et al. 2018). Eimeria have been successfully evading immune systems of hosts including chicken, turkey, pheasant, camel, goat, cattle, sheep, rabbit, mice, fish, and reptiles (Mohsin et al. 2021). Pathogenic species of Eimeria may cast negative trends to overall health and performance of affected species (Conway et al. 1990; Abbas et al. 2011). Infection may elicit symptoms of reduced appetite, lethargy, watery or pasty feces, and appearance of blood in droppings (bloody coccidiosis) (Abbas et al. 2012; Wajiha and Qureshi 2021). Some strains of poultry coccidiosis (e.g., E. maxima) may potentiate the other gut pathogens like Salmonella and Clostridium species (Immerseel et al. 2004). Mixed infection
with *Eimeria* may alter the gut microbiome altogether, damaging the mucosa of intestinal layers (Prescott et al. 2016). The prevalence of *Eimeria* and the protein meals used in feed have been shown as two risk factors working simultaneously for Clostridial infections in chicken. This protein-rich environment may predispose its host to necrotic enteritis even in the presence of a mild coccidial infection.

*Eimeria* shows resistance towards varying environmental conditions owing to the hardy nature of oocysts (Jeffers 1974; Zhu et al. 2000). The merozoite stages are the re-infective stages of *Eimeria* (Rani et al. 2021). Sporozoites invade the intestines (enterocytes) of the host, which leads to appearance of clinical signs usually during the second round of sporozoite replication in chicken.

The parasite may exhibit antigenic variants having lower cross protection among species (Blake et al. 2017). *Eimeria*, being distinguished from other apicomplexans, possesses exceptionally unique antigenic variation by virtue of retrotransposons that make them a house for several chromoviruses (Morris and Gasser 2006; Reid et al. 2014). These transposons do not have potential of horizontal transfer as the chicken host’s genome lack chromoviruses. This is important as it could help explore *Eimeria* biology using genomic screens. Moreover, attempts have been made to develop attenuated vaccine wherein issues with being specific in sequence targets were reported (Su et al. 2012).

The natural antigenic diversification and associated disease challenge has been countered by inclusion of more than one strain in the vaccine preparations. Also, *E. maxima* being the most immunogenic species of *Eimeria* is a potential vaccine candidate option for antigen isolation and research (Tang et al. 2018). Also, the profilines of *Eimeria tenella* have been exploited to develop vaccine adjuvants, capable of enhancing immune protection against *Toxoplasma gondii* (apicomplexan parasite) (Hedhli et al. 2009).

A detailed study on the connection of antigenic, genetic, and population diversity of *Eimeria tenella* with vaccine development revealed the disparity of genetic diversity in other closely related apicomplexans (Blake et al. 2015). Sampling from commercial chicken farms representing 5 different continents suggested high level of *Eimeria* interbreeding despite great genetic variation between the geographical regions, signposting the lack of *Eimeria* movement and/ or balancing selection. The diversity of nucleotides within immune mapped protein 1 (IMP-1) gene of *E. tenella* in the USA, UK, China, and India was found to be low (Kundu et al. 2017). The source of nucleotide diversity was owing to repetition of cag triplets and 5 substitutions, 3 of them being non-synonymous. The study is indicative of variable genetic diversity of different *Eimeria* species from various geographical regions, owing to some contraction/expansion in the nucleotide sequences. Moreover, based on the genetic exploration and likely promise of apical membrane antigen-1 (AMA1) as vaccine candidates against *E. tenella* infections, the study suggested to employ the combination of 2 or more antigens for targeting each *Eimeria* specie. Reports of unforeseen differences in the genome have increased research focus to comprehend and design vaccines against *Eimeria* (Blake et al. 2020b). Similarly, a detailed account regarding the genetic diversity of other *Eimeria* species and its relevance in field challenge on a global level is required.

**The host-parasite immunological relationship**

A substance capable of inducing an immunological response in the host is known as antigen. Parasites also have a number of antigens, which are recognizable by the host. *Eimeria* like other pathogens goes through nonspecific immune response as well as specific. The non-specific consists of physical barriers, and cellular response—leukocytes, phagocytes, and complements. Specific immune response consists of either antibody production (humoral) or cell-mediated response (cellular) against specific antigen of pathogen. The latter involves T lymphocytes, natural killer cells, and phagocytes (Kim et al. 2019). Owing to cross-reactivity, there are several immunoglobulin responses that are not restricted to *Eimeria* antigens only. Experiments have demonstrated the stimulation of lymphocytes with complex oocyst antigen preparations that were produced after *Eimeria* infection (Yun et al. 2000). Analysis for species specificity suggests that the adaptive immune response can identify multiple antigens and the species cross-reactive epitopes may be non-protective (Khalafla et al. 2011). This interaction is not very simple and depends upon factors like genetic makeup of host, parasite involved, and disease history (Mathis et al. 2018).

*Eimeria* overcomes non-specific defense mechanism of host that comprises of gastric secretions, lysozymes, mucous layer, etc. in the intestine (Figs. 1 and 2). Upon cracking the first line of defence, it faces resistance from mucosa-associated lymphoid tissue (MALT) as the second line of defence. Coupled with MALT, another important component of immunity called gut-associated lymphoid tissue hinders *Eimeria* by activating both humoral and cellular immune response. *Eimeria* in its progression invades into host cell with the help of microneme protein (Huang et al. 2015). A formal protocol of immune system starts here at the verge of immune cells like antigen-presenting cells, macrophages, and dendritic cells. These cells process and present antigenic molecules of pathogen to the host for final recognition by lymphocyte particularly T-lymphocytes (Min et al. 2013). Briefly, the process of production of immune response further finds humoral response in the form of Ig M, Ig Y, and Ig A which is in the case of *Eimeria*, a weak response. Cell-mediated immunity thus becomes a source of control of this disease (Yun et al. 2000). In this situation, cytokines like
Cell-mediated Immunity due to Eimeria infection/vaccination

Cellular immune response priming takes place when Eimeria is taken to antigen-presenting cells. Antigenic proteins from Eimeria parasite are converted into peptides that bind with the cytokine primed cytotoxic T cells. These cytotoxic T cells either get activated and start inoculating the cells for death or may lead to formation of memory T cells.

Humoral Immunity due to Eimeria infection/vaccination

Humoral immune response priming takes place when Eimeria is taken to antigen-presenting cells. Antigenic proteins from Eimeria parasite are converted into peptides that bind with the cytokine primed helper T cells. The cytokine release help in B cell activation and release of antibodies or they may form memory cells for future immunological response on Eimeria challenge.
interferon molecules and interleukins help mediate differentiation of resting T-helper cells (Th0) into Th1 and Th2. Th1 is specific for resolving intracellular pathogens whereas Th2 is specific for extracellular (López-Osorio et al. 2020). Th1 cells produce interferons like IFN-γ that kills parasites and limits its multiplication. Th2 cells produce interleukin like IL2, IL4, and IL10. IL2 is a growth factor for different cells and an increased amount has been found in E. acervulina infection. Similarly, other immune modulators produced by T cells are IL6, IL8, IL12-13, and IL15-18; out of these, IL-17 has been highlighted recently (Kim et al. 2019). Also, the granulocyte–macrophage colony-stimulatory factor, tumor necrosis factor (TNF)-α, T-reg cells, lipopolysaccharide-induced TNF-α factor, TNF-α super family 15, transforming growth factor (TGF)-β, and TLR4 and TLR15 have been found to play roles in immunological responses (Zhou et al. 2013). Recently, a decoy receptor namely the tumor necrosis factor receptor superfamily member-6B has been shown to play a vital role in promoting inflammatory and other immune pathways in chicken against coccidiosis (Guo et al. 2020).

Typically, the toll-like receptors bind antigen, leading to cellular pathway activation that subsequently activates innate immune players like interleukins and interferons (Mansilla and Capozzo 2017). The Eimeria genome encodes for thousands of proteins at a single time, rendering the entire profile and prediction of complete immunogenicity difficult (Reid et al. 2014). Phenomenon of host-parasite interaction in Eimeria is therefore highly unpredictable and isolation of the protective antigens remains elusive. It is mainly because the bioassays are limited to extracellular stages of the parasite and partially owing to lack of immunoprotection against some proteins that are considered useful. However, these issues are not restricted to Eimeria species only, and the development of vaccine against certain viruses, bacteria, and protozoa faces a similar problem of antigenic complexity. A study identified four novel, two known, and one unknown gene that encode for immune-protective antigens of E. maxima (Yang et al. 2017). The strategy revolves around genetics, and immunology, it focuses on candidate Eimeria antigens showing immunoprotection in the host and a CDNA library resource intended for screening T cells that influence target antigens in this study.

**Eimeria** vaccination challenges and other alternative vaccine designs: a recap

Non-judicious use of anticoccidial treatments resulted in resistance development in poultry (Abbas et al. 2015). Also, the sub-clinical infections in poultry can cast a sharp decrease in the production and performance parameters of the birds (Abbas et al. 2019). The pathogenic potential of Eimeria encourages the need for application of vaccine at an early age in chicken. The registration and production of first live vaccine named CocciVac® was achieved in 1952, on a commercial scale in the USA. For immunization of chickens, two types of vaccines are commonly used, i.e., attenuated and virulent vaccines. These vaccines target different Eimeria species and have different routes of administration. Attenuated vaccines have a lower reproducibility and immunogenic potential. Additionally, the production cost of attenuated vaccines is higher from the commercial standpoint. The virulent vaccines on the other hand may be anticoccidial sensitive strains. They may have the ability to decrease level of resistance in diverse coccidial populations owing to lesser genetic selection pressure in contrast with the recombinant vaccines (Blake et al. 2017). On the other hand, the virulent vaccines may lead to the clinical appearance of the disease or may exacerbate other pathogens. One of the major constraints in producing live chicken coccidiosis vaccines is the need to add controlled oocyst doses from all pathogenic species of Eimeria, making vaccine production demanding (labour and technicality wise) and non-economical (Soutter et al. 2020). Attenuated vaccines have been conventionally used to prevent chicken Eimeria in commercial settings. Cost of production, time- and labor-intensive passaging, associated disease challenge, and probability of developing resistance, however, limit their utilization. Moreover, the time and resources required for quality assurance to make vaccine batches of the same standards further limit the practicality of attenuated vaccines.

Immunization with live, attenuated vaccine has been a traditional way of Eimeria prevention compared with other prophylactic or therapeutic options (Panebra and Lillehoj 2019). However, non-attenuated vaccines are more popular in some countries like the USA. It is accompanied by the risk of unwanted infection, lower reproduction index of Eimeria, variable immune response, and limited production capacity (Blake 2015). Substitutive vaccination approach is shown by subunit protein vaccines. Experiments have been conducted to identify and purify immunogenic proteins from different stages of Eimeria life cycle and immunogenic trials have given variable results (Dal-loul and Lillehoj 2006). A major hurdle in subunit vaccine preparation is the absence of cross-immunity against other Eimeria species (Ahmad et al. 2016). Proteins isolated from one species are unable to protect against others. Therefore, third-generation vaccines featuring DNA or RNA material are being prioritized over second-generation (sub-unit) and first-generation (live/attenuated/killed) vaccines for Eimeria control (Mathis et al. 2018). Production and use of DNA vaccines is depicted in Fig. 3. The studies on molecular basis of immunological mediators in the face of coccidiosis challenge are warranted (Rothwell et al. 2004). This approach can bring forward the promising innate vaccine adjuvants as future recombinant vaccines.
against homologous or heterologous *Eimeria*. As indicated by the pitfalls of attenuated vaccines, recombinant vaccine research and development for chicken *Eimeria* is required. Various *Eimeria* antigens have been isolated and cloned from different parts and life-cycle stages of the pathogenic strains including AMA-1, cSZ-JN1, cSZ-JN2, EF-1α, Em6, Em8, *Eimeria* gametocyte antigens, EMHP-1, EMHP-2, EmCKRS, EmJS-1, Em14-3-3, EMRP, EmSAG, EtMIC1, EtMIC2, Gam 82, GAPDH, IMPI, LDH, MICs, MIF, NA-4, NPmz19, rEtSO7, TA4, etc. for testing as recombinant *Eimeria* vaccine candidates (Williams 2002; Song et al. 2015a; Lin et al. 2017; Jenkins et al. 2018). A brief overview of chicken *Eimeria* vaccines, from the past 10 years, is summarized in Table 1. Attenuated vaccines have lower practicality in terms of maintenance of cold storage line throughout rendering the costs of production and administration higher. Live vaccines have multiple risks like development of severe reaction among birds and alteration of resistance in coccidial species (Mathis et al. 2018). Since live vaccines replicate within host cells, they may result in subclinical coccidiosis. The immune response is augmented by re-infection in the case of live vaccine administration. Attenuated vaccines may exhibit lower pathogenicity, but usually high costs are incurred during development. Combination regime of administering attenuated and non-attenuated species has sorted out these problems to some extent (Jenkins et al. 2018). However, the need to chalk out the promising strategy for control of coccidiosis still remains inconclusive.

Live vaccines harness the ability of host cell-mediated immune response for replication and subsequent protection (Wajiha and Qureshi 2021). Normally, live oocyst vaccine consists of non-attenuated and attenuated parasites. When given orally, these vaccines give coccidial infection of low grade that generates mild immune response (Venkatas and Adeleke 2019). This protection is increased upon re-infection of parasite. Use of non-attenuated live vaccine for the *Eimeria* control has a major drawback of systemic reaction in poultry that may be associated with decreased bird performance. DNA vaccine has an advantage of stability because of its structural and chemical character and hence attracts scientists to direct their efforts in DNA vaccinology (Blake et al. 2017). The production process is easier with a negligible difference of quality among multiple vaccine batches (Song et al. 2017; Li et al. 2017). DNA vaccines are capable of inducing strong cellular and humoral immune response (Panebra and Lillehoj 2019; Rafiqi et al. 2019). Cytokine administration as adjuvants can increase their potential of inducing long-lasting and broader immunity. However, immunity is restricted to homologous strains and there is lack of cross-protective immune response among different strains prevalent over different geographical regions.
### Table 1 Summary of chicken *Eimeria* vaccines during previous decade

| Target *Eimeria* specie (E.) | Antigen/ Antibody/ Protein | Type of Vaccine | Route of Administration | Vaccinal Response | References |
|------------------------------|-----------------------------|-----------------|-------------------------|-------------------|------------|
| *E. acervulina*              | LDH, 3-1E & MIF             | Multivalent, Subunit | Intramuscular           | Partial protection | Song et al. 2015a |
|                             | LDH                         | DNA             | Intramuscular           | Protective immunity | Song et al. 2010a, b |
|                             | Profillin & QCDC            | Recombinant     | Subcutaneous            | Protective immunity | Lee et al. 2010  |
|                             | Profillin with IMS 1313 or ISA 71 | Recombinant-nano vaccine | Oral                           | Protective immunity | Jang et al. 2011  |
|                             | 3-1E and chicken IL-15      | Recombinant     | Intramuscular           | Partial protection | Ma et al. 2011  |
|                             | cSZ-JN1, cSZ-JN2            | Recombinant DNA | Intramuscular           | Partial protection | Zhu et al. 2012a, b |
|                             | EaMIC5                      | DNA             | Subcutaneous            | Partial protection | Zhang et al. 2014 |
|                             | ADF-3-1E                    | Recombinant DNA | Intramuscular           | Partial protection | Zhao et al. 2014 |
|                             | EF-1α/chIL-7 with Montanide Gel 01 adjuvant | DNA | Intramuscular | Protective immunity | Panebra and Lillehoj 2019 |
| *E. maxima*                 | Em6 & Em8                   | Multivalent, Subunit | Intramuscular           | Partial protection | Song et al. 2015b |
|                             | NP-EMax1IMPI                | Nano-vaccine    | Oral                    | Protective immunity | Jenkins et al. 2018 |
|                             | Profillin & QCDC            | Recombinant     | In-ovo                  | Protective immunity | Lee et al. 2010  |
|                             | Gam82                       | Recombinant     | Intramuscular, Oral     | Protective immunity | Jang et al. 2010  |
|                             | Gam56                       | DNA             | Intramuscular           | Protective immunity | Xu et al. 2013  |
|                             | EmMIC7                      | DNA             | Intramuscular           | Protective immunity | Huang et al. 2015 |
|                             | EmSAG                       | Recombinant     | Intramuscular           | Moderate immunity | Liu et al. 2018b |
|                             | Et-EmAMA1 and/or Et-EmIMP1  | Vector          | Oral                    | Enhanced immune protection | Tang et al. 2019 |
| *E. necatrix*               | NA4 & NPmz19                | Multivalent, Subunit | Intramuscular           | Partial protection | Song et al. 2015a |
|                             | Gam22                       | Recombinant Subunit | Oral                    | Protective immunity | Liu et al. 2014  |
| *E. tenella*                | TA4 & SO7 genes             | Multivalent, Subunit | Intramuscular           | Partial protection | Song et al. 2015a |
|                             | SO7 gene                    | DNA             | Intramuscular           | Protective immunity | Yang et al. 2010  |
|                             | TA4 & Chicken IL-2          | Chimeric DNA    | Intramuscular           | Protective immunity | Song et al. 2017 |
|                             | cSZ-2 (*E. acervulina* antigen) | Recombinant     | Intramuscular           | Partial protection | Shah et al. 2010 |
|                             | rBCG co-expressing rhomboid and chIL-2 gene | Recombinant     | Intra-nasal and Subcutaneous | Protective immunity | Wang et al. 2014 |
|                             | Profillin                   | Recombinant nano-vaccine | Subcutaneous              | Protective immunity | Zhang et al. 2012a |
|                             | SO7 & Chicken IL-2          | Chimeric DNA    | Oral                    | Protective immunity | Song et al. 2013 |
|                             | Rhomboid                    | Recombinant     | Injection followed by oral | Protective immunity | Liu et al. 2013  |
|                             | DC-derived exosomes (CD80, flotillin & HSP70, MHC-I and MHC-II) | Monovalent | Intramuscular | Protective immunity | del Cacho et al. 2011 |
|                             | EtHSP70                     | Subunit         | Subcutaneous            | Enhanced protection | Zhang et al. 2012b |
|                             | EtHSP70+EtMIC2              | Recombinant     | Intramuscular           | Partial protection | Qi et al. 2013  |
|                             | rEtMIC-1                    | Subunit         | Subcutaneous            | Protective immunity | Yin et al. 2013  |
|                             | IMP1                        | Subunit         | Subcutaneous            | Protective immunity |             |
The fact that viral proteins are expressed in *E. tenella* and recognized by immune system of birds has shown chances of developing multivalent vaccine vector (Marugan-Hernandez et al. 2016). The recent trends of research on *Eimeria* focus on transcriptomic, proteomic, and genomic analysis along with genotype diversity and phylogenetic mapping (Blake et al. 2015). These tools can help understand the parasite biology and the genetic diversity and help expand the spectrum of promising vaccine candidates. A newly discovered IMP-1 of *E. maxima* species is an effective immunogen (Blake et al. 2017). Vaccination against coccidiosis, using immune mapped protein 1 of *E. tenella* (EtIMP1), reduced the oocyst output up to 60%. Recently, the genome-wide transcriptomic analysis of highly virulent strains of *E. tenella* has revealed the upregulation of certain rhoptry kinases responsible for overwhelming signaling pathways and immunological responses in these virulent strains (Ribeiro et al. 2021). The transcriptomic data derived from the diverse antigenic profile of *Eimeria* species would be helpful in designing more efficient vaccine candidates. Additionally, it is imperative to look up for the cost of vaccine production, and vaccine response with a larger number of chicken when commercialization is the target of a certain research trial (Abbas et al. 2017). Vector vaccines also offer an option against coccidiosis. Development of vector vaccine covers three steps: (1) recombinant *Eimeria* selection system, (2) exogenous antigen-specific immune response, and (3) protection against heterologous pathogen. In first
step, transfected sporozoites or merozoites are transferred to cloaca of chick, intravenous route, or meaningfully to the site where sporozoites or merozoites invade the intestine (Duan et al. 2019). It was found that within 5 generations, using single plasmid can give rise to more than 90% of transgenic bacteria. The process of several selection produces stable expression of exogenous gene. This exogenous protein can be integrated into genome of parasites. Although there exist some challenges, success rate is evident (Qin et al. 2014). For the second step, EYFP as model antigen has been applied for lymphocyte proliferation and expression of interferon gamma in CD4 and CD8 T cells, analyzed in transgenic Eimeria-immunized chickens. The findings in terms of EYFP-specific lymphocyte proliferation and stronger IgA production represents that heterologous antigen in recombinant Eimeria influences the immune system. Hence, heterologous recombinant antigen can be considered a significant factor for immune response. In the final step, it is desired from the recombinant vector vaccine that it can elicit higher level immune response against heterologous pathogen infection. In a study, E. tenella expressed Campylobacter jejuni vaccinal candidate (CjαA) was evaluated in chickens. The study concludes 91% protection against C. jejuni. Similarly, recombinant heterologous with Toxoplasma gondii also produced promising results. Hence, Eimeria heterologous vaccine can provide cross-protection (Tang et al. 2016; Clark et al. 2012).

Eimeria vector vaccine faces following major hurdles: (a) parasite hardly finishes its life cycle in in vitro tissue culture; (b) compared to other apicomplexans like Toxoplasma and Plasmodium, the genome of Eimeria is more diverse. Development of vector vaccine gets proof of plasmid-based transfection and successful expression of exogenous lacZ gene by Et mic-1 promoter. However, limitation of sensitivity of 5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside (X-Gal) detection system delayed this process for 10 years after getting proof of successful transfection dynamics. This issue was resolved with development of fluorescent proteins (Hao et al. 2007).

Nano-vaccines are a new generation of modern vaccines which have nano-sized (less than 100nm) adjuvants and/or carriers. In recent times, markedly successful nano-vaccines had been developed and commercialized to counter COVID-19 pandemic, for instance Pfizer and Moderna vaccines (Thi et al. 2021). Similarly, Eimeria nano-vaccines (nanoparticles functionalized Eimeria antigens) have also shown a large promise as they have the ability to elicit both systemic and mucosal immunological responses, following administration via mucosa (Jenkins et al. 2018). Nano-vaccines may supersede the conventional approaches for vaccine development by virtue of efficient immunomodulation, easier engineering, and better immune coverage. It is required to further trial this approach for multivalent Eimeria vaccines.

Adjuvants for coccidia vaccines

Several novel vaccine adjuvants have been trialed for effective immune coverage in chicken coccidiosis (Lin et al. 2020). Studies have shown CD40 ligand (CD40L) as a strong immunological adjuvant. It is expressed on mast cells, activated T cells, and basophils of poultry. The CD40-CD40L interactions activate antigen-presenting cells, upregulate co-stimulatory molecules, and affect T cell–mediated effector function (Yin et al. 2015). The use of plant-mediated adjuvants including saponins, polysaccharides, lectins, and heat shock proteins against coccidia and other apicomplexan parasites may offer safer alternative to conventional adjuvants (Sander et al. 2019). The plant bio-actives have an innate ability to elicit a sustained immune response in the host against target specie. However, there is a knowledge gap in the comparative efficacy of different plant-derived adjuvants against various Eimeria strains. Recently, an oral, yeast-based sub-unit vaccine for E. tenella has been shown to decrease Eimeria replication (Soutter et al. 2022). Nano-vaccine adjuvants in apicomplexan vaccines can offer a sustained immune protection, compared to other adjuvants. Antigen delivery systems utilizing immunostimulatory complexes and virus-like particles, self-assembling polypeptide nanoparticles, have also been shown to enhance immune protective coverage of several recombinant vaccines (Collins et al. 2017). Chicken cytokine genes including IL-1β, IL-2, IL-8, IL-15, IFN-α, IFN-γ, TGF-β4, and lymphotactin have also been shown to confer protective immunity in the face of coccidiosis challenge (Tian et al. 2017; Rafiqi et al. 2019; Venkatas and Adeleke 2019). The use of ISA 71 (oil based), IMS 1313 (nano-particle based), and adjuvant complex including saponin, cholesterol, Quil A, Carbopol and dimethyldioctadecyl-ammonium bromide, and profilin plus QCDC adjuvant have shown a large promise, and exhibited protective immunity when used with Eimeria antigens (Lee et al. 2010; Jang et al. 2011; Kim et al. 2012). Moreover, chicken genetic/molecular adjuvants like truncated flagellin and interleukin-2 (IL-2) have also been shown to confer protective immunity in the face of coccidiosis challenge (Song et al. 2015a; Yan et al. 2018).

Plant-derived adjuvants for Eimeria vaccine development have also shown promising results (Sander et al. 2019). It could be an eco-sustainable approach if being potentialized and upscaled in collaboration with commercial industry. The innate animal adjuvants like cytokines from chicken have a limitation of rapid degradation and quick clearance from the host, when administered in vivo. To overcome these challenges, the approaches focusing more sustained release are being trialed to efficiently overcome the disease (Wang and Suo 2020). Furthermore, the chimeric and nano-vaccines have opened a gateway to scale-able preparations, possessing excellent physiochemical properties and relatively
stable immune responses. Similar vaccines have proven a large promise in other apicomplexan parasites (Collins et al. 2017). The robust immune response shown by virtue of humoral and cell-mediated immunity needs further validation and standardization. It is imperative to uniformly compare and declare a set of “gold-standard” study design and the parameters, evaluating the efficacy of new-generation vaccines at studies on in vitro and in vivo scale (Soutter et al. 2020).

**Criteria for efficacy of *Eimeria* vaccines**

The parasite load, reduction of intestinal lesions, or increased weight gain are important metrics to consider for vaccine efficacy. All the other parameters are immunological parameters and do not hold a clear-cut association with the level of host immunity. Performance parameters of birds like reduced feed conversion ratios, decreased oocyst shedding in feces, body weight gains, reduction in severity of intestinal lesions, and higher survivability rates as compared to the control birds can also be taken into account (Chen et al. 2015; Liu et al. 2018a, b; Yan et al. 2018). Serum biochemistry, post-mortem lesion scoring, histopathology, and microscope-assisted visualization can help validate these parameters.

Elevated levels of reactive antibodies (e.g., IgG, IgA) and the increased levels of IFN-γ, IL-2, IL-6, IL-17, and TGF-β; higher levels of CD4+/CD3+ and CD8+/CD3+ T lymphocytes and enhanced IgY antibodies producing cells; and higher antigen-driven proliferation of cells, compared to the control birds, are some of the important immunological indicators (del Cacho et al. 2011; Huang et al. 2018). Patterns of T cells are considered to be more significant criteria for immunogenesis within intestinal mucosa (Min et al. 2013).

A standard set of guidelines based on the modern vaccines in poultry *Eimeria* is lacking. Most importantly, the animal welfare should be prioritized while experimentation of immunogenicity/pathogenicity trials and guidelines regarding chicken rearing and euthanasia/slaughter must be developed. The line of the chickens and the regimen used for vaccine in research trials may act as confounding factors increasing the statistical variability among different studies (Soutter et al. 2020). The safety and efficacy of vaccines on the basis of bird’s type, age, route of administration, uniform uptake and immunogenesis, cross-protection, and drug sensitivities must be critically evaluated. This is a significant step for distinguishing the type and need of a specific vaccine preparation for particular poultry flocks. Moreover, the safety evaluation standard guidelines could help both manufacturer and farmers in choosing the right preparation for their flocks.

**Opportunities (the way forward)**

Macro-scale epidemiological investigation is needed to understand the diversified antigenic variants of *Eimeria* globally. Genome-wide analysis of *Eimeria* species is essential as its screening may bring forward more promising vaccine candidates. High-throughput sequencing tools may be made available at commercially viable scale. Recombinant *Eimeria* vaccines featuring use of biological agents (BCG, fowl pox virus) can act as vaccine vectors, offering protective immunity (Wang et al. 2014; Tang et al. 2018). The stable transfection by employing *E. acervulina* expressing the multiple copies of extracellular domain (M2e) of H9N2 influenza virus has been reported (Zhang et al. 2021). These studies encourage more research on further exploration of *Eimeria* as live vaccine vectors.

Similarly, the genome editing tools (e.g., clustered regularly-inter-spaced palindromic repeats (CRISPR)) that have shown a large promise in other species of apicomplexan parasites may also be utilized to probe basic genetics of indigenous coccidial strains (Hu et al. 2020). For instance, epistasis studies on *Toxoplasma* with the help of CRISPR screens have aided in better understanding of genetic interactions, and the genes that enable the parasite to thrive within a variety of different hosts (Young et al. 2019). Similarly, the CRISPR-aided screening has helped in the determination of functional genes in Plasmodium and related therapeutic and vaccine candidates in this apicomplexan (Thiam et al. 2022). CRISPR has been employed to edit the germ cell lines in chicken, in order to enhance desirable traits related to meat and egg production (Khawteng & Nahashon 2021). A single gene-based editing by using CRISPR tool for probing systematic analysis of gene functions in *E. tenella* revealed that the cellular distribution of secreted proteins varies in different life cycle stages (Hu et al. 2020). Another study reported the application of CRISPR for gene function study in *E. tenella* (Tang et al. 2020). Similarly, a study deploying zinc finger–like proteins revealed partial immune-protective effects of AN-1 like proteins of *E. tenella* (Zhao et al. 2020).

The multiepitope DNA vaccines could be a game changer to the current scenario of anticoccidial vaccine development. Well-organized cocktail of antigens from different life cycle stages of *Eimeria* have shown to offer promising immune protection. Recently, a multiepitope vaccine employing cholera toxin adjuvant was designed by immunoinformatic method (Madlala et al. 2021). In this scenario, development of a vaccine comprised of multiple antigens from different species of chicken *Eimeria* is signposted. One of the potential issues with a multi-valent vaccine against *Eimeria* would be the confounding factors, which might be eliminated by evaluating the immune responses of each of the component species separately (Soutter et al. 2020).
Conclusion

Coccidiosis is one of the major issues of commercial poultry production worldwide that needs to be prevented in an effective way. *Eimeria* in chicken reared at open or semi-open houses is still a huge fiscal burden to farm economy. Lessons learnt from other apicomplexan vaccines, employing the most recent approaches, need to be utilized to produce a vaccine that offers wider coverage in terms of both cellular-mediated and humoral immunity. Also, it is recommended to look up for indigenous strains of *Eimeria* and prepare multivalent vaccines accordingly to manage the drawback of variation with respect to geographical distribution. Most importantly, there’s need to rationalize the use of commercially viable and tailor-made (catering specific bird type and prevalent *Eimeria* species) new-generation vaccines.

Declarations

Competing interests The authors declare no competing interests.

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