Parasitology Research (2022) 121:2749–2763
https://doi.org/10.1007/s00436-022-07612-6

IMMUNOLOGY AND HOST-PARASITE INTERACTIONS - REVIEW

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

Tean Zaheer[1] · Rao Zahid Abbas[1] · Muhammad Imran[1] · Asghar Abbas[2] · Ali Butt[1] · Sarfraz Aslam[3] · Jameel Ahmad[3]

Received: 22 May 2021 / Accepted: 24 July 2022 / Published online: 4 August 2022

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; Gyorke 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

Section Editor: Berit Bangoura

✉ Rao Zahid Abbas
raouaf@hotmail.com

[1] Department of Parasitology, University of Agriculture, Faisalabad, Pakistan

[2] Faculty of Veterinary Science, Muhammad Nawaz Shareef University of Agriculture Multan, Multan, Pakistan

[3] Institute of Physiology, Pharmacology and Pharmaceutics, University of Agriculture, Faisalabad, Pakistan

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*), Coccivac ®, Livacox ®, Paracox ®, etc.

Many vaccine candidates for *Eimeria* have been validated in both lab and animal trials (Ma et al. 2011; Gerhold 2016; Venkatas and Adeleke 2019; Khater et al. 2020). "Paracox® 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, Sophia, 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 Coccivac® (Chapman et al. 2002). Coccivac® consists of *E. acervulina*, *E. maxima*, *E. maxima*, *E. mivati*, *E. praecox*, and *E. tenella*; Advent™ comprised of *E. acervulina*, *E. maxima*, and *E. tenella*; Inovocox™ was prepared from *E. acervulina*, *E. maxima*, *E. maxima*, 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 profilins 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 distances 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 (Khalafalla 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

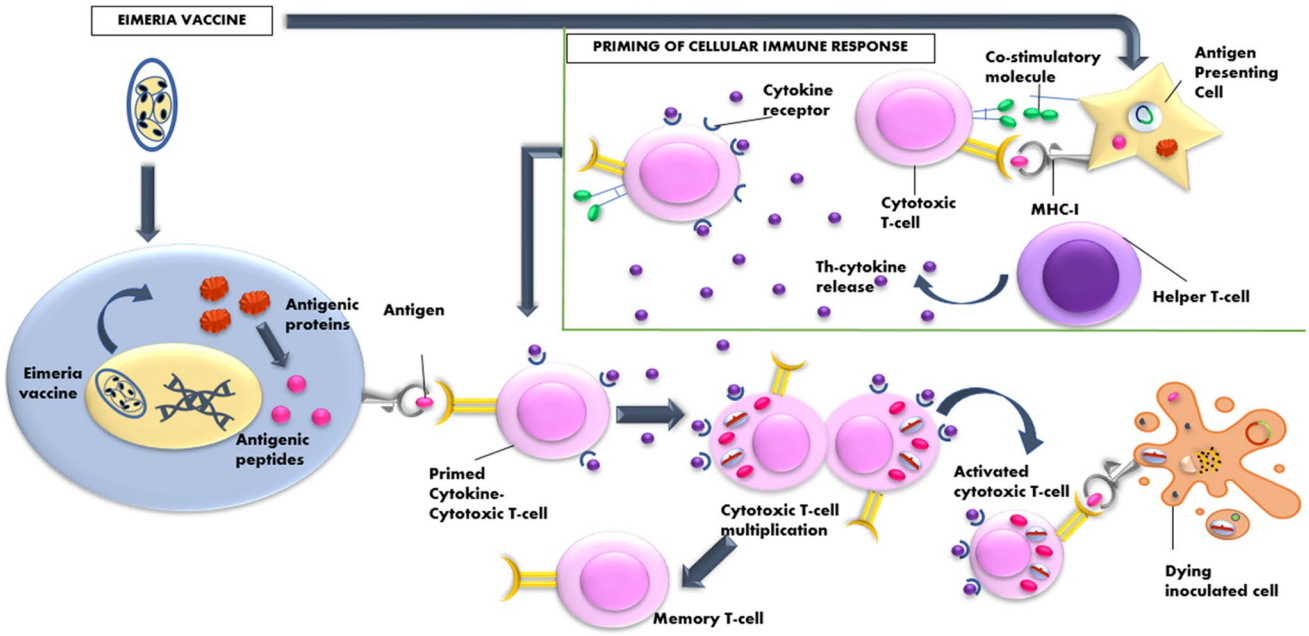

Fig. 1 Cell-mediated immunity due to *Eimeria* infection/vaccination. Cellular immune response priming takes place when *Eimeria* is taken to antigen-presenting cells. Antigenic proteins from *Eimeria* parasite are converted into peptides that bind with the cytokine primed cytotoxic T cells. These cytotoxic T cells either get activated and start inoculating the cells for death or may lead to formation of memory T cells

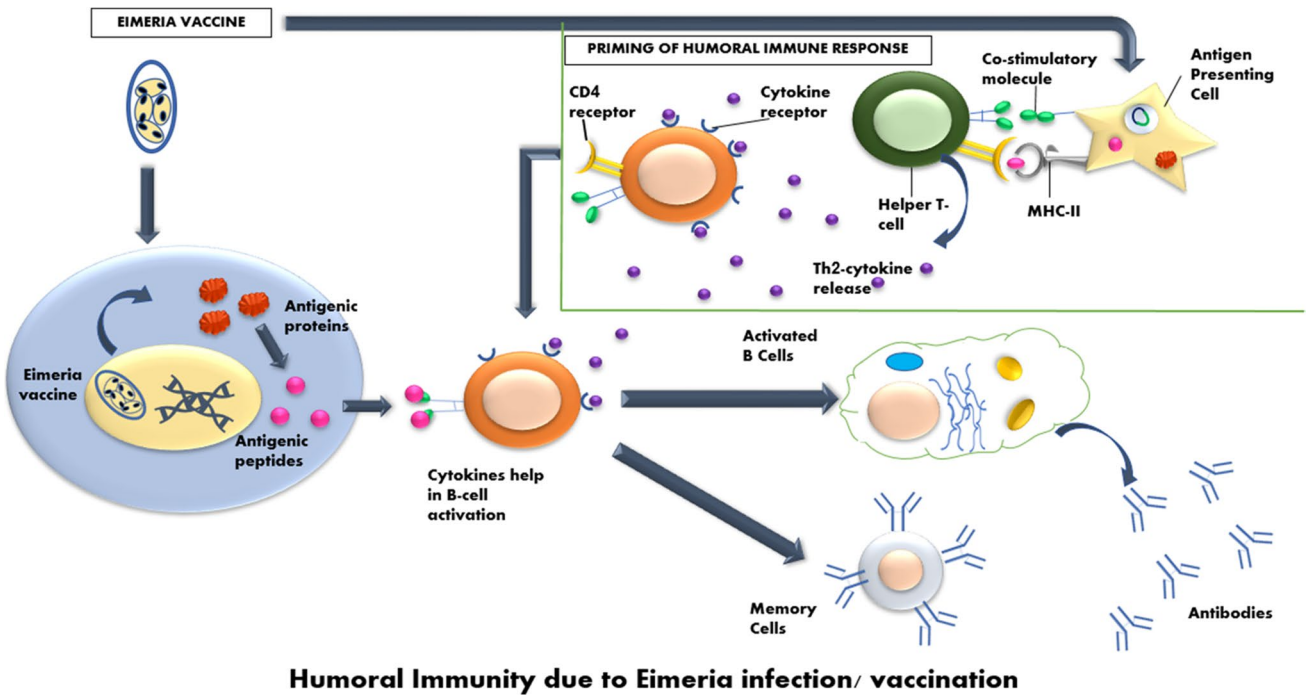

Fig. 2 CD4/T-helper immunity in Eimeriosis. Humoral immune response priming takes place when *Eimeria* is taken to antigen-presenting cells. Antigenic proteins from *Eimeria* parasite are converted into peptides that bind with the cytokine primed helper T cells. The cytokine release help in B cell activation 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 immunogenic ability difficult (Reid et al. 2014). Phenomenon of host-parasite interaction in *Eimeria* is therefore highly unpredictive 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 protozoan 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 (Dalloul 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

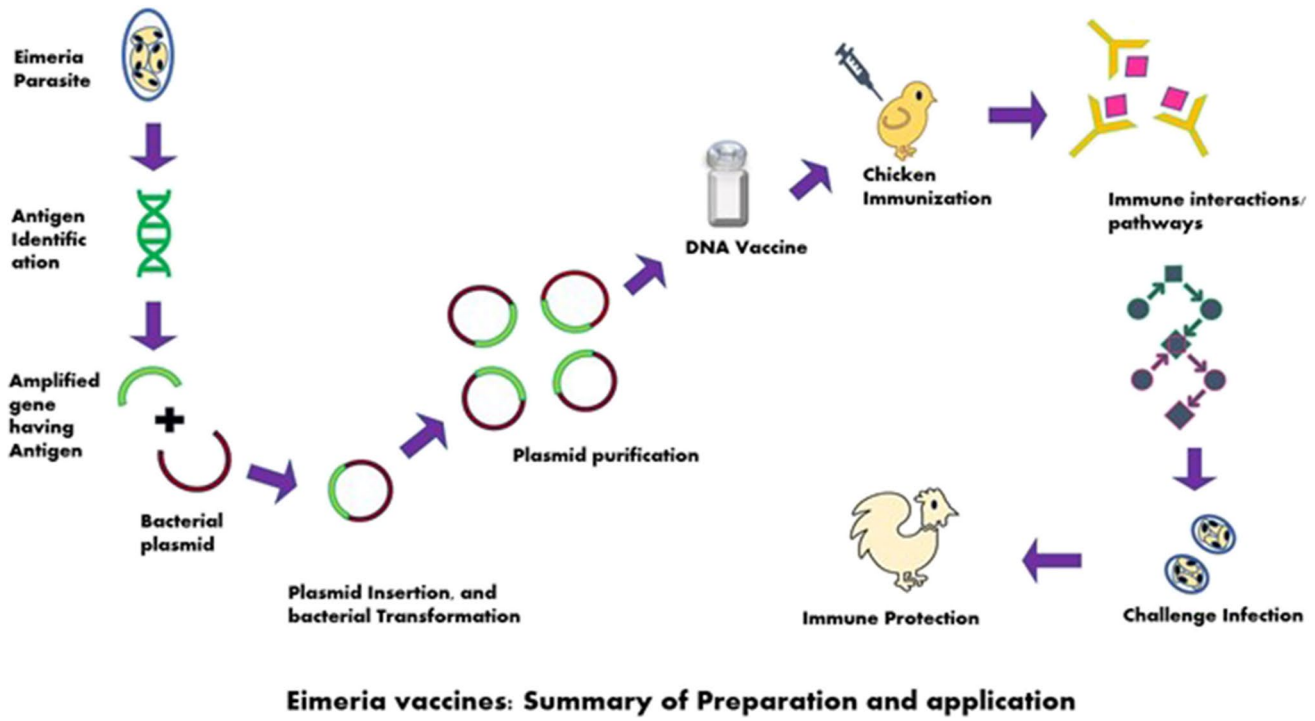

Fig. 3 *Eimeria* vaccines: summary of preparation and application. The genes from target antigen are amplified, introduced to the plasmid. Insertion and bacterial transformation take place. The transgenic plasmid is purified to form a vaccine. Chickens are immunized with the dose of vaccine. Immunological interactions take place within host body and provide immune protection in the face of disease

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 adjutants 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
	Profilin & QCDC	Recombinant	Subcutaneous	Protective immunity	Lee et al. 2010
	Profilin 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	Protective immunity	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-EMaxIMP1	Nano-vaccine	Oral	Protective immunity	Jenkins et al. 2018
	Profilin & 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
	Profilin	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				
	rEtMIC-1	Recombinant	Intramuscular	Partial protection	Qi et al. 2013
	IMP1	Subunit	Subcutaneous	Protective immunity	Yin et al. 2013
	IMP1 with FliC				

Table 1 (continued)

Target *Eimeria* specie (E.)	Antigen/ Antibody/ Protein	Type of Vaccine	Route of Administration	Vaccinal Response	References
	EtMIC-1 (polypeptides-I, II, III)	Recombinant	Oral	Protective immunity	Chen et al. 2015
	3-1 E (protein)	Recombinant	Oral	Protective immunity	Lin et al. 2015
	5401(surface antigen) and chicken IFN-γ or IL-2	Chimeric DNA	Intramuscular	Partial protection	Song et al. 2015a
	Serum exosomes	Serum derived	Intramuscular	Protective immunity	del Cacho et al. 2016
	EtCHP559	Recombinant	Intramuscular	Protective immunity	Zhai et al. 2016
	EtMIC3	Recombinant	Intramuscular	Protective immunity	Wang et al. 2017
	EtAMA1	Recombinant	Oral	Protective immunity	Li et al. 2019
	Profilin (*E. maxima*)	Recombinant Vector	Cloacal Inoculation	Protective immunity	Tang et al. 2018
	EtSO7	Recombinant	Subcutaneous	Protective immunity	Rafiqi et al. 2018
	MIC-2	Recombinant	Intramuscular	Partial protection	Yan et al. 2018
	EtAMA1 with L & C binding peptides	Recombinant	Oral	Partial protection	Ma et al. 2019
	EtGam22	Recombinant	Subcutaneous	Protective immunity	Rafiqi et al. 2019
	EtAN1-ZnFP	Recombinant	Subcutaneous	Partial protection	Zhao et al. 2020
Mixed Infections *(E. acervulina, E. maxima, E. necatrix& E. tenella)*	TA4-1 and LDH-2-	Multivalent, epitope DNA	Intramuscular	Protective immunity	Song et al. 2015a
	TA4-1-LDH-2 and IL-2	Multivalent, epitope DNA	Intramuscular	Protective immunity	Song et al. 2015b
Mixed infections (*E. tenella*, *E. maxima*, & *E. acervulina)*	Dendritic Cell derived exosomes	Polyvalent	Intramuscular	Protective immunity	del Cacho et al. 2012
	Tachyzoite gene (*E. tenella* & *E. acervulina)* and gametocyte gene of *E. maxima*	Multi-epitope DNA	Intramuscular	Partial protection	Ding et al. 2012
	GAPDH	Multivalent DNA	Intramuscular	Protective immunity	Tian et al. 2017
	14–3-3 antigen	Multivalent DNA	Intramuscular	Protective immunity	Liu et al. 2018a
E. tenella & *E. maxima*	EF-1α (*E. tenella*)	Subunit	Subcutaneous	Protective immunity	Lin et al. 2017

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 (CjaA) 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 promotor. 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 exhibited better immune responses (compared with *Eimeria* antigens alone) in injected birds (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 (Khwatenge and 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.

References

Abbas A, Abbas RZ, Khan MK, Raza MA, Mahmood MS, Saleemi MK, Hussain T, Khan JA, Sindhu ZUD (2019) Anticoccidial effects of *Trachyspermum ammi* (Ajwain) in broiler chickens. Pak Vet Journal 39:301–304. https://doi.org/10.29261/pakvetj/2019.056

Abbas A, Iqbal Z, Abbas RZ, Khan MK, Khan JA (2015) *In vitro* anticoccidial potential of *Saccharum officinarum* extract against *Eimeria* oocysts. Latin American Caribbean Bulletin of Medicinal and Aromatic Plants 14(6):456–461

Abbas A, Iqbal Z, Abbas RZ, Khan MK, Khan JA (2017) Immunomodulatory activity of *Pinus radaiata*against coccidiosis in broiler. Pak Vet J 37:145–149

Abbas RZ, Iqbal Z, Blake D, Khan MK, Saleemi MK (2011) Anticoccidial drug resistance in fowl coccidia: the state of play revisited. Worlds Poult Sci J 67(2):337–335. https://doi.org/10.1017/S004393391100033X

Abbas RZ, Iqbal Z, Blake D, Khan MK, Saleemi MK (2012) Botanicals: an alternative approach for the control of avian coccidiosis. Worlds Poult Sci J 68(2):203–215. https://doi.org/10.1017/S0043933912000268

Abu SM, Monira N, Asmaul H, Mahbub EATM, Mustafa KAH, Masudur RM (2022) Seroprevalence of Newcastle disease in layer chickens and pathology in clinically affected chickens at Gazipur. Bangladesh. Continental Vet J 2(1):35–41

Ahmad TA, El-Sayed BA, El-Sayed LH (2016) Development of immunization trials against *Eimeria* spp. Trials in Vaccinol 5:38–47. https://doi.org/10.1016/j.trivac.2016.02.001

Awais MM, Akhtar M, Anwar MI, Khaliq K (2018) Evaluation of *Saccharum officinarum* L. bagasse-derived polysaccharides as native immunomodulatory and anticoccidial agents in broilers. Vet Parasitol 249(15):74–81. https://doi.org/10.1016/j.vetpar.2017.11.012

Blake DP (2015) Eimeria genomics: where are we now and where are we going? Vet Parasitol 212(1-2):68–74. https://doi.org/10.1016/j.vetpar.2015.05.007

Blake DP, Clark EL, Macdonald SE, Thenmozhi V, Kundu K, Garg R, Jatau ID, Ayoade S, Kawahara F, Moftah A, Reid AJ, Adebambo AO, Álvarez Zapata R, Srinivasa Rao AS, Thangaraj K, Banerjee PS, Dhinakar-Raj G, Raman M, Tomley FM (2015) Population, genetic, and antigenic diversity of the apicomplexan Eimeria tenella and their relevance to vaccine development. Proc Natl Acad Sci USA 112(38):E5343–E5350. https://doi.org/10.1073/pnas.1506468112

Blake DP, Knox J, Dehaeck B, Huntington B, Rathinam T, Ravipati V, Ayoade S, Gilbert W, Adebambo AO, Jatau ID, Raman M, Parker D, Rushton J, Tomley FM (2020a) Re-calculating the cost of coccidiosis in chickens. J Vet Res 51(1):1–14. https://doi.org/10.1186/s13567-020-00837-2

Blake DP, Pastor-Fernández I, Nolan MJ, Tomley FM (2017) Recombinant anticoccidial vaccines - a cup half full? Infect Genet Evol 55:358–365. https://doi.org/10.1016/j.meegid.2017.10.009

Blake DP, Worthing K, Jenkins MC (2020b) Exploring Eimeria genomes to understand population biology: recent progress and future opportunities. Genes 11(9):1103–1116. https://doi.org/10.3390/genes11091103

Bosila MA, Mekky HM, Fedawy HS, Elbayomi KM, Amer MM (2021) Histopathological lesion of arthritis in Mycoplasma synoviae naturally infected breeder chicken in Egypt. Int J Vet Sci 10(1):72–74. https://doi.org/10.47278/journal.ijvs/2020.006

Brown-Jordan A, Blake D, Beard J, Beharry A, Serrette L, Soleyn A, Oura C (2018) Molecular identification of *Eimeria* species in broiler chickens in Trinidad. West Indies. Vet Sci 5(1):12–21. https://doi.org/10.3390/vetsci5010012

Chapman HD, Cherry TE, Danforth HD, Richards G, Shirley MW, Williams RB (2002) Sustainable coccidiosis control in poultry production: the role of live vaccines. Int J Parasitol 32(5):617–629. https://doi.org/10.1016/S0020-7519(01)00362-9

Chen P, Lv J, Zhang J, Sun H, Chen Z, Li H, Wang F, Zhao X (2015) Evaluation of immune protective efficacies of *Eimeria tenella* EtMic1 polypeptides with different domain recombination displayed on yeast surface. Exp Parasitol 155:1–7. https://doi.org/10.1016/j.exppara.2015.04.020

Clark EL, Tomley FM, Blake DP (2017) Are Eimeria genetically diverse, and does it matter? Trends Parasitol 33(3):231–241. https://doi.org/10.1016/j.pt.2016.08.007

Clark JD, Oakes RD, Redhead K, Crouch CF, Francis MJ, Tomley FM, Blake DP (2012) Eimeria species parasites as novel vaccine delivery vectors: anti-*Campylobacter jejuni* protective immunity induced by Eimeria tenella-delivered CjaA. Vaccine 30(16):2683–2688. https://doi.org/10.1016/j.vaccine.2012.02.002

Collins KA, Snaith R, Cottingham MG, Gilbert SC, Hill A (2017) Enhancing protective immunity to malaria with a highly immunogenic virus-like particle vaccine. Sci Rep 7(1):1–15. https://doi.org/10.1038/srep46621

Conway DP, McKenzie ME, Dayton AD (1990) Relationship of coccidial lesion scores and weight gain in infections of *Eimeria acervulina*, *E. maxima* and *E tenella* in broilers. Avian Pathol 19(3):489–496. https://doi.org/10.1080/03079459008418702

Craig AD, Khattak F, Hastie P, Bedford MR, Olukosi OA (2020) The similarity of the effect of carbohydrase or prebiotic supplementation in broilers aged 21 days, fed mixed cereal diets and challenged with coccidiosis infection. PloS One 15(2):e0229281. https://doi.org/10.1371/journal.pone.0229281

Dalloul RA, Lillehoj HS (2006) Poultry coccidiosis: recent advancements in control measures and vaccine development. Expert Rev Vaccines 5(1):143–163. https://doi.org/10.1586/14760584.5.1.143

Davou KP, Lawal I, Joseph A, Lawal S (2018) Some attempted strategies towards the control of avian coccidiosis: a review. SOJ Immunol 6(2):1–11. https://doi.org/10.15226/2372-0948/6/2/00169

del Cacho E, Gallego M, Lee SH, Lillehoj HS, Quilez J, Lillehoj EP, Sánchez-Acedo C (2011) Induction of protective immunity against *Eimeria tenella* infection using antigen-loaded dendritic cells (DC) and DC-derived exosomes. Vaccine 29(21):3818–3825. https://doi.org/10.1016/j.vaccine.2011.03.022

del Cacho E, Gallego M, Lee SH, Lillehoj HS, Quilez J, Lillehoj EP, Sánchez-Acedo C (2012) Induction of protective immunity against *Eimeria tenella*, *Eimeria maxima*, and *Eimeria acervulina* infections using dendritic cell-derived exosomes. Infect Immun 80(5):1909–1916. https://doi.org/10.1128/IAI.06413-11

del Cacho E, Gallego M, Lillehoj HS, Quilez J, Lillehoj EP, Sánchez-Acedo C (2016) Induction of protective immunity against experimental *Eimeria tenella* infection using serum exosomes. Vet Parasitol 224:1–6. https://doi.org/10.1016/j.vetpar.2016.04.043

Ding J, Lillehoj HS, Quiroz MA, Bevensee E, Lillehoj EP (2012) Multi-epitope recombinant vaccine induces immunoprotection against mixed infection of *Eimeria* spp. Parasitol Res 110(6):2297–2306. https://doi.org/10.1007/s00436-011-2764-y

Dkhil MA, Al-Quraishy S (2016) Nanoparticles against Eimeriosis. In: Nanoparticles in the Fight Against Parasites. Parasitol Res 8(1):207–210. https://doi.org/10.1007/978-3-319-25292-6_11

Duan CH, Hu DD, Tang XM, Suo JX, Wang S, Zhang SX, Tao GR, Li C, Wang CY, Gu XL, Tang XL, Huang GP, Xiang BQ, Wu SQ, Ben Mamoun C, Suo X, Liu XY (2019) Stable transfection of Eimeria necatrix through nucleofection of second generation merozoites. Mol Biochem Parasitol 228:1–5. https://doi.org/10.1016/j.molbiopara.2019.01.002

Gerhold RW (2016) Overview of coccidiosis in poultry". Available from http://www.merckvetmanual.com/poultry/coccidiosis/overview-of-coccidiosis-in-poultry (Accessed: June 11, 2022)

Guo L, Huang W, Tong F, Chen X, Cao S, Xu H, Luo W, Li Z, Nie Q (2020) Whole transcriptome analysis of chicken bursa reveals candidate gene that enhances the host's immune response to coccidiosis. Front Physiol 11:573676. https://doi.org/10.3389/fphys.2020.573676

Guven E, Beckstead RB, Kar S, Vatansever Z, Karaer Z (2013) Molecular identification of *Eimeria* species of broiler chickens in Turkey. Ank Üni Vet Fak Derg 60(4):245–250. https://doi.org/10.1501/Vetfak_0000002587

Gyorke A, Pop L, Cozma V (2013) Prevalence and distribution of Eimeria species in broiler chicken farms of different capacities. Parasite 20(1):50. https://doi.org/10.1051/parasite/2013052

Hao LL, Liu XY, Zhou XY, Li JD, Suo X (2007) Transient transfection of Eimeria tenella using yellow or red fluorescent protein as a marker. Mol Biochem Parasitol 153(2):213–215. https://doi.org/10.1016/j.molbiopara.2007.02.005

Hedhli D, Dimier-Poisson I, Judge JW, Rosenberg B, Mévélec MN (2009) Protective immunity against Toxoplasma challenge in mice by coadministration of *T. gondii* antigens and *Eimeria* profilin-like protein as an adjuvant. Vaccine 27(16):2274–2281. https://doi.org/10.1016/j.vaccine.2009.01.100

Hinsu AT, Thakkar JR, Koringa PG, Vrba V, Jakhesara SJ, Psifidi A, Guitian J et al (2018) Illumina next generation sequencing for the analysis of *Eimeria* populations in commercial broilers and indigenous chickens. Front Vet Sci 5:176–1. https://doi.org/10.3389/fvets.2018.00176

Hu D, Tang X, Mamoun CB, Wang C, Wang S, Gu X, Duan C et al (2020) Efficient single-gene and gene family editing in the apicomplexan parasite *Eimeria tenella* using CRISPR-Cas9 . Front Bioeng Biotechnol 8 : 28 . https://doi.org/10.3389/fbioe.2020.00128

Huang J, Zhang Z, Li M, ong X,Yan R, Xu L, Li X (2015) Immune protection of microneme 7 (EmMIC7) against *Eimeria maxima* challenge in chickens. Avian Pathol 44(5):392-400. https://doi.org/10.1080/03079457.2015.1071780

Huang X, Liu J, Tian D, Li W, Zhou Z, Huang J, Song X, Xu L, Yan R, Li X (2018) The molecular characterization and protective efficacy of microneme 3 of *Eimeria mitis* in chickens. Vet Parasitol 258:114–123. https://doi.org/10.1016/j.vetpar.2018.06.020

Huvepharma NV (2016a) Huveguard Start Product Label. Huvepharma NV Huveguard Start Product Label, Huvepharma NV Antwerp, Belgium

Huvepharma NV (2016b) Huveguard Plus Product Label, Huveguard Plus Product Label, Huvepharma, NV, Antwerp, Belgium

Idris M, Abbas RZ, Masood S, Rehman T, Farooq U, Babar W, Hussain R, Raza A, Riaz U (2017) The potential of antioxidant rich essential oils against avian coccidiosis. Worlds Poult Sci J 73(1):89–104. https://doi.org/10.1017/S0043933916000787

Immerseel FV, Buck JD, Pasmans F, Huyghebaert G, Haesebrouck F, Ducatelle R (2004) *Clostridium perfringens* in poultry: an emerging threat for animal and public health. Avian Pathol 33(6):537–549. https://doi.org/10.1080/03079450400013162

Iraqi M, Nasef SA, El-Enbaawy M (2021) Phenotypic and genotypic characteristics of antimicrobial and disinfectant resistance gram-negative bacteria involved in early broiler chick mortality. Int J Vet Sci 10(2):129–134. https://doi.org/10.47278/journal.ijvs/2020.033

Jang SI, Lillehoj HS, Lee SH, Lee KW, Lillehoj EP, Bertrand F, Dupuis L, Deville S (2011) Montanide TM IMS 1313 N VG PR nanoparticle adjuvant enhances antigen-specific immune responses to profilin following mucosal vaccination against *Eimeria acervulina*. Vet Parasitol 182(2-4):163–170. https://doi.org/10.1016/j.vetpar.2011.05.019

Jang SI, Lillehoj SH, Lee HS, Lee KW, Park MS, Chaa S, Lillehoj EP, Subramanian M, Sriraman R, Srinivasan VA (2010) *Eimeria maxima* recombinant Gam82 gametocyte antigen vaccine protects against coccidiosis and augments humoral and cell-mediated immunity. Vaccine 28(17):2980–2985. https://doi.org/10.1016/j.vaccine.2010.02.011

Jeffers TK (1974) *Eimeria acervulina* and *E. maxima*: incidence and anticoccidial drug resistance of isolants in major broiler-producing areas. Avian Dis 18:331–342. https://doi.org/10.2307/1589101

Jenkins M, Klopp S, Ritter D, Miska K, Fettere R (2010) Comparison of *Eimeria* species distribution and salinomycin resistance in commercial broiler operations utilizing different coccidiosis control strategies. Avian Dis 54(3):1002–1006. https://doi.org/10.1637/9137-111109-Reg.1

Jenkins MC, Stevens L, O'Brien C, Parker C, Miska K, Konjufca V (2018) Incorporation of a recombinant *Eimeria maxima* IMP1 antigen into nanoparticles confers protective immunity against *E. Maxima* challenge infection. Vaccine 36(8):1126–1131. https://doi.org/10.1016/j.vaccine.2017.11.014

Kadykalo S, Roberts T, Thompson M, Wilson J, Lang M, Espeisse O (2018) The value of anticoccidials for sustainable global poultry production. Int J Antimicrob Agents 51(3):304–310. https://doi.org/10.1016/j.ijantimicag.2017.09.004

Khalafalla RE, Daugschies A, Dyachenko V (2011) Cross-reactivity of anti-Eimeria tenella antibody fragments on merozoites and sporozoites of different chicken Eimeria species. Parasitol Res 108(3):745–749. https://doi.org/10.1007/s00436-010-2171-9

Khater HF, Ziam H, Abbas A, Abbas RZ, Raza MA, Hussain K, Younis EZ, Radwan IT, Selim A (2020) Avian coccidiosis: recent advances in alternative control strategies and vaccine development. Agrobiol Rec 1:11–25

Khwatenge CN, Nahashon SN (2021) Recent advances in the application of CRISPR/Cas9 gene editing system in poultry species.

Front. Genet. 12:627714. https://doi.org/10.3389/fgene.2021.627714
Kim DK, Lillehoj HS, Lee SH, Dominowski P, Yancey RJ, Lillehoj EP (2012) Effects of novel vaccine/adjuvant complexes on the protective immunity against *Eimeria acervulina* and transcriptome profiles. Avian Dis 56(1):97–109. https://doi.org/10.1637/9720-031711-Reg.1
Kim WH, Chaudhari AA, Lillehoj HS (2019) Involvement of T cell immunity in avian coccidiosis. Front Immunol 10:2732. https://doi.org/10.3389/fimmu.2019.02732
Kundu K, Garg R, Kumar S, Mandal M, Tomley FM, Blake DP, Banerjee PS (2017) Humoral and cytokine response elicited during immunisation with recombinant immune mapped protein-1 (EtIMP-1) and oocysts of *Eimeria tenella*. Vet Parasitol 244:44–53. https://doi.org/10.1016/j.vetpar.2017.07.025
Lee SH, Lillehoj HS, Jang SI, Lee KW, Yancey RJ, Dominowski P (2010) The effects of a novel adjuvant complex/Eimeria profilin vaccine on the intestinal host immune response against live *E. acervulina* challenge infection. Vaccine 28(39):6498–6504. https://doi.org/10.1016/j.vaccine.2010.06.116
Lee Y, Lu M, Lillehoj HS (2022) Coccidiosis: recent progress in host immunity and alternatives to antibiotic strategies. Vaccines 10(2):215–239. https://doi.org/10.3390/vaccines10020215
Li J, Wang F, Ma C, Huang Y, Wang D, Ma D (2017) Recombinant *Lactococcus lactis* expressing *Eimeria tenella* AMA1 protein and its immunological effects against homologous challenge. Exp Parasitol :1–8. https://doi.org/10.1016/j.exppara.2018.05.003
Li C, Yan X, Lillehoj HS, Oh S, Liu L, Sun Z, Gu C, Lee Y, Xianyu Z, Zhao H (2019) Eimeria maxima-induced transcriptional changes in the cecal mucosa of broiler chickens. Parasit Vector 12(1):1–9. https://doi.org/10.1186/s13071-019-3534-4
Lin RQ, Lillehoj HS, Lee SK, Oh S, Panebra A, Lillehoj EP (2017) Vaccination with *Eimeria tenella* elongation factor-1α recombinant protein induces protective immunity against *E. tenella* and *E. maxima* infections. Vet Parasitol 243:79–84. https://doi.org/10.1016/j.vetpar.2017.06.003
Lin X, Mohsin M, Abbas RZ, Li L, Chen H, Huang C, Li Y, Goraya MU, Huang Z, Yin G (2020) Evaluation of immunogenicity and protective efficacy of *Eimeria maxima* immune mapped protein 1 with EDA adjuvant in chicken. Pak Vet J 402209–213. https://doi.org/10.29261/pakvetj/2020.043
Lin Z, Shi Y, Deng B, Mao X, Yu D, Li M (2015) Protective immunity against *Eimeria tenella* infection in chickens following oral immunization with *Bacillus subtilis* expressing *Eimeria tenella* 3-1E protein. Parasitol Res 114:229–3236. https://doi.org/10.1007/s00436-015-4539-3
Liu Y, Zheng J, Li J, Gong P, Zhang X (2013) Protective immunity induced by a DNA vaccine encoding *Eimeria tenella* rhomboid against homologous challenge. Parasitol Res 11(1):251–257. https://doi.org/10.1007/s00436-012-3132-2
Liu D, Cao L, Zhu Y, Deng C, Su S, Xu J, Jin W, Li J, Wu L, Tao J (2014) Cloning and characterization of an *Eimeria necatrix* gene encoding a gametocyte protein and associated with oocyst wall formation. Parasit Vector 7(1):27. https://doi.org/10.1186/1756-3305-7-27
Liu J, Liu L, Li L, Tian D, Li W, Xu L, Yan R, Li X, Song X (2018a) Protective immunity induced by *Eimeria* common antigen 14–3-3 against *Eimeria tenella, Eimeria acervulina* and *Eimeria maxima*. BMC Vet Res 14(1):1–11. https://doi.org/10.1186/s12917-018-1665-z
Liu T, Huang J, Li Y, Ehsan M, Wang S, Zhou Z, Song X, Yan R, Xu L, Li X (2018b) Molecular characterization and the protective immunity evaluation of Eimeria maxima surface antigen gene. Parasit Vector 11(1):1–12. https://doi.org/10.1186/s13071-018-2906-5
Liu J, Tuo W, Wu X, Xiong J, Yu E, Yin C, Ma Z, Liu L (2020) Immunoproteomic and mass spectrometric analysis of *Eimeria acervulina* antigens recognized by antisera from chickens infected with *E. acervulina, E. tenella* or *E. necatrix*. Parasit Vector 13(1):93. https://doi.org/10.1186/s13071-020-3965-y
López-Osorio S, Chaparro-Gutiérrez JJ, Gómez-Osorio LM (2020) Overview of poultry Eimeria life cycle and host-parasite interactions. Front Vet Sci 7:384. https://doi.org/10.3389/fvets.2020.00384
Ma D, Huang Y, Ma C, Zhang L, Wang J, Wang D, Li J, Dallou RA (2019) *Eimeria tenella*: specific EtAMA1-binding peptides inhibit sporozoite entry into host cells. Poult Sci 9(10):4480–4491. https://doi.org/10.3382/ps/pez298
Ma D, Ma C, Pan L, Li G, Yang J, Hong J, Cai H, Ren X (2011) Vaccination of chickens with DNA vaccine encoding *Eimeria acervulina* 3-1E and chicken IL-15 offers protection against homologous challenge. Exp Parasitol 127(1):208–214. https://doi.org/10.1016/j.exppara.2010.07.015
Madlala T, Adeleke VT, Fatoba AJ et al (2021) Designing multiepitope-based vaccine against *Eimeria* from immune mapped protein 1 (IMP-1) antigen using immunoinformatic approach. Sci Rep 11(1):1-17. https://doi.org/10.1038/s41598-021-97880-6
Mansilla FC, Capozzo AV (2017) Apicomplexan profilins in vaccine development applied to bovine neosporosis. Exp Parasitol 183:64–68. https://doi.org/10.1016/j.exppara.2017.10.009
Marugan-Hernandez V, Cockle C, Macdonald S et al (2016) Viral proteins expressed in the protozoan parasite *Eimeria tenella* are detected by the chicken immune system. Parasit Vector 9(1):1–14. https://doi.org/10.1186/s13071-016-1756-2
Mathis GF, Newman LJ, Fitz-Coy S, Lumpkins B, Charette R, Fuller L (2018) Comparison of breeder/layer coccidiosis vaccines: part 1-precocity and pathogenicity. J Appl Poult Res 27(1):33–37. https://doi.org/10.3382/japr/pfx037
Min W, Kim WH, Lillehoj EP, Lillehoj HS (2013) Recent progress in host immunity to avian coccidiosis: IL-17 family cytokines as sentinels of the intestinal mucosa. Dev Comp Immunol 41:418–428. https://doi.org/10.1016/j.dci.2013.04.003
Mohsin M, Li L, Huang X, Aleem MT, Habib YJ, Shehata AI, Afzal MZ, Abbas RZ, Abbas A, Yin G (2021) Immunogenicity and protective efficacy of probiotics with EtIMP1C against Eimeria tenella challenge. Pak Vet J 41(2):274–278. https://doi.org/10.29261/pakvetj/2021.009
Morris GM, Gasser RB (2006) Biotechnological advances in the diagnosis of avian coccidiosis and the analysis of genetic variation in Eimeria. Biotechnol Advanc 24(6):590–603. https://doi.org/10.1016/j.biotechadv.2006.06.001
Moryani AA, Rajput N, Naeem M, Shah AH, Jahejo AR (2021) Screening of the herbs and evaluation of their combined effects on the health and immunity of coccidiosis challenged broiler chickens. Pak Vet J 41(2):228–234. https://doi.org/10.29261/pakvetj/2021.005
Panebra A, Lillehoj HS (2019) *Eimeria tenella* elongation factor-1α (EF-1α) coadministered with chicken IL-7 (chIL-7) DNA vaccine emulsified in Montanide Gel 01 adjuvant enhanced the immune response to *E. acervulina* infection in broiler chickens. Avian Dis 6(2):342–350. https://doi.org/10.1637/11976-092418-reg.1
Peek HW, Landman WJM (2011) Coccidiosis in poultry: anticoccidial products, vaccines and other prevention strategies. Vet Q 31:143–161. https://doi.org/10.1080/01652176.2011.605247
Prescott JF, Smyth JA, Shojadoost B, Vince A (2016) Experimental reproduction of necrotic enteritis in chickens: a review. Avian Pathol 45(3):317–322. https://doi.org/10.1080/03079457.2016.1141345

Price KR (2012) Use of live vaccines for coccidiosis control in replacement layer pullets. J. Appl. Poult. Res. 21:679692. https://doi.org/10.3382/japr.2011-00486

Qi NS, Wang YY, Liao SQ (2013) Partial protective of chickens against *Eimeria tenella* challenge with recombinant EtMIC-1 antigen. Parasitol Res 112:2281–2287. https://doi.org/10.1007/s00436-013-3389-0

Qin M, Liu XY, Tang XM, Suo JX, Tao GR, Suo X (2014) Transfection of Eimeria mitis with yellow fluorescent protein as reporter and the endogenous development of the transgenic parasite. PLoS One 9(12):e114188. https://doi.org/10.1371/journal.pone.0114188

Rafiqi SI, Garg R, Ram H, Reena KK, Asari M, Kumari P, Kundave VR, Singh M, Banerjee PS (2019) Immunoprophylactic evaluation of recombinant gametocyte 22 antigen of *Eimeria tenella* in broiler chickens. Parasitol Res 118(3):945–953. https://doi.org/10.1007/s00436-018-06198-2

Rafiqi SI, Garg R, Reena KK, Ram H, Singh M, Banerjee PS (2018) Immune response and protective efficacy of *Eimeria tenella* recombinant refractile body protein, EtSO7, in chickens. Vet Parasitol 258:108–113. https://doi.org/10.1016/j.vetpar.2018.06.013

Rani Z, Abbas RZ, Abbas A, Saeed Z, Rehman T, Hussain MK, Rehman A, Hussain K (2021) In vitro and in vivo anticoccidial effects of butyric acid and its impact on blood and serum chemistry of broiler chickens. Kafkas Univ Vet Fak Derg 27(5):583–588. https://doi.org/10.9775/kvfd.2021.25907

Rashid M, Akbar H, Bakhsh A, Rashid MI, Hassan MA, Ullah R, Hussain T, Manzoor S, Yin H (2019) Assessing the prevalence and economic significance of coccidiosis individually and in combination with concurrent infections in Pakistani commercial poultry farms. Poult Sci 98(3):1167–1175. https://doi.org/10.3382/ps/pey522

Reid AJ, Blake DP, Ansari HR, Billington K, Browne HP, Bryant J, Dunn M et al (2014) Genomic analysis of the causative agents of coccidiosis in domestic chickens. Genome Res 24(10):1676–1685. https://doi.org/10.1101/gr.168955.113

Ribeiro E, Silva A, Sausset A, Bussière FI, Laurent F, Lacroix-Lamandé S, Silvestre A (2021) Genome-wide expression patterns of rhoptry kinases during the *Eimeria tenella* life-cycle. Microorganisms 9(8):1621–1634. https://doi.org/10.3390/microorganisms9081621

Rothwell L, Young JR, Zoorob R, Whittaker CA, Hesketh P, Archer A, Smith AL, Kaiser P (2004) Cloning and characterization of chicken IL-10 and its role in the immune response to *Eimeria maxima*. J Immunol 173(4):2675–2682. https://doi.org/10.3390/microorganisms9081621

Sander VA, Corigliano MG, Clemente M (2019) Promising plant-derived adjuvants in the development of coccidial vaccines. Front Vet Sci 6:20–35. https://doi.org/10.3389/fvets.2019.00020

Shah MAA, Yan R, Xu L, Song X, Li X (2010) A recombinant DNA vaccine encoding *Eimeria acervulina* cSZ-2 induces immunity against experimental *E. tenella* infection. Vet Parasitol 16(1-2):185–189. https://doi.org/10.1016/j.vetpar.2009.12.035

Shahid SRA, Shah MA, Riaz A, Malik AM, Hasan MU, Xiangrui L, Babar W, Shahid SKA (2020) Identification and molecular characterization of *Eimeria tenella* based on EtMic5 gene in Pakistan. Pak Vet J 40(4):443–448. https://doi.org/10.29261/pakvetj/2020.063

Song H, Xu X, Yan R, Shah MAA, Li X (2010a) Changes of cytokines and IgG antibody in chickens vaccinated with DNA vaccines encoding *Eimeria acervulina* lactate dehydrogenase. Vet Parasitol 173(3-4):219–227. https://doi.org/10.1016/j.vetpar.2010.06.030

Song H, Yan R, Xu L, Song X, Shah MAA, Zhu H, Li X (2010b) Efficacy of DNA vaccines carrying *Eimeria acervulina* lactate dehydrogenase antigen gene against coccidiosis. Exp Parasitol 126(2):224–231. https://doi.org/10.1016/j.exppara.2010.05.015

Song H, Qiu B, Yan R, Xu L, Song X, Li X (2013) The protective efficacy of chimeric SO7/IL-2 DNA vaccine against coccidiosis in chickens. Res Vet Sci 94(3):562–567. https://doi.org/10.1016/j.rvsc.2012.11.006

Song X, Gao Y, Xu L, Yan R, Li X (2015a) Partial protection against four species of chicken coccidia induced by multivalent subunit vaccine. Vet Parasitol 212(3–4):80–85. https://doi.org/10.1016/j.vetpar.2015.08.026

Song X, Ren Z, Yan R, Xu L, Li X (2015b) Induction of protective immunity against *Eimeria tenella, Eimeria necatrix, Eimeria maxima and Eimeria acervulina* infections using multivalent epitope DNA vaccines. Vaccine 33(24):2764–2770. https://doi.org/10.1016/j.vaccine.2015.04.052

Song X, Zhao X, Xu L, Yan R, Li X (2017) Immune protection duration and efficacy stability of DNA vaccine encoding *Eimeria tenella* TA4 and chicken IL-2 against coccidiosis. Res Vet Sci 111:31–35. https://doi.org/10.1016/j.rvsc.2016.11.012

Soutter F, Werling D, Nolan M, Küster T, Attree E, Marugán-Hernández V, Kim S, Tomley FM, Blake DP (2022) A novel whole yeast-based subunit oral vaccine against Eimeria tenella in chickens. Front Immunol 13:809711–809725. https://doi.org/10.3389/fimmu.2022.809711

Soutter F, Werling D, Tomley FM, Blake DP (2020) Poultry coccidiosis: design and interpretation of vaccine studies. Front Vet Sci 7:101–112. https://doi.org/10.3389/fvets.2020.00101

Su H, Liu X, Yan W, Shi T, Zhao X, Blake DP, Tomley FM, Suo X (2012) *piggyBac* transposon-mediated transgenesis in the apicomplexan parasite *Eimeria tenella*. PLoS ONE 7:e40075. https://doi.org/10.1371/journal.pone.0040075

Tahir A, Khan MA, Bibi K, Bibi S, Rauf F, Ayaz F (2021) Prevalence of colibacillosis in young broiler chicks and antibiogram of Escherichia coli in different areas of Hazara Region. Adv Life Sci 8(3):238–240

Tang X, Suo J, Li C, Du M, Wang C, Hu D, Duan C, Lyu Y, Liu X, Suo X (2018) Transgenic *Eimeria tenella* expressing profilin of *Eimeria maxima* elicits enhanced protective immunity and alters gut microbiome of chickens. Infect Immun 86(9):e00888–e00817. https://doi.org/10.1128/IAI.00888-17

Tang X, Suo J, Liang L, Duan C, Hu D, Gu X, Yu Y, Liu X, Cui S, Suo X (2020) Genetic modification of the protozoan Eimeria tenella using the CRISPR/Cas9 system. Vet Res *51*(1):1–5. https://doi.org/10.1186/s13567-020-00766-0

Tang X, Wang C, Liang L, Hu D, Zhang S, Duan C, Suo J, Liu X, Suo X, Cui S (2019) Co-immunization with two recombinant *Eimeria tenella* lines expressing immunoprotective antigens of *E. maxima* elicits enhanced protection against *E. maxima* infection. Parasit Vector 12(1):1–8. https://doi.org/10.1186/s13071-019-3605-6

Tang XM, Yin GW, Qin M, Tao GR, Suo JX, Liu XY, Suo X (2016) Transgenic Eimeria tenella as a vaccine vehicle: expressing TgSAG1 elicits protective immunity against Toxoplasma gondii infections in chickens and mice. *Sci Rep* 6(1):1–9. https://doi.org/10.1038/srep29379

Thi TTH, Suys EJ, Lee JS, Nguyen DH, Park KD, Truong NP (2021) Lipid-based nanoparticles in the clinic and clinical trials: from cancer nanomedicine to COVID-19 vaccines. Vaccines 9(4):359

Thiam LG, Mangou K, Ba A, Mbengue A, Bei AK (2022) Leveraging genome editing to functionally evaluate Plasmodium diversity. Trends Parasitol. 38(7):558–571. https://doi.org/10.1016/j.pt.2022.03.005

Tian L, Li W, Huang X, Tian D, Liu J, Yang X, Liu L et al (2017) Protective efficacy of coccidial common antigen glyceraldehyde 3-phosphate dehydrogenase (GAPDH) against challenge with three *Eimeria* species. Front Microbiol 18(8):1245–1257. https://doi.org/10.3389/fmicb.2017.01245

Venkatas J, Adeleke MA (2019) A review of *Eimeria* antigen identification for the development of novel anticoccidial vaccines.

Parasitol Res 118(6):1701–1710. https://doi.org/10.1007/s00436-019-06338-2

Wajiha, Qureshi NA (2021) In vitro anticoccidial, antioxidant activities and biochemical screening of methanolic and aqueous leaves extracts of selected plants. Pak Vet J 41(1):57–63. https://doi.org/10.29261/pakvetj/2020.071

Wang S, Suo X (2020) Still naïve or primed: Anticoccidial vaccines call for memory. Exp Parasitol 216:107945–107950. https://doi.org/10.1016/j.exppara.2020.107945

Wang Q, Chen L, Li J, Zheng J, Cai N, Gong P, Li S, Li S, Zhang X (2014) A novel recombinant BCG vaccine encoding *Eimeria tenella* rhomboid and chicken IL-2 induces protective immunity against coccidiosis. Korean J Parasitol 52(3):251–25. https://doi.org/10.3347/kjp.2014.52.3.251

Wang XQ, Wu LL, Gao Y, Zhang Y, Weng YB, Lin RQ (2017) Evaluation of the protective effect of pVAX-EtMIC3-recombined plasmid against *E. tenella* in chicken. Parasitol Res:116

Williams RB (2002) Fifty years of anticoccidial vaccines for poultry (1952-2002). Avian Dis 46:775–802. https://doi.org/10.1637/0005-2086(2002)046[0775:FYOAVF]2.0.CO;2

Xu J, Zhang Y, Tao J (2013) Efficacy of a DNA vaccine carrying *Eimeria maxima* Gam56 antigen gene against coccidiosis in chickens. Korean J Parasitol 51(2):147–154. https://doi.org/10.3347/kjp.2013.51.2.147

Yan M, Cui X, Zhao Q, Zhu S, Huang B, Wang L, Zhao H et al (2018) Molecular characterization and protective efficacy of the microneme 2 protein from *Eimeria tenella*. Parasite 25:60–70. https://doi.org/10.1051/parasite/2018061

Yang G, Wang C, Hao F, Zhao D, Zhang Y, Li Y (2010) Studies on construction of a recombinant *Eimeria tenella* SO7 gene expressing *Escherichia coli* and its protective efficacy against homologous infection. Parasitol Int 59(4):517–523. https://doi.org/10.1016/j.parint.2010.06.010

Yang X, Li M, Liu J, Ji Y, Li X, Xu L, Yan R, Song X (2017) Identification of immune protective genes of Eimeria maxima through cDNA expression library screening. Parasit Vector 10(1):1–10. https://doi.org/10.1186/s13071-017-2029-4

Yin G, Lin Q, Qiu J, Qin M, Tang X, Suo X, Huang Z, Liu X (2015) Immunogenicity and protective efficacy of an *Eimeria* vaccine candidate based on *Eimeria tenella* immune mapped protein 1 and chicken CD40 ligand. Vet Parasitol 210(1-2):19–24. https://doi.org/10.1016/j.vetpar.2015.03.012

Yin G, Qin M, Liu X, Suo J, TangX TG, Han Q, Suo X, Wu W (2013) An Eimeria vaccine candidate based on *Eimeria tenella* immune mapped protein 1 and the TLR-5 agonist *Salmonella typhimurium* FliC flagellin. Biochem Biophys Res Commun 440(3):437–442. https://doi.org/10.1016/j.bbrc.2013.09.088

Young J, Dominicus C, Wagener J et al (2019) A CRISPR platform for targeted in vivo screens identifies Toxoplasma gondii virulence factors in mice. Nat Commun 10:3963. https://doi.org/10.1038/s41467-019-11855-w

Yun CH, Lillehoj HS, Lillehoj EP (2000) Intestinal immune responses to coccidiosis. Dev Comp Immunol 24(2-3):303–324. https://doi.org/10.1016/s0145-305x(99)00080-4

Zhai Q, Huang B, Dong H, Zhao Q, Zhu S, Liang S, Li S, Yang S, Han H (2016) Molecular characterization and immune protection of a new conserved hypothetical protein of *Eimeria tenella*. PloS one 11(6):e0157678–e0157698. https://doi.org/10.1371/journal.pone.0157678

Zhang DF, Xu H, Sun BB, Li JQ, Zhou QJ, Zhang HL, Du AF (2012a) Adjuvant effect of ginsenoside-based nanoparticles (ginsomes) on the recombinant vaccine against *Eimeria tenella* in chickens. Parasitol Res 110(6):2445–2453. https://doi.org/10.1007/s00436-011-2784-7

Zhang L, Ma L, Liu R, Zhang Y, Zhang S, Hu C, Song M, Cai J, Wang M (2012b) *Eimeria tenella* heat shock protein70 enhances protection of recombinant microneme protein MIC2 subunit antigen vaccination against *E. tenella* challenge. Vet Parasitol 188(3-4):239–246. https://doi.org/10.1016/j.vetpar.2012.03.025

Zhang Z, Huang J, Li M, Sui Y, Wang S, Liu L, Xu L, Yan R, Song X, Li X (2014) Identification and molecular characterization of microneme 5 of *Eimeria acervulina*. PLoS One 9(12):e115411–e115419. https://doi.org/10.1371/journal.pone.0115411

Zhang S, Tang X, Wang S, Shi F, Duan C, Bi F, Suo J, Hu D, Liu J, Wang C, Suo X, Liu X (2021) Establishment of recombinant Eimeria acervulina expressing multi-copies M2e derived from avian influenza virus H9N2. Vaccines 9(7):791–101. https://doi.org/10.3390/vaccines9070791

Zhao Y, Xu R, Zhang Y, Ji X, Zhang J, Liu Y, Bao Y, Qin J (2014) Protective efficacy in chickens of recombinant plasmid pET32a(+)-ADF-3-1E of *Eimeria acervulina*. Parasitol Res 113(8):3007–3014. https://doi.org/10.1007/s00436-014-3963-0

Zhao H, Zhao H, Zhao O, Zhu S, Huang B, Lv L, Liu G et al (2020) Molecular characterization and immune protection of an AN1-like zinc finger protein of *Eimeria tenella*. Parasitol Res 119(2):623–635. https://doi.org/10.1007/s00436-019-06545-x

Zhou Z, Wang Z, Cao L, Hu S, Zhang Z, Qin B, Guo Z, Nie K (2013) Upregulation of chicken TLR4, TLR15 and MyD88 in heterophils and monocyte-derived macrophages stimulated with *Eimeria tenella in vitro*. Exp Parasitol 133:417–433. https://doi.org/10.1016/j.exppara.2013.01.002

Zhu H, Xu L, Yan R, Song X, Tang F, Wang S, Li X (2012a) Identification and characterization of a cDNA clone-encoding antigen of *Eimeria acervulina*. Parasitol 139(13):1711–1719. https://doi.org/10.1017/S0031182012001163

Zhu H, Yan R, Wang S, Song X, Xu L, Li X (2012b) Identification and molecular characterization of a novel antigen of *Eimeria acervulina*. Mol Biochem Parasitol 186(1):21–28. https://doi.org/10.1016/j.molbiopara.2012.09.002

Zhu JJ, Lillehoj HS, Allen PC, Yun CH, Pollock D, Sadjadi M, Emara MG (2000) Analysis of disease resistance-associated parameters in broiler chickens challenged with *Eimeria maxima*. Poult Sci 79(5):619–625. https://doi.org/10.1093/ps/79.5.619