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Advancements in understanding chicken coccidiosis: from *Eimeria* biology to innovative control strategies

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Abstract

Coccidiosis, an intestinal disease caused by *Eimeria* protozoan parasites, affects various animal species, and especially poses a significant threat to the poultry industry. The current primary control methods include anticoccidial drugs and vaccines. However, emerging challenges such as drug resistance and vaccine efficacy issues are rooted in the complex life cycle and species diversification of *Eimeria*. In this review, we first consolidate recent breakthroughs in understanding *Eimeria* biology, focusing on the parasite development and its intricate interactions with the host, notably its relationships with host immune cells and the gut microbiota. Furthermore, we provide an extensive summary of current control strategies for *Eimeria* infections. This includes an in-depth analysis of anticoccidial drugs, their mechanisms of resistance, and the increasing utilization of diverse anticoccidial vaccines to combat these challenges. Finally, we highlight the latest innovative strategies leading the way in coccidiosis control. Through an exploration of cutting-edge techniques, we also provide insights into future directions for effectively combating this disease. In conclusion, the future of coccidiosis control lies in the use of a multifaceted approach, integrating advanced biological insights with innovative therapeutic strategies. This review not only serves to enhance our understanding of *Eimeria* biology but also provides a valuable resource for researchers involved in developing and implementing strategies to manage and control coccidiosis, ensuring the health and productivity of poultry worldwide.

Keywords *Eimeria*, Parasite development, Drugs, Vaccines, Innovative strategies

Brief overview of coccidiosis and its significance in poultry

Coccidiosis is an intestinal disease caused by protozoan parasites of the *Eimeria* genus, which are obligate intracellular parasites and highly host-specific members of the phylum Apicomplexa. This phylum also includes pathogens responsible for various human and animal diseases, such as *Plasmodium* spp., *Toxoplasma gondii*, *Babesia* spp., and *Cryptosporidium* spp. [1]. Coccidiosis affects a wide range of animals, from birds and mammals to reptiles and amphibians, and poses a significant threat to the poultry industry. Chickens are paramount in the agricultural economy, with over 70 billion raised in 2020, accounting for a third of global meat production and producing over 1.6 trillion eggs for human consumption [2]. In the upcoming years, as the production of chicken meat and eggs continues to rise, tackling pathogens such as coccidiosis will become increasingly vital for ensuring global food security and sustaining a robust agro-economy.

Modern poultry farming practices, which typically involve raising large numbers of birds in confined spaces with high stocking densities, often on accumulated litter,

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create optimal conditions for *Eimeria* transmission [3]. *Eimeria* spreads when birds consume food or water contaminated with sporulated oocysts. This leads to the release and invasion of sporozoites into the bird's gastrointestinal tract. The oocysts, which are the transmission stage of the parasite, possess a resilient multilayered wall that resists many standard disinfectants. This durability allows oocysts to survive and remain infectious in moist environments for extended periods, ranging from months to years [4, 5]. Consequently, wherever poultry are raised, the persistent nature of oocysts ensures the continuous presence of these parasites.

The severity of coccidiosis and its impact on individual chickens and overall flock productivity can vary widely. Factors influencing this risk include the specific parasite species, the infectious dose, and the age and immune status of the host. Infected chickens experience overgrowth of *Eimeria* spp. in their intestinal lining, leading to significant economic repercussions [1]. Globally, the cost of coccidiosis and its control in chickens rose from US$ 0.8 billion in 2002 to US$ 3 billion in 2006 [6–9]. By 2020, using an updated estimation model by Blake et al., who considered current poultry production and disease prevalence, the global cost was recalculated to be around £ 10.4 billion annually [10].

Given its substantial economic repercussions and implications for animal welfare, avian coccidiosis has been ranked among the top three poultry diseases in the UK [11] and listed among the top ten veterinary diseases affecting impoverished populations in South Asia [12]. A 2019 survey of broiler veterinarians in the US identified coccidiosis, particularly *Eimeria maxima*, as the foremost disease concern. Similarly, a poll by the US Association of Veterinarians in Egg Production (AVEP) revealed that coccidiosis was the most critical disease for cage-free replacement layers and the second most significant disease for those raised in cages [13]. In China, over 16.1 billion poultry (mainly chickens), which produce 24.43 million tons of meat and 34.56 million tons of eggs, were raised in 2022 [14] and avian coccidiosis was the top epidemic animal disease. The highest number of cases and deaths were reported between April and September 2023, accounting for 69.8% – 90.7% and 38.9% – 71.1% of the total reported cases and deaths, respectively [15].

Eimeria parasites and their interactions with the host

Eimeria species and their specific characteristics

Eimeria spp. are responsible for the debilitating intestinal ailment known as coccidiosis. Over 1900 *Eimeria* species have been identified so far, and this count is still on the rise [3]. The documentation of *Eimeria* affecting chickens dates back over a century [16], initially identifying 9 potential species, which were later consolidated into 7 well-recognized *Eimeria* spp., i.e., *E. acervulina, E. brunetti, E. maxima, E. mitis, E. necatrix, E. praecox,* and *E. tenella* [17, 18]. Recently, the discovery of three cryptic *Eimeria* operational taxonomic units (OTUs) in chickens from various continents have attracted great interest from researchers [19–21]. Based on their genotypic and phenotypic characteristics, these strains were recently classified as distinct parasite species, named *E. lata, E. nagambie,* and *E. zaria* [22] (Table 1).

Each chicken *Eimeria* species exhibits a distinct preference for specific regions within the gastrointestinal tract [33]. These species can be differentiated based on the pathology they cause, visible macroscopic lesions, oocyst morphology, minimum time required for sporulation, and the minimum prepatent period (the interval between a bird's infection with a sporulated oocyst and the first shedding of oocysts via feces). Other distinguishing factors include the size of the schizont and the specific location of parasite development within the intestinal epithelium [34–36]. Immunity against a particular *Eimeria* species in chickens is typically highly specific to that species, offering minimal to no cross-protection against other species [37]. The recently identified species, namely *E. lata, E. nagambie,* and *E. zaria* possess the capacity to hinder chicken bodyweight gain and escape the immunity induced by current commercially available anticoccidial vaccines, all of which adversely affect chicken production. Current live vaccines designed to combat coccidiosis offer limited or no protection against these new species [22, 38]. Consequently, these parasites challenge the effectiveness of vaccines, posing risks to food security and animal welfare in systems dependent on anticoccidial vaccination. This indicates the potential for increased pathogenic and economic threats ahead.

The regulation of *Eimeria* parasite development

The life cycle of *Eimeria* encompasses both asexual and sexual reproduction. Infection initiates when sporulated oocysts are ingested through the fecal–oral route via contaminated food or water [39]. The wall of sporulated oocysts is mechanically ruptured in the gizzard and sporozoites are released from sporocysts due to the enzymatic activity of primary chymotrypsin and the presence of bile salts in the small intestine. These sporozoites either invade epithelial cells or are taken up by intestinal immune cells, with the invasion of sporozoites signifying the commencement of their developmental journey [40].

Sporozoites possess several organelles playing pivotal roles in the invasion process, including the apicoplast, micronemes and rhoptries. The initial attachment is often reversible and relies on the recognition of surface

Table 1 *Eimeria* species that infected chickens and their main biological characteristics

Organism	Prepatent period (h)	Development site	Fecundity	Pathogenicity	Schizogony number	Genome size (Mb)	Predicted proteins	References
E. acervulina	89	Duodenum, Jejunum	+ + + + +	+ +	4	46.1	6867	Vetterling et al., 1966 [23]; Long et al., 1976 [24]; Eckert et al., 1995 [25]; Reid et al., 2014 [26]
E. brunetti	120	Cecum, Rectum	+ +	+ + + +	3	65.6	8711	Kheysin,1972 [27]; Long et al., 1976 [24]; Eckert et al., 1995 [25]; Reid et al., 2014 [26]
E. maxima	120	Jejunum, Ileum	+ +	+ + +	2–3	46.2	6057	Long et al., 1976 [24]; Eckert et al., 1995 [25]; Reid et al., 2014 [26]; Dubey and Jenkins, 2018 [28]
E. mitis	91	Ileum	+ + + +	+	4	69.5	10,077	Long et al., 1976 [24]; Novilla et al., 1987 [29]; Eckert et al., 1995 [25]; Reid et al., 2014 [26]
E. necatrix	138	Jejunum, Ileum, Cecun	+	+ + + + +	3	55.2	8627	Long et al., 1976 [24]; McDonald and Rose, 1987 [30]; Eckert et al., 1995 [25]; Reid et al., 2014 [26]
E. praecox	84	Duodenum, Jejunum	+ + + +	+	2	56.7	7635	Kheysin, 1972 [27]; Long et al., 1976 [24]; Eckert et al., 1995 [25]; Reid et al., 2014 [26]
E. tenella	132	Cecum	+ + +	+ + + +	3	53.25	7268	Kheysin, 1972 [27]; Long et al., 1976 [24]; McDonald and Rose, 1987 [30]; Eckert et al., 1995 [25]; Aunin et al., 2021 [31]
E. lata	125–130	Duodenum, Jejunum	+ +	+ + +	na	42.9	10,309	Blake et al., 2021 [22]
E. nagambie	132	Duodenum, Jejunum	na	+ + +	na	58.0	12,777	Cantacessi et al., 2008 [32]; Blake et al., 2021 [22]
E. zaria	130–135	Duodenum, Jejunum	+ + +	+ +	na	50.6	11,705	Blake et al., 2021 [22]

Na not available

receptors by GPI-anchored surface antigens (SAGs) [41]. Subsequently, attention shifts to the micronemes, secretory organelles that congregate at the apical end of the sporozoite. There are approximately fifteen identified microneme proteins (MICs), which migrate to the parasite's apical surface during the attachment phase [42]. As invasion proceeds, a critical structure known as the moving junction (MJ) forms, establishing an intimate interaction between the parasite and the host cell. The MJ consists of rhoptry neck (RON) proteins and apical membrane antigen 1 (AMA1) [43]. Among these, RON2, RON4, and RON5 have all been localized at the MJ complex during the invasion process [43]. In a transcriptome analysis of *E. necatrix*, focusing on unsporulated oocysts (UOs) and sporozoites (SZs), a total of 73 upregulated genes in UOs and 50 upregulated genes in SZs were identified. These genes encode various crucial proteins, including microneme proteins, apical membrane antigens, rhoptry neck proteins, rhoptry proteins, dense granule proteins, heat shock proteins, calcium-dependent protein kinases, cyclin-dependent kinases, cGMP-dependent protein kinases and glycosylphosphatidylinositol-anchored surface antigens. These findings strongly implicate the proteins in the attachment to host cells and the ultimate invasion process [44]. Furthermore, isobaric tags for relative and absolute quantitation (iTRAQ)-based proteomics were performed to profile the proteomes of unsporulated oocysts, sporozoites and second-generation merozoites (MZ-2); a total of 118 differentially expressed proteins (DEPs) were identified. These findings underscore the complexity of the cellular invasion process and shed light on the intricate molecular mechanisms involved in invasion strategy of *E. necatrix* [45]. The *E. tenella Eimeria*-specific protein, known as EtEsp, exhibits variable expression levels throughout the parasite's life cycle, suggesting a pivotal role in its development and cell invasion. Notably, a polyclonal anti-rEtEsp antibody test demonstrated a significant impediment to *E. tenella*'s ability to invade host cells, highlighting rEtEsp as a promising target for the control of *E. tenella* infections in poultry [46]. Additionally, anti-EtMIC8 antibodies could significantly reduce the

sporozoites invasion rates into host cells compared to the control group [47]. Further exploration of these proteins' function could significantly enhance our understanding of the parasite's infection mechanisms and contribute to the development of novel treatment strategies.

The subsequent phase of development in *Eimeria* species encompasses two to five rounds of asexual replication, known as schizogony. During this process, nuclear divisions and cellular expansion result in the formation of a multinuclear structure called a schizont. In particular, the membrane occupation and recognition nexus protein 1 (MORN1) is associated with the posterior ring of the inner membrane complex (IMC) in the multiple daughters formed during *T. gondii* endopolygeny, as well as in schizogony in *Eimeria* and *P. falciparum* [48]. Notably, in *P. falciparum*, an orphan protein kinase (PfPK7) is linked to a reduced production of daughter merozoites per schizont, implying its role in regulating parasite proliferation and development [49]. Additionally, an essential contractile ring protein (PfCINCH) plays a critical role in controlling cell division. Parasites lacking PfCINCH develop inviable conjoined daughters containing components intended for multiple cells, further underscoring its significance in this process [50]. Through the utilization of the BLAST tool within the ToxoDB database (http://toxodb.org/toxo/), it was determined that PfCINCH shares approximately 26% sequence identity with the *EAH_00057910* gene in the *E. acervulina*. While this discovery suggested a potential role for this gene in regulating parasite development in *Eimeria*, it is imperative to conduct further research to ascertain its precise function. Furthermore, the serpentine receptor 10 (SR10) plays a pivotal role in regulating intra-erythrocytic development, impacting various processes, such as DNA replication and the ubiquitin and proteasome pathways. These functions are particularly affected when coordination with host rhythms is disrupted [51]. Additionally, SR10 exhibited sequence identities of 30%, 28% and 27% with three genes (*EfaB_PLUS_20412.g1738.t1*, *ETH2_0701600* and *ETH_00010045*) that encode transmembrane proteins in *Eimeria*, respectively. These genes also play potential roles in regulating *Eimeria* development, but their precise functions require further exploration.

Scientists have embarked on experiments aimed at unraveling the critical proteins that govern *Eimeria* parasite development. Notably, enolase and pyruvate kinase, both glycolytic enzymes, have been identified in sporozoites, and exhibit heightened expression during schizogony. While their primary role lies in glycolysis during anaerobic intracellular stages, they also actively participate in the invasion process and contribute to the control of gene regulation in *E. tenella* [52]. Comparative transcriptome analysis of *E. necatrix* second- (MZ-2) and third-generation (MZ-3) merozoites unveiled that a staggering 2053 genes exhibited differential expression during these intricate processes. In MZ-2, the upregulated genes were predominantly associated with protein degradation and amino acid metabolism, whereas the upregulated genes in MZ-3 were primarily enriched for transcriptional activity, cell proliferation, and cell differentiation [53]. Furthermore, an active rhoptry kinase (EtROP2) capable of phosphorylating cell substrates of approximately 50 kDa, has been shown to accelerate the shortening of the prepatent period and the early development of first-generation schizonts when overexpressed [54]. Conducting a dual RNA-seq transcriptome analysis of chicken cecal tissue during *E. tenella* infection revealed a significant upregulation of genes, including matrix metalloproteinases, chemokines, interferon (IFN)-γ, and IFN-stimulated genes such as guanylate binding protein (GBP), interferon regulatory factor (IRF), and radical s-adenosyl methionine domain containing 2 (RSAD2). This comprehensive examination sheds light on the dynamic gene expression profile of *E. tenella* during schizogony in cecal tissue, providing invaluable insights into the host-parasite interactions [55]. Furthermore, the documentation of proteome-wide lysine acetylation in *E. tenella* revealed dynamic changes in the lysine acetylome across different stages of the parasite's life cycle, indicating significant regulatory events during growth and stage conversion [56]. This insight into lysine acetylation adds a new dimension to our understanding of *Eimeria*'s biology and offers new strategies for identifying anticoccidiosis drugs and developing vaccines.

Following schizogony, *Eimeria* parasites universally undergo sexual development, giving rise to micro- and macro-gametocytes [57]. The molecular mechanisms governing sexual commitment in apicomplexan parasites have revealed an intricate landscape of epigenetic regulation. In *Plasmodium*, a pivotal transcriptional master regulator known as AP2-G orchestrates the sequential transcription of downstream gametocyte genes, while the expression of AP2-G is influenced by histone methylation and acetylation [58]. Notably, Chen et al. reported the remarkable discovery of a cluster of 15 AP2s exhibiting high expression levels during *E. tenella* gametocyte stage, specifically at 132 h post-infection, as per transcriptomic data. These transcription factors are believed to play a significant role in the regulation of gametocyte gene expression [59]. In *T. gondii*, sexual genes face repression by a complex known as microrchidia (MORC)/HDAC3/AP2s suppressor in tachyzoites. Suppression or knockdown of the components of this complex results in the confinement of pre-sexual development to a cat-restricted environment in vitro [60–62]. Remarkably, the presence of the MORC protein in *Eimeria* species

signifies its potential importance in shedding light on the longstanding debate surrounding whether sexual commitment in *Eimeria* is genetically predetermined [39, 63, 64] or influenced by environmental factors [65, 66].

The microgametocyte is identifiable by its front-end housing DNA and the presence of two mobile flagella that contain a specialized cytoskeletal axoneme [53]. Consequently, gene expression in microgametes primarily revolves around the constituents responsible for flagellar composition and mobility, notably encompassing dyneins, basal body proteins, and axonemal proteins [53, 67]. In contrast, the macrogametocyte, distinguished by its large rounded shape, harbors distinctive organelles, including veil-forming bodies (VFBs) and type 1 and type 2 wall-forming bodies (WFB1/WFB2) [68]. During macrogamete formation, the VFBs are released following fertilization by a microgamete. WFB1 comprises muco-proteins, mucopolysaccharides and glycoproteins like gametocyte protein 22 (GAM22) [69]. These components are subsequently relocated to the periphery with the assistance of F-actin [70], eventually contributing to the formation of the outer membrane of the oocyst. On the other hand, WFB2, which contains well-known antigens such as GAM56, GAM58 and GAM82, contributes to the development of the inner layers of the oocyst wall [71].

Regulating the formation of the oocyst wall involves the proteolysis of tyrosine-rich proteins in macrogametes into smaller tyrosine-rich peptides via specific enzymes like serine proteases, aminopeptidases and subtilisins. Subsequently, these degraded fragments of small tyrosine-rich proteins are cross-linked to create a tyrosine-rich protein matrix through enzymes like peroxidases, for example, the amiloride-sensitive amine oxidase EtAO2. Glycosylation emerges as another significant feature of *Eimeria* macrogametes [26]. Notably, gametocyte antigens GAM56 and GAM82 exhibit heavy glycosylation [72]. Additionally, the macrogamete is distinguished by an abundance of polysaccharide granules, lipid droplets and the presence of the non-nuclear genome of the apicoplast, signaling its inheritance through the maternal lineage [68].

In order to become infectious, unsporulated oocysts were excreted in the feces and then undergo sporulation in the environment, a process primarily governed by three pivotal factors: temperature, humidity and oxygen levels [73]. The rate of oocyst sporulation significantly impacts the epidemiology of infections in chicken flocks. This has led researchers to explore various methodologies aimed at inhibiting sporulation, including the use of substances like pine bark (*Pinus radiata*) [74], artemisinin [75] and essential oils [76]. Beyond the inhibition of oocyst sporulation, it is imperative to identify the specific genes responsible for orchestrating the developmental progression of *Eimeria* parasites, ultimately addressing the transmission of these parasites. AP2 transcription factors play a crucial role in regulating gene expression during transitions in the life cycle of *T. gondii* [60–62]. Through meticulous transcriptome analysis, 53 proteins containing AP2 domains within the *E. tenella* genome were identified. These proteins are organized into four distinct clusters according to their functions: cluster 1 proteins are involved in sporogony, cluster 2 proteins exhibit heightened expression during schizogony, cluster 3 proteins are characterized by increased expression in sporulated oocysts and cluster 4 proteins show elevated expression in the gametocyte stage. This comprehensive study serves as a valuable foundation for further exploration into the roles of AP2 transcription factor genes in governing the development of *Eimeria* parasites [59].

Eimeria research has yet to fully decode the complexities of life-cycle regulation, highlighting an urgent need for deeper exploration. Cutting-edge techniques like clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR-Cas9) and RNA sequencing are poised to enhance our understanding of life-cycle phases, paving the way for precise interventions. By disrupting or manipulating specific stages, we can potentially revolutionize the management of coccidiosis. This progress hinges on continued research, which is on the brink of advancing our capabilities in controlling this widespread coccidiosis.

Interactions between *Eimeria* infection and gut microbiota

The gastrointestinal tract (GIT) of chickens comprises a complex and diverse microbiota, including bacteria, viruses, archaea and fungi. Typically, these organisms have a beneficial symbiotic interaction with the host, including promoting the gut immune system maturation and thereby protecting the host from intestinal infections caused by pathogenic or opportunistic enteric microorganisms. However, it is important to note that gut microbiota may also facilitate pathogens infection—wherein pathogenic organisms exploit microbiota metabolites or the microbiota-related environment [77, 78]. Supporting this idea, studies have shown that compared with conventional chickens, germ-free chickens exhibit resistance to *E. tenella* infection, displaying much lower load of oocysts in cecal content [79]. Moreover, Gong et al. recently demonstrated that mice subjected to diverse antibiotic treatments, resulting in distinct gut microbiota profiles, exhibited varying susceptibilities to *E. falciformis* infection and showed differences in disease severity [80]. These studies highlight the crucial role of gut microbiota in *E. tenella* infection.

On the other hand, *Eimeria* infection can alter the composition of gut microbiota, leading to dysbiosis. *Eimeria*

parasites infiltrate the intestinal epithelial cells, causing intestine structure damage and inducing mucosal inflammation. These changes in the intestinal environment result in alterations of gut microbiota, characterized by a decline in beneficial bacteria and a surge in harmful ones, jeopardizing the host's equilibrium [81, 82]. Meanwhile, these reshaped microbial landscape by *Eimeria* infection amplifies the presence of pathogens like *Clostridium perfringens*, *Streptococcus* spp., *Salmonella enterica*, and *Campylobacter jejuni*, while diminishing immune-regulating bacteria such as *Lactobacillus, Faecalibacterium, Candidatus*, Ruminococcaceae, and Firmicutes [83–85]. Furthermore, it was demonstrated that when *Eimeria* coinfected with *C. perfringens*, it can escalate to necrotic enteritis (NE), and its associated microbiota alteration including a spike in *Clostridium* sensu*, Escherichia, Shigella,* and *Weissella*, and a decline in *Lactobacillus* in the jejunum [86, 87].

In addition to altering commensal bacteria in the intestine, *Eimeria* infection, particularly *E. maxima* infection, is a key factor predisposing chickens to necrotic enteritis, facilitating the overgrowth of pathogenic bacteria such as *C. perfringens* and contributing to aggregate enteritis [88]. Mechanistically, the damage induced by *Eimeria* infection to the intestinal epithelium creates an environment conducive to *C. perfringens* colonization. Specifically, this damage causes serum leakage into the gut, spurring mucus production and offering a nutrient-rich environment for *C. perfringens* growth [89]. Moreover, coccidiosis-induced damage exposes specific collagens in the extracellular matrix [90, 91], which serve as binding sites for *C. perfringens*, leading to the production of potent toxins like NetB by *C. perfringens* [92, 93]. Both NE and coccidiosis are prevalent enteric diseases in poultry, imposing significant economic burdens on the industry. For years, chemoprophylaxis and sub-therapeutic antimicrobial doses in feed effectively managed these diseases. However, global shifts toward limiting antibiotics in animal feed have seen a resurgence in diseases like NE and coccidiosis, especially in broilers, drawing industry attention [94]. Considering the important roles of gut microbiota in both *Eimeria* and *C. perfringens* infection and their induced disease, strategies to modulate the gut microbiota and/or its metabolites may serve as alternative approaches to control NE and coccidiosis.

Immune responses of chickens against *Eimeria* infection

Innate immunity

The innate immune system serves as the first line of host defense against *Eimeria* infection in chickens. It's composed of physical barriers, innate immune molecules, as well as cellular components, including macrophages, dendritic cells, and natural killer cells [95]. Upon invasion by *Eimeria*, the intestinal epithelium undergoes structural changes, leading to recruitment and activation of immune cells, which can detect pathogen-associated molecular patterns (PAMPs) using pattern recognition receptors (PRRs) [96]. This detection results in the release of diverse soluble factors [97]. While the importance of profilin recognition by Toll-like receptor 11/12 (TLR11/12) in resisting *T. gondii* is known, the initial detection of *Eimeria*-derived profilin by PRRs remains unreported [98]. However, PRRs such as TLR1LA, TLR4, TLR5, TLR7 and TLR21, along with cytokines like IFN-α, IFN-β, IFN-γ, IL-1β, IL-12 and IL-22, are up-regulated in specific immune cells after exposure to *E. tenella* sporozoites or in tissues from infected chickens. This suggests TLR-mediated pathways detect *Eimeria* infections [99–101]. The potential roles of dendritic cells, macrophages, intraepithelial lymphocytes and other innate immune cells in defending against *Eimeria* infections have been proposed [96, 102, 103]. Yet, the details on how *Eimeria* antigens are presented by innate immunity to elicit adaptive immunity against *Eimeria* infection remains limited.

Adaptive immunity

Adaptive immune responses are crucial for long-term protection against *Eimeria* infection in chickens. This arm of the immune system involves the activation of T and B lymphocytes, which recognize specific antigens. Upon activation, T cells differentiate into effector cells, including $CD4^+$ T helper cells and $CD8^+$ cytotoxic T cells [104, 105]. B lymphocytes differentiate into plasma cells that produce antibodies specific to *Eimeria* antigens. These antibodies can neutralize the parasites and prevent their invasion of host cells [106]. Both humoral and cell-mediated immune (CMI) responses play roles against *Eimeria* infection [107, 108]. The mechanism of action of humoral immunity in combating *Eimeria* infections has not been definitively confirmed. It is speculated that antibodies may play a role in mediating cross-presentation of *Eimeria* antigens as a study indicated that the surface displayed Fc fragment of immunoglobulin in recombinant *Eimeria* elicits enhanced protective immunity against subsequent infection [109]. On the other hand, development of antigen-specific memory upon pathogen exposure is a hallmark of the adaptive immune system. The immune system has the capacity to retain a memory of previously encountered pathogens, enabling swift and potent responses upon subsequent exposure [110]. This finding forms the basis of vaccination. In immunized chickens, sporozoites are frequently found within or adjacent to $TCR1^+$ $CD8^+$ cells and $TCR2^+$ $CD8^+$ cells [111], which are probably memory γδ and αβ $CD8^+$ T cells, respectively. αβ T cells play a crucial

role in memory responses against *Eimeria* infection, as TCRβ$^{-/-}$ mice are highly susceptible to secondary infection by the highly immunogenic parasite *E. vermiformis* [112, 113]. Furthermore, this memory immune response is mediated by a specific subset of cells known as tissue-resident memory T (T_{RM}) cells, which are localized in the tissues [114, 115]. These cells possess the remarkable ability to rapidly inhibit the proliferation and progression of pathogens. Upon reinfection, they quickly proliferate at the site of infection, effectively controlling the growth of the pathogen. Shi et al. conducted a study to investigate the role of $CD8^+$ T_{RM} cells in providing immunity against reinfection with *Eimeria* [116]. Researchers have observed that $CD8^+$ T_{RM} cells quickly up-regulated effector genes and inhibited the maturation of early-stage schizonts, a critical phase of *Eimeria* infection. In contrast, naïve $CD8^+$ T cells took days to respond effectively, allowing parasite proliferation. Interestingly, researchers have shown that transferring a small quantity of $CD8^+$ T_{RM} cells (10^6) provides a level of protection comparable to transferring a larger quantity of gut-associated MLN $CD8^+$ T cells (10^7), highlighting the potency of T_{RM} cells [116, 117]. The intricate immune response in the gut, as elucidated by Shi et al., carries significant implications for the development of vaccines targeting *Eimeria* and related diseases. These findings have implications for the development of live oocyst-based vaccines against *Eimeria* and potentially other protozoan diseases. Recent discoveries highlight the crucial role of T stem cell memory (TSCM) cells in various human diseases, underscoring their exceptional longevity, robust potential for immune reconstitution and therapeutic promise in enhancing vaccine efficacy [118–120]. However, the role of T_{SCM} has limited exploration in parasitic diseases [121].

Eimeria infection controls and their innovations

Anticoccidial drugs

In the context of coccidiosis control in poultry, prophylaxis primarily relies on the use of anticoccidial drugs. This approach found its origins in 1948 with the introduction of sulfaquinoxaline, marking the inception of the concept of anticoccidial drugs. Over time, a diverse array of these drugs has been developed and integrated into poultry health management (Fig. 1A).

Anticoccidial drugs can be broadly categorized into three main groups:

1) Synthetic compounds

These compounds demonstrate a broad spectrum of activity against various parasites. They encompass chemically diverse substances, including quinolones, pyridones, alkaloids, thiamine analogues, and triazine derivatives [122, 123]. Despite limited recent efforts in developing new anticoccidial drugs, ethanamizuril stands out as a novel triazine compound recently approved in China [124]. Synthetic drugs function through specific modes that target the metabolism of parasites. This includes actions such as inhibiting parasite mitochondrial respiration, disrupting the folic acid pathway, and competitively inhibiting thiamine uptake [123]. The efficacy of these synthetic compounds in combating coccidiosis highlights their diverse mechanisms, which interfere with essential parasite metabolic pathways. While the development of new drugs in this category has been limited, the approval of ethanamizuril underscores ongoing efforts to innovate and expand the arsenal of anticoccidial treatments for safeguarding poultry health.

2) Ionophores: aiding in coccidiosis control

Polyether antibiotics, also known as ionophores, are derived from microorganisms like *Streptomyces* spp. and *Actinomadura* spp.. These compounds, classified based on their structure, can be grouped into monovalent ionophores (salinomycin, monensin, and narasin), monovalent glycosidic ionophores (maduramicin and semduramycin), and divalent ionophores (lasalocid) [125]. Ionophores have emerged as pivotal choices for combatting coccidiosis, primarily due to their advantageous characteristics, including the slow development of resistance and their ability to partially inhibit parasite development [126]. This partial inhibition confers a unique advantage by allowing the host to develop immunity while simultaneously preventing the disease. Ionophores exhibit effectiveness against both the asexual and sexual stages of *Eimeria* infection, disrupting the regular transport of ions, such as Na^+ and K^+, across the membranes of sporozoites and early trophozoites [123, 127].

3) Phytotherapy: exploring herbal solutions for coccidiosis.

Phytotherapy, also known as herbal medicine, explores the utilization of plants and their extracts in the treatment and prevention of coccidiosis. Several herbal compounds, including betaine and citric acid extracts, have undergone extensive scrutiny for their potential efficacy [125, 128]. Unfortunately, despite promising research findings, there is a dearth of replication studies and limited practical implementation of these compounds on a large scale. Further research and development efforts are necessary to harness the full potential of phytotherapy in coc-

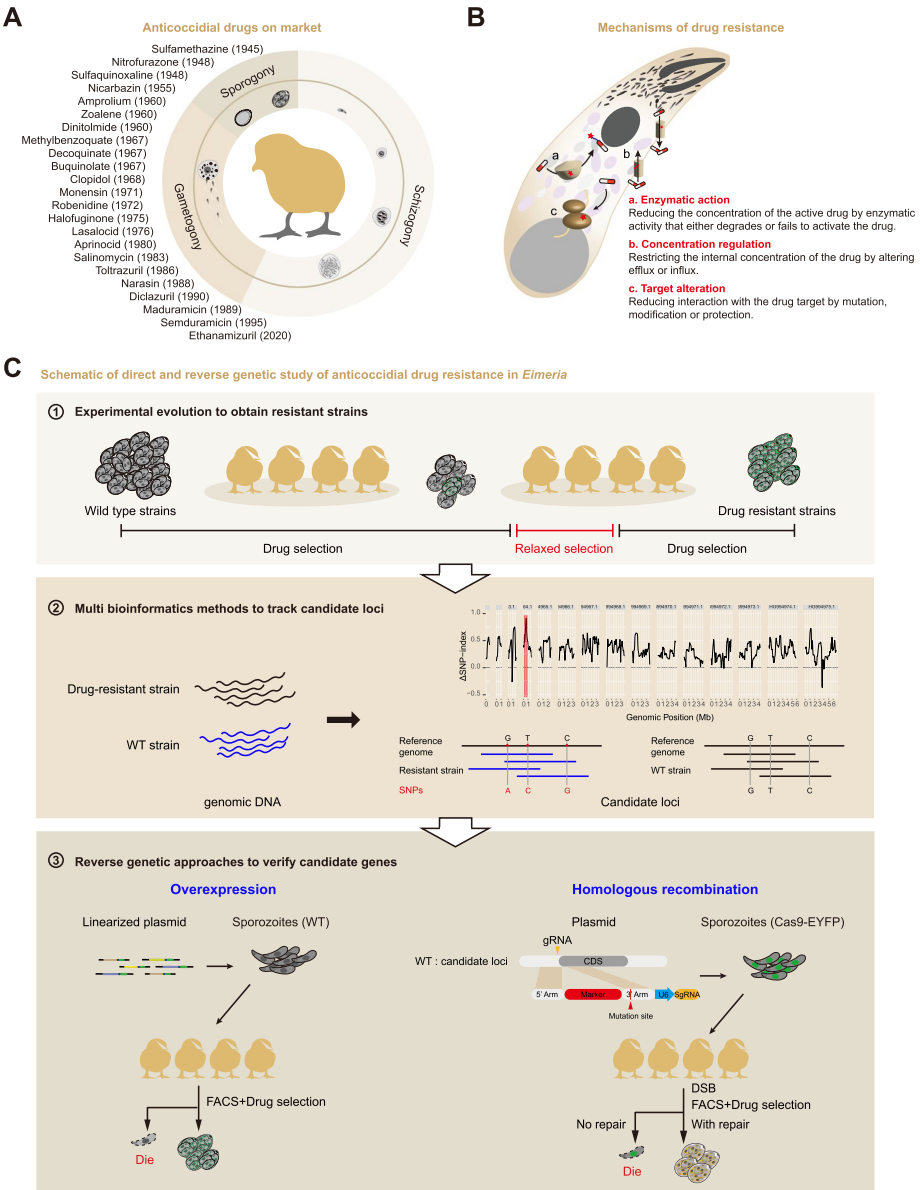

Fig. 1 Overview of anticoccidial drugs and drug resistance mechanisms. **A** Anticoccidial drugs introduced into and used in the poultry industry. **B** The diagram of the three major mechanisms of drug resistance. a. enzymatic action: drugs' effectiveness is reduced either because an enzyme degrades it or fails to activate it. b. concentration regulation: internal concentration of the drug is regulated, either by restricting its entry (influx) or enhancing its exit (efflux). c. target alteration: drugs' effectiveness is compromised because the target molecule of the drug in the organism undergoes mutations or modifications, making the drug unable to bind or act effectively. Star, mutation. **C** Schematic of direct and reverse genetic study of anticoccidial drug resistance in *Eimeria*. ① Experimental evolution to obtain resistant strains: Starting with a wild-type strain, the organism is exposed to a drug for the selection of drug-resistant strains. The procedure includes periods of drug exposure and relaxation (no drug). ② Multi bioinformatics methods to track candidate loci: The genomic DNA of drug-resistant strains is analyzed to pinpoint areas (loci) in the genome that might be responsible for resistance. These candidate loci are highlighted by Single Nucleotide Polymorphisms (SNPs), which are variations in a single DNA building block. ③ Reverse genetic approaches to verify candidate genes: To validate the identified candidate genes, two methods are depicted: Overexpression: Overexpression involves inserting one or more copies of a specific gene to evaluate if its increased presence produces or enhances drug resistance. This method aims to assess how amplifying gene copies or its expression influences the organism's ability to combat drug stress; Homologous recombination: Homologous recombination via CRISPR/Cas9 involves creating targeted DNA breaks, enabling the replacement of a normal gene with a mutated one. This precise editing method induces drug resistance in parasites carrying the altered gene. DSB: double-strand break. HR, homologous recombination. FACS: fluorescence-activated cell sorting

cidiosis management, potentially providing sustainable and natural solutions for poultry health.

Development of anticoccidial drug resistance

The prolonged and widespread use of anticoccidial drugs has led to the emergence of drug resistance, affecting nearly all major drugs extensively employed in the poultry industry. Notably, resistance has been reported across various categories of these drugs. *E. tenella* has been a primary focus of study concerning drug resistance, but investigations have also extended to *E. acervulina* and other species, often isolated from mixed infections in natural settings. To gain insights into the epidemiology and mechanisms of drug resistance in *Eimeria* parasites, researchers have pursued the acquisition of drug-resistant *Eimeria* strains.

Two principal strategies have been employed to achieve this objective. The first involves the direct isolation of drug-resistant parasites from field samples, while the second method entails a progressive dose-escalation approach to cultivate parasites with heightened drug tolerance. A third innovative technique has emerged, simulating the natural development of drug-resistant strains. This method leverages drug-sensitive *Eimeria* parasites and medicated chickens, facilitating the rapid generation of resistant strains in a significantly shorter timeframe [129].

Initially, research on anticoccidial drug resistance predominantly revolved around epidemiological studies. Subsequently, the exploration of resistance mechanisms took shape through morphological observations and the use of biochemical methods [130]. An early study unveiled that decoquinate and clopidol could inhibit electron transport in mitochondria isolated from unsporulated oocysts [131]. As the field of eimerian parasite research embraced cutting-edge technologies, proteomics-based biomarker discovery played a crucial role in identifying proteins strongly associated with resistance mechanisms [132]. Furthermore, RNA-Seq analysis allowed for a comparative examination of differentially expressed genes between drug-sensitive and -resistant strains [133]. These investigations collectively suggested that common resistance mechanisms hinge on alterations in drug targets, diminished intracellular drug concentrations, and the inactivation or inability to activate the drug (see Fig. 1B).

Unveiling the molecular mechanisms of anticoccidial drug resistance

Directed evolution, coupled with whole-genome sequencing, has proven successful in pinpointing resistance targets and pathways for various antiparasitic compounds within apicomplexan parasites, such as *P. falciparum* and *T. gondii* [134–137]. Building upon these groundbreaking discoveries, our recent research has delved into unraveling the resistance mechanisms of several drugs in *E. tenella*, employing advanced genetic methodologies. Notably, we have made significant strides in comprehending the molecular intricacies of halofuginone resistance in *E. tenella*. Through our investigations, forward and reverse genetic approaches were successfully used to identify the A1852G mutation in the cytoplasmic prolyl-tRNA synthetase gene (*EtcPRS*), directly impacting the drug's binding activity to this protein and markedly reducing the susceptibility of *E. tenella* to halofuginone [129]. In a parallel study, we have verified mutations in cytochrome b, a protein localized in the mitochondrion, are associated with decoquinate resistance [138]. Our ongoing endeavors encompass the exploration of resistance mechanisms against salinomycin, maduramycin, monensin and diclazuril [139].

By identifying the genes and mechanisms linked to resistance, we can foster innovation in the development of anticoccidial drugs. Strategies such as targeting specific cellular components, modifying existing drug structures, and combining drugs with contrasting selection pressures on a target can effectively counteract resistance [140, 141], ultimately aiming to mitigate the global prevalence of *Eimeria*. With the advent of new technologies, candidate genes exhibiting a resistance phenotype can be authenticated through gene-editing techniques like overexpression and CRISPR/Cas9 [59, 142, 143]. Building upon our prior investigations, integrating forward and reverse genetic approaches can unravel the candidate genes responsible for the resistance phenotype. This holistic approach has been instrumental in exploring the molecular markers of anticoccidial drugs (Fig. 1).

Anticoccidial vaccines

As the challenges of drug resistance in *Eimeria* and drug residues in the animal-derived products intensify [1], anticoccidial vaccines have emerged as pivotal tools in the control of coccidiosis. At present, commercial anticoccidial vaccines include virulent strain-based vaccines, precociously (or less commonly) embryo-adapted attenuated vaccines and transmission-blocking subunit vaccines [144].

Virulent strain-based vaccines

The virulent vaccines are composed of specific ratios of wild-type *Eimeria* strains that were not attenuated [145], notable examples being Immucox® and Coccivac® [146]. While the virulent vaccines can provide robust protection against coccidiosis, their virulence poses risks. Improper immunization can lead to coccidiosis and even

further induce the occurrence of other diseases such as necrotic enteritis and cause mortality [147, 148].

Attenuated vaccines

Attenuated vaccines are mainly made up of *Eimeria* strains attenuated by selection for precocity, with some being attenuated by serial parasite passage through chicken embryonated eggs [149]. Compared with virulent vaccine, the pathogenicity of attenuated vaccine decreased significantly while maintaining immunogenicity [150]. Currently, several versions of the commercial precociously attenuated anticoccidial vaccines including Paracox®, Neca™ and SCOCVAC® have been widely used to vaccinate both breeder and broiler chickens, Livacox® having both precocious and chicken embryo-adaptation attenuated strains [149]. However, there are also two shortcomings: (1) The fecundity of the attenuated *Eimeria* strains is significantly lower than that of the parent strain, resulting in an increase in development cost [145]; (2) There are some potential risks such as unstable attenuated performance and reversion to virulence [149].

Transmission-blocking subunit vaccine

Transmission-blocking vaccine, known as CoxAbic®, is the first commercially available subunit anticoccidial vaccine. It is composed of affinity-purified gametocyte antigens from *E. maxima* [151]. The vaccine is mainly used to immunize hens before laying eggs to protect their offspring with maternal antibodies [150, 152]. However, the production of the CoxAbic® vaccine relies on the affinity purification of native gametocyte antigens from parasites, a method encumbered by the drawbacks of being expensive, time-consuming and labor-intensive.

Alternative control strategies

Traditional anticoccidial drugs and vaccines have been the primary approaches for the control of poultry coccidiosis [147]. However, the ban on antibiotics necessitates the exploration of alternative control methods. Several herbal remedies have demonstrated effectiveness in coccidiosis control [153, 154]. For instance, oregano oil, derived from the oregano herb, when added in small quantities to the diet, reduces oocyst output and aids in preventing chicken coccidiosis [155]. Commercially available oregano oil preparations are promoted as natural coccidiostat alternatives [156].

While alternative strategies currently play a limited role in coccidiosis prevention, their potential merits attention. Research indicates that IL-2 and INF-α can boost chicken immunity against coccidiosis [157, 158]. Notably, a recombinant chicken IL-2 injection, MIAOZUO®, has received market approval, and recombinant chicken INF-α injection is undergoing clinical trials. These developments introduce novel avenues for coccidiosis control [157, 158].

Innovative control strategies

Multi-omics

High-throughput omics technologies, including genomics, epigenomics, transcriptomics, proteomics, microbiome and metabolomics have revolutionized research in *Eimeria* biology and control studies. Integration of multiple types of omics data (multi-omics) greatly facilitates our knowledge in understanding the molecular biology of *Eimeria*.

With the development of sequencing technology, including the utilization of High-through chromosome conformation capture (Hi-C) and third-generation sequencing techniques like PacBio single-molecule real-time sequencing and Nanopore sequencing technology, researchers have applied these methodologies to the genome assembly and full-length transcriptional analysis in *Eimeria*. These sequencing technologies enable direct sequencing of super long reads without GC content bias, which is very common in *Eimeria* genome [26] and results in difficulties in genome assembly. By using these techniques, high-quality chromosomal genomes of *E. tenella* Houghton strain [31] and *E. acervulina* Beijing strain (unpublished data) were assembled. These high-quality genomes provide basic and insights of molecular biology of *Eimeria* species, provide some evidence and citations concerning genetic markers for virulence and drug resistance.

To understand gene expression during the *Eimeria* life cycle, RNA-seq is considered to be solid and budget friendly for the analysis of global transcription profile. In *E. necatrix*, a comparative transcriptome analysis has been conducted between second- and third-generation merozoites [53]. Additionally, the full-length transcriptomes of *E. necatrix* have been characterized through PacBio sequencing [44]. With respected to *E. tenella*, our team has provided comprehensive transcriptome profiles of seven stages of *E. tenella*, including unsporulated oocysts, partially sporulated oocysts, completely sporulated oocysts, sporozoites, merozoites (108 hpi and 120 hpi) and gametocytes [59]. To date, this is the most complete gene expressing profile in *Eimeria*. Understanding the expression pattern of *Eimeria* genes greatly promotes the identification of potential targets for vaccine development.

Dual RNA-seq was also used to understand host-parasite interactions with biological samples containing both species. In the *E. tenella* infection model, host immune factors, such as matrix metalloproteinases, chemokines and IFN-γ related genes, were elevated after early infection, while parasite genes involved in protein expression

and energy metabolism were up-regulated and genes for DNA and RNA processing were down-regulated [55]. In *E. falciformis* infection, immune reaction and tissue repair related genes (e.g., TGF-β, EGF, TNF, IL-1 and IL-6) were enriched after infection, while dynamic parasite gene expression was observed in early and late infections [159]. These comprehensive transcriptomic data not only present parasite gene expression patterns, but also imply dynamic characteristics of the host immune response to *Eimeria* infection.

From the perspective of protein expression, quantitative mass spectrometry proteomics methods were applied to *Eimeria* studies, including iTRAQ and 4D label-free quantitative techniques. Gao et al., compared the protein abundances of unsporulated oocysts, sporozoites and second-generation merozoites in *E. necatrix* by iTRAQ [45], which identifies key proteins involved in parasite invasion. Post-translational lysine acetylome of *E. tenella* proteins was comprehensively analyzed among five life stages by 4D label-free quantitative proteomics, which reveals a widespread distribution and dynamic changes of lysine acetylation [56]. Analyzing *Eimeria* proteome and its interaction with the host accelerates the identification of immunogenic proteins as vaccine candidates against coccidiosis.

Gene-editing in *Eimeria*

Before the advent of CRISPR/Cas9, other techniques such as zinc finger nucleases (ZFNs) and transcription activator-like effector nuclease (TALENs) mediate site-specific genome editing [160]. The easy-to-handle property of the adjusted CRISPR gene editing system makes it possible for its board application in both model and non-model organisms [161].

Eimeria genome encodes around 8000 genes, but the function of most remain unknown, especially for the key proteins involved in the parasite virulence and development, have not been determined. Currently, our team adapted *Streptococcus pyogenes* CRISPR-Cas9 gene editing tool to *E. tenella* in different strategies. Tang et al. (2020) [143] reported CRISPR/Cas9 mediated *Eimeria* gene double-strand DNA breaks (DSBs) in vitro, and successfully tagged the endogenous microneme protein 2 (EtMic2) by co-transfecting wild-type *E. tenella* sporozoites with Cas9-gRNA plasmid and a donor fragment in vivo. Hu et al. (2020) [142] generated an *E. tenella* strain constitutively expressing codon usage optimized Cas9 inside the parasite nuclei. This strain showed comparable phenotypes to the wild-type strain, including growth and virulence. This Cas9-expressing parasite showed a cleavage efficiency of ~29% in vivo. By transfecting this parasite with a donor fragment with homologous recombinant flanks, homologous recombination mediated endogenous gene knock-out and gene tagging were demonstrated successfully. Meanwhile, a preliminary loss-of-function screening of 33 *E. tenella* AP2s was performed using this parasite. Later on, an unsporulated oocyst stage specific AP2 gene was deleted using this system [59]. Additionally, a *Francisella novicida* FnCas12a/crRNA ribonucleoprotein (RNP) mediated genome editing was adapted in *E. tenella* by Cheng et al. (2020) [162]. Histone 4 gene has been knocked out by transfecting with 30 μg of FnCas12a and crRNA (1:1) and incubated at 41°C for 15 min, and a housekeeping actin has been tagged by YFP with the same strategy [162]. These tools may greatly facilitate research on *Eimeria* biology and also identification of the functions of vital genes (Fig. 2). More importantly, gene editing in *Eimeria* makes it possible for the knockout of virulence genes or regulators to create attenuated strains for the development of vaccines in this parasite. Moreover, gene editing combined with transgenetics will greatly facilitate the creation of transgenic parasites for the development of *Eimeria* as a vaccine delivery vector for other intestinal diseases.

The synergy of probiotics, prebiotics, and phytochemicals

Probiotics, a distinct class of microorganisms, play a pivotal role in regulating intestinal flora, bolstering immunity, and fending off pathogens [163]. Recent studies underscore the efficacy of *Lactobacillus plantarum* in inhibiting the infection of *E. tenella* [164]. Adding *Lactobacillus* (*L. salivarius* and *L. jhonsonii*) and *Saccharomyces cerevisiae* to chicken feed has been observed to increase levels of antioxidant enzymes and tight junction proteins, thereby amplifying the resistance of chickens to coccidiosis [165]. Prebiotics, substances that foster the growth of beneficial intestinal microorganisms, when combined with probiotics, manifest enhanced probiotic effects. This synergistic combination has been noted to markedly alleviated the symptoms in chickens infected with *E. tenella* [166]. Phytochemicals, emerging as potent anti-inflammatory agents [167], have long been explored for their anti-coccidiosis efficacy. Notably, phytochemicals like saponins, tannins have been demonstrated the ability to inhibit the invasion of the parasite and expedite the repair of epithelial injuries by *E. tenella* sporozoites [168]. The combination of probiotics, prebiotics, and phytochemicals has been found to bolster the resilience of chickens during coccidiosis infection [169]. Given their promising attributes, these substances and their combinations hold immense potential for future applications in coccidiosis prevention and treatment.

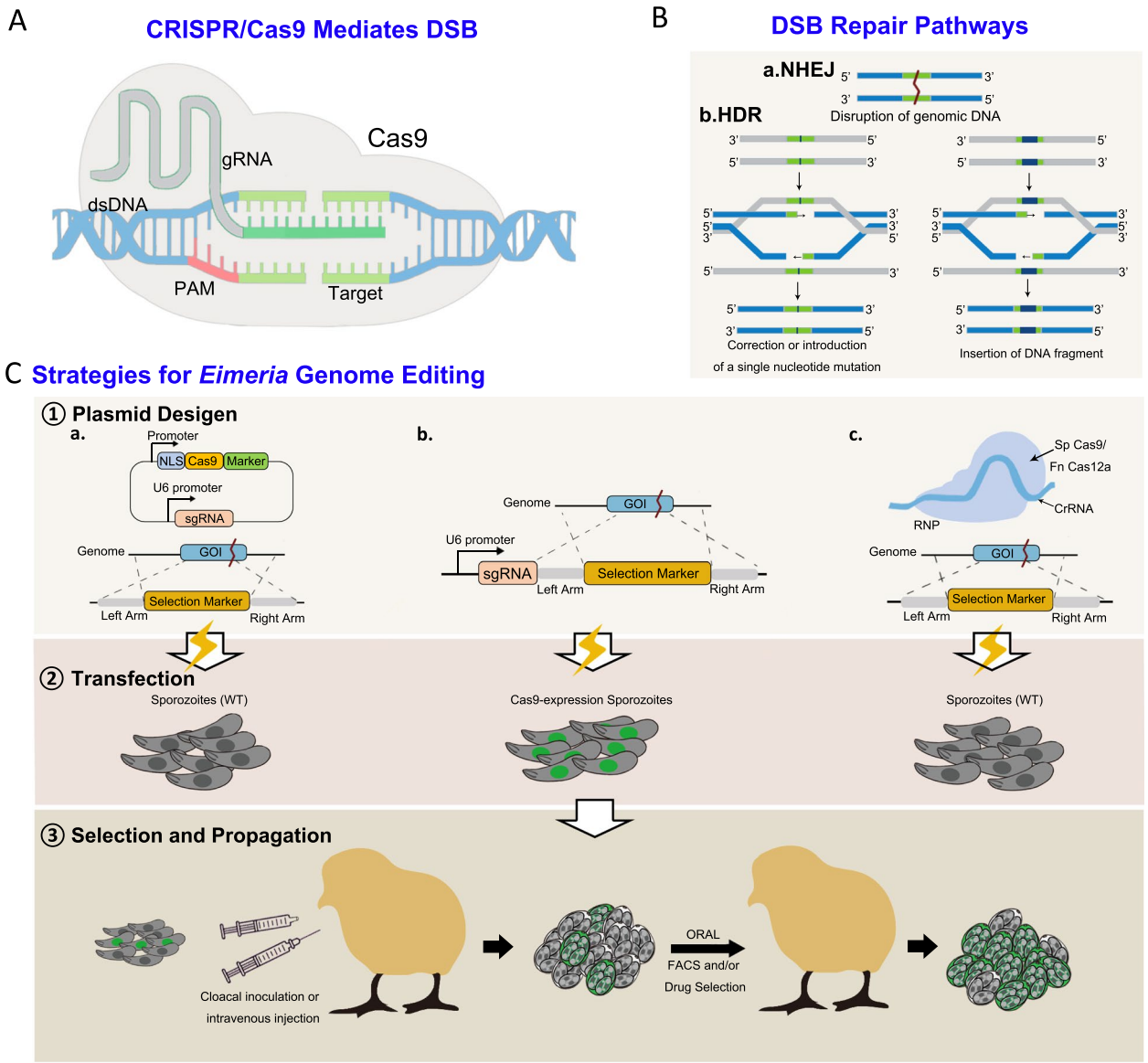

Fig. 2 *Eimeria* genome editing using CRISPR/Cas system and its application. **A** DNA double-strand breaks (DSB) mediated by CRISPR/Cas system: Illustration of DSB principle induced by CRISPR/Cas system. **B** Two repair modes of DSB. a. Non-homologous end joining (NHEJ): Causes insertion/deletion mutations in the targeted locus. b. Homologous direct repair (HDR): Occurs with a DNA repair template, enabling single nucleotide correction/introduction or DNA sequence insertion. **C** Strategies for *Eimeria* genome editing each with three steps: Strategy Design and Plasmid/Repair Template Construction ① Begin by designing the editing strategy and constructing plasmids or repair templates. a. Strategy 1: Utilizes Cas9, sgRNA expression cassettes, circular plasmids, and repair template DNA. Wide applicability but limited by low efficacy in multi-plasmids co-transfection. b. Strategy 2: Employs a single repair template DNA with an sgRNA expression cassette. Applicable only to stable Cas9 expression strain. c. Strategy 3: RNP-Mediated Genome Editing: Wide applicability but lower efficacy in vitro. Transfection ② Optimize transfection conditions for efficient delivery of editing components into Eimeria cells. Selection and Propagation ③ Enrich and propagate the modified *Eimeria* population. Utilize drug-mediated selection or other techniques to isolate edited recombinants. Transfected sporozoites or merozoites were inoculated via cloacal (suitable to *E. tenella*, *E. mitis* and *E. necatrix*) or intravenous injection (suitable to *E. acervulina*) and genome modified *Eimeira* oocyst were selected by fluorescent protein activated cell sorting (FACS) and/or drug(s) pressure. GOI: Gene of Interest; Selection Marker: Fluorescent protein and/or drug-resistance genes

Innovative anticoccidial vaccines

Precocious line-based gene knockout vaccines

Currently, the CRISPR/Cas9 system and other advanced genetic manipulation techniques have been effectively implemented in *Eimeria* [142, 143, 162, 170]. These cutting-edge methods are anticipated to offer more efficient

avenues for creating the next wave of anticoccidial vaccines rooted in the precocious line [171, 172]. Targeting and deactivating virulence or developmental regulatory genes within these lines to construct vaccine strains with developmental anomalies is a compelling avenue of research. Vaccines based on gene knockouts in the precocious line enhance safety [149, 172, 173]. However, this endeavor demands in-depth exploration and analysis of key regulators in *Eimeria* development, signifying a substantial journey ahead.

Vector-vaccines

Several immunodominant antigens offer partial protection against coccidiosis [174, 175]. Traditional inoculation methods for these antigens involve intramuscular or subcutaneous injections [176]. These methods differ significantly from the natural infection pathway of *Eimeria* in terms of antigen recognition and presentation [172]. To enhance the protective effects of *Eimeria* antigens, it is essential to discover new immunodominant antigens and develop a compatible antigen delivery system. The delivery mechanism plays a pivotal role in determining the strength and nature of immune response [116, 172, 177].

Live vector vaccines emulate the natural infection process, prompting animals to generate a robust protective immune response [177, 178]. Currently, live vectors like probiotics, yeast, attenuated *Salmonella*, fowlpox virus and transgenic coccidia are employed to deliver protective antigens of *Eimeria*, achieving partial immunoprotection in animals [177–183]. Given that *Eimeria* is an intracellular pathogen, the live intracellular vectors such as attenuated *Salmonella,* fowlpox virus, adenovirus and transgenic *Eimeria* are more promising in the development of innovative anticoccidial vaccines [172, 177, 178]. Transgenic *Eimeria*, a live eukaryotic vaccine vector, is now at the forefront of the next-generation anticoccidial vaccine research. Future vaccines may focus on enhancing immunogenicity by incorporating molecular adjuvants like IL-2, Fc, profilin into the vaccine strains, thereby reducing the required vaccine dose [172, 184, 185]. Another approach involves expressing immunoprotective antigens of heterogeneous species in one species of *Eimeria* [182, 183], streamlining the vaccine formulation.

Other vaccines

Structural vaccinology signifies a revolutionary shift in vaccine development. By leveraging in-depth knowledge of a pathogen's structure and the requisite immune response for protection, scientists can now design more effective vaccines. This methodology has been successful implementations in creating vaccines against formidable pathogens such as influenza virus and HIV [186–188]. However, the primary emphasis of this approach has been on augmenting antibody responses to structural epitopes. To adapt this strategy for coccidiosis vaccine development, there is a need for heightened focus on bolstering T-cell responses. Nanoparticle-based vaccines have demonstrated immense potential, generating enhanced immune responses against specific antigens [189]. The intrinsic capability to manipulate nanomaterials—adjusting their size, shape, surface charge, and chemical properties—provides a unique advantage. This malleability facilitates the creation of nanoparticles tailored to deliver antigens efficiently to target cells, amplify immune reactions, and augment vaccine stability. On the other hand, dendritic cell-targeting vaccines harness the distinct attributes of dendritic cells to evoke a powerful immune response against particular antigens [190, 191]. Such vaccines primarily aim at delivering antigens to dendritic cells, being achieved either directly or through intermediaries, instigating their activation and the subsequent presentation to T cells [190, 191].

Perspective

Coccidiosis challenges the poultry sector, impacting chicken health and threatening global food safety. While anticoccidial drugs and vaccines help, drug resistance and vaccine safety concerns arise, especially for young chickens. New strategies are essential, and we spotlight advanced methods to combat chicken coccidiosis.

Advancements in understanding *Eimeria* biology

The study of *Eimeria* biology has witnessed significant breakthroughs in recent years. With the advent of advanced molecular techniques, we now have a deeper understanding of the *Eimeria* lifecycle, its interactions with the host, and the intricate balance it maintains with the gut microbiota. Cutting-edge technologies such as CRISPR/Cas and Multiomics hold promise in identifying novel drug targets and developing highly effective vaccines, ensuring broad safety in agricultural settings.

Innovative control strategies with new-generation vaccines

The future of coccidiosis vaccine research looks bright, driven by technological and theoretical breakthroughs. The introduction of the CRISPR/Cas9 gene-editing tool marks a significant turning point, allowing for exact alterations in *Eimeria* and signaling the onset of advanced anticoccidial vaccines. Future vaccine strategies may focus on enhancing immunogenicity of protective antigens by incorporating molecular adjuvants like IL-2, Fc, profilin, and flagellin. This could potentially reduce the required vaccine dosage and boost safety. Structural vaccinology utilizes the processes of structural biology and

computational modeling, aiming for vaccines that are more precise, effective and safe. This methodology should be further refined to boost T-cell responses in anticoccidial vaccine formulations. Another novel tactic is the co-expression of protective antigens from multiple species within a single species, simplifying vaccine compositions and elevating safety standards. An emerging research direction is crafting vaccine strains with developmental anomalies by deleting crucial regulatory or virulence genes. However, this endeavor necessitates a profound grasp of important regulations directing *Eimeria* development. Overall, the overarching goal of a successful anticoccidial vaccine is to trigger protective memory responses [192], particularly resident memory and/or stem cell memory responses against *Eimeria*. Comprehensive insights into enhancing memory responses against *Eimeria* infection may be achieved through interdisciplinary approaches that integrate molecular biology, immunology, genetics, and computational sciences.

Innovative strategies involving the synergy of probiotics, prebiotics, and phytochemicals

The gut ecosystem, with its complex interplay of host cells, pathogens and commensal microorganisms, offers a rich avenue for innovative control strategies. Consequently, the management of gut health is multifaceted, requiring a synergistic use of probiotics, prebiotics, and phytochemicals, along with intermittent use of anticoccidial drugs, leading to a holistic approach to coccidiosis control. By modulating the gut microbiota, enhancing the host's immune responses, and directly targeting *Eimeria* parasites, these strategies aim to establish a fortified defense against coccidiosis. The potential of these natural compounds, especially when used in combination, is vast and warrants further exploration.

In conclusion, the future of coccidiosis control lies in a multifaceted approach, integrating advanced biological insights with innovative therapeutic strategies. As we continue to deepen our understanding of *Eimeria* biology and its interactions with the host, we move closer to a future where coccidiosis can be effectively managed, ensuring the health and productivity of poultry worldwide.

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Authors' contributions
Conception and study design: X.S., X.L.; Figure design and drawing: P.S., X.T.; Statistical analysis: D.H., F.S., W.Y.; Preparation of first draft: Y.G., S.Z., X.Y., T.S., S.W., J.Z., G.Y.; Critical review of the manuscript: X.S., H.D.; Reading and approval of the final version of the manuscript: all authors.

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Declarations

Ethics approval and consent to participate
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Competing interest
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