Securing poultry production from the ever-present *Eimeria* challenge

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Abstract

The intestinal disease coccidiosis, caused by protozoan parasites of the genus *Eimeria*, is one of the most important livestock diseases in the world. It has a high impact in the poultry industry where parasite transmission is favoured by high-density housing of large numbers of susceptible birds. Coccidiosis control in poultry is achieved by careful husbandry combined with in-feed anticoccidial drugs or vaccination with live parasites. However, outbreaks of coccidiosis still occur and sub-clinical infections, which impact significantly on productivity and food security, are common due to widespread drug resistance, high parasite prevalence, and environmental persistence. Herein, we review some recent approaches for the production of cheaper third generation vaccines, based on robust methods for identification of immunoprotective antigens and the use of transgenic *Eimeria.*

Sustainable poultry production in the face of *Eimeria* challenge

Global poultry production has tripled in the last 20 years and the world's chicken flock is estimated at approximately 21 billion, producing 1.1 trillion eggs and approximately 90 million tonnes of meat (equivalent to ~ 60 billion carcasses) each year (www.faostat.fao.org). Expansion is predicted to continue for at least 30 years with Africa and Asia accounting for the most growth [1]. Commercial poultry production is possible only with the support of effective pathogen control, including good animal husbandry, chemoprophylaxis, and vaccination. A major and recurring problem is coccidiosis [2-4], an enteric disease caused by protozoan *Eimeria* species (see Glossary), which are parasitic coccidians with homoxenous faecal-oral life cycles (Figure 1) that are closely related to *Toxoplasma*, *Neospora*, and *Isospora* and more distantly related to other apicomplexans including *Babesia*, *Plasmodium*, and *Theileria* [5, 6]. Seven species of *Eimeria* infect the chicken with absolute host-specificity, causing haemorrhagic (*Eimeria brunetti*, *Eimeria necatrix,* and *Eimeria tenella*) or malabsorptive (*Eimeria acervulina*, *Eimeria maxima*, *Eimeria mitis*, and *Eimeria praecox*) disease. These parasites are highly prevalent and can persist for long periods in the environment, including in faeces and litter (bedding/substrate).Thus, most chicken flocks in the world are exposed and many chickens become infected. Uncontrolled outbreaks cause high morbidity and mortality, and if infections are only partially controlled then sub-clinical disease is common and economically relevant because it causes poor feed conversion, reduced egg production, and failure to thrive. Comparison of the costs incurred by veterinary infectious diseases in the UK has highlighted coccidiosis as a leading problem in terms of total cost, including the cost of control [7]. The global economic impact of coccidiosis is unclear but has been estimated to be in excess of $3 billion USD per annum due to production losses combined with costs of prevention and treatment [8, 9]. Additional risk has been noted in much of the developing world where *Eimeria* can undermine the rapid expansion of poultry production and exert a profound impact on local poverty [10]. Costs in other livestock sectors where *Eimeria* parasites are also prevalent are less well defined but likely to be similarly high [11]. Links between eimerian infection and increased intestinal colonisation of bacterial pathogens such as *Clostridium perfringens* and *Salmonella enterica* serovars Typhimurium and Enteritidis increases risk to food security and raises concerns of zoonotic food-borne disease [12-14]. The global distribution of the *Eimeria* species, complemented by their ability for environmental survival as oocysts and their propensity for drug resistance, poses a serious threat to secure production of poultry-derived food products. The control of *Eimeria* remains as important now as it has ever been with the development of cost-effective, scalable vaccines required urgently.

Current options for control

Successful commercialisation of poultry production in housed, free-range, and organic systems relies on effective control of *Eimeria* parasites. Good husbandry plays a key role in limiting oocyst sporulation and recycling through measures such as restricting bird access to faeces, maintaining litter quality, controlling house temperature, ventilation, and moisture levels, and thorough cleaning between flocks. However, husbandry alone is inadequate for control, and prophylactic anticoccidial drugs (ionophores and other chemicals) are crucial for sustainability of most production systems. In the UK more than 40% of all antimicrobials sold for use in food and non-food animals are classified for the control of coccidial parasites (277 tonnes of active ingredient in 2011; mostly for control of *Eimeria*) with ionophores representing more than 70% of these [15]. Resistance to anticoccidial drugs has been recognized for decades and regarded as ubiquitous [16] with wide acceptance that drugs remain effective in the field only because they suppress parasite growth sufficiently to allow birds to develop natural immunity [17]. The speed at which resistance can appear, combined with legislative restrictions on the use of many in-feed drugs in some countries and consumer concerns regarding chemical residues in food products has discouraged most attempts to develop new anticoccidials.

The major alternative option for coccidiosis control is vaccination, which is used effectively to protect egg-laying and breeder chickens but applied more rarely within the majority broiler sector. Anticoccidial vaccines are available in some countries for use with turkeys (for example Coccivac-T®), but vaccines targeting *Eimeria* species that parasitise mammals are yet to be commercialized. With one exception, all of the anticoccidial vaccines that are currently available are based on varied formulations of multiple live species of *Eimeria*. The development and compositions of these vaccines have been reviewed thoroughly in recent years and will not be described in depth here [3, 8, 18]. Briefly, live anticoccidial vaccines fall into two major groups. The first generation, based on formulations of live wild-type parasites, includes vaccines such as Coccivac®-B and -D and Immucox®, all of which are still currently available. First introduced in 1952, live wild-type vaccines rely on the controlled exposure of chickens to small numbers of virulent oocysts that initiate infection and induce a natural protective immune response. While effective and still widely used in some sectors of the poultry production industry [3], these vaccines have a requirement for “even” vaccine distribution across the flock to avoid occurrence of disease. Ingestion of too-high a dose can cause a direct negative impact on feed conversion or even clinical symptoms of haemorrhagic or malabsorptive coccidiosis, while ingestion of too-small a dose leaves birds unprotected against large numbers of recycled vaccine oocysts excreted by other flock

members under anything but the very best flock management [19]. Recognition of the risk posed by vaccinal pathogenicity prompted development of second generation live attenuated vaccines. Most of these vaccines contain parasites derived by selection for the trait of "precocious" development (i.e., more rapid completion of the life cycle with a reduced pre-patent period, lower reproductive capacity and consequential reduction in pathogenicity). Parasite lines selected through multiple rounds of *in vivo* passage to be capable of completing their life cycles 13-33 hours faster than their wild-type progenitor retain full immunoprotective capacity while losing one, or even two, rounds of schizogony [20, 21], making them safer, and attenuated vaccines. A small number of attenuated lines have also been developed by serial passage of parasites through embryonating eggs [21-23], a process that results in parasites that are significantly less pathogenic for chickens than their wild-type progenitor [24]. Second generation vaccines were introduced nearly 40 years after the first wild-type vaccines and include Paracox® (attenuated by selection for precocity, registered 1989) and Livacox® (attenuated by selection for precocity or egg adaptation, registered 1992), which remain highly successful with > 1 billion doses of the former sold each year [25]. The marketing of these major second generation products has in turn stimulated the generation of several more attenuated lines from parasites around the world as the basis of regional vaccines (e.g., [26-28]). These successes have been tempered, however, by inherent production limitations. *Eimeria* parasites cannot yet be propagated efficiently *in vitro* [29, 30], which means all vaccine lines have to be grown in chickens. The relatively low reproductive index of attenuated compared to wild-type vaccine lines increases production, and thus retail, costs (retail between UK £0.04 and £0.30 per dose depending on formulation; http://www.animal-meds.co.uk/), and perhaps more importantly imposes practical limitations on the number of doses that can be produced. For these reasons, live-attenuated vaccine usage is confined mainly to the breeding and egg-laying sectors of the poultry industry. Attempts to develop live vaccines attractive to the broiler sector, where economic margin per bird is much lower, have included simplified formulations containing fewer parasite species such as Coccivac®-B, Paracox®-5, and Livacox® T, but uptake remains low with only about 5-10% of the poultry produced each year receiving an anticoccidial vaccine. To make a significant impact a third generation of cheaper, and more readily up scalable, anticoccidial vaccines is required.

What has happened to the third generation anticoccidial vaccines?

Throughout the 1980s and 1990s, numerous efforts were made to identify parasite antigens and the genes that encode them as novel targets in the development of third generation subunit anticoccidial vaccines (reviewed extensively in [3]). Unfortunately progress has been

extremely limited to date, reflecting the general difficulty experienced in the identification of antiprotozoal or antiparasite vaccine candidates and the limitations of testing for immunogenicity, rather than ability to induce immune protection [31]. The likely requirement for more than one antigen if subunit vaccination is to become a viable alternative to chemotherapy has added further complication. Commercial problems including the cost of developing and licensing new vaccines, protecting intellectual property and the risk of resistance developing to any vaccine based on a small number of immunoprotective antigens served to further dampen enthusiasm within the industry. To date only CoxAbic®, a subunit vaccine prepared from *E. maxima* affinity purified gametocyte antigens (APGA), has been successfully commercialized [32, 33]. While CoxAbic® has been reported to be effective in the field [32, 34], in common with the live anticoccidial vaccines it relies on parasite propagation in chickens for its production and remains limited by production capacity and relative expense, further complicated by the need for antigen purification [30].

Vaccine development strategies have included the testing of recombinant protein, DNA, dendritic cell-derived exosome, and vectored subunit vaccines (Table 1), all relying on the identification of one or more appropriate antigens. Early studies focused on antigens identified on the basis of host immune recognition, and led to the identification of several proteins involved in host-parasite interaction and invasion. Microneme proteins 1 (also identified as Etp100 and TFP100), 2 and 4 (TFP250) were tested as recombinant proteins [35-37]; more recently a rhomboid-like protease linked to invasion has received attention [38], and GPI-linked surface antigens including EtSAG1 (also identified as TA4) have been re-visited and widely tested with mixed results [39-41]. Other immunogenic parasite molecules such as α-tubulin have been tested as recombinant protein [42]. A putative fibronectin was investigated using monoclonal antibodies [43] and various proteins from the sporozoite refractile bodies including SO7 (also identified as RB1 and GX3262), refractile body protein 1A, and lactate dehydrogenase have been tested using multiple approaches [40, 44-47]. Profilin (also identified as 3-1E) has been tested more than any other eimerian antigen as both an anticoccidial vaccine candidate and an adjuvant [48-54]. Attempts have also been made to define the immunoprotective component of the APGA CoxAbic® vaccine through investigations into gametocyte antigens gam56 and gam82 [55-57]. Unfortunately, while partially protective effects have been reported in many of these experimental challenge studies, progress towards vaccine commercialization has not followed for any of them. Efforts to improve efficacy and reproducibility have included production of hybrid proteins, DNA, and synthetic vaccines [40, 58, 59], testing of novel adjuvants including a range of Montanide oil-based adjuvants, ginsenosides and plant saponins [36, 53, 60], and co-

administration of avian cytokines including interleukin (IL)-1β, IL-2, IL-8, IL-15, interferon (IFN)α, IFNγ, transforming growth factor (TGF)-β4, and lymphotactin [49]. The reasons why none of these approaches have reached field-testing or commercialization remain obscure but clearly indicate lack of reproducible, efficacious vaccine protection. This could be due to inherent insufficiencies in the immunoprotective capacities of the tested antigens or reflect limitations in the vaccine delivery strategies used. Many parasite antigens exposed to the host during *in vivo* replication are highly immunogenic, but the majority of these do not induce immune responses that block re-infection. Ultimately it is highly likely that more than a single antigen will be needed to induce solid immune protection against each *Eimeria* species, suggesting that co-vaccination with multiple existing or new vaccine candidates should be tested as part of future vaccine development programmes.

New approaches

Recent changes to legislation governing the use of anticoccidials in many countries such as those within the EU and a general expansion of interest in food security issues have stimulated renewed interest in the development of third generation anticoccidial vaccines. Given the paramount importance of identifying effective vaccine candidates, attention has focused on robust strategies for their identification. Improved understanding of molecules that are essential to host-parasite interaction can highlight new candidates. For example, the use of cell-based and carbohydrate microarray assays to investigate the role played by *E. tenella* microneme protein 3 (EtMIC3) in site-specific invasion of the chicken intestine suggested that vaccines based on the microneme adhesive repeat regions (MARR) from this protein may be effective at blocking invasion [61]. Testing single, multiple, and/or hybrid MARR as recombinant protein and DNA vaccines demonstrated significant immune protection against *E. tenella* infection in a series of small-scale challenge studies [61].

Transcriptome-wide screening approaches have been used recently to identify novel vaccine candidates both *in vivo* and *in silico*. Zhu and colleagues used the eukaryotic expression vector pVAX1.0 to create and test an *E. acervulina* sporozoite expression library [62]. Sequential rounds of screening by vaccination identified two candidate cDNA clones (cSZ-JN1 and cSZ-JN2), each of which, when tested individually, induced immune protection in terms of weight gain, lesion score, and oocyst output [62, 63]. While useful in this example the potential for confounding factors should be noted including the risk of combining antigens which induce antagonistic and protective immune responses in the same pool, as described for *Leishmania donovani* [64], as well as the need to choose an immunologically relevant life cycle stage. *In silico* approaches to the same problem rely on access to good quality

datasets. Klotz and colleagues generated a cDNA library from *E. tenella* sporozoites and sequenced 191 clones [65]. Working on the hypothesis that many parasite proteins expected to interact with the host immune system are likely to be secreted, all open reading frames predicted to encode a signal peptide sequence were examined, and six were tested for vaccinal potential, highlighting two surface antigen (EtSAG)-like sequences and a putative *Plasmodium falciparum* Pfs40 homologue [65]. The recent expansion in genomic and transcriptomic resources for *Eimeria* species (Reid et al., unpublished) (see http://www.genedb.org/Homepage/Etenella) now finally provides resources appropriate for larger, more systematic transcriptome-wide *in silico* analyses.

Recognizing the importance of immune protection, rather than immune stimulation, also prompted the creation of genome-wide genetic mapping studies to identify vaccine candidates. Building on previous observations that strain-specific immune selection of genetically distinct *E. maxima* strains conferred a highly selectable phenotype [66], genomic regions whose inheritance conferred susceptibility to immune selection were mapped using a 'hitch-hiker' mapping strategy (Figure 2) [67]. Differences in the inheritance of strain-specific genetic markers by progeny of multiple crosses between antigenically distinct *E. maxima* strains, with or without strain-specific immune selection, identified six genomic regions likely to encode immunoprotective antigens. Detailed fine mapping of two of these regions has identified apical membrane antigen-1 (EmAMA-1) and immune mapped protein-1 (EmIMP-1) as vaccine candidate antigens. *In vivo* testing of these two candidates using DNA and recombinant protein vaccination has confirmed their annotation as valid anticoccidial vaccine candidates. Amongst the strengths of the genetic mapping approach is the removal of confounding factors such as antigenic dominance, stage-specific protein expression, or site of protein localization, and the fact that immune killing is used as an absolute readout of immunoprotective antigenicity. Again, availability of genomic resources for *Eimeria* species combined with next-generation sequencing technologies promotes extension of genetics-led strategies for vaccine development and improved understanding of other selectable traits.

Beyond the identification of effective vaccine candidates, the context and environment in which antigens are delivered to hosts are of fundamental importance. DNA and recombinant protein vaccinations have become valuable tools, but the current labour-intensive routes of application suggest that they are unlikely to be accepted as mainstream commercial vaccines for the mass poultry markets. The scale and tight economic margins of poultry production indicate a requirement for low input, easily administered vaccines. For third

generation anticoccidial vaccines a number of vectored approaches have been tested with many of the antigens described above. Fowlpox virus (FWPV) and herpes virus of turkeys (HVT) are effective vectors for delivery of *E. acervulina* refractile body transhydrogenase and lactate dehydrogenase under experimental conditions [46]. Both of these viruses are licensed for use in poultry, and a small number of recombinant FWPV and HVT vaccines are commercially available, offering protection against diseases including Newcastle disease, infectious bursal disease, and infectious laryngotracheitis. Several bacterial vectors have also been tested with *Eimeria* antigens under experimental conditions including *Escherichia coli* [68], *Salmonella* Typhimurium [69-72], and *Mycobacterium bovis* [73], all of which offer plausible commercial possibilities. Also offering commercial potential are plant vector systems, in which vaccination can theoretically be incorporated into routine dietary components. Preliminary trials in which the microneme proteins EtMIC1 and EtMIC2 have been expressed in the tobacco plant *Nicotiana tabacum* have yielded promising results [74, 75]. In other trials expression of single chain variable fragment (scFv) antibodies with affinity for EtSAG1 expressed in seeds of the fodder pea ('Eiffel' variety) reduced *E. tenella* replication in birds following forced feeding [76].

Standardising vaccine assessment and development

Over the last 30 years many antigens have been tested as novel anticoccidial vaccine candidates, generating a vast amount of new data on different aspects of parasite biology and identifying potential targets for intervention. Unfortunately lack of standardisation between experiments, research groups, government agencies, and industry has hampered meaningful comparisons. Experimental variables such as the breed/line of chicken and parasite species/strain used [66, 77] as well as considerations including the choice of DNA vaccine vector or recombinant protein expression system, the type of buffer or adjuvant, the dose, frequency and site of immunization, and the specific methods used to measure vaccine efficacy, all exert profound influences on experimental outcomes. Variation in diet has also been shown to significantly influence the results of anticoccidial vaccination using a recombinant protein where phytonutrient concentration affected the type and magnitude of immune response during challenge as well as weight gain and parasite replication [78]. While phytotherapy and pre-/probiotics are yet to become established as convincing anticoccidial strategies [79], it is clear that environmental variation has an impact on eimerian replication and is highly likely to influence responses to vaccination. While this problem is not specific to research with *Eimeria*, the establishment of harmonised guidelines for the testing of anticoccidial subunit vaccine candidates, drugs and diagnostics, as has already been described for live vaccines [80], would support best practice and community

recognition of the most effective antigens, and could provide a relevant scale of efficacy for new contenders. Ultimately any third generation anticoccidial vaccine will require rigorous testing comparable to that specified by the British and European Pharmacopoeia for live coccidiosis vaccines [81] before it can be registered.

New opportunities

Broader biological work that has been ongoing with *Eimeria* parasites is now providing opportunities for improved control of coccidiosis. Protocols supporting genetic complementation and modification by transgenesis are immensely powerful tools for cell biological and genetics studies in several genera of apicomplexan parasites. Recent progress in this field with *Eimeria* allows creation of transgenic parasite lines expressing one or more foreign proteins [82, 83], prompting the notion that *Eimeria* species could be used as host-specific vaccine delivery vectors. Preliminary tests indicated that *E. tenella* is capable of expressing enhanced yellow fluorescent protein (EYFP) as a model antigen that stimulates a range of humoral and cell-mediated immune responses in the chicken following oral vaccination with genetically modified oocysts [84]. More recently, experimental oral vaccination of chickens with *E. tenella* parasites expressing *Campylobacter jejuni* antigen A (CjaA) was found to stimulate an anti-*C. jejuni* immune response and reduce *C. jejuni* colonization of the gut by between 86% and 91% compared to controls [85]. Successful genetic complementation of other *Eimeria* species including *E. acervulina*, *E. maxima*, and *E. praecox*, as well as the rat-specific *Eimeria nieschulzi* [29, 67, 86], support the potential use of these less pathogenic species and strains as vectors to develop novel types of anticoccidial vaccines that may induce immunoprotection against other veterinary or zoonotic pathogens such as *C. jejuni* or avian influenza [87].

Changing focus to the host, a number of independent genetics studies have compared and quantified *Eimeria* replication in genetically distinct lines of inbred and outbred chicken, revealing between two- and fourfold variations in overall susceptibility to different parasite species [77, 88]. Recent availability of detailed genomic resources for the chicken, including a full draft genome sequence and high-density single nucleotide polymorphism (SNP) arrays [89] now permit the detailed genetic mapping, sequencing, and testing of loci and genes associated with resistance phenotypes.

Concluding remarks

Coccidiosis caused by parasitic *Eimeria* species remains one of the greatest burdens on the economics of production of poultry and poultry-derived products. While control is widely

available through the use of anticoccidial drugs, and live first and second-generation vaccines, neither of these are completely satisfactory, and new strategies are urgently required. A third generation of more cost-effective anticoccidial vaccines seems to be feasible with several candidate antigens described and many reports of partial protection in experimental settings. However, none of these approaches has progressed into commercial development despite more than 25 years of research [90, 91]. Comparison with progress for other apicomplexans, including high profile human pathogens such as *P. falciparum*, make it clear that whilst vaccines are far from straightforward, they are at least technically possible [92]. Despite many apparently promising candidates falling by the wayside there is now a small panel of realistic vaccine contenders. Future challenges will include identification of optimal delivery strategies and how we respond to parasite evolution in the face of vaccine selection. Of great importance, the genomes of all seven *Eimeria* species that infect the chicken are now sequenced and undergoing analysis and annotation (Reid et al., unpublished) (see http://www.genedb.org/Homepage/Etenella). Such resources will be invaluable in the discovery of novel targets for anticoccidial vaccine or drug intervention and identification of putative homologues in each species.

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561

Figure legends

Figure 1. A generalised life cycle for parasites within the *Eimeria* genus.

1. Ingestion of a sporulated oocyst initiates the endogenous phases of the *Eimeria* life cycle. 2. For avian *Eimeria* species the tough oocyst wall is disrupted mechanically during passage through the crop or gizzard, releasing four sporocysts from each sporulated oocyst. For *Eimeria* which infect mammals, enzymatic digestion is likely to be more important as the oocyst traverses the stomach and proximal intestine. 3. Exposure to digestive enzymes allows the sporozoite to escape the sporocyst as it passes through the intestine. The sporozoite continues to pass through the intestinal lumen until it attaches to and invades the epithelial layer. The exact site of invasion varies for each *Eimeria* species [2, 3]. 4. Inside the epithelial cell the sporozoite rounds up into a trophozoite before undergoing schizogony (asexual multiple fission), resulting in the production of multiple first generation merozoites which rupture and leave the host cell. 5. Each first generation merozoite invades another epithelial cell prior to entering a second round of schizogony, leading to production of second generation merozoites. One, two or more rounds of schizogony may follow. 6. After a parasite species-specific finite number of schizogonies the final generation of merozoites differentiate into gametes as the sexual phase of the life cycle begins, forming macrogametocytes, which develop into uninucleate macrogametes (♀), or microgametocytes which produce large numbers of motile, biflagellated microgametes by multiple fisson (♂). 7. Mature microgametes leave the host cell and penetrate neighbouring cells, fertilising mature macrogametes to form zygotes. 8. After fertilisation the macrogamete forms a resistant wall as it transforms into an oocyst which escapes from the host cell into the intestinal lumen to be excreted into the environment to initiate the exogenous phase. 9. The unsporulated (non-infectious) oocyst undergoes sporulation in the environment requiring warmth, oxygen and moisture as it undergoes sequential meiotic and mitotic nuclear division to become a sporulated oocyst. The sporulated oocyst, which contains four sporocysts, each of which contain two sporozoites, is now infectious.

Each stage of the *Eimeria* life cycle within the host is known to be immunogenic, with the early life cycle stages considered to be most important in the induction of a protective immune response [33, 93, 94]. Few of the current anticoccidial vaccine candidates are expressed throughout the *Eimeria* life cycle and it is likely that multiple antigens will be required if an effective subunit vaccine is to be established.

Figure 2. Genetic mapping of loci encoding essential immunoprotective antigens as novel vaccine candidates [67]. **(a)** Antigenically distinct *Eimeria maxima* red and blue strains genotyped at a panel of marker loci (twelve used in this example), two of which are absent from the red strain, three absent from the blue strain. Marker red-6 (highlighted in black) is closely linked to a gene that encodes a strain-specific immunoprotective antigen. **(b)** In the absence of immune selection each hybrid progeny population will contain every marker, which defines either parent within a pool of multiple, genetically heterogeneous clones arising from meiotic segregation and recombination. **(c)** Under red strain-specific immune selection all progeny parasites expressing the red strain-specific antigen will be killed, removing or severely reducing the occurrence of genetically linked marker red-6. Thus, genes closely associated with marker 6 within the red strain genome will be assessed for vaccine candidacy.

Box 1. Outstanding questions

- Which of the 8,000-9,000 antigens encoded within each eimerian genome is capable of stimulating a protective immune response?

  A small number of defined subunit vaccine candidates have been identified, but none have reached clinical development.

- If antigens appropriate for use as defined subunit vaccines are identified, how can they be delivered to poultry in a cost effective manner?

- How can immunisation be optimised in young, immunologically naive chickens?

  The identification of optimal delivery mechanisms and adjuvants will be crucial.

- To what extent are *Eimeria* capable of evolving to avoid subunit vaccine-induced immune killing, and how can we minimise this risk?

Tables

Table 1. Summary of the most widely tested anticoccidial subunit vaccine candidates.

	Form of delivery tested		
Vaccine candidate	Protein[a]	DNA[b]	Vectored[c]
Apical membrane antigen-1	✓ [67]	✓ [67]	✓ [67]
gam56	✓ [57]	✓ [58]	n/d
gam82	✓ [56]	n/d	n/d
Microneme protein 2	✓ [95]	✓ [96]	✓ [75]
Microneme protein 3	✓ [61]	✓ [61]	n/d
Immune mapped protein-1	✓ [67]	✓ [67]	✓ [67]
Lactate dehydrogenase	✓ [47]	✓ [97]	✓ [46]
Profilin (3-1E)	✓ [78]	✓ [49]	n/d
Rhomboid-like proteins	✓ [98]	✓ [98]	✓ [73]
SO7	✓ [99]	✓ [65]	✓ [69]
TA4	✓ [39]	✓ [58]	✓ [72]

[a]Protein vaccination: antigen expressed as a recombinant protein (e.g. bacterial expression) prior to vaccination, usually delivered by subcutaneous or other site injection supplemented with an adjuvant such as Freund's Incomplete Adjuvant.

[b]DNA vaccination: complete or partial antigen sequence presented within a eukaryotic expression vector under control of a strong promoter designed to drive transcription and translation of the vaccine antigen *in vivo*, usually delivered by intra-muscular or other site injection.

[c]Vectored vaccination: use of genetically modified virus, bacteria, parasite, yeast or plant to express a vaccinal antigen and deliver it to the vaccinated animal either in a live or killed formulation.

Abbreviations: ✓, tested and found to be effective; n/d, not determined.

Glossary

Anticoccidial chemicals: anticoccidial drugs produced by synthesis, distinct from the ionophores. Examples include decoquinate, diclazuril and robeindine.

Antigenic dominance: immunogenic antigens that stimulate a strong immune response and overwhelm other, potentially immunoprotective, antigens. In this example screening immune responses of convalescent animals can reveal the dominant immunogenic, but not necessarily immunoprotective, antigens providing potentially false leads in subunit vaccine development.

Attenuated anticoccidial vaccine: live vaccines containing one or more *Eimeria* species parasites attenuated by selection for precocious development or serial passage in embryonated eggs. Attenuation results in reduced reproductive capacity and consequentially a reduced risk of clinical or sub-clinical disease.

Eimeria: genus of apicomplexan parasite. Seven species are recognized to infect the chicken.

Gametocyte: the sexual stages of the *Eimeria* life cycle.

Haemorrhagic coccidiosis: disease caused by *Eimeria brunetti*, *Eimeria necatrix* and *Eimeria tenella*, characterized by haemorrhagic enteritis.

Hitch-hiker genetic mapping: population-based genetic mapping strategy to identify genes which associate with a selectable phenotype through detection of changes in the occurrence of polymorphic, but potentially neutral, genetic markers that are physically linked to a causative locus.

Homoxenous faecal-oral life cycle: Single host parasite life cycle, transmitted by ingestion of faecally contaminated environmental material (e.g. food, water, bedding, preening).

Ionophores: lipid soluble antimicrobials produced by fermentation. The major drug class used to control *Eimeria*. Examples include monensin, narasin and salinomycin.

Malabsorptive coccidiosis: disease caused by *Eimeria acervulina*, *Eimeria maxima*, *Eimeria mitis* and *Eimeria praecox*, characterized by mucoid enteritis.

Poultry production systems:

- Breeder: chicken produced to breed future generations of broiler, layer or other chickens.
- Broiler: chicken produced for meat production.

- Free-range: chickens produced for meat and/or eggs that are allowed freedom to roam for food, usually within an enclosed area but with provision for extensive movement in the open air.
- Housed: chickens produced for meat and/or eggs enclosed within a building ('house'). House design varies between regions, usually featuring two or more wire walls in tropical and hot regions but enclosed within solid walls in more temperate and colder regions.
- Layer: chicken produced and maintained for egg production.
- Organic: chicken production for meat and/or eggs in any form of accommodation achieved without the use of synthetic products, including drugs or growth promoters for the chickens or their food, or genetic modification.

Phytotherapy: The use of extracts from natural sources such as plants as medicinal or health-promoting products.

Oocyst: a cyst formed by a protozoan parasite. For *Eimeria* this is the environmentally-resistant stage of the life cycle and the infectious unit.

Schizogony: asexual reproduction by multiple fission.