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Review

Recombinant anticoccidial vaccines - a cup half full?

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ABSTRACT

Eimeria species parasites can cause the disease coccidiosis, most notably in chickens. The occurrence of coccidiosis is currently controlled through a combination of good husbandry, chemoprophylaxis and/or live parasite vaccination; however, scalable, cost-effective subunit or recombinant vaccines are required. Many antigens have been proposed for use in novel anticoccidial vaccines, supported by the capacity to reduce disease severity or parasite replication, increase body weight gain in the face of challenge or improve feed conversion under experimental conditions, but none has reached commercial development. Nonetheless, the protection against challenge induced by some antigens has been within the lower range described for the ionophores against susceptible isolates or current live vaccines prior to oocyst recycling. With such levels of efficacy it may be that combinations of anticoccidial antigens already described are sufficient for development as novel multi-valent vaccines, pending identification of optimal delivery systems. Selection of the best antigens to be included in such vaccines can be informed by knowledge defining the natural occurrence of specific antigenic diversity, with relevance to the risk of immediate vaccine breakthrough, and the rate at which parasite genomes can evolve new diversity. For *Eimeria*, such data are now becoming available for antigens such as apical membrane antigen 1 (AMA1) and immune mapped protein 1 (IMP1) and more are anticipated as high-capacity, high-throughput sequencing technologies become increasingly accessible.

1. Introduction

Eimeria have been recognised as important intestinal parasites of poultry for more than 100 years (Chapman, 2014). During this period understanding of the parasite life-cycle, their global distribution and environmental stability, has become well established (Shirley et al., 2005). Risks associated with uncontrolled coccidial infection include failure of chickens to thrive, increased susceptibility to diseases such as necrotic enteritis, compromised feed conversion and, for some species of parasite, high levels of mortality (Shirley et al., 2005; Williams, 1999). In response, prophylactic anticoccidial drugs are routinely used to control *Eimeria*, and live parasite vaccines are popular in some sectors of the industry (Blake and Tomley, 2014). Diagnosis commonly relies on a combination of pathology (lesion scoring) and detection of oocysts in faeces or litter and has not changed fundamentally in more than 50 years (Nolan et al., 2015). Nonetheless, parasite identification remains largely subjective and differentiation of strains and genotypes is impossible without detailed laboratory analysis. Approaches considered routine for many bacterial pathogens to define drug resistance profiles, or identify the presence of specific virulence factors (Cosentino et al., 2010; Fluit et al., 2001), are not available for *Eimeria*. Oocysts defined by genotypes that confer drug resistance, enhanced virulence or even immunological escape from vaccine-induced protection, appear identical to other oocysts, leaving the farmer, veterinarian and scientist in the dark (Peek and Landman, 2003; Williams et al., 2009). At present such variant parasites are identifiable only by expensive testing *in vivo*, for example determining drug resistance profiles by anticoccidial sensitivity testing (ASTs) (Naciri et al., 2003).

Attempts to develop next-generation recombinant anticoccidial vaccines have led to the identification of many potential vaccine antigens. However, until recently the extent of naturally occurring allelic diversity in the genes encoding these antigens has been unknown (Blake et al., 2015). The relevance of such diversity, and predicting the effects it will have on immune escape and subsequent vaccine failure, is crucial and can help to inform selection of the optimal antigens for inclusion in recombinant vaccine formulations. Pre-existing antigenic diversity in field populations of parasites can immediately limit vaccine efficacy with those expressing allelic variants escaping full control, as has been described in other apicomplexans such as *Plasmodium falciparum* with the antimalarial vaccine candidates apical membrane antigen 1 (AMA1) and merozoite surface antigen 1 (MSP1) (Arnott et al., 2014; Takala and Plowe, 2009). Even where allelic diversity associated with immune escape is low, or occurs/emerges at very low frequency, the selection pressure to survive and replicate in the face of immunity is potent,

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favouring genetic diversity and the rapid emergence of resistance.

Selection pressure is particularly profound for *Eimeria* which infect chickens due to a combination of features of the parasite (functionally haploid, highly fecund and rapid replication) and the host (high numbers, intense farming, rapid turnover), as amply demonstrated by the speed with which resistance to chemical anticoccidial drugs emerges (Chapman, 1997). In contrast, live coccidiosis vaccines have been used for more than 50 years with little evidence of parasite evolution towards resistance/immune escape. This is probably attributable to each parasite expressing between 6000 and 9000 antigens during completion of their life-cycle (Reid et al., 2014; Shirley et al., 2005), many of which contribute to robust immune protection. Recombinant vaccines based on small numbers of antigens are likely to exert considerably more focused genetic selection pressure, as is the case for anticoccidial drugs, and parasite genomes will require fewer mutations to achieve immune escape. A key question is whether new anticoccidial vaccines will suffer the same fate as most anticoccidial drugs, with swift selection for vaccine-resistant parasite populations that undermine vaccine value? Without such understanding, the decades of research into recombinant anticoccidial vaccines may be undone within months of vaccine release. In this targeted review we summarise the existing options for control of *Eimeria* species parasites which infect chickens, explore the candidates available for inclusion in sub-unit or recombinant anticoccidial vaccines and discuss the current understanding of genetic diversity for these antigens.

2. Coccidiosis

Eimeria are protozoan parasites of the phylum Apicomplexa. Close relatives of *Toxoplasma gondii* and the *Plasmodium* species, *Eimeria* can cause the disease coccidiosis in all livestock although most species of the genus are strictly host-specific (Kvicerova and Hypsa, 2013; Vrba and Pakandl, 2015). Coccidiosis is a significant economic burden to commercial cattle and sheep production, but the greatest losses occur within the poultry industry, where the disease has been estimated to incur annual deficits in excess of £2 billion (Dalloul and Lillehoj, 2006; Lassen and Ostergaard, 2012; Williams, 1999). Clinical disease manifests as a haemorrhagic or malabsorptive enteritis caused by *Eimeria tenella*, *Eimeria necatrix* or *Eimeria brunetti*, and *Eimeria acervulina*, *Eimeria maxima*, *Eimeria mitis* or *Eimeria praecox*, respectively (Long et al., 1976; Williams et al., 2009). Subclinical infection is common, influencing key production parameters such as food conversion ratio (FCR), average daily gain (ADG), and days to slaughter (Williams, 1999). All *Eimeria* species follow homoxenous faecal-oral life-cycles, offering opportunities for control of transmission in the environment, as well as replication *in vivo*. Husbandry measures including maintenance of dry litter, influenced by variables such as stocking density, quality of housing and ventilation, diet and occurrence of other enteric pathogens, can reduce *Eimeria* transmission, but additional anticoccidial control is essential in modern poultry production.

3. Current anticoccidial control

Ten different active ingredients are currently available in anticoccidial products licensed for prophylactic use with poultry in the European Union, plus one additional therapeutic coccidiocide (http://www.noahcompendium.co.uk/). In the UK between 240 and 300 tonnes of these active ingredients are sold for use in livestock production every year, with the vast majority being used in the poultry sector (Eckford et al., 2014). The drugs available can be divided into chemical and ionophore groups, produced by synthesis or fermentation, respectively (Blake and Tomley, 2014). At present the ionophores dominate the anticoccidial market, representing more than 70% of the drugs used (Eckford et al., 2014), although their status as antibiotics in countries such as the US is beginning to restrict their application. The success of the ionophores has been at least partially attributed to the incomplete anticoccidial protection they provide, even against naïve field isolates, at doses that are not toxic to chickens. This allows the parasite to continue replicating at a low level in the face of treatment (reviewed elsewhere (Chapman, 1999)). For example, in one early study parasite replication and disease pathology caused by apparently susceptible *E. tenella* was not completely blocked in chickens given monensin or lasalocid treatment at 125 ppm. At this dose, equivalent to concentrations used in current commercial applications such as Elancoban® G200 and Avatec® 150G, oocyst output was reduced by 82–97% (monensin), while weight gain was reduced by 10% and 12% (monensin and lasalocid, respectively) and average caecal lesions of 1.2 per group were still recorded (both ionophores) (Chapman, 1976). Subsequently, in trials with monensin at 125 ppm *E. maxima* oocyst output was reduced by 40–92% compared to unmedicated controls (challenge, doses 1000–10 sporulated oocysts per bird, respectively), and *E. brunetti* by 53–98% (Chapman, 1978). Equivalent studies in modern field isolates would be expected to reveal similar or even greater parasite escape as a consequence of drug resistance (Djemai et al., 2016). Furthermore, immunity was found to develop in medicated birds following sequential trickle exposure to *E. maxima*, *E. brunetti* and *E. tenella* (Chapman, 1978). While the comparison between many trials is hindered by experimental variation, low-level parasite escape from ionophore treatment has been a frequent observation (Bafundo et al., 2008; Karlsson and Reid, 1978; Ruff et al., 1980). Indeed, it is accepted that the 'leakiness' of these important anticoccidial drugs is a key factor in their success. Ionophores allow chickens to be exposed to low levels of replicating *Eimeria* that induce natural immune protection that is of huge value to the bird when drugs are withdrawn prior to slaughter, or at the onset of egg production (Chapman, 1976, 1999). Incomplete parasite killing also reduces selective pressure towards drug resistance which probably has extended the active commercial life of the ionophores. Nonetheless, resistance has been described among *Eimeria* to every drug currently available, often appearing within one year of release (Chapman, 1997). Such a swift response against the potent effect of lethal drug selection suggests the occurrence of pre-existing genetic diversity within *Eimeria* genomes and/or a high ability for genome plasticity and diversification.

Anticoccidial vaccination using formulations of live *Eimeria* parasites offers an effective alternative to chemoprophylaxis. Robust immune protection is achieved following ingestion and re-cycling of controlled doses of vaccine oocysts. Anticoccidial vaccine uptake in the poultry industry has been limited by the need for multiple vaccine lines of parasites to be produced by independent passage in chickens. This places some practical limitations on manufacturing capacity and means that vaccines can cost significantly more than anticoccidial drugs (Blake and Tomley, 2014). The first live anticoccidial vaccines such as Coccivac®, and more recently Immucox®, included oocysts of unmodified wild-type *Eimeria* (Williams, 2002). Non-attenuated vaccines are widely used in many parts of the world and offer good vaccine protection; importantly their manufacturing yields are much higher than those of live-attenuated vaccines, so they are considerably cheaper (Chapman and Jeffers, 2014). Because they replicate with high efficiency, live wild-type vaccine parasites also contribute to restoring anticoccidial sensitivity to commercial poultry farms that have drug-resistant populations of *Eimeria*, thus extending the 'life' of several important drugs (Chapman and Jeffers, 2015; Jenkins et al., 2010; Peek and Landman, 2006). However, there are significant drawbacks to the use of non-attenuated vaccines; because the parasites are fully virulent, they carry associated risks of vaccine-induced disease, which has limited their uptake, most notably in Europe where they are not currently licensed. Subsequently, a second generation of live attenuated vaccines was produced, incorporating parasite lines that are selected for early (precocious) development or (in one example) adaptation to growth in embryonic chicks (reviewed in detail by Williams (Williams, 2002)). These second-generation vaccines replicate to lower levels and have superb safety combined with efficacy. However, reduced replication