Review

Parasites ramble on: Focus on food security

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

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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 subclinical infections, which significantly impact 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 past 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 subclinical disease is common and

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Glossary

Anticoccidial chemicals: anticoccidial drugs produced by synthesis, distinct from the ionophores. Examples include decoquinate, diclazuril, and robenidine.
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 subclinical disease.
Eimeria: genus of apicomplexan parasite. Seven species are recognised 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*, characterised 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*, characterised by mucoid enteritis.
Oocyst: a cyst formed by a protozoan parasite. For *Eimeria* this is the environmentally resistant stage of the life cycle and the infectious unit.
Phytotherapy: the use of extracts from natural sources such as plants as medicinal or health-promoting products.
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.

Schizogony: asexual reproduction by multiple fission.

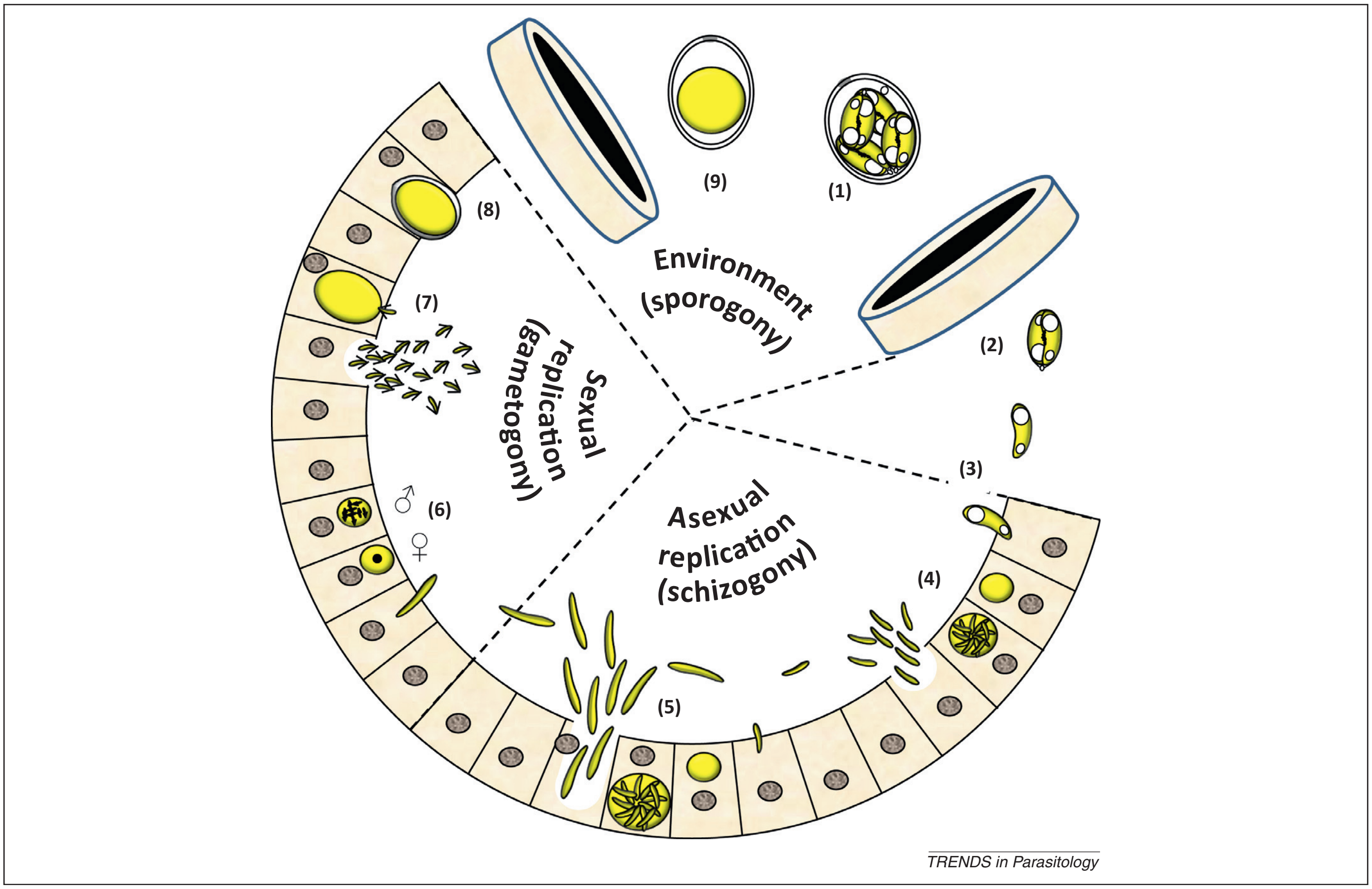

Figure 1. A generalised life cycle for parasites within the *Eimeria* genus. **(1)** Ingestion of a sporulated oocyst initiates the endogenous phases of the *Eimeria* life cycle. **(2)** For avian *Eimeria* species, the tough oocyst wall is disrupted mechanically during passage through the crop or gizzard, releasing four sporocysts from each sporulated oocyst. For *Eimeria* that 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 fission (♂). **(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 probable that multiple antigens will be required if an effective subunit vaccine is to be established.

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 owing 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 foodborne 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, moisture levels, and thorough cleaning between flocks. However, husbandry alone is inadequate for control,