NON-TECHNICAL SUMMARY

Enhancing control of poultry diseases

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This project is intended to generate new data and knowledge about (1) fundamental biology and genetics of coccidial parasites and intestinal bacteria, (2) host immune responses, and (3) the interactions and co-interactions of parasites and bacteria with the chicken.

Infectious diseases of chickens can compromise meat and egg production, cause severe welfare problems, and be a source of human zoonotic disease. There is a requirement for new approaches to control pathogens that infect chickens for several reasons including pathogen resistance to antimicrobials, legislative restrictions on the use of some in-feed drugs, and consumer concerns about chemical residues in meat. New types of control (i.e. vaccines and alternative compounds rn c a requir

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vaccines via the oral route. Parasites produced here will also be used in *in vitro* studies wherever possible to accelerate vaccine development while reducing the use of live chickens.

WP3: Validation of novel anticoccidial feed or water additives

As demands for alternatives to anticoccidial drugs intensify interest in feed or water additives such as botanicals, botanical extracts, enzymes and other molecules is increasing. We currently use an *in vitro* model as a screening tool to identify candidates, but final efficacy will be verified using chickens reared under field conditions.

WP4: Understanding *Eimeria* population biology

The widespread occurrence of anticoccidial drug resistance combined with strong legislative and consumer driven requirements for reduced antimicrobial use in livestock production has increased demand for vaccines to protect against *Eimeria*. Seven *Eimeria* species have long been recognized to infect chickens; however recent detection of three new *Eimeria* species circulating in chickens across much of the southern hemisphere that can escape from current vaccines has revealed unexpected levels of complexity. The consequences of vaccine breakthrough by cryptic parasites can include very high prevalence of sub-clinical infection as well as outbreaks of clinical disease. The occurrence, characteristics and genetic flexibility of *Eimeria* field populations is unclear. We will employ new sequencing technologies and traditional parasitology to improve *Eimeria* genome resources and characterise *Eimeria* field populations, exploring the impact of drug or vaccine selection and mixed infections to improve control of disease.

WP5: The genetic basis of susceptibility/resistance to coccidiosis

Genetic resistance of chickens to infectious disease is well documented, including coccidiosis caused by *Eimeria*. Understanding genetic resistance provides a possible means to breed flocks that are naturally protected against disease. We aim to explore resistance/susceptibility traits during infection including body weight gain, intestinal structure, circulating immune cells and proteins and parasite replication, plus chicken activity and behaviour to identify genetic, biological and visual markers that can be used in selective breeding and routine husbandry of chickens to improve resistance to disease.

WP6: Understanding the consequences of pathogen interaction and impact on gut bacterial populations

Eimeria infection of chickens can alter, and be influenced by, the composition of the intestinal microflora, in some examples modifying colonisation of specific pathogens such as *Campylobacter jejuni*. We aim to use next-generation sequencing techniques to further define these interactions to (i) improve poultry health in terms of altered colonisation and/or pathogenesis of bacterial pathogens, (ii) reduce the risk of zoonotic transmission to humans through the food chain, (iii) explore the host genetic contribution to variation, and (iv) evaluate interactions in the presence of known anticoccidials or alternative additives.

WP7: Explore the use of embryonated chicken eggs as replacements for hatched chicks in studies with *Eimeria*

A small number of lines of *Eimeria* have previously been adapted to replicate in embryonated chicken

- farmers, reducing costs of pathogen control, improving ease of vaccine or feed additive administration

- animal health companies, maintaining a competitive position in markets for control of coccidiosis

- consumers, reducing the cost and improving availability of poultry products

- the environment, reducing use of antimicrobial and anticoccidial drugs.

Commercial development of new anticoccidial vaccines based on vectored proteins would have many benefits including use of none or fewer birds for production of the parasites used in existing live vaccines, cheaper vaccines, and wider uptake. The UK leads the world in manufacture of live attenuated coccidiosis vaccines but there are major issues associated with the need to incorporate many ‘lines’ of parasites in order to induce immune protection. Figures from industry indicate that more than 2 billion doses of Paracox are sold each year requiring sacrifice of >250K birds for parasite production. A prototype multi-valent vaccine will ensure that the UK animal health industry has a solid foundation from which to retain a leading position on coccidiosis control, contributing to overall wealth creation. Inclusion of vaccine antigens protective against other pathogens such as *Campylobacter jejuni* can improve health and welfare further, while reducing the number of vaccinations required. Consideration of new and emerging parasite types may be essential in the absence of drug-based prophylaxis.

Identification of markers that can be used in selective breeding of chickens that are more naturally resistant to *Eimeria*, or respond better to vaccination, offer benefits to poultry, poultry consumers and producers, and the environment, as outlined above. Reducing the occurrence of ill health in chickens will lower the overall cost of poultry products, benefiting consumers as well as production and distribution networks.

Understanding interactions between *Eimeria* and bacterial zoonoses can improve poultry product quality, reducing risks to consumers and increasing confidence in food supply. Understanding interactions with the 'healthy' gut bacteria found in chicken intestines can improve chicken health, welfare and productivity. Improved broiler chicken gut health is expected to lower demand for antimicrobial intervention, reducing drug use in livestock production. Lower antimicrobial consumption will reduce selection for antimicrobial resistance in enteric and environmental microbial populations, and reduce antimicrobial flow into environments around chicken production systems.

Indirect benefits include staff and students working on the project who will receive training in laboratory and simulated farm level settings, including a range of protocols that can only be applied with live animals and can also be used to answer a variety of experimental questions beyond the remit of this work. The national and international scientific community will benefit from improved understanding of *Eimeria*, their interactions with bacteria, and provision of improved vaccine vectors for poultry.

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All data produced from these studies will be published in Gold Open Access peer reviewed journals, as mandated and supported by the funding bodies. In addition to data, protocols and standards developed or applied will be described, providing resources and benchmarks for comparative studies. Data such as DNA or RNA sequences (e.g. defining bacterial populations or genes that are active) will be

submitted to open repositories, specifically the European Nucleotide Archive (ENA), linked to the DNA Data Bank of Japan (DDJB) and GenBank. Published studies will include results of null or unassociated measures to share awareness. Results will be shared with peer audiences through national and international conferences (e.g. British and World Veterinary Poultry Association meetings, British Society for Parasitology).

Results and progress will also be reported in industry journals and magazines, as well as live events such as the Pig and Poultry Show, to ensure dissemination to relevant target audiences.

A series of collaborations with partners in industry and academia will enhance outputs for the work, including links to researchers in Asia, Africa and Central/South America, as well as Europe and North America.

- Domestic fowl (Gallus gallus domesticus): 5500

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Chickens will be used throughout these studies, recognising that they are the target animal and not just a model. A range of chicken types will be used, including (i) specific pathogen free (SPF) chickens to permit optimal parasite production and accurate assessment of measures with minimal background variation, (ii) commercial broiler and layer chickens, representing target populations and providing real-life examples that can be reared under simulated industry conditions, and (iii) genetic knockout (KO) chicken lines lacking specific immune functions to assess the impact of the host immune response on parasite replication, pathogenicity and vaccine response. Chicks will typically be received at day of hatch for vaccination and equivalent characterisation studies, or 2-3 weeks of age for parasite production and selection studies. Studies with embryonated chicken eggs will conclude prior to hatching.

Chicks will typically be used up to six weeks of age (maximum of ten weeks old), recognising that coccidiosis is primarily a problem during the early phase of chicken growth.

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Experiments will usually fall into one of four types, with some variation around specific procedures.

1. Parasite amplification, selection or characterisation

SPF chickens will typically be used for these studies, although a range of inbred or genetically modified chickens may be used (e.g. the range available at the National Avian Research Facility [NARF]).

Eimeria

considerably, especially in hybrid commercial chickens, possibly reaching ~5%.

Industry data indicate that 10-30% of pedigree and commercial broiler chickens will experience enteric dysbiosis by five weeks of age when reared under standard commercial conditions in the absence of antimicrobial prophylaxis. Chickens that experience dysbiosis will be removed from the study, not retained and treated.

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Eimeria parasites do not grow productively in cell culture and can only be obtained from infection of live animals. Similarly, studies of anti-parasite control and host-pathogen interactions require use of live animals to assess effects on parasite growth and consequences of infection. Over the past two decades our lab has significantly improved propagation of *Eimeria* in cell culture, and we routinely use immortalised cell lines for the study of early invasion events in the parasite's lifecycle and effects of potential interventions, significantly reducing the use of chickens. We will continue to use cell culture whenever possible, but studies beyond the first step in parasite replication cannot be supported in this manner. We will also continue to test new methods for full propagation of the parasite life cycle in cell culture or tissue explant systems. However, currently the only way to amplify parasites, perform genetic crosses, maintain pure strains, study pathology and host immunity, generate novel transgenic lines of parasite, or evaluate vaccine efficacy is to carry out infections of animals.

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For comparative studies such as definition of vaccine or feed additive efficacy, immune responses, cross-protection between *Eimeria* species and impact on gut bacterial populations, animal numbers