Are *Eimeria* genetically diverse, and does it matter?

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Abstract

Eimeria pose a risk to all livestock species as a cause of coccidiosis, reducing productivity and compromising animal welfare. Pressure to reduce drug use in the food chain makes development of cost-effective vaccines against *Eimeria* essential. For novel vaccines to be successful, understanding genetic and antigenic diversity in field populations is key. *Eimeria* species that infect chickens are most significant, with *Eimeria tenella* among the best studied and most economically important. Genome-wide single nucleotide polymorphism-based haplotyping has been used to determine population structure, genotype distribution, and potential for cross-fertilization between *E. tenella* strains. Here, we discuss recent developments in our understanding of diversity for *Eimeria* in relation to its specialized lifecycle, distribution across the globe, and the challenges posed to vaccine development.

Genome sequences pave new roads for anticoccidial vaccine development

Eimeria species, protozoan parasites that can cause the damaging intestinal disease coccidiosis, pose a significant risk to global poultry production [1, 2]. Members of the phylum Apicomplexa, the genus *Eimeria* encompasses at least 1 200 species, almost all of which are restricted to a single host [3]. Seven *Eimeria* species are recognized to infect the chicken, causing a considerable disease burden across the globe [2, 4, 5]. Similarly, several species are considered to be highly pathogenic in turkeys (reviewed in [6]). Whilst the pathology associated with each *Eimeria* species infecting chickens has long been understood [7, 8], parasite population structures and the extent of genetic diversity in field populations are only now emerging. Interest in parasite occurrence, diversity and epidemiology is driven by a global need for cheap and effective vaccines as alternatives to anticoccidial drugs. Details of regional variation in *Eimeria* species prevalence, distribution of genetically and antigenically distinct strains, and the frequency at which polymorphic strains cross-fertilize, all provide valuable knowledge that can underpin rational vaccine design and development. In particular the recent availability of genome sequence resources for all seven *Eimeria* species of the chicken [9] provides opportunities to define many of the variables outlined above [10].

Here we review and discuss recent findings relating to the genetic and antigenic diversity of *Eimeria* species which infect chickens in the context of vaccine development and the potential for future successes based on new sequencing technologies and the search for novel vaccine candidate antigens.

Current control strategies for *Eimeria* which infect chickens

More than 60 billion chickens are produced in the world every year, yielding 1.1 trillion eggs and more than 90 million tonnes of meat [11]. The poultry industry in the United States of America (USA) alone is worth in excess of $38.1 billion, which includes the combined production value of chickens and turkeys [12]. Consequently, effective means of controlling pathogens which infect chickens are essential and of increasing importance as trends for expansion and intensification of global poultry production are maintained (Grace *et al*., 2012; https://cgspace.cgiar.org/bitstream/handle/10568/21161/ZooMap_July2012_final.pdf).

Control of coccidiosis in poultry relies predominantly on chemoprophylaxis, although resistance to anticoccidial drugs is common in *Eimeria* field populations [13-15]. Prior to the year 2000, anticoccidial drugs were used in ~95% of flocks where anticoccidial control was employed, including ~99% of commercial broiler flocks [16]. More recently, a study from the USA has reported that this percentage has fallen to between 60 and 99%, depending upon the time of year [16]. While anticoccidial drugs remain essential to chicken production and these trends are not yet reflected in much of the world, reductions in drug application throughout the food chain driven by legislative and consumer pressure is encouraging alternatives for coccidiosis control [13, 17]. The use of live oocyst vaccines comprising mixes of species of non-attenuated (formerly wild-type) or attenuated parasites [2] are well established. Oral exposure to controlled numbers of vaccine oocysts is designed to result in low grade coccidial infection, inducing a protective immune response that is boosted by re-infection as the live vaccine re-circulates through the chicken house. However, vaccine production costs and the requirement for multiple parasite lines in each vaccine have been

significant barriers to the widespread use of live vaccines in the majority broiler production sector [2]. Nonetheless, non-attenuated vaccines are now included in anticoccidial rotation programs by 35-40% of commercial broiler companies in the USA [16].

Recombinant subunit vaccines have been considered as potential alternatives for coccidiosis control for many years, and the concept has returned to the fore in the past decade with the discovery and testing of many partially immunoprotective antigens and expansion of the number of vaccine delivery systems available for use in chickens. Low genetic variability in the target antigen(s) is a key requirement for success precisely because recombinant vaccines rely on the expression of a single, or a small number of antigens [18, 19]. Vaccination using such a small subset of antigens from a complex parasite such as *Eimeria* may provide a significant driving force for immune selection, which could lead to the rapid appearance and dissemination of alleles which confer vaccine-escape (resistance) [10]. The phylum Apicomplexa encompasses a number of parasites important for human and/or animal health including *Plasmodium falciparum* and *Toxoplasma gondii*. The well-characterized population structures and genetic diversity of these parasites have shown that there are numerous barriers to the success of subunit vaccines, but have inspired relevant vaccine development (e.g. [20-22]). In contrast, rather little is known of the genetic diversity and structure of field populations of *Eimeria* parasites, the potential for mixing between genotypes or the selective pressures imposed on loci which encode immunoprotective antigens, highlighting the numerous challenges posed to the development of novel subunit vaccines (reviewed in [11]).

Defining genetic diversity within *Eimeria* species

Eimeria parasites have been recognized for more than a century [7]. Early approaches to understanding parasite diversity focused on parasite (mainly oocyst) morphology, lifecycle (location and timing of development in the gut) and pathogenicity [23]. Differences in the mobility of specific metabolic enzymes during starch gel electrophoresis by isoelectric focusing permitted discrimination between *Eimeria* species and some strains [24], but it was only with the application of techniques that visualize DNA such as pulsed field gel electrophoresis to examine chromosomes, and amplified fragment length polymorphism to examine polymorphisms, that genetic variation began to be explored (reviewed in [25]). Now, advances in molecular biology permit the detailed definition of genetic diversity at specified loci of interest and across whole genomes (Box 1).

Assessing genetic diversity of *Eimeria* using defined locus sequencing

Sequencing short genomic regions, such as internal transcribed spacer (ITS) or mitochondrial cytochrome oxidase subunit 1 (COX1) loci, has been used widely to infer the relatedness of *Eimeria* isolates, particularly those collected from the field. The technique is relatively inexpensive, can be carried out with limited laboratory resources, and is supported by a published sequence archive with ~1 000 and ~100 sequences currently available for ITS and COX1 respectively (GenBank; accessed 7th June, 2016 http://www.ncbi.nlm.nih.gov/pubmed using the 'nucleotide' menu).

ITS sequencing has been the molecular technology used most widely for assessing *Eimeria* occurrence in field populations. Initially, studies focused largely on separate countries or continents with examples including Australia, India, Africa and

124 the USA [5, 26-28]. The most comprehensive survey of *Eimeria* field isolates was
125 published recently in which 512 pooled faecal samples were surveyed from poultry
126 farms situated in 20 countries across five continents [29]. Here, ITS1-5.8S-ITS2
127 sequence analysis revealed some interesting aspects of population structure. The
128 genetic signatures of *Eimeria acervulina* and *Eimeria mitis* indicated that regular
129 interbreeding occurs between genotypes, while *Eimeria tenella* exhibited a more
130 restricted population structure [10, 29]. The inclusion of sequences derived from
131 laboratory reference strains that are progenitors to many vaccine parasites in the
132 comparison suggested that the samples collected were representative of wild-type
133 field strains, not re-sampling of vaccinal lines [29]. It was suggested that the faster
134 generation time and greater fecundity of *E. acervulina* and *E. mitis* compared to *E.*
135 *tenella* (~33% shorter prepatent period and 2.5-4 times more oocysts produced per
136 oocyst ingested [30, 31]) could account for the observed differences in population
137 structure. As a consequence, *E. acervulina* and *E. mitis* parasites have greater
138 opportunity for co-infection and hybridization and their genomes may evolve more
139 rapidly.

140 Analysis of ITS sequence datasets has also led to the discovery of three new
141 *Eimeria* 'operational taxonomic units' (OTUs) [5, 26, 29, 32]. Initially, ITS2 sequencing
142 of isolates from Australia provided the first definition of the three *Eimeria* OTU
143 genotypes termed OTUx, OTUy and OTUz [26], which have been supported by
144 subsequent ITS1 and ITS2 sequencing of isolates covering a greater geographical range
145 [29, 32, 33]. These divergent parasites appear to be restricted at present to southern
146 regions of the world below 30^{o}N latitude [29, 34], although future human and trade
147 movements risk the expansion of their range. The spread of parasites with these novel

148 genotypes may have significant consequences for vaccine development and
149 application. At present it is unclear whether these variants can evade the immune
150 protection offered by live vaccines, although Morris and colleagues have provided one
151 example of escape from the field [35]. Sequence comparison currently suggests that
152 OTUx is most closely related to *Eimeria maxima*, with *Eimeria brunetti* the closest link
153 to OTUy [32]. Comparison of ITS1-5.8S rDNA-ITS2 sequences has revealed the greatest
154 divergence for OTUz with distinct long and short forms, as described previously for *E.*
155 *maxima* and *E. mitis* [27, 29].

156 The development of next generation sequencing technologies has moved
157 analysis of genomic diversity from the single gene to genome wide levels, vastly
158 increasing available genetic and genomic resources for *Eimeria*. For example, fully
159 resolving the phylogenetic relationships between *Eimeria* species which infect
160 chickens and turkeys has proven difficult based on COX1 and 18S rDNA sequences
161 alone [36-38]. The robustness of separation of the seven *Eimeria* species recognized
162 to infect chickens was greatly improved using whole-genome phylogenies [9] and may
163 prove beneficial in future analyses of field isolates. Mitochondrial genome sequencing
164 has also been used effectively to separate *Eimeria* species which infect domestic
165 turkeys [39] (Table 1). The addition of genome sequences resources for cloned OTU x,
166 y and z lines are a high priority and should resolve the cryptic status of these
167 genotypes.

168

169 **A Dynamic and Adaptable Genome?**

170 Beyond the resolution of phylogenetic debate, whole genome sequencing has
171 revealed interesting aspects of genome structure for *Eimeria* (refer to Table 1 for a

summary of resources). Initial analysis of *E. tenella* chromosome 1, sequenced following purification from pulse field gel electrophoresis-resolved karyotypes, revealed alternating regions of repeat-poor (P) and repeat-rich (R) sequences [40]. More recently, Illumina-based genome sequencing and assembly demonstrated that the P and R structure was not limited to chromosome 1, but was conserved in all chromosomes of *E. tenella*, and across the genomes of *E. acervulina*, *E. brunetti, E. maxima*, *E. mitis*, *E. necatrix*, and *E. praecox*, as well as *Eimeria falciformis* (a parasite specific to the mouse) [9, 41]. Interestingly, differences were observed in repeat content between species. *Eimeria tenella*, for example, has fewer R regions than the other six species which infect chickens, while *E. necatrix* was more repeat rich across its genome, most notably in regions syntenic with *E. tenella* [9]. This P/R structure does not appear in the genomes of other coccidia such as *Neospora caninum* or *T. gondii* [9, 42], although it has been detected in the more closely related *Cyclospora cayetanensis* genome [43]. Ling and colleagues have suggested that the unusual genome organization might pose an evolutionary advantage to the parasite by facilitating rapid evolution and diversification. Variation in restriction fragment length polymorphism (RFLP) fragment size between different strains of *E. tenella* associated with R-, but not P-regions, lending some support for genome plasticity [9, 40, 44]. A disproportionately high repeat content in protein coding sequences could confer some evolutionary advantage, although their effects on protein structure appear to be neutral and genes known to be integral to host–parasite interaction were relatively free of repeats [9]. The seven *Eimeria* species which infect poultry do not appear to possess the sub-telomeric regions which in *P. falciparum* contain a set of plastic genes involved in host immune system evasion [9, 40, 45]. Telomere-like repeats are,

however, dispersed throughout the R-segments, suggesting there are complexities in the structure of the genome that we do not fully yet understand. Telomere-like repeats have previously been described in the *Plasmodium knowlesi* genome where they associate with variant antigen families, although a similar linkage has not been described for *Eimeria* [46]. The impact of the segmented *Eimeria* genome structure on the appearance and extent of genetic diversity is yet to be determined, although it may well associate with hotspots of genetic recombination. The implications of such hotspots on vaccine development are similarly unclear.

The importance of population structure

Genetic mapping has been useful in establishing the population structure of some apicomplexans (reviewed in [47]), as have other molecular tools (reviewed in [25]). Population structure varies across the Apicomplexa. *Plasmodium falciparum* has been shown to exhibit signatures of panmictic or clonal population structures, influenced in part by regional transmission rates [48, 49]. *Toxoplasma gondii* is commonly clonal in much of the world, with a small number of dominant genotypes described, although a higher level of genetic diversity has been detected in regions such as South America where population mixing appears to occur at a greater frequency [21, 50]. For *E. tenella*, comparison of haplotype occurrence and diversity defined following multiplex single nucleotide polymorphism (SNP) genotyping revealed that north Indian and north African field populations were characterized by a limited number of distinct haplotypes and significant linkage disequilibrium (Figure 1), resembling the region specific population structure of *T. gondii* [10]. This population structure suggests that limited opportunities exist for cross-fertilization

220 and genetic recombination, and that the expansion of a small number of haplotypes
221 might be common although not necessarily clonal. In contrast, a greater haplotype
222 diversity was reported in southern India and Nigeria with multiple haplotypes
223 appearing, all at a very low frequency, indicating that co-infection with heterologous
224 isolates and cross-fertilization is common during sexual reproduction, and that genetic
225 diversity is likely to be greater than estimated by current sampling [10]. These findings
226 suggest there are numerous opportunities for recombination in the field in these
227 regions.

228 The regional differences in population structure observed for *E. tenella* may
229 have several underlying causes. In southern India there is a greater poultry density
230 than found in the north (Grace *et al*., 2012;
231 https://cgspace.cgiar.org/bitstream/handle/10568/21161/ZooMap_July2012_final.p
232 df), and therefore more opportunity for parasite co-infection and cross-fertilization.
233 Further, the climate in south India is commonly more humid than in north India [51],
234 likely favoring higher levels of oocyst sporulation and increased parasite survival in the
235 poultry house environment as reported in comparisons of rainy versus dry seasons
236 [52]. Higher rates of transmission commonly associate with elevated levels of
237 outcrossing and increased genotype abundance for other apicomplexans such as *P.*
238 *falciparum* [49]. Importantly, co-infection of a single host with two or more genetically
239 distinct *Eimeria* isolates does not guarantee cross-fertilization. The *Eimeria* life cycle
240 includes a single, transiently diploid phase during sexual reproduction and oocyst
241 maturation [53], so timing of the co-infection has to be essentially simultaneous for
242 genetic recombination to occur. Additionally, the *in vivo* phase of the *Eimeria* life cycle
243 is predominantly self-limiting, with features such as prepatent period and the number

244 of rounds of schizogony stable, unless subjected to deliberate selection for
245 developmental rate [10, 54, 55]. Studies using major histocompatibility complex
246 (MHC) class I or II knockout mice suggest little or no role for the host immune response
247 in the conclusion of parasite replication [56]. Thus, gametes of each genotype must
248 mature in parallel for cross-fertilization to take place. *In vivo* experiments using
249 laboratory strains of *E. tenella* have shown that, given the opportunity, cross-
250 fertilization is common, highlighting the potential that *E. tenella* has to hybridize in
251 field populations and indicating the ease with which vaccine or drug-resistant alleles
252 could propagate in field parasite populations [10]. Combined, these factors emphasize
253 the importance of considering region specific environmental and social variables in
254 implementation of novel control strategies for *Eimeria* species. Fornace and
255 colleagues demonstrated that the diversity of species present in small-scale
256 production systems in Africa was directly linked to profitability [5]. However, there
257 have been few similar studies and the potential is there to link population structure
258 and the burden of coccidiosis to profitability in particular regions of the globe.
259

260 **The relevance of antigenic diversity**

261 Selection of candidate antigens for vaccine development has proved to be a
262 significant barrier to progress in other Apicomplexa such as *T. gondii* and the
263 *Plasmodium* species [57-59]. Differentiating immunogenicity from 'true' immune
264 protection can be difficult, making selection of protective antigens problematic [19].
265 In one example, homologs of apical membrane antigen 1 (AMA-1) have been shown
266 to be protective in a range of apicomplexan parasites including *E. maxima* [19], *E.*
267 *tenella* [60] and *P. falciparum* [61-63], and it has been widely proposed as a candidate

for subunit vaccine development. However, extensive allelic diversity has limited development of *P. falciparum* AMA-1, with more than 60 polymorphic amino acid residues detected and more than 200 haplotypes within even a single population [61, 62, 64, 65]. Despite such discouraging reports from *P. falciparum*, AMA-1 has shown promise as a vaccine candidate for *E. tenella*, with a potent inhibitory effect on parasite invasion [60]. More recently, genotyping *E. tenella* field isolates collected from Africa and India suggested that polymorphisms in the EtAMA-1 locus are lower than expected in field populations with largely neutral signatures of selection. The functionality of AMA-1 may outweigh the potential benefit to the parasite of immune evasion, which may be of limited value in the self-limiting eimerian life cycle [10]. Similarly, just four nucleotide polymorphisms exist between EmAMA-1 coding sequences from the *E. maxima* Houghton and Weybridge laboratory strains, two causing non-synonymous changes, one situated in the putative pro-domain and one located in domain 1 [9, 66]. Nonetheless, despite such limited diversity within the coding region of at least one vaccine candidate, strain specific immune escape has been reported *in vivo* for *E. acervulina* [67, 68], *E. mitis* [69], *E. maxima* [70] and *E. tenella* [71-73]. Comparison of *E. tenella* isolates collected from chickens reared in British and Indian poultry houses revealed incomplete immune protection between isolates, most notably following low-level primary exposure [10]. Despite these reports, there is no evidence that vaccine resistance has evolved in response to whole live parasite vaccination [2, 11]. One possible explanation for this is that throughout its lifecycle each *Eimeria* species expresses between 6 000 and 9 000 proteins [9], exposing the host to a complex portfolio of antigens. Selection targeting multiple immunoprotective antigens in parallel during replication in the chicken is likely to limit

292 the capacity for any individual parasite to evade the host immune response as a
293 consequence of diversifying selection. Thus, the complexity of the antigenic repertoire
294 might explain why resistance to live parasite vaccination has not yet developed [10]
295 [11]. Incorporating multiple antigens, in addition to AMA-1, in novel subunit vaccines
296 would therefore be likely to extend their potential for long term success by buffering
297 the effects of diversifying selection on a single target antigen.
298
299 **Life cycle stage-specific antigen expression and immune selection**
300 Each *Eimeria* life cycle features a series of extra- and intracellular stages within
301 the definitive host as the parasite undergoes successive rounds of asexual, and then
302 sexual replication [9, 53]. Throughout this process *Eimeria* expresses many of its genes
303 in a stage-specific manner which can impact on the development of novel vaccines. In
304 *T. gondii*, for example, vaccination with life cycle stage-specific antigens leads to stage-
305 limited protection [57, 58]. In *Eimeria*, the early life cycle stages are important to the
306 induction of protective immunity during natural infection [9, 11, 60]. Importantly,
307 vaccine candidates such as AMA-1 are primarily expressed by a single life cycle stage
308 and are unlikely to be subjected to a protein-specific adaptive immune response
309 during primary infection given the absence of protracted colonization [10, 54, 60].
310 Thus, the large oocyst output resulting from even low dose primary infections results
311 in considerable environmental contamination with parasites which have never been
312 exposed to immune selection.
313
314 **Future directions**

315 A clear direction for future work is to expand our understanding of population
316 structure to other *Eimeria* species in the field as has been reported recently for *E.*
317 *tenella* [10]. Elucidating the population structure and potential for mixing is key in the
318 development of novel control strategies for *Eimeria*. Understanding the possible
319 biological, environmental, industrial and social drivers which underpin the observed
320 diversity may be even more important, demanding detailed epidemiological
321 interrogation. Opportunities to develop medium/high throughput tools such as
322 Sequenom-based genotyping, and new high-throughput sequencing technologies
323 such as restriction site associated DNA (RAD) sequencing, will facilitate the move away
324 from ITS sequencing to genome wide analysis of genetic diversity with particular
325 relevance to field samples. *Eimeria* genomic resources have increased greatly in recent
326 years (reviewed in [74]). Additionally, since the cost of sequencing a genome the size
327 of *E. tenella* is now relatively modest (51.8 Mb DNA in the current genome assembly
328 [9]), the opportunity exists to build on the available genomic resources with whole
329 genome sequencing of other *Eimeria* strains and species. Parasites of the three OTU
330 genotypes are obvious candidates, with species which infect other livestock species
331 further priorities. The genomes of non-target species can yield clues as to the
332 structure and function of other closely related species. Comparative analysis of the *E.*
333 *falciformis* genome with *T. gondii* revealed a shared emergence and diversification
334 across the Coccidia of gene families associated with motility and invasion [41]. Building
335 on information from whole genome sequencing, another relatively new technology,
336 RNA sequencing (RNASeq) can be used for transcriptomic profiling of other key
337 antigens of interest and is likely to offer clues as to their function and suitability as
338 vaccine targets. RNASeq has already been used successfully to define transcriptomes

339 from several *Eimeria* life cycle stages [9, 53]. Indeed in the near future Isoform
340 sequencing (IsoSeq), which at present generates transcripts >3Kb [75], could be
341 utilized to sequence the entire transcriptome of a single parasite in full length
342 fragments. Genome editing techniques such as the CRISPR/Cas system have huge
343 potential and could be used, for example, to switch allelic type for a small number of
344 target antigen coding genes. The CRISPR/Cas system has been used successfully in *P.*
345 *falciparum* [76, 77] and *T. gondii* [78, 79], but is not yet available for *Eimeria*. These
346 tools should improve the molecular definition of diversity, expand our understanding
347 of parasite evolution and host evasion, and highlight regions of the genome that show
348 promise in the development of novel sub-unit vaccines.
349
350 **Concluding Remarks**
351 There are several key challenges posed by population, genetic and antigenic
352 diversity of *Eimeria* parasites to the development of novel vaccines (see Outstanding
353 Questions). How genetic, particularly antigenic, diversity influences pathogenicity,
354 vaccine specificity and epidemiology, and the implications of this for effective
355 intervention and control, are important questions that need to be answered for all
356 apicomplexan parasites. Recent studies have revealed a polarized global occurrence
357 for genetically divergent *Eimeria* strains, and possibly even new species, that may be
358 capable of replicating within chickens vaccinated using current generation vaccines.
359 These parasites pose a significant risk to vaccine efficacy, and thus food security and
360 animal welfare, in production systems which rely on anticoccidial vaccination.
361 Considering social and environmental variables in novel control strategies is of great
362 importance, with factors including choice of production system, geographic

separation of farms and climatic conditions likely to influence parasite population dynamics. The recent expansion in genetic and genomic resources available for *Eimeria* has dramatically improved our ability to genotype parasites recovered from field populations and begin to assess how many of these variables will affect genetic diversity, and whether that diversity will impact on vaccine efficacy and longevity.

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Figure legends

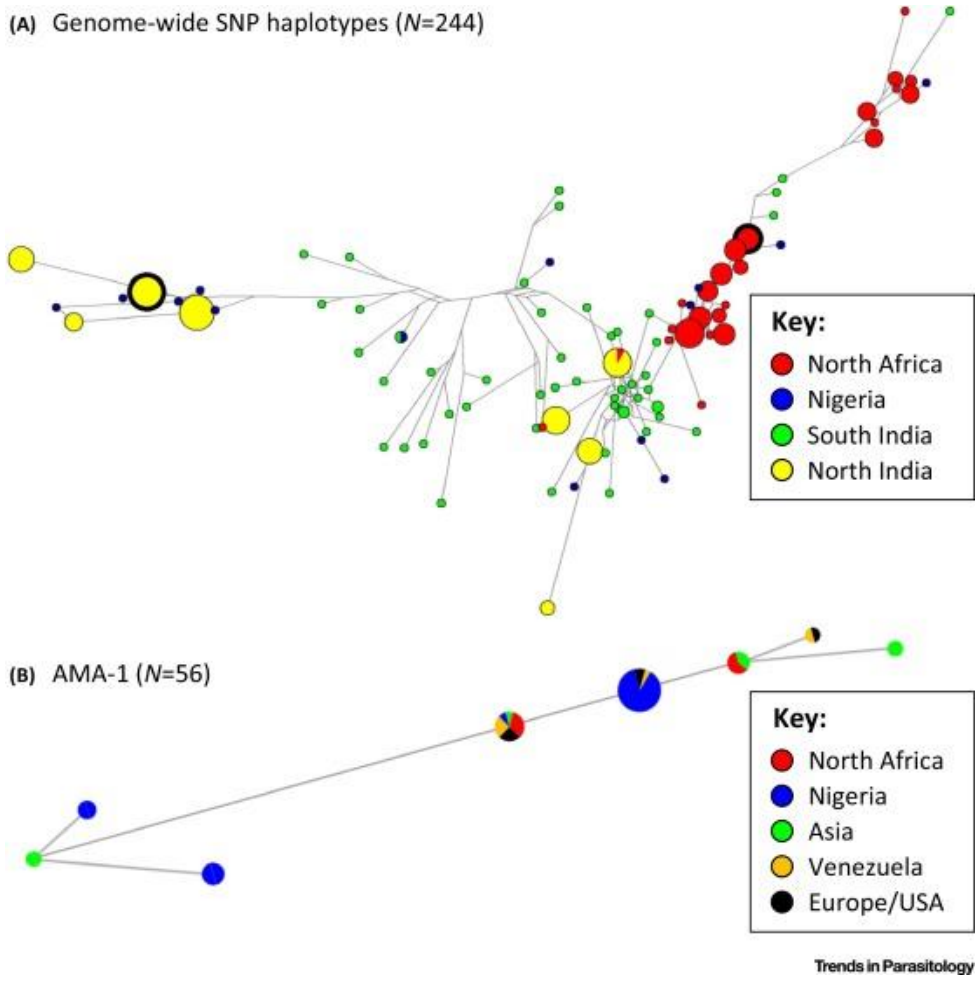

Figure 1. Median-joining phylogenetic NETWORKs illustrating genome-wide and antigen specific diversity reported for *Eimeria tenella*. A. The influence of geographic origin on *E. tenella* single nucleotide polymorphism (SNP) haplotype occurrence and complexity. Parasite populations from Nigeria and south India presented high haplotype diversity and apparent panmixia, compared to more restricted variation in north African and north Indian populations. Node size indicates the frequency of haplotype occurrence. Figure reproduced from [10]. B. Coding sequence polymorphism within the apical membrane antigen 1 (AMA-1) locus. Eight allelic types were detected with less geographic specificity than described for genome haplotypes. Figure derived from data presented in [10].

606 **Table 1. Genome size and available genetic resources for *Eimeria* species in comparison with the apicomplexan species**
607 ***Toxoplasma gondii* and *Plasmodium falciparum*[a].**
608

Species	Host	Genome Size (Mb)	Reference Genome	Mitochondrial Genome	RNASeq	Defined Locus Sequencing	SNP Arrays	Proteomics
Eimeria falciformis	Mouse	43.67	●			●		
Eimeria acervulina	Chicken	45.83	●	●		●		
Eimeria brunetti	Chicken	66.89	●	●		●		
Eimeria maxima	Chicken	45.98	●	●		●		
Eimeria mitis	Chicken	72.24	●	●		●		
Eimeria necatrix	Chicken	55.01	●	●		●		
Eimeria praecox	Chicken	60.08	●	●		●		
Eimeria tenella	Chicken	51.86	●	●	●	●	●	●
Eimeria adenoeides	Turkey	-		●		●		
Eimeria dispersa	Turkey	-		●		●		
Eimeria gallopavonis	Turkey	-		●		●		
Eimeria innocua	Turkey	-		●		●		
Eimeria meleagridis	Turkey	-		●		●		
Eimeria meleagrimitis	Turkey	-		●		●		
Toxoplasma gondii GT1	Cat, others	63.95	●	●	●	●	●	●
Plasmodium falciparum	Mosquito, human	23.3	●	●	●	●	●	●

609 [a]Table adapted from the *Toxoplasma* Genetics Resource *ToxoDB* (http://www.toxodb.org/toxo/showApplication.do, accessed 15th June,

610 2016), supplemented by [80-87].

611 [b]● = sequence resource available.

612

613

Box 1. The utility of SNP genotyping assays in determining parasite population dynamics in the field.

Defined locus sequencing has been widely used to genotype *Eimeria* field isolates since it is cost effective and relatively quick to accomplish. However, with the advent of new high throughput sequencing technologies analysis of genetic diversity across whole genomes is now possible. When whole genome sequencing first became available the associated costs were prohibitive, but this is changing rapidly as the technology becomes cheaper. Reference genome sequence assemblies are now available for the seven *Eimeria* species that infect chickens [9]. Large-scale genome re-sequencing of field isolates will soon be possible but is not yet affordable. In the interim period sequencing a small number of additional strains for comparison with the relevant reference genome provides a resource to design genotyping tools based on specific single nucleotide polymorphisms (SNPs). Custom SNP-based assays are a cost effective method of genotyping parasites which can be applied effectively to large-scale collections of field isolates [10]. SNP genotyping technologies provide useful tools to assess the level of cross-fertilization and genetic recombination in field populations. Such knowledge can be employed to improve the prospects of future subunit vaccines being effective in the field.