1 Introduction – Setting the Scene

Abstract

This phylum of parasitic protozoa is named for the group of organelles they all share, that enable infective stages to enter their host cells. They vary in the risk they pose to economical livestock production, native wildlife survival and public health. Based on their life histories, this review arbitrarily groups them into those with a direct life cycle, those with an intermediate or prey host and those that have an arthropod vector. Evolving imaging technology, augmented by immunological and molecular advances, has seen more than a century of development in the way these parasites are identified and taxonomically classified. The latter two technologies have also enabled the identification of previously unrecognized antigenic proteins, many of which are shared between genera, and some of which hold immunogenic potential, and novel putative targets for management intervention using chemical control.

The Apicomplexa share a complex of organelles that were first recognized by transmission electron microscopy (TEM) (Chapman, 2014). These organelles (micronemes, rhoptry, dense granules and conoid) at the apex of each infective stage (sporozoite, tachyzoite) enable host cell adherence, invasion and colonization by the orderly sequential secretion of their contents (Gaji *et al*., 2021; Koreny *et al*., 2021; Pinto and Vinayak, 2021). The micronemes are secreted first, assisting the parasite to attach firmly to the host cell. The rhoptries are the next to release their contents, assisting the parasite to penetrate the host cell. Dense granule proteins are secreted by the parasite after host cell invasion, and are deemed critical to acquiring nutrients from the host cell (Gaji *et al*., 2021). The genera differ in their configuration of the different elements of the apical complex at different life cycle stages. Some examples are shown schematically in Fig. 1.1 (Koreny *et al*., 2021).

The Apicomplexa are globally prevalent and their taxonomical classification has been in flux for some time, from being a sub-phylum (Sporozoa; producing cysts) containing the Orders Coccidia and Haemosporidia (Soulsby, 1968) to being formally named the Apicomplexa, a sub-phylum containing the Classes Sporozoea (forming cysts or 'spores'), with single ('monoxenous) or multi-host (heteroxenous) life cycles, and Piroplasmea, with ticks as the known vectors (Levine, 1970). By 1987, the Apicomplexa had been elevated to the status of Phylum (Levine, 1988).

They vary greatly in their morphology, host ranges, pathogenicity and modes of transmission. Previously assumed high synteny, conserved or shared genomic loci, between *Neospora caninum* and *Toxoplasma gondii* have since been shown not to exist (Berná *et al*., 2021). Another curiosity not shared by all the Apicomplexa is the apicoplast or plastid, an extrachromosomal genome of 27–35 kb shown to be a remnant plastid or chloroplast genome derived from green algae. It does not have any photosynthetic activity, but plays an essential role in lipid metabolism (Gleeson, 2000; Gaji *et al*., 2021). It remains an essential organelle to those parasites that have retained it (Mitchell, 2008), and is proposed as the site of action for some therapeutic chemicals (Gleeson, 2000; Dirikolu *et al*., 2013). Those same chemicals might, however, also be effective against Apicomplexa without a plastid (Dirikolu *et al*., 2013; Chapter 7, this volume).

Malaria is the best-known human disease the Apicomplexa cause (mosquito-borne *Plasmodium* spp.), but they also cause several economically significant livestock diseases, such as coccidiosis in various hosts, and babesiosis (Red Water, Tick Fever) and East Coast Fever in cattle. In addition,

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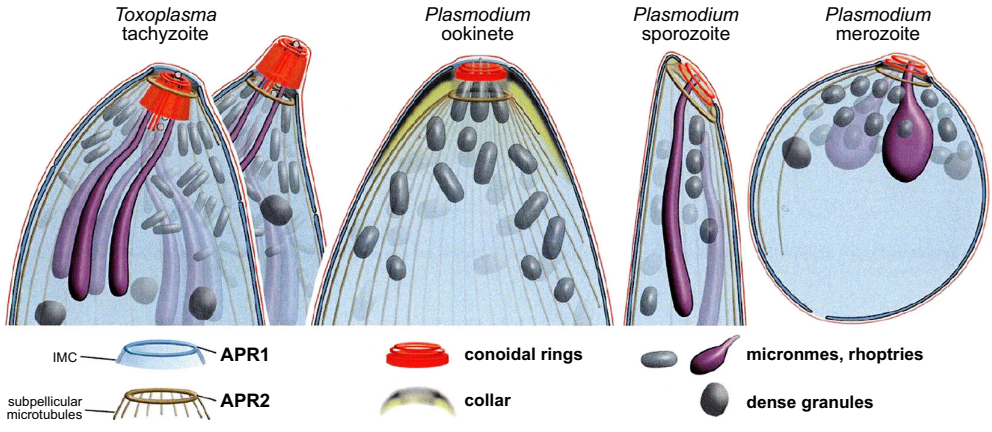

Fig. 1.1. Variations in the configuration and composition of the apical complex between *T. gondii* tachyzoites and different stages of *Plasmodium*. (© 2021 Koreny *et al*., accessed under the terms of the Creative Commons Attribution Licence.)

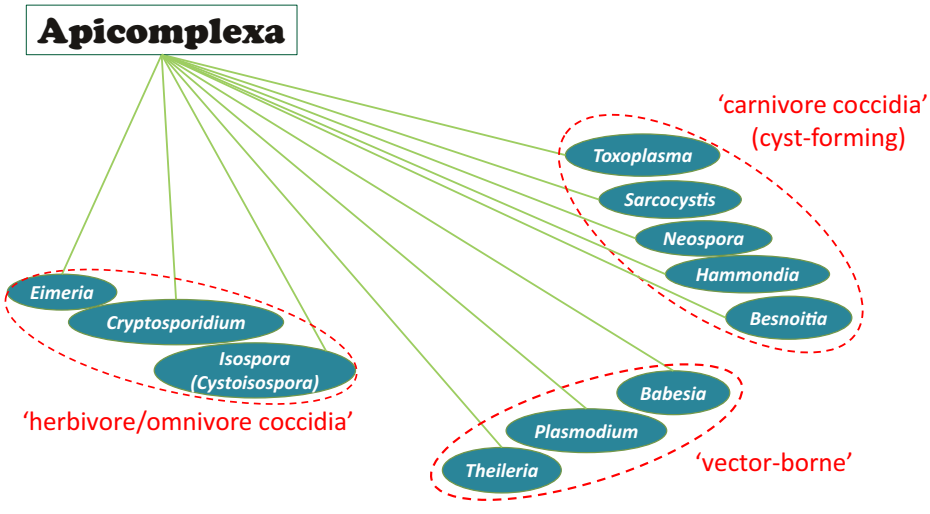

Fig. 1.2. An organizational 'mind map' of the Apicomplexa. (Author's own work, created in Microsoft Powerpoint 2010.)

a number of Apicomplexa are zoonotic, with potentially grave public health consequences. The European Commission recognized the importance of these parasites by supporting the Cooperation in Science and Technology (COST) Action 857 'Apicomplexan Biology in the Post-Genomic Era', which has six working groups (Beck *et al*., 2009).

The diagrammatic conceptual grouping of the genera, representing the similarities and differences between the Apicomplexa, as shown in Fig. 1.2, is followed in the layout of the initial chapters. As far as the available information allows, each parasite is discussed in terms of its life history, epizootiology, pathophysiology, diagnosis/recognition, treatment and prophylaxis. The latter three aspects are dealt with more extensively in separate, subsequent chapters.

Confirmation of the presumptive clinical diagnosis of Apicomplexa infections started out

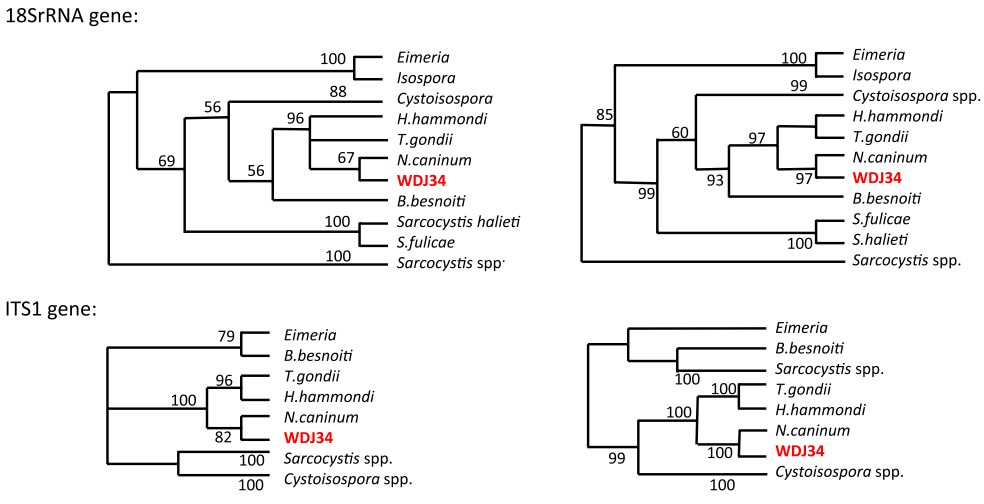

Fig. 1.3. Four dendrograms constructed from two gene sequences from an apicomplexan isolated from an Australian wild dog (WDJ34), using neighbour-joining and Bayesian analyses. (Adapted from Davidson *et al*., 2022.)

with visual recognition of various life cycle stages under a light microscope, with the parasites identified based on their morphology (shape, size), and host and organ preference. It is probably only older parasitologists who will mourn the replacement of beautifully colourful microscopic images in Romanowsky-stained blood films or Haematoxylin & Eosin (H&E)-stained histological sections by the less romantic, but powerful and data-rich, highly technical new methods. Transmission and scanning electron microscopy enabled closer scrutiny of morphological features (Shkap *et al.*, 1988; Mehlhorn *et al.*, 2009), while serological techniques added sophistication, allowing differentiation of species and strains that were morphologically identical (Cortes *et al.*, 2006; Wang *et al.*, 2010). Serology is used for both antigen recognition, e.g. in immunohistochemistry (Dubey and Hamir, 2000; Sánchez *et al.*, 2009; Uzêda *et al.*, 2013), or for demonstrating an immune reaction testifying to a prior (and possibly ongoing) exposure to the parasite (Schares *et al.*, 2010).

The advent of molecular biology meant that nucleic acid extracted from minute specimens could be amplified by techniques such as the polymerase chain reaction (PCR) or isothermal loop amplification (LAMP). This introduced a completely new approach to taxonomy and diagnosis. Molecular biological techniques helped to unravel the phylogeny of genera and species that were morphologically and antigenically indistinguishable (Ellis *et al.*, 1999; Jenkins *et al.*, 1999; Ogedengbe *et al.*, 2016; Ryan *et al.*, 2017). Depending on the portion of the genome which is sequenced, the evolutionary relationships of the genera can be presented schematically in different phylogenetic trees or dendrograms. In Fig. 1.3, the 18S rRNA and ITS1 genes of an isolate of *Neospora caninum* were sequenced, and four dendrograms constructed, using two different analytical methods (Davidson *et al.*, 2022).

Molecular technology does, however, also present the pitfall of misinterpretation of arthropod vector potential, thus contributing to the accumulation of misleading information in the scientific literature. Isolating an organism from a sanguiverous arthropod taken off a host does not of itself justify the conclusion of a vector relationship. A multitude of host–pathogen–vector and abiotic factors needs to be considered (Estrada-Peña *et al.*, 2013).

The term ‘diagnosis’ is used in this volume mainly to refer to identification of the pathogen rather than clinical diagnosis of the disease (Chapter 5).

Although host- and organ-specificity continue to play a role in the taxonomy of the Apicomplexa, certain life cycle stages of the cyst-forming genera (*Besnoitia, Hammondia, Neospora, Sarcocystis, Toxoplasma*) seem to be catholic and opportunistic in their choice of host (Heydorn *et al.*, 1984; Dubey and Hamir, 2000; Cheadle *et al.*, 2001a, b, c; Dubey *et al.*, 2001a, b; Tanhauser *et al.*, 2001; Monteiro *et al.*, 2008; Miller *et al.*, 2010; Reichel, 2013).

Another noteworthy aspect of their life history is the ability of many Apicomplexa to be transmitted vertically, with the clinical outcome of foetal/neonatal death, teratogenesis, inapparent signs, dependent on factors such as the stage of pregnancy when the transmission occurs. Examples of intra-uterine acquisition of the infection have been described for *Neospora*, *Toxoplasma* (Chapter 3), *Babesia* and *Theileria* species (Chapter 4).

The review in this book merely scratches the surface of the abundant scientific literature on research into the Apicomplexa of livestock. It is realistic, rather than cynical, to believe that expected return on monetary investment is the strongest driver of this research. A disease that is seen to pose a bigger threat to profitable production, or to offer a bigger promise of profitable investment in prophylaxis and/or treatment, is likely to benefit from more concerted research efforts. In the case of the Apicomplexa, *Eimeria* infection in intensive poultry production in decades past was a major driver in elucidating the pathophysiology and life cycles of the various species, and in the search for effective and safe anticoccidial chemicals (Johnson and Reid, 1970; Chapman, 1998, 2014). In contrast, infections whose economic impacts are more difficult to quantify are bound to be neglected in the consideration of concerted research efforts. This can be because they mostly occur in extensive production systems (Smith, 1961, Smith, 1962; O'Donoghue and Ford, 1986; Savini *et al.*, 1992; Kirkland *et al.*, 2012; Fordyce *et al.*, 2013; Moloney *et al.*, 2017; Clune *et al.*, 2021) or seem to cause problems only sporadically (Plant *et al.*, 1972, 1974; Nurse and Lenghaus, 1986; Boulton *et al.*, 1995; Atkinson *et al.*, 2000; Kul *et al.*, 2009; Vangeel, 2012) or do not offer a clear pathway to resolution (Plant *et al.*, 1972; Vermunt, 1994; Yildiz *et al.*, 2009; Ryan, 2016; Jacobson *et al.*, 2020).

It is this monetary incentive that gave rise to benefit–cost analyses of surveillance and management of animal diseases, as have been done in Australia (Abdalla *et al.*, 2000; Sackett *et al.*, 2006; Lane *et al.*, 2015; Dal Grande *et al.*, 2021; Shephard *et al.*, 2022) and on a global scale, analogous to similar investigations of the burden of human disease (Rushton *et al.*, 2018; Dieleman *et al.*, 2020). The economic impact of a human disease can be estimated and calculated by various means. Criteria such as cost of disease, illnesses, hospitalizations and mortalities are used, but health-adjusted life years (HALYs) are widely quoted. These are expressed as disability-adjusted life years (DALYs; Kirk *et al.*, 2015) or quality-adjusted life years (QALYs; Batz *et al.*, 2012, 2014; Hoffmann *et al.*, 2012) and used for comparisons of the relative impact of illnesses and in economic analyses (Gold *et al.*, 2002). It is worth noting that the cost of a livestock disease is not solely its impact on animal productivity and survival but also the costs of intervention, made up of vaccines, drugs and the human resources required for mustering and husbandry (Gunn, 2003; Lane *et al.*, 2015).

It will be interesting to see if concerns about the impact of Apicomplexa on public health and human QALYs, and on animal wellbeing, influence future decisions about investment in research into their infections in livestock.

The more complex the life cycle of an apicomplexan parasite, the more apparent opportunities there are for intervention and management of the infection. In the case of the 'herbivore/omnivore coccidia' (Chapter 2), the parasite is either in the host, where it can be reached by chemical or immunological means, or in the environment, where hygiene measures (separation from excreta, disinfection of premises) can be applied. In the case of the 'carnivore coccidia' (Chapter 3), there is a second (the definitive) host to consider for the use of therapeutic chemicals or vaccines, as well as minimizing contact between the definitive host and the susceptible livestock, e.g. by feral animal control. Managing vector-borne (Chapter 4) apicomplexan infections offers an even more diverse multi-pronged

approach. In addition to addressing the parasite in the livestock stage of the life cycle chemically or immunologically, hosts can be bred and selected for resistance to, or immunized against, vector infestation (e.g. indicine vs taurine cattle breeds against ticks, cattle tick vaccines), or the vectors can be addressed directly by chemical or non-chemical means, such as insect traps and biological control.

Prevention of disease by various means of immunization, or by relying on chemicals, has not been universally successful. Vaccines have thus far been relatively crude, whereas the use of chemicals is invariably non-sustainable due to the potential problems of pollution of the environment, contamination of the human food chain, and development of resistance in the apicomplexan parasite or its arthropod vector. In addition to enabling a finer approach to taxonomy, molecular techniques also enable novel approaches to possible pathways to new drug receptors and/or subunit recombinant vaccines (Goodswen *et al.*, 2012, 2013; Sidik *et al.*, 2016; Gaji *et al.*, 2021; Pinto and Vinayak, 2021). Genomic sequencing continues to reveal commonalities in the form of conserved genes encoding for proteins that are shared by more than one genus in the phylum (Ponts *et al.*, 2008; Blake *et al.*, 2011; Koreny *et al.*, 2021; Pinto and Vinayak, 2021). It is tempting to visualize 'cross-pollination' between disciplines (Beck *et al.*, 2009) and the development of vaccines and drugs that are effective against a broad spectrum of apicomplexan parasites.

Prevention of incursion into naïve populations with biosecurity measures such as testing/screening and quarantine is difficult for vector-borne infections and virtually impossible for the more cryptic 'coccidia'. The presence of reservoirs of infection in neighbouring or sympatric wildlife populations, 'sylvatic' reservoirs, poses a constant biosecurity threat to domestic livestock and the risk of conflict between conservation and production interests.

This book attempts to highlight the similarities and differences between the various Apicomplexa infections, to identify those of greatest significance, and to suggest sustainable approaches to better management of their impact on livestock productivity and profitability. Although the outlook is from an Australian livestock perspective, with a southern hemisphere bias, global research is cited, where appropriate, in the context of biosecurity and lessons to be learnt. For example, besnoitiosis seems to be spreading in Europe, but is regarded as an exotic disease in Australia; the 'Muguga cocktail' infect-and-treat management of East Coast Fever in Africa serves as an example of success; the human impact of toxoplasmosis has thus far received more attention abroad than in Australia. Some, but not all, of the Australian research cited in the book was financed by the rural R&D corporation (www.ruralrdc.com.au). Meat & Livestock Australia (www.mla.com.au) is one of 15 rural RDCs, relying on funding from the Government of the Commonwealth of Australia, matched by red-meat producer levies and non-governmental donor contributions. 'Livestock', in the context of this book, refers to domestic animals producing food and fibre for human consumption: cattle, chickens, goats, pigs and sheep, but not camelids, horses or other poultry (turkeys, geese, ducks), which are considered 'minor species'.

Island states such as Australia and New Zealand pride themselves on freedom from many economically devastating livestock diseases but are known to harbour many of the genera of Apicomplexa that infect livestock. Their economic impact on livestock production is, however, at best an estimate and at worst unknown. Attempts to use associations between prevalence and suboptimal productivity as proof of causality or transmission do not stand up to scrutiny (Liénard *et al.*, 2011; Fordyce *et al.*, 2013; Gunn *et al.*, 2016; Ryan, 2016; Lanyon and O'Handley, 2020).

Many of the benefits accruing from knowledge of the molecular biology of parasites that were foreseen at the turn of the century have materialized for the Apicomplexa. Great advances have been made in the biology, diagnosis and identification of parasites, with high levels of sensitivity and specificity. Molecular methods have provided antigens for vaccine screening and identified receptors and enzymes for mechanism-based chemotherapy. DNA vaccines with desirable characteristics, such as sustained stimulation of the host immune system (Prichard and Tait, 2001), may not yet be a reality, but the indications are that it might just be a matter of time.

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