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East Coast fever, a neglected tropical disease with an outdated vaccine approach?

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Since its discovery, bovine theileriosis has caused major socioeconomic losses in sub-Saharan Africa. Acaricide resistance of the intermediate host, paucity of therapeutics, and lack of sufficiently cross-protective vaccines increase the risk of parasite spread due to global warming. Here, we highlight three important areas that require investigation to develop next-generation vaccines.

East Coast fever: a neglected 'tropical' disease with an increasing global burden

The protozoan parasite *Theileria* spp. belongs to the order Piroplasmida within the phylum Apicomplexa. Other widely established apicomplexan parasites include *Plasmodium* spp. and *Toxoplasma gondii*, a zoonotic parasite with increasing global importance. *Theileria parva* causes the most important tick-borne disease of cattle in sub-Saharan Africa, also known as East Coast fever (ECF). From 1999 onwards, about one million cattle died from infection every year, leading to annual economic losses of more than US $300 million[i]. From an estimated 170 million cattle in 12 sub-Saharan countries in 2020, at least 40 million were at risk[ii]. Movement of infected cattle and distribution of the tick vector allow now for a much wider spread of the parasite and introduce the disease to areas where suitable tick habitats are present. As a result, *T. parva* is spreading north into Southern Sudan and westwards into Cameroon. The parasite can therefore be considered a re-emerging pathogen and ECF a neglected tropical disease (NTD). In this article, we highlight three areas (summarised in Figure 1) that should receive more attention and funding.

To treat or not to treat? A potentially irrelevant question – the problem of increasing resistance

The repertoire and effectiveness of control measures for *T. parva* are very limited. They currently include the following. (i) Extensive acaricide treatment of cattle to prevent tick infestation despite concerns regarding their environmental toxicity and increasing resistance in tick populations. (ii) Treatment with the expensive antiprotozoal drug buparvaquone (one dose costs approximately US $10) after infection and occurrence of clinical signs. The availability of both control strategies might be limited unless new therapeutic agents are identified. (iii) ECF prevention currently relies on the 'infection and treatment method' (ITM). It combines the inoculation of live *T. parva* sporozoites and concurrent treatment with long-lasting oxytetracycline. The production of ITM stabilates is costly, difficult to standardise on a large scale, and dependent on a continuous cold chain. Immune protection through ITM-induced cell-mediated immunity can be limited, especially in infections of cattle with buffalo-derived *T. parva*. Unfortunately, ITM represents an example of market failure, which evoked recent efforts of GALVmed to commercialise this product via non-traditional routes. A parasite preparation regularly used for ITM in Kenya, Tanzania, and Malawi contains live sporozoites of three *T. parva* isolates. Despite good efficacy, the production methods and storage requirements make a geographically wider application difficult, and all of these difficulties related to ITM were recently discussed in great detail [1]. In addition, countries with *T. parva* strains other than those present in the vaccine are concerned about introducing additional foreign strains, and are unsure whether or not to register and use the Muguga cocktail. These limitations highlight a need for new technologies to sustain long-term parasite control. Successful stimulation of long-lasting immunity to ECF by ITM provides a strong rationale to develop subunit vaccines as sustainable and scalable alternatives to the live vaccine.

Current experimental vaccine approaches

Several groups have presented new vaccine strategies with well-known parasite-derived antigens to induce either antibody or $CD8^+$ T cell (CTL) responses, including new strategies as to combine the *T. parva* p67 sporozoite surface antigen with nanotechnologies. However, p67 is currently the only identified antigen for the development of an antisporozoite vaccine.

Attempts to generate vaccines that elicit protective T cell responses have focused predominantly on antigens with CTL epitopes. This has been particularly challenging due to the large proteome of *T. parva*, with more than 4000 predicted open reading frames (ORFs), and the role of highly diverse bovine MHC class I molecules in restricting the epitopes presented. This complexity is compounded by the high level of nucleotide and amino acid diversity in field populations of *T. parva* for some of these antigenic proteins and the role of sexual recombination of the parasite. This parasite diversity needs to be considered when designing new (subunit) vaccines by including multiple antigens. The focus on antibody and/or CTL responses as drivers for protection against *T. parva* was accompanied by insufficient attention to other components of the immune system in past studies.

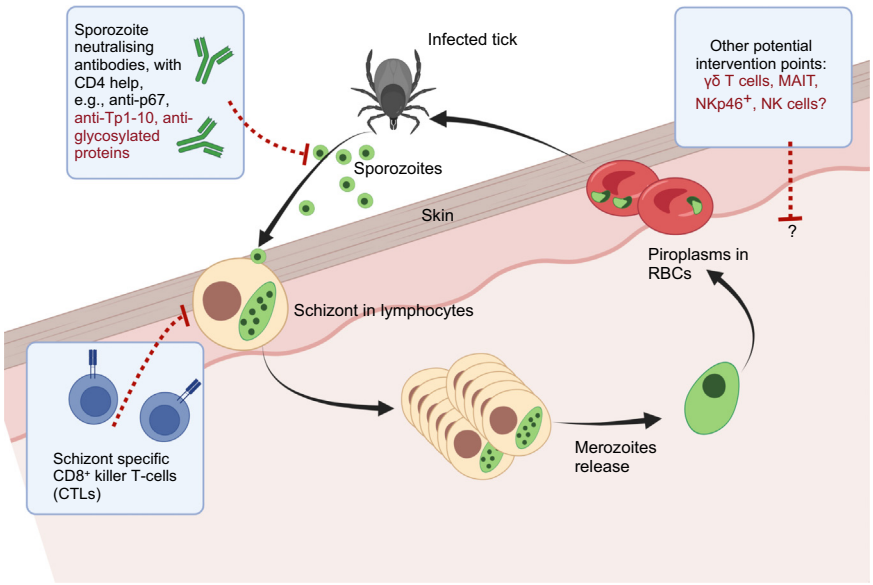

Figure 1. Summary of *Theileria parva* life cycle in cattle, indicating the different developmental stages and the immune response to them. Boxes next to specific developmental stages summarise the main points of the current article. For the indicated stages, the described knowledge gaps are written in red whereas existing knowledge is written in black. Figure created with BioRender (www.biorender.com). Abbreviations: CTL, $CD8^+$ T cell; MAIT, mucosal-associated invariant T cells; NK, natural killer cells; RBCs, red blood cells.

Possibilities for new *Theileria* vaccine strategies

In the following paragraphs we take the aforementioned new possibilities for vaccine design into account and suggest additional aspects that should be investigated to expand our current understanding of the biology of *T. parva* and ultimately develop suitable subunit vaccines.

Understanding host cell invasion

Host cell invasion by *Theileria* sporozoites is poorly understood at the molecular level and differs from mechanisms used by other Apicomplexans. It is described as 'zippering' from parasite and host cell membranes and involves unknown molecules on either side. Many other Apicomplexans utilise glycosylated proteins/lipids in host cell attachment and invasion [2,3]. These interactions are mainly established through O-linked glycans, but several C-type lectin receptors also recognise specific N-glycans [4]. Until recently, *T. parva* was thought to lack the capacity for glycosylation; however, its latest genome annotation and RNAseq analysis highlighted three glycosyltransferases that are usually involved in N-glycosylation [5]. If a functional oligosaccharyltransferase is also identified, identification of putative glycolipids/glycoproteins involved in the 'zippering' process could lead to the discovery of new vaccine targets. Such putative glycosylated antigens could aid blocking of the initial stage of host cell invasion by *T. parva* sporozoites and ultimately provide promising vaccine candidates for ECF control.

Insufficient understanding of *Theileria* protein conformation and function

Two recent transcriptomics/proteomics studies of *T. parva* identified nearly 50% of the total predicted parasite proteome, including new potential vaccine antigens [6,7]. These proteins are predicted to be orthologs of *Plasmodium falciparum* sporozoite surface molecules and invasion organelle proteins as well as proteins that may contribute to lymphocyte transformation [6]. Nevertheless, a large proportion of the proteins identified in these studies, and predicted in the latest reannotation of the *T. parva* genome [5] with RNAseq data from *T. parva* schizonts, retained a 'hypothetical/predicted' designation. Sequence comparisons against every available genome failed to highlight orthologs or homologs. A comprehensive, accurate annotation of the parasite genome aims to facilitate the identification of (i) expressed proteins, (ii) their function based on sequence and/or structural homologies, and (iii) novel (chemo)therapeutic targets for disease treatment/vaccine design. This has so far been unsuccessful for many *T. parva* proteins. Additional resources, such as the AlphaFold2 server, have not yet led to confident modelling/structural analysis of many *T. parva* proteins. However, the combination of genetic approaches with additional techniques – such as liquid chromatography–tandem mass spectrometry (LC-MS/MS), matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) analyses or biophysical methods – could fill some of these knowledge gaps and elucidate the structures and functions of *T. parva* proteins. This could be followed by the use of bioinformatic tools such as MADOKA or DALI to subsequently infer protein function. Continuous development of novel techniques and algorithms creates new opportunities to expand our understanding of *T. parva* proteomics. This could also aid the development of live-attenuated parasite vaccines using the CRISPR-Cas approach and refine our knowledge of suitable target antigens.

The role of other components of the immune system in protection against *T. parva*

The identification of new parasitic antigens should be complemented by an analysis of additional arms of the immune response against *T. parva*. Anti-p67 neutralising antibodies and CTLs against schizont

antigens are inducible by recombinant vaccines but do not confer comprehensive disease protection [8,9]. Stimulation of either antibody- or T cell-mediated immunity is unlikely to recapitulate the complexity of the immune response against the pathogen. The focus on antibody or CTL antigens as vaccine candidates has excluded assessment of the role that $CD4^+$ T cells, γδ T cells, innate-like T cell subsets such as mucosal-associated invariant T (MAIT) cells, $NKp46^+$ T cells, and natural killer (NK) cells could play in an effective immune response post-vaccination.

Although Taracha and colleagues identified how antigen-specific $CD4^+$ T cells promote CTL function in the *Theileria* model, $CD4^+$ T cell antigens have not been specifically included in vaccines that were evaluated to date [15]. Morrison and colleagues have recently provided the first description of *T. parva* proteins with $CD4^+$ T cell epitopes, which should feed into the antigen composition of future vaccines [10]. Similarly, γδ T cells recognise and lyse *T. parva*-infected cells [11], yet their role in *T. parva* immunity is unclear. Recent work demonstrates the role of human γδ T cells in response to malaria [12] and highlights it as promising. As young cattle have a large proportion of γδ T cells, it makes their role in ECF immunity even more interesting, specifically taking the various immunological functions into account [13,14]. A major advantage of γδ T cells is their independence of MHC I/MHC II presentation [11]. Similarly, other 'innate-like' immune cell subsets are unlikely to be MHC-restricted. They might polarise T cell responses post-vaccination and/or contribute directly to anti-*Theileria* immunity. Such cells include the novel $NKp46^+CD3^+$ subset of T cells, MAIT cells, and NK cells. A 'holistic' vaccine approach that targets these cell subsets to trigger a comprehensive immune response remains unexplored in *T. parva* and offers yet untested opportunities.

Concluding remarks

Decades of *T. parva* research have provided a wealth of information on the pathogen and the host immune response towards it – however, the search for an effective next-generation vaccine has to continue as resistance to acaricides, rising costs for current drugs, and limitations of the ITM regimen significantly increase the urgent need for disease protection.

Novel insights into the biology of the cattle host and the pathogen, as well as incorporating new technologies, will enable novel strategies towards identifying an affordable, stable, and effective vaccine. However, two fundamental questions still need to be addressed: (i) what ultimately stimulates protective immunity towards *T. parva in vivo,* and (ii) which antigens need to be included in a vaccine to accommodate the genetic complexity of both parasite and cattle and confer protection under field conditions?

Immune cell subsets other than CTLs might hold the answer to these questions and are worthy of detailed analyses. Similarly, unconventional ligands, such as glycans and carbohydrate-based molecules, could play an important role in host cell infection or transformation and be crucial for vaccine design. Close collaboration of *Theileria* experts with other members of the scientific community will add a broad range of expertise, innovative approaches to vaccine development, and therefore strongly benefit the successful control of ECF.

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Declaration of interests

The authors have no competing interests to declare.

Resources

[i]www.aciar.gov.au/publication/technical-publications/economic-impact-ticks-and-tick-borne-diseases-livestock-africa-asia-and-australia

[ii]www.fao.org/faostat/en/#data/QCL

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