In silico identification of Theileria parva surface proteins

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ARTICLE INFO
Keywords:
Apicomplexa
Theileria
East Coast Fever
Parasitology

ABSTRACT

East Coast Fever is a devastating African cattle disease caused by the apicomplexan parasite, Theileria parva. Little is known about the cell surface, and few proteins have been identified. Here, we take an in silico approach to identify novel cell surface proteins, and predict the structure of four key proteins.

Introduction

Theileria parva is the causative agent of East Coast Fever (ECF) a lethal, tick-borne disease of cattle in sub-Saharan Africa. T. parva is an apicomplexan parasite, closely related to Plasmodium, the causative agent of malaria. Mortality levels vary from 3 to 80% depending on parasite strain and cattle breed, killing over one million cattle each year (Nene et al., 2016). The only drug licenced to treat T. parva is buparvaquone, which is over 30 years old. Although there is no reported resistance to buparvaquone in T. parva, there is rising levels of resistance in Theileria annulata, a related cattle pathogen (Mhadhbi et al., 2015).

The main mechanism for control is cattle dipping and vaccination. However, the current vaccination model is an ‘infection and treat’ model. Large numbers of infected ticks are produced, ground up, and frozen. The tick residue is transported in liquid nitrogen and injected to the cattle to be vaccinated. As this causes disease, the cattle are then vaccinated with high dose of tetracycline to prevent the infection taking hold (MacGregor et al., 2021). This model of vaccination has significant drawbacks:

- the transport of the vaccine in liquid nitrogen is impractical in the context of rural Africa,
- it requires the use of high levels of antibiotics, thus driving the rise of antibiotic resistance,
- it requires the presence of a veterinarian, which significantly increases costs,
- regional vaccines are needed

vaccinated cattle remain lifelong asymptomatic carriers of disease and cannot be exported.

A modern vaccine is urgently required (Nene and Morrison, 2016).

As with many eukaryotic parasites, T. parva differentiate between a series of different life-stages, each characterised by distinct morphologies and patterns of gene expression. These include distinct protein composition of the parasite cell surface between life-stages. As putative vaccine targets there are two key life-stages of interest: (i) the free-living sporozoite stage, where the parasite is released from the tick’s salivary glands and passes into the cattle bloodstream, and (ii) the schizont stage, where T. parva causes cancer-like cell proliferation within the cattle lymphocytes. An ideal vaccine would target the sporozoite and/or schizont stages, giving rise to a CD4 and CD8 T cell immune response (Morrison et al., 2021).

Previous works have used in silico analysis of gene sequences from the T. parva Mugaga strain genome and proteome to identify putative surface proteins, containing both a signal peptide and putative GPI-anchor addition sequence (Nyagwange et al., 2018a). These can be considered as highly likely to reside on the cell surface, and not internal membranes, as utilization of a GPI-anchor for membrane attachment is a surface-specific feature. Here, we sought to identify a longer candidate list of putative surface proteins, using a similar theoretical approach, but to instead identify proteins that are attached to the plasma membrane with one or more transmembrane domains.

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https://doi.org/10.1016/j.tcsw.2022.100078
Received 23 February 2022; Received in revised form 19 May 2022; Accepted 20 May 2022
Available online 21 May 2022
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or apicoplast (with bipartide targeting) using TargetP, Fig. 1 (Armen pathway) and so not exposed to the external environment. We therefore removed all proteins that were predicted to localise to the mitochondria inevitably include proteins that are internal (e.g. in the endosomal peptide and a transmembrane domain were identified, Fig. 1.

For all proteins with a putative signal peptide using SignalP 5.0 (Almagro pathway) and so not exposed to the external environment. We therefore removed all proteins that were predicted to localise to the mitochondrial pathways.

Putative membrane proteins. Shortlist of putative membrane proteins with signal peptide, with experimental evidence for expression in *Theileria parva* sporozoites. *Sporozoite protome: figure given is emPAI, a measure of the relative protein abundance in sample.

| Gene name               | Gene ID       | Product name                                      | Number of TM domains | TMs wo/signal peptide | Size/ kDa | Sporozoite protome |
|-------------------------|---------------|--------------------------------------------------|----------------------|-----------------------|-----------|-------------------|
| TpMuguga_01g0016        | XP_765543.1   | Vacular protein sorting/targeting protein 10   | 1                    | 1                     | 96        | 0.71              |
| TpMuguga_01g00326       | XP_765853.1   | emp24/gp25L/p24 family/GOLD family protein      | 1                    | 1                     | 30        | 1.2               |
| TpMuguga_01g00509       | XP_766029.1   | putative integral membrane protein              | 1                    | 1                     | 66        | 1.69              |
| TpMuguga_01g00620       | XP_766141.1   | putative integral membrane protein              | 1                    | 0                     | 124       | 0.16              |
| TpMuguga_01g00921       |              | HAD ATPase P-type IC family protein             | 11                   | 12                    | 166       | 0.91              |
| TpMuguga_01g00939       | XP_766460.1   | putative integral membrane protein              | 1                    | 0                     | 36        | 3.65              |
| TpMuguga_01g011013       | XP_766534.1   | Sugar efflux transporter for intercellular exchange family protein | 7                    | 7                     | 42        | 0.4               |
| TpMuguga_01g01013       | XP_766590.1   | Sugar (and other) transporter family protein     | 12                   | 11                    | 52        | 0.43              |
| TpMuguga_01g01091       | XP_766612.1   | Tp12                                             | 1                    | 0                     | 64        | 12.02             |
| TpMuguga_01g01193       | XP_766716.1   | emp24/gp25L/p24 family/GOLD family protein      | 2                    | 1                     | 24        | 1.15              |
| TpMuguga_02g00330       | XP_764896.1   | emp24/gp25L/p24 family/GOLD family protein      | 1                    | 1                     | 24        | 1.15              |
| TpMuguga_02g00538       | XP_765104.1   | Sel1 repeat family protein                      | 1                    | 1                     | 177       | 1.34              |
| TpMuguga_02g00543       | XP_765109.1   | putative integral membrane protein              | 3                    | 3                     | 54        | 0.3               |
| TpMuguga_02g00602       | XP_765168.1   | Thiorodoxin family protein                      | 1                    | 1                     | 25        | 19.43             |
| TpMuguga_02g00655       |              | putative integral membrane protein              | 1                    | 0                     | 117       | 4.39              |
| TpMuguga_03g00068       | XP_763186.1   | putative integral membrane protein              | 1                    | 1                     | 269       | 4.18              |
| TpMuguga_03g00175       | XP_763193.1   | putative integral membrane protein              | 2                    | 1                     | 36        | 2.68              |
| TpMuguga_03g00264       | XP_763282.1   | Si/P1 Nuclease family protein                   | 2                    | 2                     | 45        | 1.05              |
| TpMuguga_03g00419       | XP_763440.1   | Thiorodoxin family protein                      | 1                    | 0                     | 62        | 33.82             |
| TpMuguga_04g00068       | XP_763703.1   | putative integral membrane protein              | 1                    | 0                     | 141       | 1.32              |
| TpMuguga_04g00399       | XP_764034.1   | putative integral membrane protein              | 1                    | 1                     | 27        | 1.39              |
| TpMuguga_04g00649       | XP_764284.1   | Gpi transamidase subunit Gpi16                  | 1                    | 1                     | 41        | 7.78              |
| TpMuguga_04g00668       | XP_764304.1   | unspecified product                              | 1                    | 1                     | 46        | 0.83              |
| TpMuguga_04g00917       | XP_764554.1   | SVSP family protein                              | 1                    | 0                     | 60        | 0.48              |

Methodology

The *T. parva* Mugaga strain genome (causative agent of ECF) (Hayashida et al., 2013; Tretina et al., 2020) was computationally screened for all proteins with a putative signal peptide using SignalP 5.0 (Almagro Armenteros et al., 2019) plus one or more transmembrane domain using HHMmer (Finn et al., 2011). 91 protein-encoding genes with a signal peptide and a transmembrane domain were identified, Fig. 1.

Inclusion of proteins that have putative transmembrane domains will inevitably include proteins that are internal (e.g. in the endosomal pathways) and so not exposed to the external environment. We therefore removed all proteins that were predicted to localise to the mitochondria or apicoplast (with bipartide targeting) using TargetP, Fig. 1 (Armenteros et al., 2019). Proteins with a putative GPI-anchor addition sequence were also excluded as these have been considered previously (Nyagwange et al., 2018a; Pierleoni et al., 2008). This led to a final long-list of 68 proteins, shown in Supplementary Table 1. These represent a maximal list of proteins of interest.

Next, we looked for proteins with evidence of expression in the sporozoite. A whole-cell proteomic dataset is available for the *T. parva* sporozoite stage, providing evidence for 2007 proteins, representing about 50% of the total predicted genes (Nyagwange et al., 2018b). Of the 68 proteins of interest (Suppl. Table 1), a total of 30 were present in the proteome dataset, Table 1.

To further refine our search, we removed all proteins predicted to contain a single transmembrane domain where it is located within the first 30 aa; these most likely represent false positive results, where the signal peptide domain is incorrectly identified as containing a transmembrane domain. However, we retained TpMuguga_01g01091 (Tp12), a known antigen (Morrison et al., 2015).

Following analysis of localization and function of homologous proteins in other species, and selection for proteins with the largest predicted external size, we selected the five most promising candidates, plus Tp12 (TpMuguga_01g01091). For each protein, we removed the signal peptide and predicted the structure using Phyre2, Swiss-model and AlphaFold (Jumper et al., 2021; Kelley et al., 2015; Waterhouse et al., 2018). The results are shown in Table 2. TpMuguga_03g00168 was too large for AlphaFold, giving rise to no hits in Phyre2 and no hits in Swiss-model. Modelling of TpMuguga_01g01091 (Tp12) gave rise to a disordered protein with little confidence in AlphaFold. The four protein structures that were able to be modelled are shown in Fig. 2.

Two proteins (TpMuguga_01g01013 and TpMuguga_01g00169) are predicted sugar transporters, and TpMuguga_01g00921 is predicted to be a cation transporting ATPase. The remaining membrane protein TpMuguga_03g00168 has unknown function. All four have external facing regions that may offer target epitopes for novel vaccines. To test this, we utilized the epitope prediction tool NetMHC 4.0 which predicts peptide-MHC Class I binding for known proteins (Jurgutis et al., 2017; Nielsen et al., 2018). Each of the four proteins were analyzed for the bovine BoLA-T2a allele, with peptide length between 8 and 14 aa. The results are shown in Table 3, giving the numbers of peptides identified as strong or weak binders. These indicate that these proteins are highly likely to contain epitopes that will be candidates for vaccination.

As many cell surface proteins contain post-translational modifications, we used bioinformatic tools to search for glycosylation (Steenbock et al., 2013) and phosphorylation sites (Wang et al., 2020), as shown in
Table 2. Short list of the six most promising membrane proteins. The results of structure prediction by AlphaFold, Phyre2 and Swiss-model are shown, together with the final model choice for modelling in Fig. 2.

| Long list of IDs | Predicted mature protein (aa#) | AlphaFold2 using ColabFold (aa#) | Average pLDDT | Potential mature | Region (aa#) | Phyre2 PDB | Swissmodel Region (aa#) | Final model | Region (aa#) | Region top hit Function top hit |
|------------------|--------------------------------|----------------------------------|---------------|----------------|--------------|-----------|----------------------|-------------|--------------|-----------------------------|
| TpMuguga_01g00921 | 18-450 not performed | – | – | – | – | – | – | – | – | – |
| TpMuguga_01g01013 | 16-379 not performed | 20-474 | 67.9 | 90.7 | 16-379 | 20-474 | 5XPD (0.38) | no model | no model | no model |
| TpMuguga_01g01069 | 144-356 | 14-453 | 61.5 | 61.5 | – | – | – | – | – | – |
| TpMuguga_02g00543 | 23-457 | – | – | – | – | – | – | – | – | – |
| TpMuguga_03g00168 | 236-1289 not performed | – | – | – | – | – | – | – | – | – |
| TpMuguga_03g010091 | 21-3568 | 19-472 | 56.3 | 61.5 | – | – | – | – | – | – |

Table 3. These indicated that all four proteins are glycosylated, while two of the four also contain phosphorylation sites. It should be noted that the training datasets for post-transcriptional modification prediction software were not obtained from *T. parva*, so these results will need to be validated experimentally.

Conclusions

Recent advances in *Plasmodium* cell biology have identified numerous surface proteins which could be part of a future multi-valent subunit vaccine against malaria. In contrast, very few surface proteins have been characterized in *T. parva*. However, many surface proteins that have been identified give rise to neutralizing antibodies, suggesting that they would be potential vaccine targets (Musoke et al., 1992; Nyagwange et al., 2018).

In the absence of a proteome-based experimental approach to identify further surface proteins, a recent *in silico* study identified 21 putative GPI-anchored surface proteins (Nyagwange et al., 2018). Of the six expressed GPI anchored surface proteins, four gave rise to sporozoite neutralizing antibodies.

Here, we took an *in silico* approach to identify transmembrane domain proteins on the *T. parva* cell surface, thus further increasing the numbers of putative surface proteins for future vaccinology attempts. Of the six most promising candidate proteins, three (TpMuguga_01g01013, TpMuguga_01g00921 and TpMuguga_01g01069) are likely to be involved in nutrient or cation uptake. This is unsurprising, as the parasite must interact with the environment.

Two of these proteins (TpMuguga_01g00921 and TpMuguga_01g01069) also have high levels of expression in the schizont stage, suggesting that in addition, they play a role in nutrient acquisition during this intracellular stage (Tonui et al., 2018). The schizont stage must have very high requirement for glucose, due to the rapid rate of cell proliferation. In *Plasmodium*, chemical inhibition of a key hexose transporter suppresses the growth of the parasite (Jiang et al., 2020). As TpMuguga_01g01069 is the only sugar transporter predicted to have a signal peptide and thus a surface localization in *T. parva*, it may also be a good drug target. A fourth protein (TpMuguga_03g00168) is a clear membrane protein with no predicted function. An experimental based approach will be required to obtain structures of TpMuguga_03g00168 and TpMuguga_01g01091 (Tp12).

Epitope prediction software suggests that these four proteins may be immunogenic, with predicted strong binding peptides by NetMHC (Jurtz et al., 2017). Although laboratory-based methodologies will be required to test these predictions, the results suggest that these proteins have potential as vaccine targets.

One of the challenges of *T. parva* research is the absence of a system for stable genetic manipulation, so it is not possible to confirm if these four proteins are essential, nor confirm the localization of proteins with tagging. An alternative approach would be to take a whole-cell spatial proteomic localization technique which would give localization information for all cellular proteins (Lundberg and Borner, 2019). An analysis of the related apicomplexan parasite *Toxoplasma* using hyperLOPIT (hyperplexed localisation of organelle proteins by isotope tagging) identified 110 integral surface proteins and 71 peripheral surface proteins (Barylyuk et al., 2020). A similar proteomic-based approach of *T. parva* would transform our understanding of this parasite, especially at the cell surface, and provide a massive leap forward in the quest to develop a modern *T. parva* vaccine.

NG was funded by a University of Nottingham Developing Solutions Masters Scholarship and by the J N Tata endowment, India.

CRediT authorship contribution statement

Nitisha Gurav: Methodology, Investigation. Olivia J.S. Macleod: Methodology, Investigation. Paula MacGregor: Conceptualization, Methodology, Writing – review & editing. R. Ellen R. Nisbet:
Table 3
Epitope and post-transcriptional modification. Epitope prediction was carried out using NetMIC 4.0, with the bovine BoLA-T2α antigen. The number of strong binding (SB) epitopes (with a rating < 0.5) and weak binding (WB) epitopes (with a rating < 2) is given for each protein. Identification of putative glycosylation was carried out using NetOGlyce 4.0, and phosphorylation sites by MustiDeep.

| Protein  | SB  | WB  | glycosylation sites | phosphorylation Sites |
|----------|-----|-----|---------------------|-----------------------|
| Tp01g00921 | 43  | 206 | 5                   | 27                    |
| Tp01g01013 | 7   | 37  | 21                  | 0                     |
| Tp02g00543 | 20  | 53  | 7                   | 7                     |
| Tp01g10169 | 13  | 51  | 1                   | 0                     |

Conceptualization, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tcsw.2022.100078.

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Fig. 2. Predicted protein structures. 01g00921 encodes a cation transporter ATPase (red; Phyre2 model shown), 01g01013 encodes a sugar transporter (green; AlphaFold2 model shown), the function of 02g00543 is unknown (orange) and 01g10169 encodes a sugar transporter (purple, modelled with AlphaFold2).
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