International Journal of Genetics and Molecular Biology Vol. 2(5), pp. 72-77, May 2010
Available online at http://www.academicjournals.org/IJGMB
ISSN 2006-9863© 2010 Academic Journals

Full Length Research Paper

Theileria parva genome and CTL candidate vaccine antigens: An overview

Nyerhovwo J. Tonukari

Department of Biochemistry, Delta State University, Abraka, Nigeria. E-mail: tonukari@gmail.com.

Accepted 12 May, 2010

The availability of complete genome sequences for a variety of organisms, coupled with genome-wide analyses that allow evaluation of the functions of thousands of genes in parallel, have the potential to greatly impact on cell biology research. The lymphocyte-transforming *Theileria parva* parasite nuclear genome (8.3 Mbp in length consisting of four chromosomes) available in GenBank reveals genes with strong similarity to other known apicomplexan homologs. Mining of sequence data has proved useful in the search for candidate vaccine antigens. Genome mining and cDNA library screening identified six antigens targeted by CD8 T cells from *T. parva*-immune cattle of different major histocompatibility complex (MHC) genotypes. These antigens are being evaluated for the development of a subunit vaccine.

Key words: *Theileria parva*, apicomplexan homologs, genome mining, cDNA library screening.

INTRODUCTION

The protozoan parasite *Theileria parva* is transmitted by *Rhipicephalus* ticks and causes an often fatal lymphoproliferative disease of cattle known as East Coast fever (ECF). The apicomplexan pathogen causes a severe lymphoproliferative disease of cattle in eastern, central, and Southern Africa (Katzer et al., 2006) leading to economic losses among smallholder farmers in Africa. It is an intracellular parasite that infects and transforms bovine lymphocytes. This disease, which kills over 1 million cattle each year in sub-Saharan Africa, results in economic losses exceeding $200 million annually (Norval et al., 1992). *T. parva*-induced T-cell transformation is the predominant mechanism underlying the pathogenesis of ECF. Upon invasion by the parasite, T cells undergo lymphoblastoid transformation (Brown et al., 1973), become independent of antigen receptor stimulation (Baldwin and Teale, 1987; Galley et al., 1997) and cease to require exogenous growth factors to proliferate (Dobbelaere et al., 1988; Brown and Logan, 1986; Heussler et al., 1999).

Efforts to control ECF are largely based on the use of acaricides to prevent infestation with infected ticks, but this approach is increasingly being compromised by the emergence of acaricide resistance in the vector tick populations. Although drugs are available to treat the disease, these are expensive and require an early diagnosis to be effective. Immunization using the live vaccine endangers solid immunity to homologous and occasionally, heterologous challenge (Radley et al., 1975). However, the high production costs coupled with the requirements for cold storage and skilled handling made this vaccine inaccessible to most poor livestock farmers who need it most. In spite of this, the demand for this vaccine is on the increase suggesting that an efficacious, safe, inexpensive and easy-to-deliver improved recombinant subunit vaccine would be readily adopted (Randolph, 2008).

The parasite has a complex life cycle (Mehlhorn and Schein, 1988), involving obligate developmental stages in mammalian and vector hosts (Figure 1). Cattle become infected by inoculation of sporozoite forms in the tick saliva. These invade lymphocytes and differentiate to multinucleate schizonts, which drive the cell into a state of continuous proliferation and divide with it, ensuring

Abbreviations: ECF, East coast fever; **MHC,** major histocompatibility complex; **MPSS,** massively parallel signature sequencing; **ORFs,** open reading frames; **MVA,** modified vaccinia virus Ankara; **vCP,** canarypox virus; **IGRs,** intergenic regions; **PCR-RFLP,** polymerase chain reaction-restriction fragment length polymorphism; **MPSS,** massively parallel signature sequencing; **BoLA,** bovine leukocyte antigen; **CTL,** cytotoxic T lymphocyte.

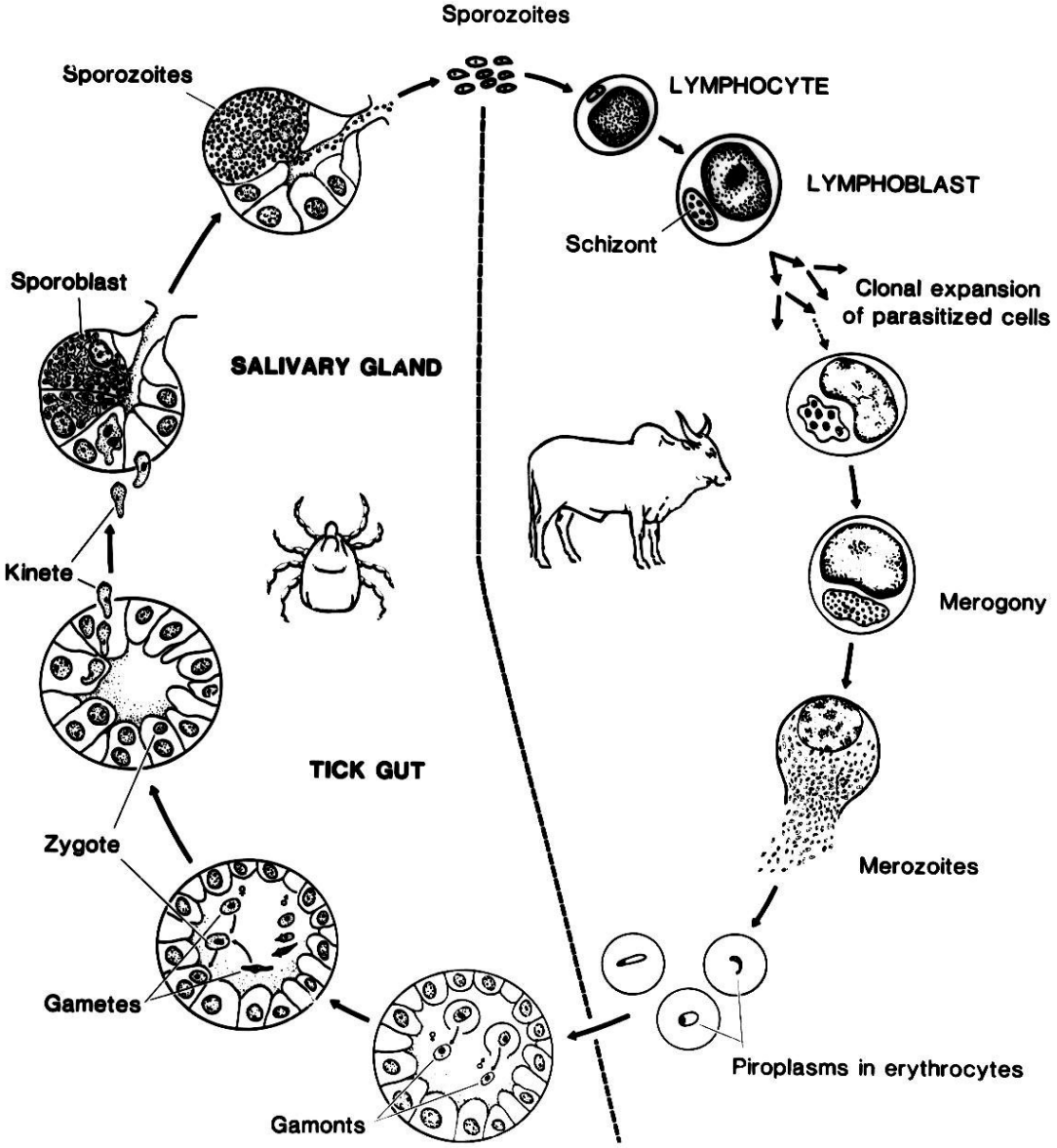

Figure 1. Life cycle of *T. parva.*
Source: Norval et al. (1992).

transmission of infection to each daughter cell. In a proportion of infected cells, schizonts undergo further differentiation to mononucleate merozoites; these are released from the dying cell and invade erythrocytes, where they develop into tick-infective piroplasm forms. Upon ingestion by a feeding tick, these are released into the gut lumen and give rise to macro and micro gametes, which undergo syngamy to form diploid zygotes. After invading gut epithelial cells, zygotes undergo reduction division to yield kinete forms, which access the hemocoel and migrate to the salivary gland, where they invade cells of type III acini. The parasite then undergoes a process of sporogony to produce cattle-infective sporozoites. The parasite therefore adopts a strategy whereby expansion is accomplished through asexual division, with an exponential phase in the case of the schizont, while genetic exchange is accommodated through a sexual phase in the tick (Neitz, 1957; Katzer et al., 2006).

Theileria parva genome

The availability of complete genome sequences for a variety of organisms, coupled with novel approaches that allow evaluation of the functions of thousands of genes in parallel, have the potential to greatly impact on cell biology research (Martin and Drubin, 2003). The increasing number of complete and nearly complete genome

sequences provides a significant amount of material for large-scale comparative genomic analysis (Ureta-Vidal et al., 2003). Genome-wide analysis is a global and systematic investigation of a gene(s) expression under specific condition(s) or the scanning of a genomic sequence for particular genes, patterns, motifs and elements using bioinformatics tools. The genome of *T. parva* was sequenced in order to facilitate research on parasite biology, assist the identification of schizont antigens for vaccine development (Malcolm et al., 2005; Graham et al., 2006), and extend comparative apicomplexan genomics, in particular with *Plasmodium falciparum*, which causes malaria.

The haploid *T. parva* nuclear genome consists of 4 chromosomes and a molecular size of 8,308,027 bp. Its total G + C content is 34.1% and the number of protein encoding genes is 4035 with a mean gene length of 1407 bp (Gardner et al., 2005). The authors also sequenced the parasite apicoplast and mitochondrial (Kairo et al., 1994) genomes. The *T. parva* chromosomes contain one extremely A + T-rich region (> 97%) about 3 kbp in length that may be the centromere. The telomeric repeats are short. The *T. parva* nuclear genome contains about 4035 protein-encoding genes, 20% fewer than *P. falciparum*, but exhibits higher gene density, a greater proportion of genes with introns and shorter intergenic regions (Gardner et al., 2005).

Guo and Silva (2008) also analyzed the evolution of non-coding sequences in the *Theileria* genome and identified conserved sequence elements that may be involved in gene regulation of these parasitic species. They reported that intergenic regions and introns in *Theileria* are short, and their length distributions are considerably right-skewed. Intergenic regions flanked by genes in 5' - 3' orientation tend to be longer and slightly more AT-rich than those flanked by two stop codons; intergenic regions flanked by genes in 3' - 5' orientation have intermediate values of length and AT composition. Intron position is negatively correlated with intron length and positively correlated with GC content.

Genome-wide searches for functional elements revealed several conserved motifs in intergenic regions of *Theileria* genomes. Two such motifs preferentially located within the first 60 base pairs upstream of the transcription start sites in *T. parva*, are preferentially associated with specific protein functional categories, and have significant similarity to known regulatory motifs in other species. These results suggest that these two motifs are likely to represent transcription factor binding sites in *Theileria*. These researchers concluded that *Theileria* genomes are highly compact, with selection seemingly favouring short introns and intergenic regions.

Three over-represented sequence motifs were independently identified in intergenic regions of both *Theileria* species, and the evidence suggests that at least two of them play a role in transcriptional control in *T. parva* (Guo and Silva, 2008). The highly compact genome of *Theileria* seems to result from selection pressure for small introns and intergenic regions (IGRs). Much like in other apicomplexan genomes, classical eukaryotic promoter elements have not been found in *Theileria;* genome-wide *de novo* searches identified several conserved sequence motifs in IGRs. Two putative *T. parva* motifs have localized distri-bution relative to the transcriptional start sites and are preferentially associated with specific protein functions, which is consistent with the hypothesis that they participate in transcriptional regulation in this eukaryotic parasite. The fact is that conserved motifs with similar sequence are found in *Plasmodium* hints at the possibility of common regulatory mechanisms across the phylum *Apicomplexa* (Guo and Silva, 2008).

It is well known that cross-protection between *T. parva* stocks is limited, but precise evaluation of genetic diversity in field populations of the parasite has been hampered by a lack of molecular markers spanning the genome. Katzer et al. (2006) recently reported 42 newly identified polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) markers distributed across the four *T. parva* chromosomes, as well as 19 new satellite markers for chromosomes 1 and 2. This brings the total number of available polymorphic markers to 141 for the 8.5 Mb genome. They have used these markers to characterize existing parasite stabilates and have also shown that passage of the parasite through naive cattle and ticks can lead to substantial changes of parasite populations in resulting stabilates. These markers have also been used to show that passage of mixed parasites through an immunized calf results in the removal of the immunizing genotype from the parasite population produced by ticks fed on this animal (Katzer et al., 2006). The markers are not evenly distributed, but instead often cluster together in certain regions of the four chromosomes. Attempts to identify markers in gaps have met with only limited success, suggesting that the regions that lack polymorphic markers may represent regions in which the *T. parva* genome is conserved across different isolates.

Bishop et al. (2005) have also described a high-resolution analysis of the genome-wide pattern of transcription within a single parasite life-cycle stage using a quanti-fiable technique based on sequence signatures whose origins in the genome are verifiable. These authors employed massively parallel signature sequencing (MPSS) to analyze the transcriptome of *T. parva* (Richard et al., 2005). In total, 1 095 000, 20 bp sequences representing 4371 different signatures were generated from *T. parva* schizonts. Reproducible signatures were identified within 73% of potentially detectable predicted genes and 83% had signatures in at least one MPSS cycle. A predicted leader peptide was detected on 405 expressed genes. The quantitative range of signatures was 4 – 52 256 transcripts per million (t.p.m.). Rare transcripts (< 50 t.p.m.) were detected from

36% of genes. Sequence signatures approximated a log-normal distribution, as in microarray. Transcripts were widely distributed throughout the genome, although only 47% of 138 telomere-associated open reading frames (ORFs) exhibited signatures.

Antisense signatures comprised 13.8% of the total, comparable with *Plasmodium*. 85 predicted genes with antisense signatures lacked a sense signature. Antisense transcripts were independently amplified from schizont cDNA and verified by sequencing. The MPSS transcripts per million for seven genes encoding schizont antigens recognized by bovine CD8 T cells varied 1000-fold (Simon et al., 2008). There was concordance between transcription and protein expression for heat shock proteins that were very highly expressed according to MPSS and proteomics. The data suggests a low level of baseline transcription from the majority of protein-coding genes (Bishop et al., 2005).

T. parva antigens

In order to identify antigens in *T. parva*, Graham et al. (2006) adopted two approaches, both dependent on screening of transiently transfected antigen-presenting cells with fully characterized CTL (Taracha et al., 1995; Goddeeris and Morrison, 1988) from live vaccine-immunized cattle of diverse bovine leukocyte antigen (BoLA) MHC class I genotypes. First, in a targeted gene approach, the authors immunoscreened genes that were predicted by using preliminary sequence data from one of the four *T. parva* chromosomes (Gardner et al., 2005) to contain a secretion signal. The approach was based on the observation that the schizont lies free in the host cell cytoplasm (Shaw, 2003) in which signal peptide-containing parasite proteins would directly access the host cell MHC class I antigen processing and presentation pathway. Analysis of proteins encoded by the predicted *T. parva* genes (Gardner et al., 2005) on chromosome 1 for the presence of signal peptides (SIGNALP-2.0; www.cbs.dtu.dk/services/SignalP-2.0) and transmembrane domains (TMHMM, www.cbs.dtu.dk/services/TMHMM) generated a set of 55 candidate antigen genes that were selected for cloning and screening amplified genes cloned into eukaryotic expression vector pTargeT (Promega) (Graham et al., 2006).

The second approach involves a random immunoscreen of schizont cDNA clones because secretion of proteins that do not contain signal sequences has been reported (Nacer et al., 2001). A unidirectional cDNA library was constructed in the eukaryotic expression plasmid vector pcDNA3 (Invitrogen) essentially as described in De Plaen et al. (1997).

Double-stranded cDNA was generated from 2 µg of total poly(A)$^+$-RNA (FastTrack 2.0 kit, Invitrogen) isolated from purified schizonts (Baumgartner et al., 1999) by using the SuperScript Choice System (Invitrogen). Plasmid DNA minipreps were prepared from 1,000 pools of bacterial cultures each containing 50 colonies and another 1,000 pools each containing 10 colonies for high-throughput immunoscreening. To resolve a positive pool of 50 cDNAs, 256 colonies were pooled into 48 clusters of 16 colonies by using a three-way matrix and screened to identify the individual clone in the three unique clusters. For the resolution of a positive pool of 10 cDNA, plasmid DNA was prepared from 48 colonies and tested to identify the individual clone. Positive cDNA clones were end-sequenced, and sequence information was used to identify the complete open reading frames (ORFs) from the *T. parva* genome sequence database (www.ncbi.nlm.nih.gov/sutils/blast_table.cgi?taxid=Protozoa) (Graham et al., 2006).

To screen the cloned genes, an assay (IFN-g ELISpot) that measures IFN-g released from *T. parva* specific cytotoxic T lymphocyte (CTL) in response to BoLA class I cDNA-expressing COS-7 cells or transformed autologous skin fibroblasts transiently transfected with *T. parva* cDNA was developed (Graham et al., 2006). In addition to releasing INF-g, CTL lines used to identify these antigens were shown to recognize and lyse COS-7 cells and autologous fibroblasts, transiently transfected with these genes. To evaluate the relevance of these CTL antigens in immunity to ECF, live vaccine-immunized animals were re-challenged with live *T. parva* sporozoites and the kinetics of CD8+ T cell-mediated IFNg responses to these antigens analyzed (Mwangi et al., 2007) (Table 1). In the targeted approach, only one antigen (Tp2) was identified, while in the random approach, the same Tp2 and five additional antigens (Tp1, Tp4, Tp5, Tp7, and Tp8) were obtained. Graham et al. (2006) also reported that the six antigens are targets of BoLA MHC class I-restricted, IFN-γ-secreting, and lytic CD8$^+$ T cells, indicating that the live vaccine primes a CTL response against them.

The identification of schizont antigens provides an opportunity not only to explore vaccination, but also to dissect further the specificity of CD8 T-cell responses in order to understand better the basis of the strain specificity of immunity to *T. parva*. Graham et al. (2008) reported the identification of antigenic epitopes in the six CD8 T-cell target antigens and their MHC restriction specificities. By examining responses of cattle to immunization with live parasites or a cocktail of the CD8 T-cell target antigens, they provided evidence that these antigens are highly dominant in animals with the corresponding MHC genotypes.

ECF SUB-UNIT VACCINE: The quest

Previous efforts towards the development of an ECF recombinant subunit vaccine focused on the infective stage (sporozoite) of the parasite in the tick and yielded a

Table 1. List of *T. parva* CTL target antigens identified through immune-screening of selected genes and random cDNA library.

Antigen	Identity	Signal peptide	Anchor motiff	Mw (kD)
Tp1	Hypothetical protein	1-19	Yes	62
Tp2	Hypothetical protein	1-23	No	19
Tp4	TCP-1	No	Yes	63
Tp5	eIF-1A	No	No	18
Tp7	Hsp90	No	Yes	84
Tp8	Cysteine proteinase	No	Yes	50

TCP-1, eta subunit of T complex protein 1; eif-1A, translation elongation initiation factor 1A; Hsp90, heat shock protein 90. Adapted from Mwangi et al. (2007).

'first generation' prototype recombinant vaccine, based on one antigen, p67, whose effectiveness was suboptimal (Nene et al., 1996; Musoke et al., 2005). However, immunity against the bovine intracellular protozoan parasite *T. parva* has been shown to be mediated by CD8 T cells. Six antigens targeted by CD8 T cells from *T. parva*-immune cattle of different MHC genotypes have been identified, raising the prospect of developing a subunit vaccine (Graham et al., 2006; Mwangi et al, 2007). Five of these CTL target antigens have been formulated in proprietary technologies including plasmid DNA, modified vaccinia virus Ankara (MVA), and canarypox virus (vCP), evaluated in cattle and shown to induce CTL responses that correlated with resistance against lethal challenge in a proportion of animals (Graham et al., 2006). Also, CTL lines from one of four calves, BY120, responded specifically to cells infected with MVA expressing the antigen Tp2 and synthetic peptides were employed to map a new CTL epitope on this antigen (Akoolo et al., 2008).

To facilitate further dissection of the specificity of protective CD8 T-cell responses and to assist in the assessment of responses to vaccination, Graham et al. (2008) set out to identify the epitopes recognized in these *T. parva* antigens and their MHC restriction elements. Nine epitopes in six *T. parva* antigens, together with their respective MHC restriction elements, were successfully identified. Five of the cytotoxic-T-lymphocyte epitopes were found to be restricted by products of previously described alleles, and four were restricted by four novel restriction elements. Analyses of CD8 T-cell responses to five of the epitopes in groups of cattle carrying the defined restriction elements and immunized with live parasites demonstrated that, with one exception, the epitopes were consistently recognized by animals of the respective genotypes. The analysis of responses was extended to animals immunized with multiple antigens delivered in separate vaccine constructs. Specific CD8 T-cell responses were detected in 19 of 24 immunized cattle. All responder cattle mounted responses specific for antigens for which they carried an identified restriction element. By contrast, only 8 of 19 responder cattle displayed a response to antigens for which they did not carry an identified restriction element. These data demonstrate that the identified antigens are inherently dominant in animals with the corresponding MHC genotypes (Graham et al., 2008).

REFERENCES

Akoolo L, Pellé R, Saya R, Awino E, Nyanjui J, Taracha EL, Kanyari P, Mwangi DM, Graham SP (2008). Evaluation of the recognition of Theileria parva vaccine candidate antigens by cytotoxic T lymphocytes from Zebu cattle. Vet. Immunol. Immunopathol., Feb 15; 121(3-4): 216-21.

Baldwin CL, Teale AJ (1987). Alloreactive T cell clones transformed by *Theileria parva* retain cytolytic activity and antigen specificity. Eur. J. Immunol., 17: 1859-1862.

Baumgartner M, Tardieux I, Ohayon H, Gounon P, Langsley G (1999). The use of nocodazole in cell cycle analysis and parasite purification from *Theileria parva*-infected B cells. Microbes Infect., 1: 1181-1188.

Brown CG, Stagg DA, Purnell RE, Kanhai GK, Payne RC (1973). Infection and Transformation of Bovine Lymphoid Cells *in vitro* by Infective Particles of *Theileria parva*. Nature, 245: 101-103.

Brown WC, Logan KS (1986). Bovine T-cell clones infected with *Theileria parva* produce a factor with IL 2-like activity. Parasite Immunol., 8: 189-192.

De Plaen E, Lurquin C, Lethe B, van der Bruggen P, Brichard V, Renauld JC, Coulie P, van Pel A, Boon T (1997). Identification of genes coding for tumor antigens recognized by cytolytic T lymphocytes. Methods, 12: 125-142.

Dobbelaere DA, Coquerelle TM, Roditi IJ, Eichhorn M, Williams RO (1988). *Theileria parva* infection induces autocrine growth of bovine lymphocytes Proc. Natl. Acad. Sci. USA, 85: 4730-4734.

Galley Y, Hagens G, Glaser I, Davis WC, Eichhorn M, Dobbelaere DAE (1997). Jun NH2-terminal kinase is constitutively activated in T cells transformed by the intracellular parasite *Theileria parva.* Proc. Natl. Acad. Sci., 94: 5119-5124.

Goddeeris BM, Morrison WI (1988). Techniques for the generation, cloning, and characterization of bovine cytotoxic T cells specific for the protozoan Theileria parva. J. Tissue Cult. Methods, 11: 101-110.

Graham SP, Pelle R, Honda Y, Mwangi DM, Tonukari NJ, Yamage M, Glew EJ, De Villiers EP, Shah T, Bishop R, Abuya E, Awino E, Gachanja J, Luyai AE, Mbwika F, Muthiani AM, Ndegwa DM, Njahira M, Nyanjui JK, Onono FO, Osaso J, Saya RM, Wildmann C, Fraser CM, Maudlin I, Gardner MJ, Morzaria SP, Loosmore S, Gilbert SC, Audonnet JC, van der Bruggen P, Nene V, Taracha EL (2006). *Theileria parva* candidate vaccine antigens recognized by immune bovine cytotoxic T lymphocytes. Proc. Natl. Acad. Sci. USA, 103: 3288-3291.

Guo X, Silva CJ (2008). Properties of non-coding DNA and identification of putative cis-regulatory elements in *Theileria parva*. BMC

Genomics, 9: 582.
Heussler TV, Joel MJ, Paula CF, Catherine B, Chao-Guang C, Martin JP, Dirk AED (1999). The intracellular parasite *Theileria parva* protects infected T cells from apoptosis. PNAS 96(13): 7312-7317.
Kairo A, Fairlamb AH, Gobright E, Nene V (1994). A 7.1 kb linear DNA molecule of *Theileria parva* has scrambled rDNA sequences and open reading frames for mitochondrially encoded proteins. 1994 February 15; EMBO J., 13(4): 898-905.
Katzer F, Daniel N, Chris O, Richard PB, Evans LN, Taracha ARW, Declan JM (2006). Extensive Genotypic Diversity in a Recombining Population of the Apicomplexan Parasite Theileria parva. Infect. Immun., 74(10): 5456-5464.
Malcolm JG, Richard B, Trushar S, Etienne PV, Jane MC, Neil H, Qinghu R, Ian T, Arnab P, Matthew B, Robert JMW, Shigeharu S, Stuart AR, David JM, Zikai X, Shamira JS, Janice W, Lingxia J, Jeffery L, Bruce W, Azadeh S, Alexander RD, Delia W, Jonathan C, Jennifer RW, Brian H, Samuel VA, Todd HC, Charles L, Bernard S, Joana CS, Teresa RU, Tamara VF, Mihaela P, Jonathan A, William CN, Evans LNT, Steven LS, Owen RW, Henry AF, Subhash MJ, Craig V, Claire MF, Vishvanath N (2005). Genome sequence of *Theileria parva*, a Bovine Pathogen that Transforms Lymphocytes. Science, 309(5731): 134-137.
Martin AC, Drubin DG (2003). Impact of genome-wide functional analyses on cell biology research. Curr. Opin. Cell Biol. 15: 1-6.
Mehlhorn H, Schein E (1998). Redescription of *Babesia equi* Laveran, 1901 as *Theileria equi.* Parasitol. Res., 84(6): 467-475.
Musoke R, Nduati R, Barasa K (2005). PMCT Training Curriculum, Module 3; Child Nutrition. pp 98. Kenya PMCT Project. Accessed at www.popcouncil.org September 8, 2005.
Mwangi DM, Graham SP, Pellé R, Honda Y, Tonukari NJ, Yamage M, Glew EJ, de Villiers EP, Shah T, Bishop R, Abuya E, Awino E, Gachanja J, Luyai AE, Mbwika F, Muthiani AM, Ndegwa DM, Njahira M, Nyanjui JK, Onono FO, Osaso J, Saya RM, Wildmann C, Fraser CM, Maudlin I, Gardner MJ, Morzaria SP, Loosmore S, Gilbert SC, Audonnet JC, van der Bruggen P, Nene V, Taracha ELN (2007). Exploiting host immunity and parasite genomics to develop a robust sub-unit vaccine against east coast fever in cattle – where are we? In the Proceedings of the 4th All Africa Conference on Animal Agriculture and the 31ST Annual Meeting of the Tanzania Society for Animal Production (TSAP) 20-24 September 2005: The role of biotechnology in animal agriculture to address poverty in Africa. Opportunities and challenges. pp 187-191. Arusha, Tanzania. J.E.O. Rege, A.M. Nyamu and D. Sendalo, (eds). 2006.
Nacer A, Berry L, Slomianny C, Mattei D (2001). *Plasmodium falciparum* signal sequences: Simply sequences or special signals? Int. J. Parasitol., 31: 1371-1379.
Neitz WO (1957). Theileriosis, gonderiosis and cytauxzoonosis: a review. Onderstepoort J. Veterinary Res., 27: 275-430.
Nene AV, Hermens HJ, Zilvold G (1996). Paraplegic locomotion: a review. Spinal Cord, 34: 507-524.
Norval RAI, Perry BD, Young AS (1992). The epidemiology of *Theileria* in Africa. Academic Press Ltd, London.
Radley DE, Young AS, Brown CGD, Burridge MJ, Cunningham MP, Musisi FL, Purnell RE (1975). East coast fever: 2. Cross-immunity trials with a Kenya strain of *Theileria lawrencei.* Vet. Parasitol., 1(1): 43-50.
Randolph SE (2008). Dynamics of tick-borne disease systems: minor role of recent climate change. Rev. sci. tech. Off. int. Epiz., 27(2): 367-381.
Richard B, Trushar S, Roger P, David H, Terry P, Lee H, Andrew B, Helen H, Simon PG, Evans L, Taracha N, Simon K, Charles L, Brian H, Jennifer W, Owen W, Malcolm JG, Vishvanath N, Etienne PV (2005). Analysis of the transcriptome of the protozoan *Theileria parva* using MPSS reveals that the majority of genes are transcriptionally active in the schizont stage. Nucleic Acids Res., 33(17): 5503-5511.
Shaw MK (2003). Cell invasion by Theileria sporozoites. Trends Parasitol., 19: 2–6.
Simon PG, Roger P, Mat Y, Duncan MM, Yoshikazu H, Ramadhan SM, Etienne PV, Evelyne A, Elias A, James G, Ferdinand M, Anthony MM, Cecelia M, John KN, Fredrick OO, Julius O, Victor R, Rosemary MS, Shirley AE, Declan JM, Niall DM, Sarah CG, Jean-Christophe A, Ivan MW, Pierre VB, Evans LNT (2008). Characterization of the Fine Specificity of Bovine CD8 T-Cell Responses to Defined Antigens from the Protozoan Parasite *Theileria parva.* Infection Immunity, 76(2): 685-694.
Taracha ELN, Goddeeris BM, Teale AJ, Kemp SJ, Morrison WI (1995). Parasite strain specificity of bovine cytotoxic T cell responses to *Theileria parva* is determined primarily by immunodominance. J. Immunol., 155: 4854-4860.
Ureta-Vidal A, Ettwiller L, Birney E (2003). Comparative genomics: genome-wide analysis in metazoan eukaryotes. Nat. Rev. Genet., 4: 251-262.