Identification of *Theileria parva* vaccine candidate genes using a Bioinformatics approach

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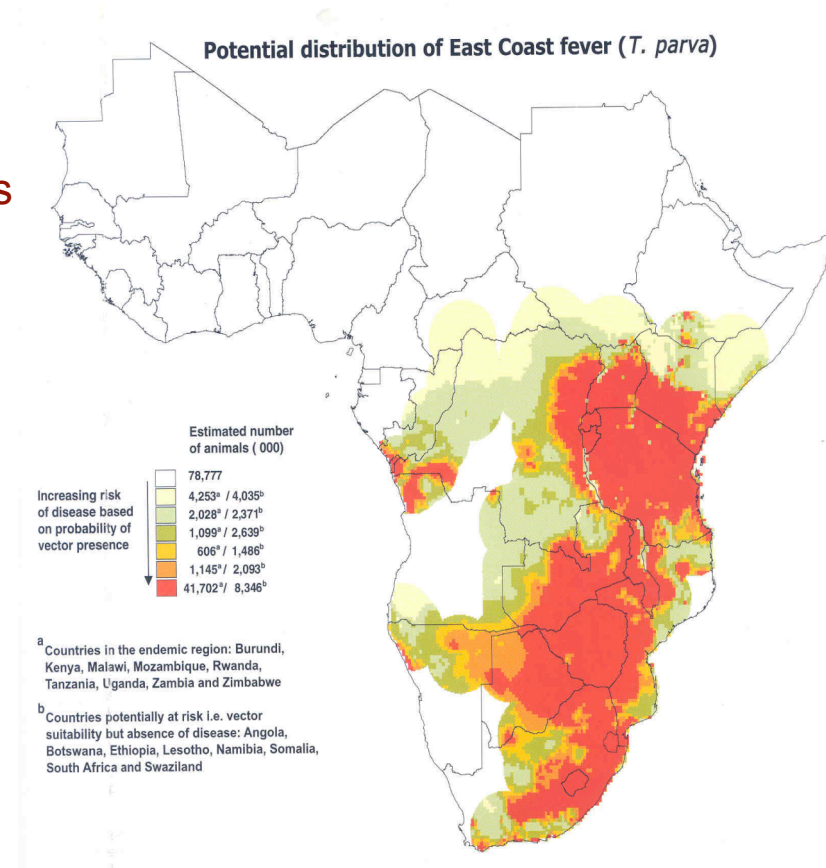
Nairobi, Kenya

East Coast Fever

- East Coast Fever (ECF) is a disease of cattle that affects the lymphocytes & transforms them to a cancerous state
- Caused by Apicomplexan parasite *Theileria parva*
    - *Plasmodium* (malaria)
    - *Toxoplasma* (toxoplasmosis)
    - *Cryptosporidium* (diarrhoea)
    - *Babesia* (cattle, humans)
    - *Theileria* (ruminants)
    - *Eimeria* (poultry)
- Transmitted by Brown ear tick (*Rhipicephalus appendiculatus)*
- Clinical signs are high fever, swelling of the lymph node, anorexia, respiratory distress, loss of condition
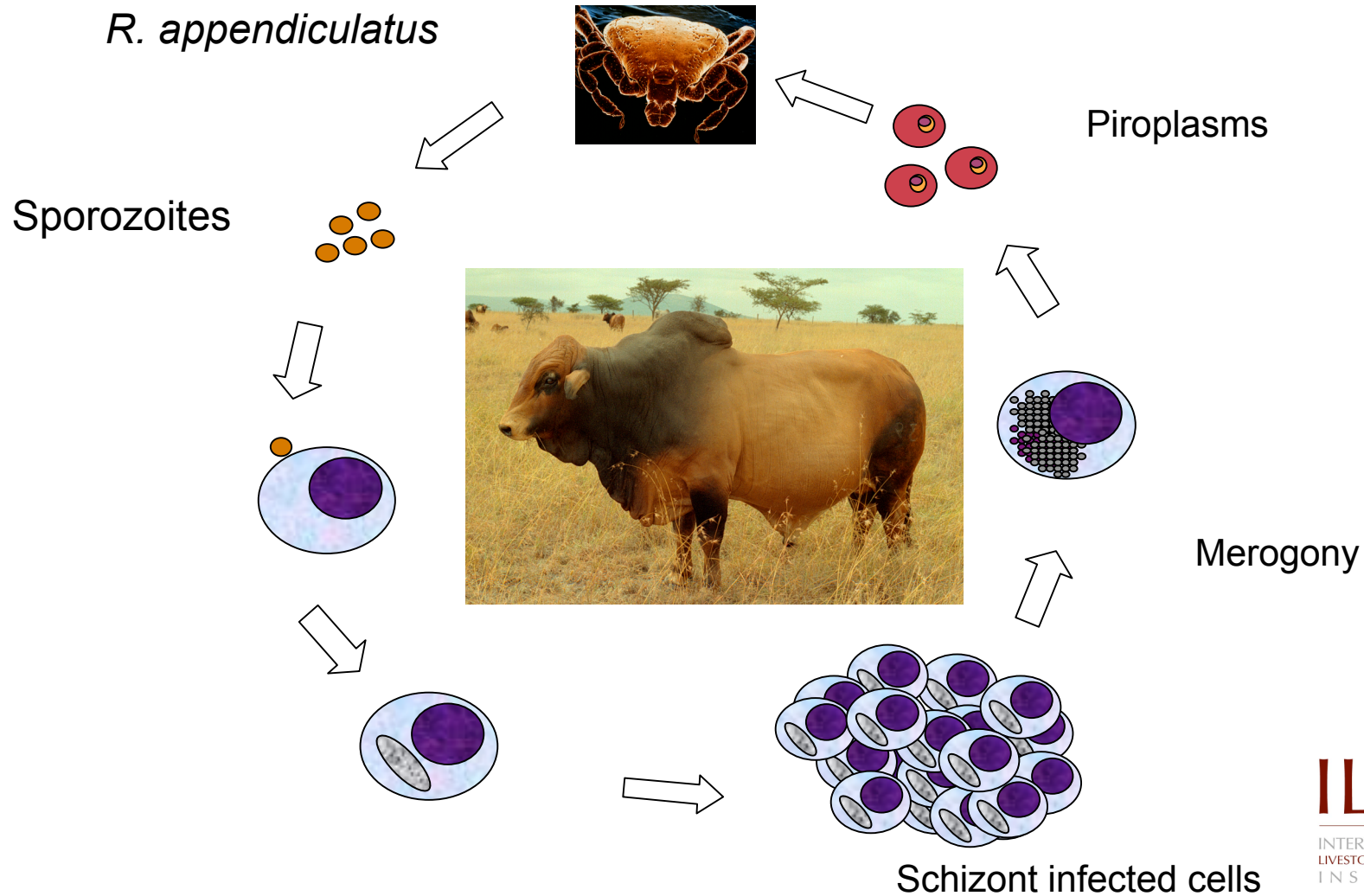- Death is associated with Lung failure due to accumulated fluid
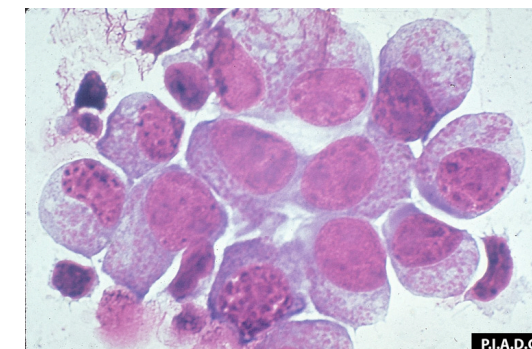
East Coast Fever

• Economic loss approximately US$300m annually
  – Control of disease, Mortalities
  – Research, training, extension services
  – Animals products (Beef, milk, hides)

• 28m cattle at risk of disease of which1m die/year.

• Mortality rate varies
(exotic; 100%, local; 0-50%)

Potential distribution of East Coast fever (*T. parva*)

Estimated number of animals (000)

Increasing risk of disease based on probability of vector presence

	78,777
	4,253[a] / 4,035[b]
	2,028[a] / 2,371[b]
	1,099[a] / 2,639[b]
	606[a] / 1,486[b]
	1,145[a] / 2,093[b]
	41,702[a] / 8,346[b]

[a] Countries in the endemic region: Burundi, Kenya, Malawi, Mozambique, Rwanda, Tanzania, Uganda, Zambia and Zimbabwe

[b] Countries potentially at risk i.e. vector suitability but absence of disease: Angola, Botswana, Ethiopia, Lesotho, Namibia, Somalia, South Africa and Swaziland

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Theileria parva life cycle

R. appendiculatus

Piroplasms

Sporozoites

Merogony

Schizont infected cells

Current Control of ECF

- Vector Control

- Chemotherapy -Butalex (Buparvaquone)

- Vaccination - Infection and treatment

Infection and Treatment Method (ITM) for vaccinating against ECF

- Inoculate animal with live sporozoites and treat with tetracycline (LA)

- Animals show mild reaction & develops a protective immunity to subsequent infection

- Protective immunity mediated by parasite specific class 1MHC restricted CD8+ **Cytotoxic T Lymphocytes (CTL) lyses schizont infected cells**

- Short comings
  - Cold chain
  - Trained personal
  - Some animals may develop clinical signs
  - Carrier state
  - New strains

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ECF Immunity
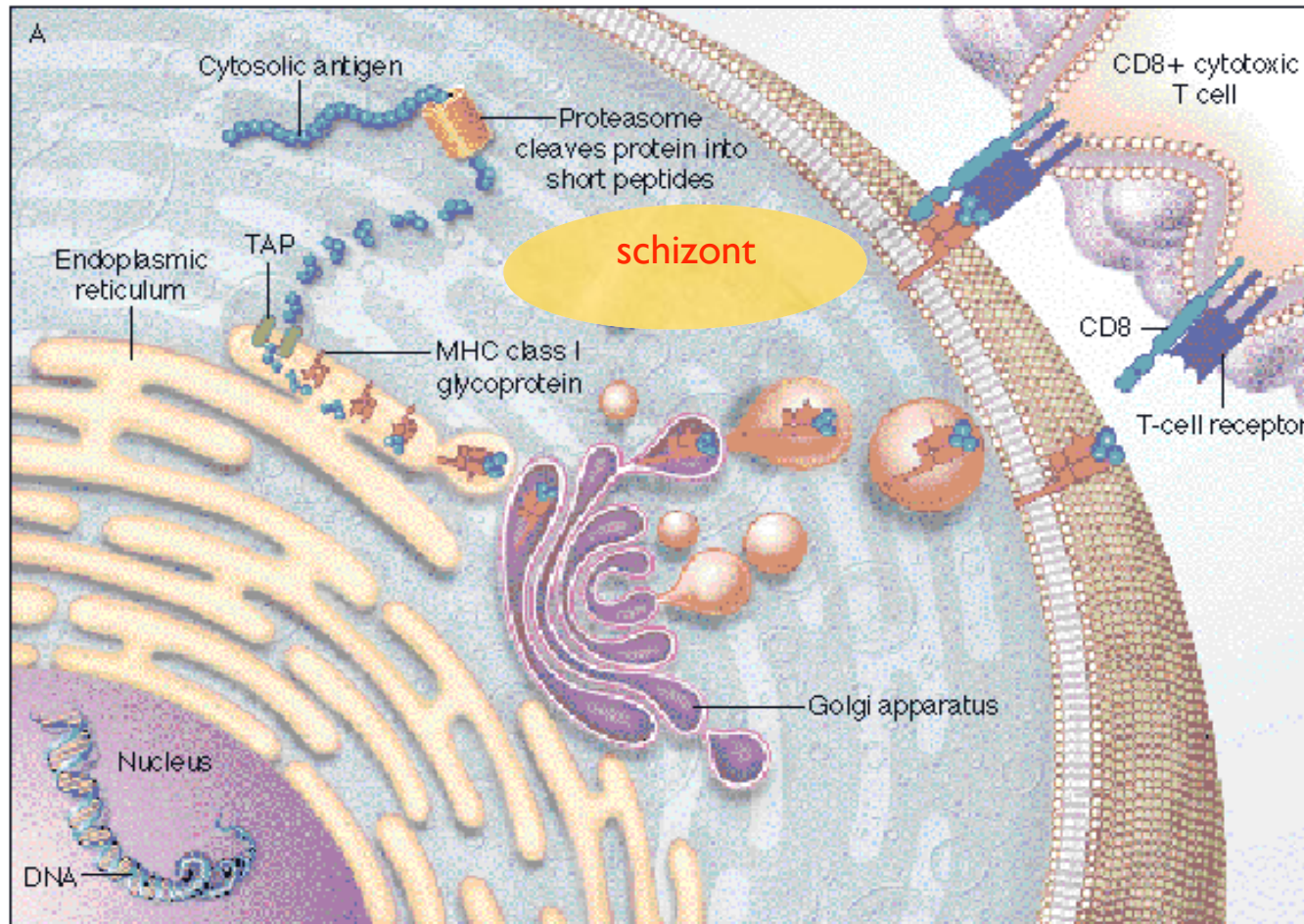
Exploitation of genome data for vaccine development

- Hypothesis:
  - Secreted proteins are vaccine candidates
  - Accessible to host immune response (T-cells)

- Methodology:
  - Sequence parasite genome
  - Identify genes encoding secreted proteins
  - Assay proteins in T-cell assay

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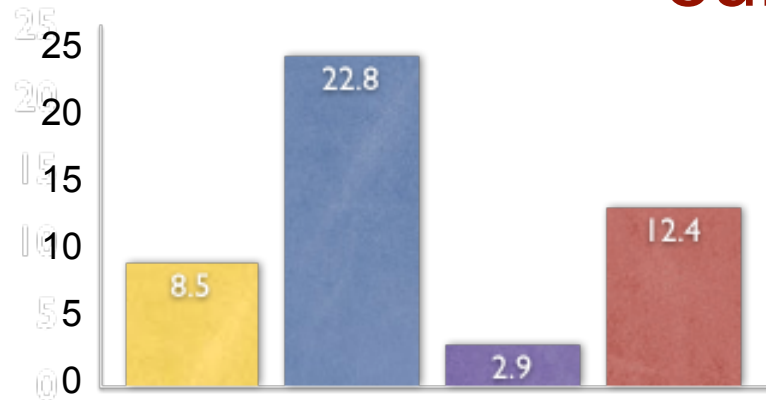INSTITUTE

Methodology for ECF vaccine development

T. parva genome sequence

Bioinformatics tools

Predict ~ 4000 gene sequences & list candidate vaccine antigens

Microarrays to test expression

Random cDNA screen

Proteomics

Filter genes via immunological assays

Clone genes of vaccine interest

Assess immune responses in cattle

Comparative analysis with other livestock and human parasitic diseases (Tropical theileriosis, babesiosis and malaria)

Test experimental vaccine under laboratory conditions

PROOF-OF-CONCEPT ESTABLISHED

Theileria parva genome project

- Whole genome sequenced by random shotgun approach
- TIGR contracted for shotgun sequencing, assembly and genome closure
- Annotation and genome analysis done by African scientists at ILRI
- Genome data:
  - 4 chromosomes
  - Genome size of 8.3 Mbp
  - 4035 genes

Gardner *et al*., 2005. Science

Chromosome 2
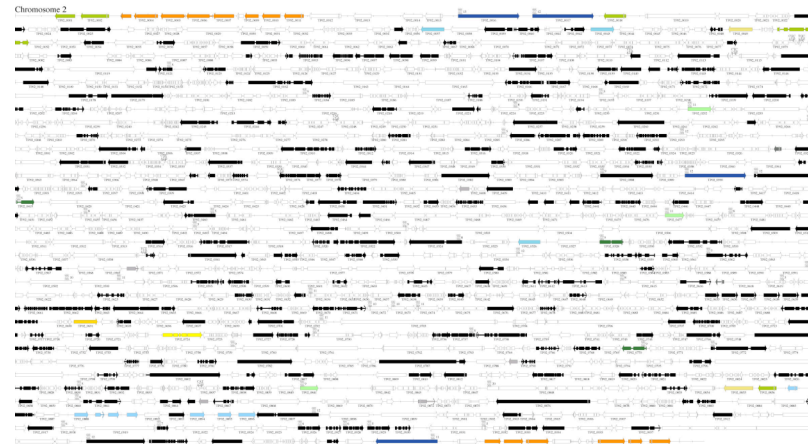
Comparison of *T. parva* to other eukaryotes

Genome size (Mb): T. parva 8.5, P. falciparum 22.8, E. cuniculu 2.9, S. pombe 12.4

Base composition (%GC): T. parva 34.2, P. falciparum 19.4, E. cuniculu 47.3, S. pombe 36.0

Number of genes: T. parva 4,058, P. falciparum 5,268, E. cuniculu 1,997, S. pombe 4,929
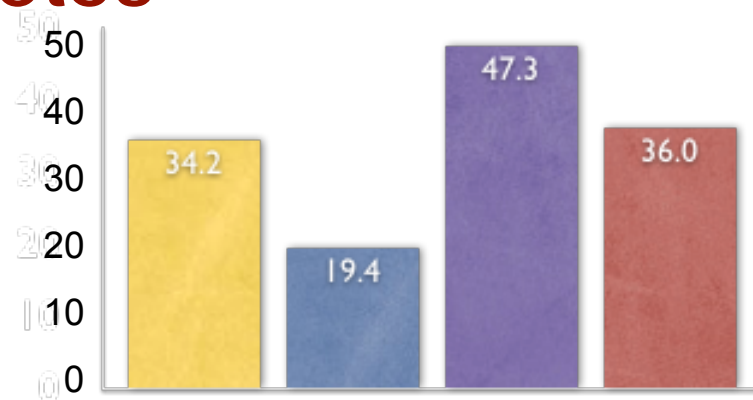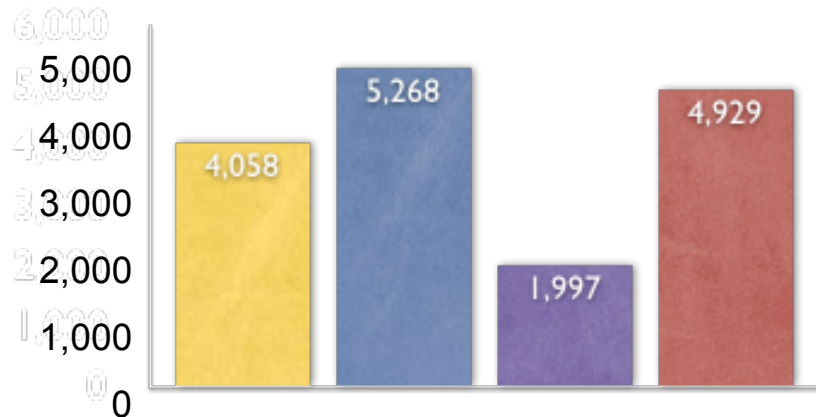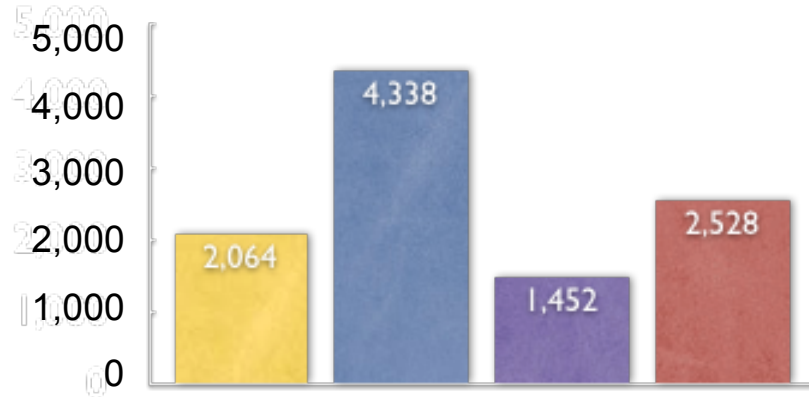
Gene density (nt per gene): T. parva 2,064, P. falciparum 4,338, E. cuniculu 1,452, S. pombe 2,528

Legend: *T. parva*, *P. falciparum*, *E. cuniculu*, *S. pombe*

Secretory proteins
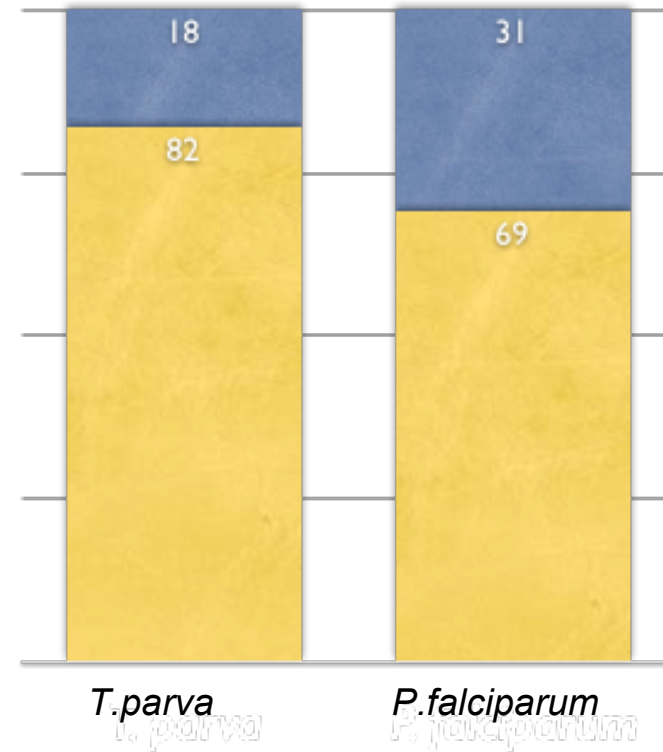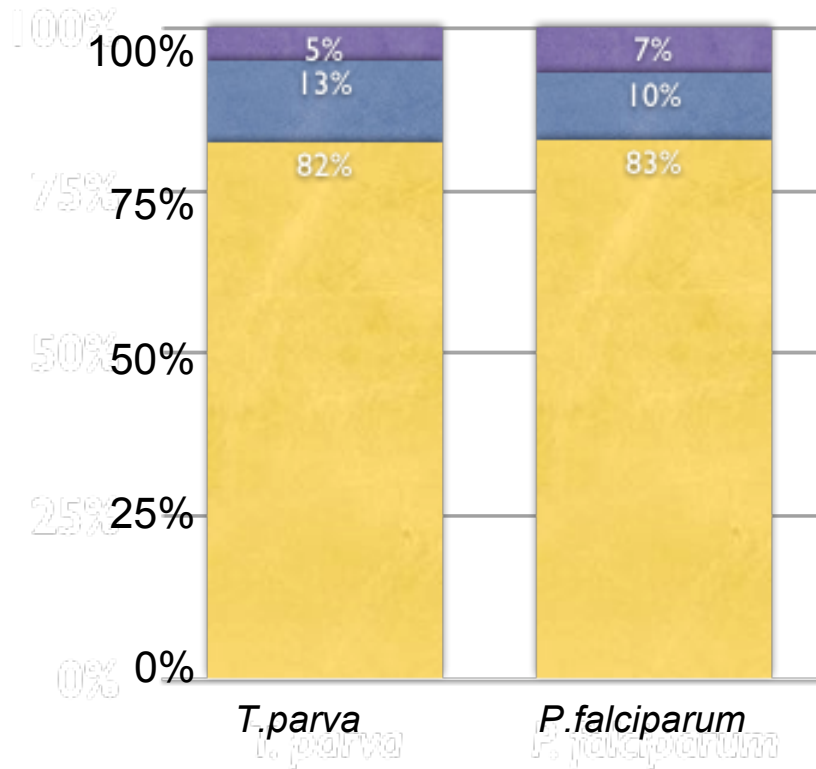
Genome alignment of *T. parva* and *T. annulata* chromosome 3

Tpr locus

TP-Chr3   TA-Chr3

T.parva and *T.annulata* display nearly complete synteny across all chromosomes

Unique matches are indicated by lines

• Red - same orientation

• Blue - reverse orientation

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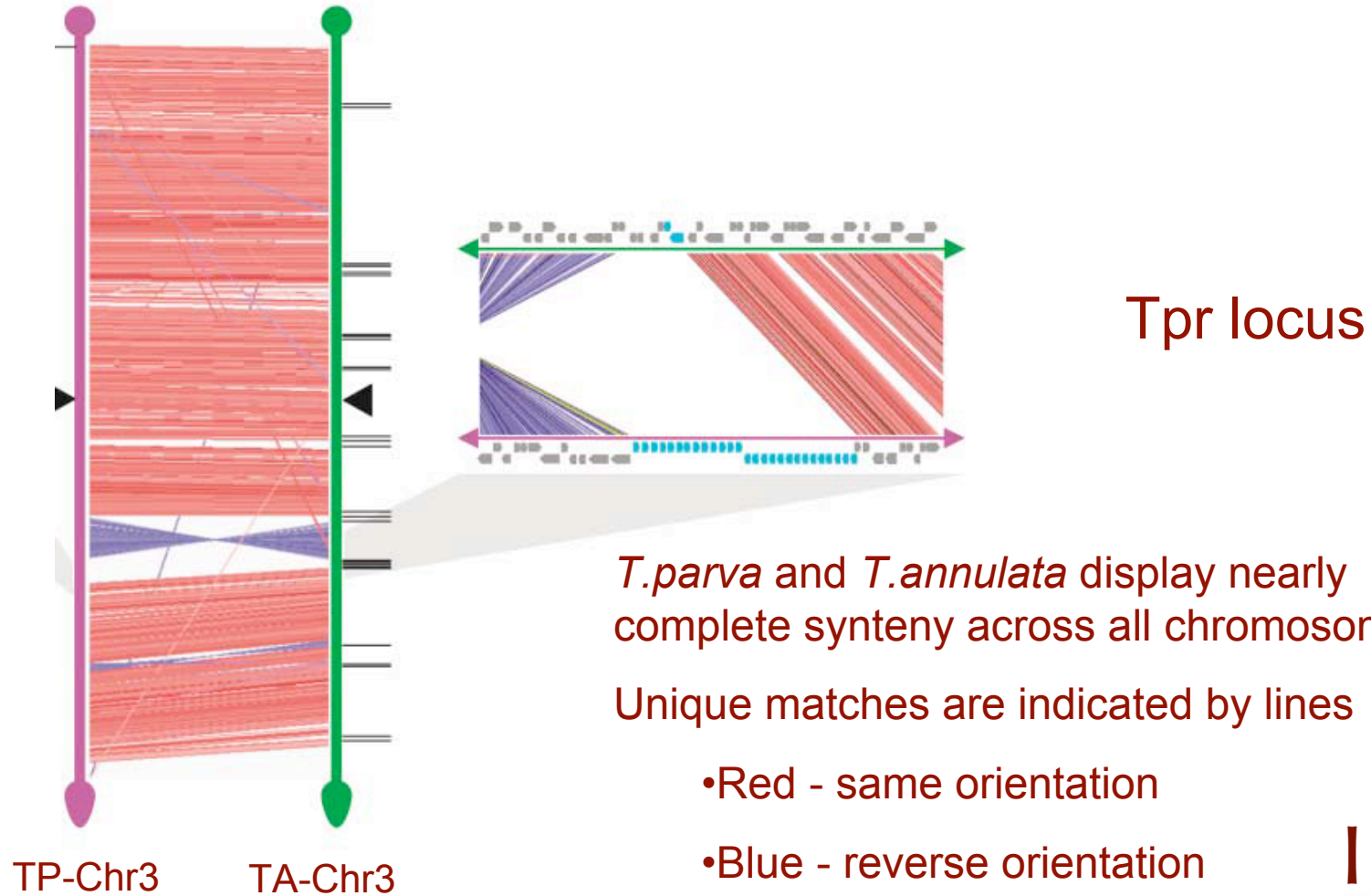
Regions of microsynteny between *T. parva* and *P. falciparum*
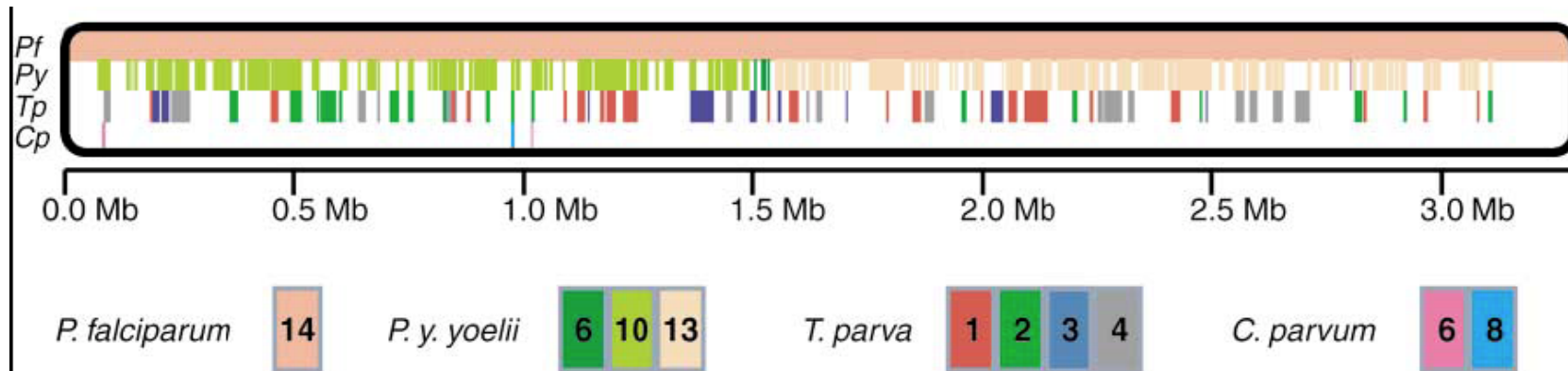
Top row, *P. falciparum* chromosome 14 proteins.
Second row, *P. y. yoelii* orthologs from *P. y. yoelii* chromosomes 6, 10, and 13.
Third row, *T. parva* orthologs from *T. parva* chromosomes 1, 2, 3, and 4.
Fourth row, *C. parvum* orthologs from *C. parvum* chromosomes 6 and 8.

Pain *et al*., 2005. Science

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Theileria parva transcriptome

- The *T. parva* transcriptome was analysed using Massively Parallel Signal Sequencing (MPSS)

- Majority of genes are transcriptionally active in the schizont stage

- Used to annotate the genome

Bishop *et al*., 2005. NAR
Shah *et al*., 2006. Gene
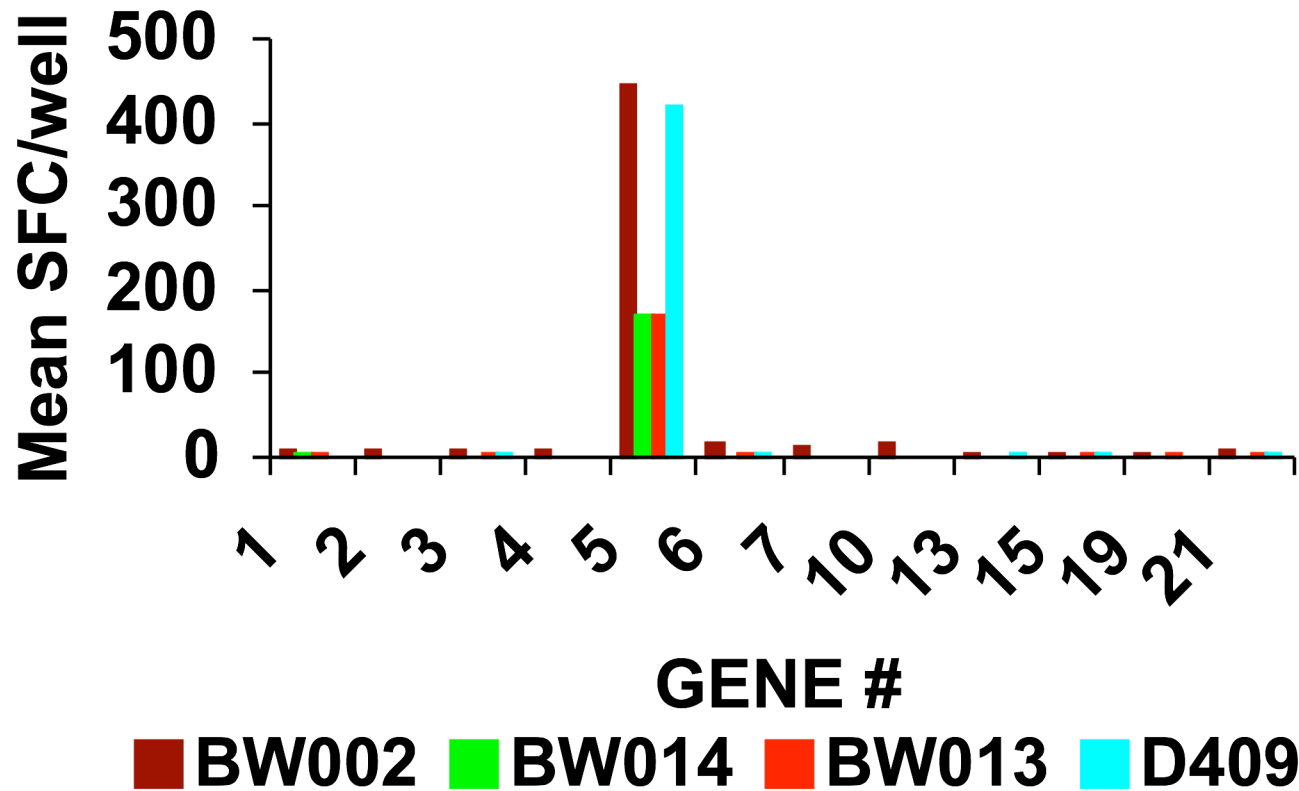
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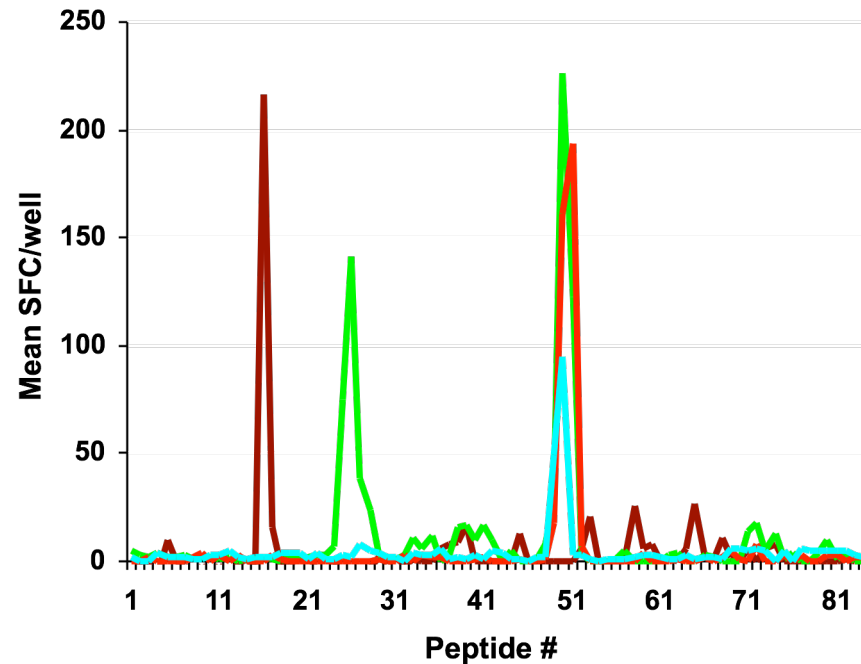LIVESTOCK RESEARCH
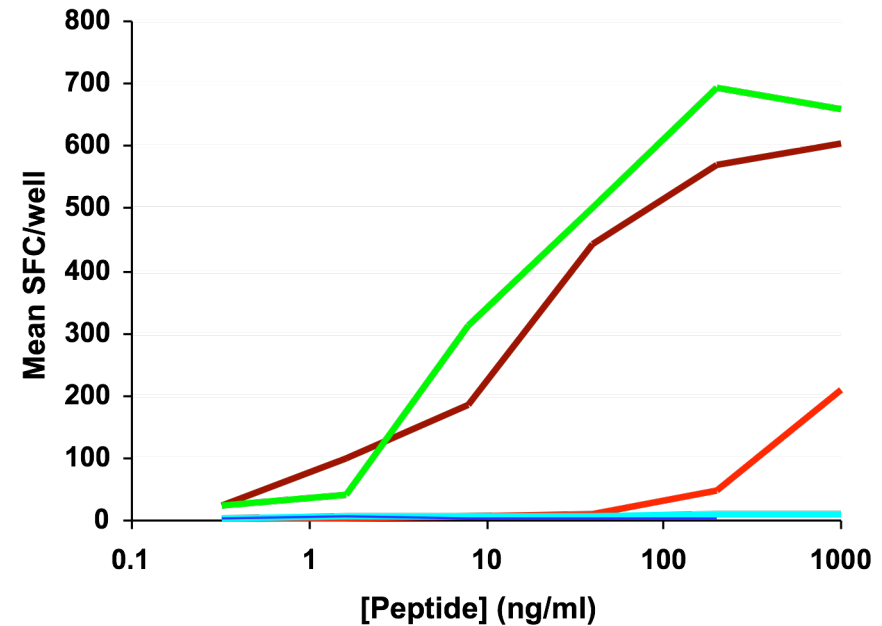INSTITUTE
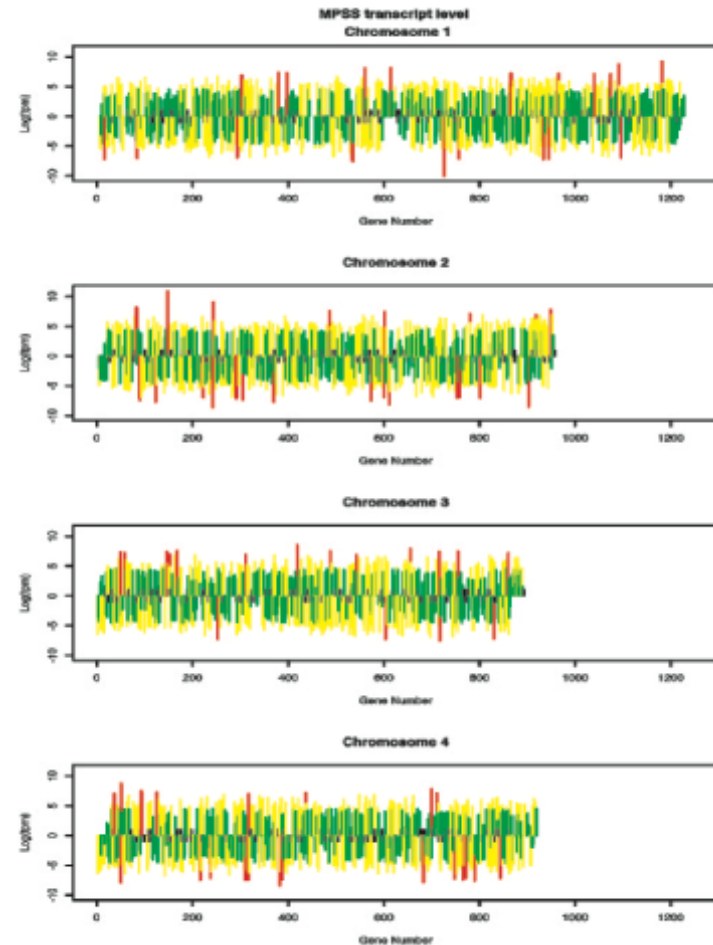
IFN-γ ELISpot based immunoscreen
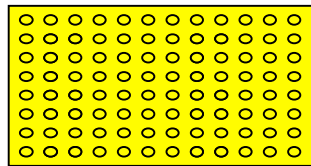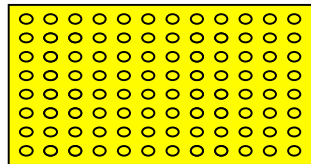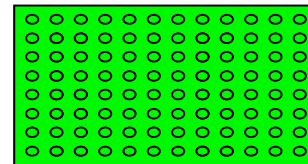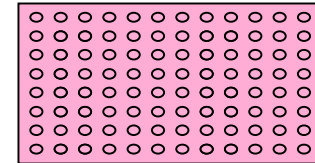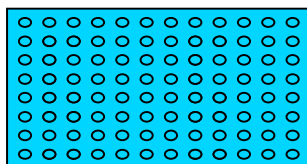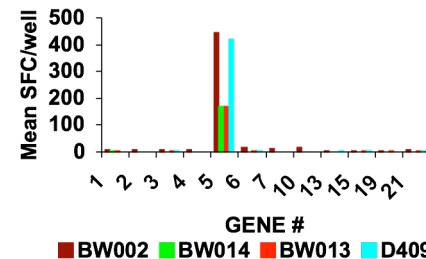
Screening of targeted genes to identify Tp2

Confirmation that protein Tp2 encode CTL target antigens

Tp2 responses during protective immunity
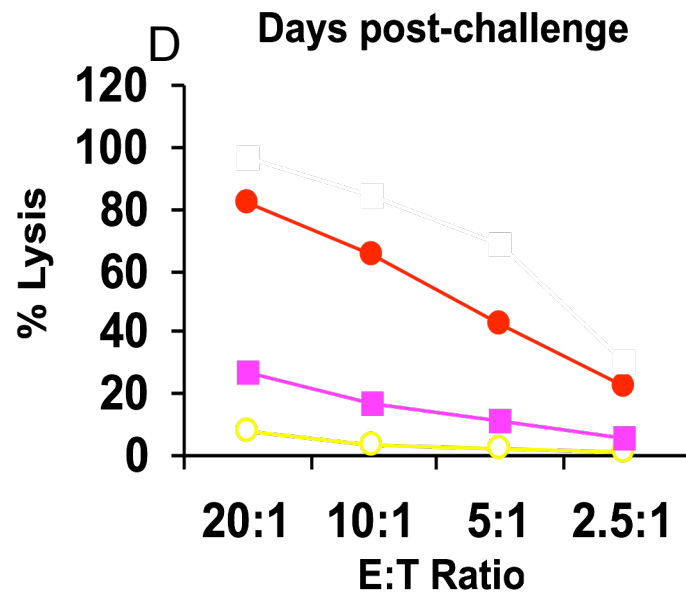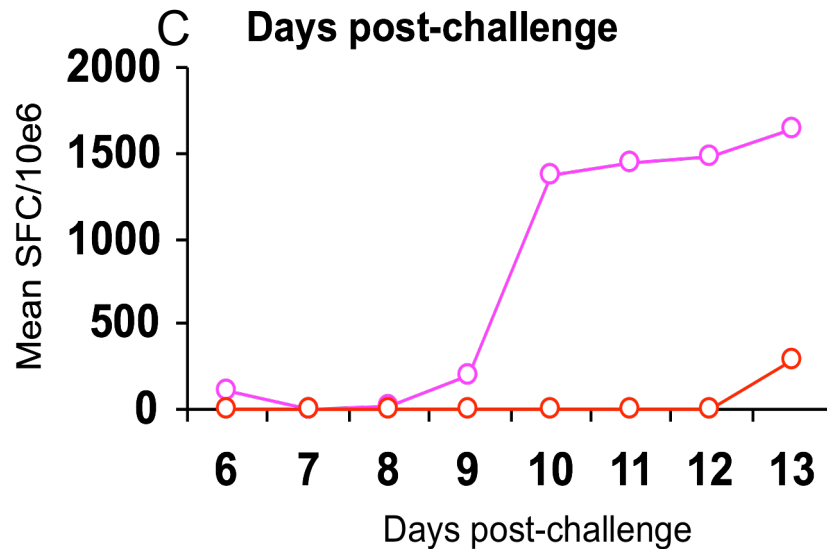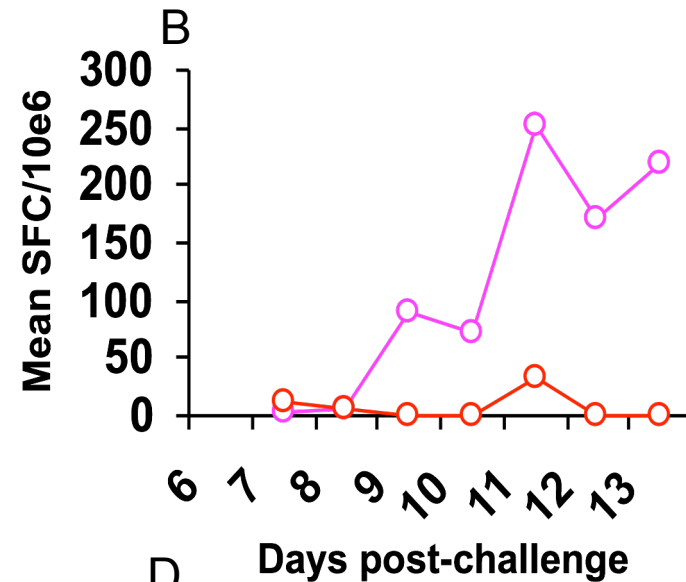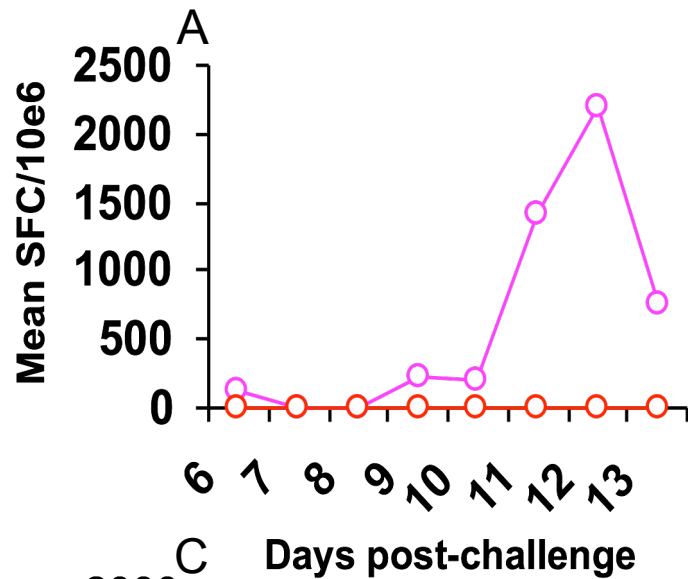
T.parva antigen orthologs in other apicomplexan parasites

T. parva antigen	T. annulata	Plasmodium falciparum	Cryptosporidium Parvum
Tp1	CAI76045 (49.0)	ND	ND
Tp2	CAI73971 (58.4)	NP_705130 (37.3)	ND
Tp4	CAI75431 (95.0)	NP_473202 (60.2)	EAK87917 (58.2)
Tp5	CAI74223 (98.0)	NP_701303 (74.8)	EAK89502 (75.4)
Tp7	CAI74741 (97.2)	NP_704028 (70.8)	EAK89246 (70.8)
Tp8	CAI74839 (89.3)	ND	ND
Tp9	CAI74089 (50.0)	ND	ND
Tp10	CAI76781 (93.0)	NP_701852 (31.0)	EAK90417 (31.0)

Conclusion

- This study validates the exploitation of genomic data to identify vaccine candidate antigens from an Apicomplexan parasite using CTL derived from the natural ruminant host.

- Extension of this approach to the complete genome of *T. parva* is likely to result in identification of further vaccine candidates.

- Tp2 is an attractive candidate for inclusion in a CTL targeted sub-unit vaccine.

- An in-depth evaluation of this antigen in cattle involving a range of antigen delivery technologies is in progress in order to assess the immunogenicity and protective efficacy of Tp2 against clinical East Coast fever.

Graham et al., 2006. PNAS

Graham et al., 2007. Immunome Res.

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Acknowledgements