

# Proteomics of *Theileria parva* sporozoites

James Nyagwange<sup>1,3</sup>, Nicola Ternette<sup>2</sup>, Edwin Tijhaar<sup>3</sup>, Roger Pelle<sup>4</sup>, Vish Nene<sup>5</sup>  
<sup>1,4,5</sup> International Livestock Research Institute, P. O. Box 30709, Nairobi, Kenya  
<sup>2</sup> The Jenner Institute, Nuffield Department of Medicine, University of Oxford, UK  
<sup>3</sup> Cell Biology and Immunology Group, Wageningen University, The Netherlands

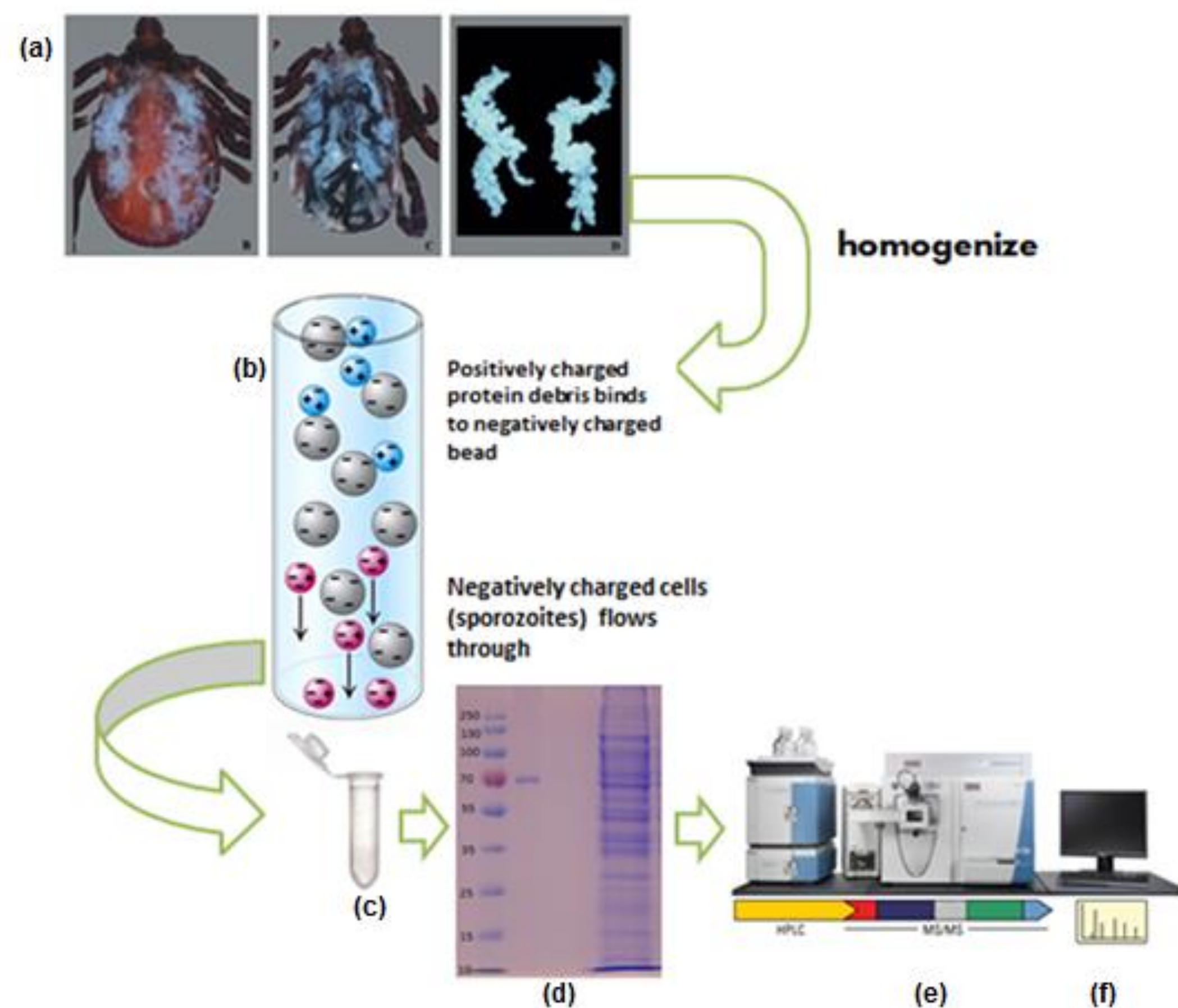
## Introduction

East Coast fever (ECF) is a lymphoproliferative disease caused by the protozoan parasite *Theileria parva*. It kills about one million cattle annually in Africa. The sporozoite stage of this parasite, harbored in the salivary glands of the tick *Rhipicephalus appendiculatus*, invades and establishes infection in the bovine lymphocytes during tick feeding. However, little is known about the parasite molecules involved in this infection process. It is therefore necessary to elucidate the protein composition of the sporozoites to identify novel targets for blocking invasion. Blocking this initial stage of invasion presents a promising vaccine strategy for the control of ECF.

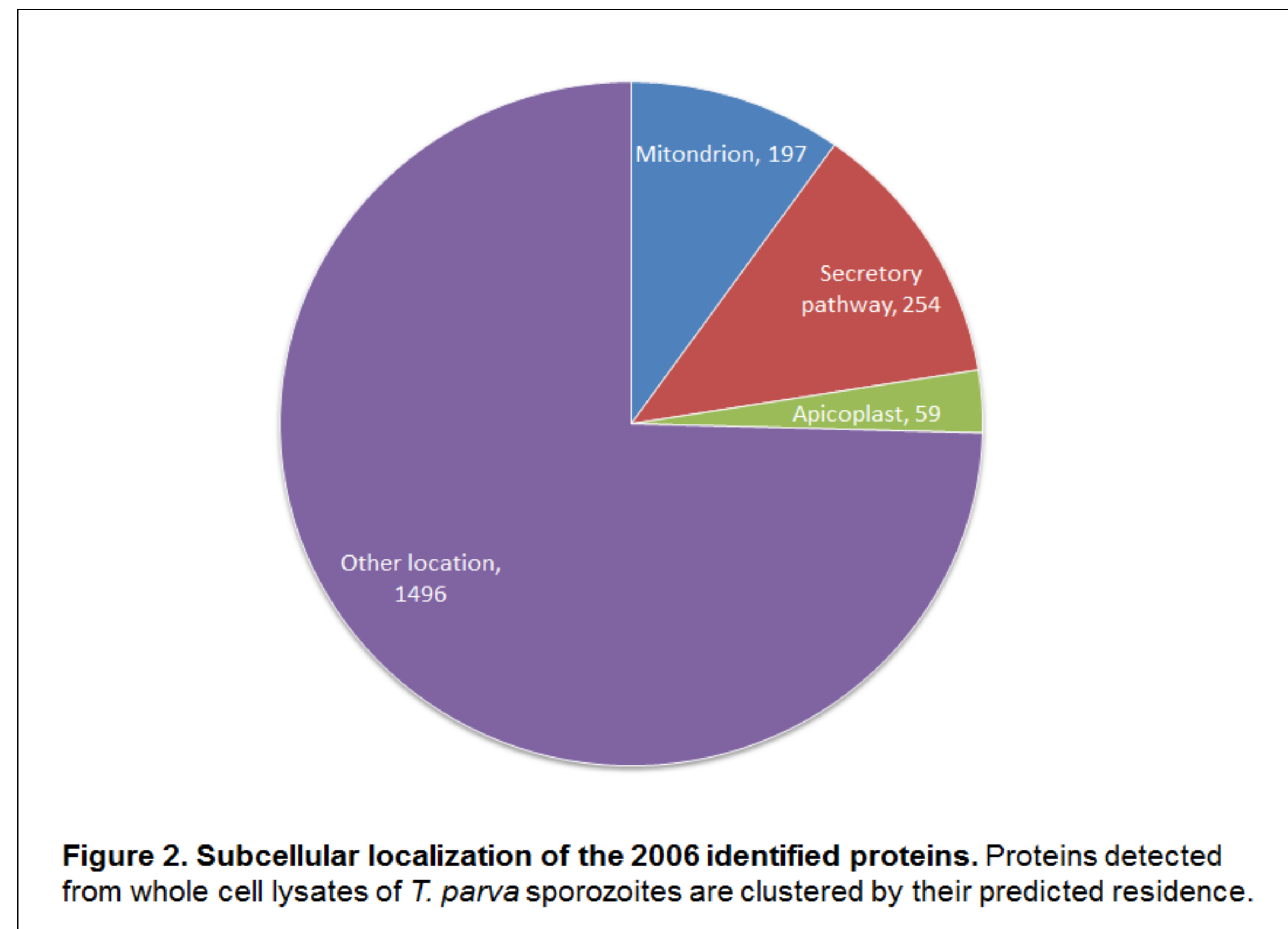
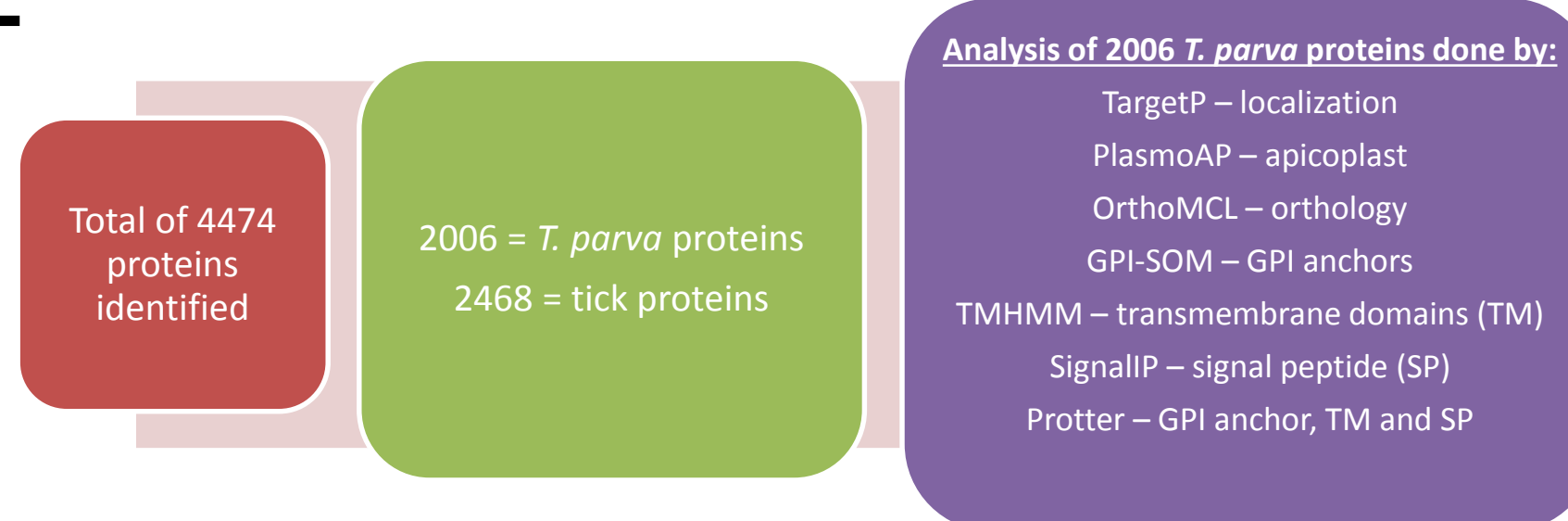
## Aim

To (i) establish the protein expression profile of *T. parva* sporozoites and (ii) identify proteins involved in host cell entry and invasion

## Method



## Results



**Table 1. Invasion organelle proteins.** Proteins detected from whole cell lysates of *T. parva* sporozoites are aligned by orthology with *P. falciparum* sporozoites proteins into orthologous groups (OGs).

OGs	Organism name	ORF locus tag	Gene name	Annotation
<b>Rhoptry neck proteins</b>				
OG5_142870	<i>Plasmodium falciparum</i>	PF14_0495	PIRON2	Rhoptry neck protein 2
	<i>Theileria parva</i>	TP01_0014	-	Hypothetical protein
OG5_153587	<i>Plasmodium falciparum</i>	MAL8P1.73	-	Conserved Plasmodium protein, unknown function
	<i>Theileria parva</i>	TP01_1161	-	Hypothetical protein
<b>Rhoptry Proteins</b>				
OG5_153563	<i>Plasmodium falciparum</i>	MAL13P1.237	-	Conserved Plasmodium protein, unknown function
	<i>Theileria parva</i>	TP02_0645	-	Hypothetical protein
OG5_128020	<i>Plasmodium falciparum</i>	PF11570c	PIM18AAP	M18 aspartyl aminopeptidase
	<i>Theileria parva</i>	TP01_1150	-	Aspartyl aminopeptidase
OG5_145111	<i>Plasmodium falciparum</i>	PF10295c	-	RNB-like protein, putative
	<i>Theileria parva</i>	TP01_0396	-	Hypothetical protein
OG5_141731	<i>Plasmodium falciparum</i>	MAL13P1.308	-	Conserved Plasmodium protein, unknown function
	<i>Theileria parva</i>	TP01_0092	-	Hypothetical protein
<b>Microneme Proteins</b>				
OG5_147452	<i>Plasmodium falciparum</i>	PF11_0344	AMA1	Apical membrane antigen 1, AMA1
	<i>Theileria parva</i>	TP01_0650	-	Apical membrane antigen 1
OG5_134280	<i>Plasmodium falciparum</i>	PFL0805w	-	MAC/ Perforin, putative
	<i>Plasmodium falciparum</i>	PF08_0052	-	Perforin like protein 5
	<i>Theileria parva</i>	TP01_0164	-	Hypothetical protein
	<i>Theileria parva</i>	TP02_0166	-	Hypothetical protein
OG5_171217	<i>Plasmodium falciparum</i>	PF10800c	CaTOS	CaTOS, putative
	<i>Theileria parva</i>	TP01_0232	-	Hypothetical protein
OG5_135185	<i>Plasmodium falciparum</i>	PF0585w	-	Leu/Phe-tRNA protein transferase, putative
	<i>Theileria parva</i>	TP02_0627	-	Leucyl/phenylalanyl-tRNA protein transferase
<b>Dense Granules/ Exonemes</b>				
OG5_126706	<i>Plasmodium falciparum</i>	MAL8P1.69	-	14-3-3 protein, putative
	<i>Theileria parva</i>	TP02_0607	-	Hypothetical protein

**Table 2. Proteins involved with calcium signaling.** Proteins detected from whole cell lysates of *T. parva* sporozoites are aligned by orthology with *P. falciparum* sporozoites proteins

OGs	Organism name	ORF locus tag	Gene name	Annotation
OG5_147452	<i>Plasmodium falciparum</i>	PF11_0344	AMA1	Apical membrane antigen 1, AMA1
	<i>Theileria parva</i>	TP01_0650	-	Apical membrane antigen 1
OG5_126800	<i>Plasmodium falciparum</i>	PF14_0323	-	Calmodulin
	<i>Theileria parva</i>	TP02_0717	-	Calmodulin
OG5_129380	<i>Plasmodium falciparum</i>	PFA_0345w	PICEN1	Centrin-1
	<i>Theileria parva</i>	TP01_0227	-	Centrin
OG5_152981	<i>Plasmodium falciparum</i>	PF11_0411	-	Conserved Plasmodium protein
	<i>Theileria parva</i>	TP02_0412	-	Hypothetical protein
OG5_146625	<i>Plasmodium falciparum</i>	PF11_0239	CDPK6	Calcium-dependent protein kinase, putative
	<i>Plasmodium falciparum</i>	PF07_0072	PICDPK4	Calcium-dependent protein kinase 4
	<i>Plasmodium falciparum</i>	PF13_0211	CDPK5	Calcium-dependent protein kinase, putative
	<i>Theileria parva</i>	TP01_0983	-	Calcium-dependent protein kinase
	<i>Theileria parva</i>	TP01_1073	-	Calmodulin-domain protein kinase
	<i>Theileria parva</i>	TP02_0399	-	Calcium-dependent protein kinase
OG5_133188	<i>Plasmodium falciparum</i>	PF11_0395	-	Guanylyl cyclase
	<i>Theileria parva</i>	TP02_0848	-	Guanylyl cyclase
OG5_141757	<i>Plasmodium falciparum</i>	PFL2225w	MTIP	Myosin A tail domain interacting protein
	<i>Theileria parva</i>	TP01_0513	-	Myosin light chain
OG5_126577	<i>Plasmodium falciparum</i>	MAL13P1.148	-	Myosin
	<i>Theileria parva</i>	TP01_0774	-	myosin
OG5_126674	<i>Plasmodium falciparum</i>	PFL0590c	ATPase4	Non-SERCA-type Ca <sup>2+</sup> -transporting P-ATPase
	<i>Plasmodium falciparum</i>	PFA_0310c	-	Calcium-transporting ATPase, putative
	<i>Theileria parva</i>	TP01_0720	-	P-type ATPase
	<i>Theileria parva</i>	TP02_0524	-	Calcium-transporting ATPase
OG5_127599	<i>Plasmodium falciparum</i>	PF14_0346	PKG	cGMP-dependent protein kinase
	<i>Theileria parva</i>	TP02_0378	-	cAMP-dependent protein kinase
OG5_126635	<i>Plasmodium falciparum</i>	PFL2250c	PKB	Rac-beta serine/threonine protein kinase, PIPKB
	<i>Theileria parva</i>	TP02_0727	-	cAMP-dependent protein kinase catalytic subunit

**Table 3. Surface proteins.** Proteins detected from whole cell lysates of *T. parva* sporozoites are aligned by orthology with *P. falciparum* sporozoites proteins

OGs	Organism name	ORF locus tag	Gene name	Annotation
OG5_127135	<i>Plasmodium falciparum</i>	PFB0210c	HT1	Hexose transporter, PIHT1
	<i>Theileria parva</i>	TP01_1069	-	Monosaccharide transporter
OG5_126744	<i>Plasmodium falciparum</i>	PF11_0351	-	Heat shock protein hsp70 homologue
	<i>Plasmodium falciparum</i>	PF08_0054	-	Heat shock 70 kDa protein
	<i>Plasmodium falciparum</i>	MAL13P1.540	-	Heat shock protein 70 (hsp70), putative
	<i>Theileria parva</i>	TP01_0479	-	Hypothetical protein
	<i>Theileria parva</i>	TP02_0148	-	Heat shock protein 70
	<i>Theileria parva</i>	TP02_0753	-	Heat shock protein 70
OG5_127061	<i>Plasmodium falciparum</i>	PFD0305c	-	Vacuolar ATP synthase subunit b
	<i>Theileria parva</i>	TP01_0389	-	ATP synthase F1 subunit beta
OG5_126628	<i>Plasmodium falciparum</i>	PF14_0598	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
	<i>Theileria parva</i>	TP02_0858	-	Glyceraldehyde-3-phosphate dehydrogenase
OG5_143227	<i>Plasmodium falciparum</i>	PF14_0065	-	Conserved Plasmodium membrane protein, unknown function
	<i>Theileria parva</i>	TP01_0761	-	Hypothetical protein
OG5_147452	<i>Plasmodium falciparum</i>	PF11_0344	AMA1	Apical membrane antigen 1, AMA1
	<i>Theileria parva</i>	TP01_0650	-	Apical membrane antigen 1
OG5_126595	<i>Plasmodium falciparum</i>	PF11_0114	-	Actin-like protein homolog, ALP1 homolog
	<i>Plasmodium falciparum</i>	PFL2215w	ACT1	Actin I
	<i>Plasmodium falciparum</i>	PF14_0124	-	Actin II
	<i>Theileria parva</i>	TP01_0789	-	Actin
	<i>Theileria parva</i>	TP02_0056	-	Actin
	<i>Theileria parva</i>	TP02_0903	-	Actin

**Table 4. Additional surface proteins.** Proteins detected from whole cell lysates of *T. parva* sporozoites predicted to contain either GPI anchor, transmembrane domain or signal peptide.

ORF locus tag	Coverage (%)	# Unique Peptides	Description	# TM Domains	GPI-linked	Signal peptide	# TM Domains	GPI-linked	Signal peptide
TP01_0012	42	6	14 kDa zinc-binding protein	1	1	0	1	0	0
TP01_0438	24	34	Hypothetical protein	0	1	1	0	0	1
TP01_0554	10	3	Hypothetical protein	0	1	0	0	0	0
TP01_0655	5	3	E3 ubiquitin-protein ligase synoviolin	6	1	0	5	0	0
TP01_0715	10	4	Hypothetical protein	2	1	0	2	0	0
TP01_0939	34	12	Hypothetical protein	1	1	1	1	0	1
TP01_0972	16	4	Hypothetical protein	1	1	1	1	0	1
TP01_1056	39	12	Merozoite Antigen	0	1	1	0	0	1
TP01_1066	22	5	Hypothetical protein	5	1	0	5	0	0
TP02_0412	11	5	Hypothetical protein	0	1	1	0	0	1
TP02_0500	4	3	Sodium-dependent phosphate transporter 1-B	9	1	0	9	0	0
TP02_0538	23	31	Hypothetical protein	1	1	1	1	0	1
TP02_0553	30	6	Hypothetical protein	1	1	1	1	0	1
TP02_0950	5	2	Hypothetical protein	1	1	1	1	0	1
TP01_0876	16	1	Hypothetical protein	1	0	0	1	0	1
TP03_0245	2	1	Hypothetical protein	9	1	0	3	0	0
TP03_0287	68	53	Sporozoite P67 surface antigen	1	1	1	1	0	1
TP03_0512	38	12	Tartrate-resistant acid phosphatase type 5	2	1	0	1	0	0
TP04_0437	53	60	104 kDa microneme/rhoptry antigen	1	1	1	0	1	1
TP04_0615	7	2	Probable N-acetylglucosaminyl-phosphatidylinositol de-N-acetylase	0	1	1	0	0	1

## Conclusion

In total 2006 *T. parva* sporozoite proteins have been confirmed to be expressed representing approximately 50% of the total predicted *T. parva* protein expression profile. Potential surface proteins and possible proteins involved in host entry and invasion have been identified using bioinformatic tools.