

CHAPTER 6

Analysis of the gene encoding the *Theileria parva* polymorphic immunodominant molecule (PIM) reveals evidence of the presence of cattle-type alleles in South Africa

"There's two possible outcomes: if the result confirms the hypothesis, then you've made a discovery. If the result is contrary to the hypothesis, then you've made a discovery." **Enrico Fermi**



6.1 Abstract

Analysis of two T. parva genes coding for the antigenic proteins p67 and p104, revealed the presence of cattle-type alleles identical to those of T. parva Muguga (a stock that causes ECF in Kenya) from T. parva samples collected from three cattle from a farm in Ladysmith. In addition, variants of p67 allele 1 and p104 allele 1, characteristic of the cattle-type alleles, were identified from T. parva samples obtained from buffalo from four game parks in South Africa. Consequently, polymorphic immunodominant molecule (PIM) PCR-RFLP profiles and inferred amino acid sequences were analyzed to confirm the presence of cattle-type alleles in T. parva samples obtained from cattle and buffalo in South Africa. PIM PCR-RFLP profiles similar to that of the T. parva Muguga stock were obtained from three of the six cattle samples from the Ladysmith farm and the inferred amino acid sequences of the PIM gene from two of these samples (Lad 02 and Lad 10) were almost identical to the T. parva Muguga PIM sequence. This finding supports recent studies in which p67 and p104 alleles similar to those of the T. parva Muguga stock were identified from the same Ladysmith samples. None of the PIM gene sequences obtained from T. parva field samples characterized in this study were identical, providing further evidence that the PIM gene evolves at an extremely high rate. Cattle-type PIM alleles were not identified from buffalo T. parva samples. In addition to sequences similar to known PIM alleles, for the first time, 'mixed' alleles consisting of cattleand buffalo-type amino acid motifs were identified. The significance of T. parva parasites carrying 'mixed' PIM alleles will have to be established and their risk to cattle evaluated. With the extent of genetic diversity that has been demonstrated by the three characterization studies presented in this thesis, the epidemiology of theileriosis in South Africa needs to be further investigated.

6.2 Introduction

The tick transmitted apicoplexan protozoan parasite, *Theileria parva*, is by far the most pathogenic and economically significant *Theileria* sp. in eastern, central and southern Africa (Mukhebi *et al.*, 1992). Infections by *T. parva* are associated with high mortality, primarily in exotic and crossbred cattle, but also in indigenous calves and adult cattle in endemically stable areas (Perry and Young, 1995). This places major constraints on cattle production and the expansion of the dairy industry. The Cape buffalo (*Syncerus caffer*) is the natural reservoir host of the parasite.



Tick transmission of the parasite from infected buffalo to susceptible cattle results in a disease syndrome called Corridor disease, while *T. parva* parasites that can circulate in cattle cause two disease syndromes, East Coast fever (ECF) and January disease (Theiler, 1904; Neitz, 1955; Lawrence, 1992). To distinguish between the different types of *T. parva* parasites, parasites that circulate in cattle and cause ECF and January disease are referred to as cattle-derived *T. parva* and parasites that originate from buffalo and cause Corridor disease are known as buffalo-derived *T. parva* (Perry and Young, 1993). East Coast fever was introduced into South Africa at the end of the 19th century and eradicated in the early 1950s (Anonymous, 1981). Although ECF was eradicated in southern Africa, its principal vector, the three-host ixodid tick *Rhipicephalus appendiculatus*, is still widespread. Corridor disease is a controlled disease in South Africa and sporadic outbreaks still occur.

Studies on two *T. parva* genes, p67 and p104, presented in the previous two chapters, revealed the presence of cattle-type alleles identical to those of *T. parva* Muguga (a stock that causes ECF in Kenya) from *T. parva* samples collected from three cattle from a farm in Ladysmith, South Africa (Chapter 4; Chapter 5; Sibeko *et al.*, 2010). In addition, variants of p67 allele 1 and p104 allele 1, characteristic of the cattle-type alleles, were identified from *T. parva* samples obtained from buffalo from four game parks in South Africa, namely, Kruger National Park, Hluhluwe-iMfolozi, Ithala and Mabalingwe (Collins, 1997; Chapter 4; Sibeko *et al.*, 2010). Unless these alleles can be associated with parasites that can cause fatal disease, their importance remains unclear. Consequently, another *T. parva* gene encoding an antigenic protein, the polymorphic immunodominant molecule (PIM), was investigated.

The PIM gene has previously been successfully used to differentiate between cattle- and buffalo-derived *T. parva* stocks (Geysen *et al.*, 1999; Bishop *et al.*, 2001). Although PIM is expressed by both the sporozoite and the schizont stages of the parasite, it is expressed predominantly by the schizont stage and is thus believed to play a role as a target antigen in the induction of the cytotoxic T cell response (Shapiro *et al.*, 1987; Toye *et al.*, 1991; Katende *et al.*, 1998; Shaw, 2003; Graham *et al.*, 2007). It is also capable of inducing sporozoite-neutralizing antibodies and has been exploited in discriminatory assays for *T. parva* isolates because of its highly conserved termini flanking a variable region with a highly polymorphic central region (Toye *et al.*, 1995a; 1995b; Bishop *et al.*, 2001; Geysen *et al.*, 1999; 2004; De Deken *et al.*, 2007). The variations in the central region of the PIM gene produce the polymorphism that has been exploited for discrimination between *T. parva* stocks (Geysen *et al.*, 1999; Bishop *et al.*, 2001).



In this study, the PIM gene was characterized to confirm the presence of cattle-type alleles in *T. parva* samples obtained from cattle and buffalo in South Africa. The diversity of the parasite populations circulating in buffalo and cattle in South Africa was also demonstrated.

6.3 Materials and methods

6.3.1 Sample collection

Blood samples were collected from buffalo from different game parks, and from cattle from farms with suspected theileriosis. The samples were collected in EDTA vaccutainer tubes and stored at -20 °C, for long term storage, or 4 °C for short term storage, before extraction of DNA.

6.3.2 DNA isolation and selection of *T. parva*-positive samples

Total DNA was extracted from 200 μ l of EDTA blood using the High Pure PCR Template Preparation kit (Roche Diagnostics, Mannheim, Germany), according to the method described by the manufacturer, except that extracted DNA was eluted in 100 μ l elution buffer. Extracted DNA was stored at 4°C until further analysis. The presence of *T. parva* DNA was determined using the real-time PCR assay as previously described (Chapter 3; Sibeko *et al.*, 2008). One hundred and nine *T. parva*-positive samples consisting of 101 field samples collected from buffalo from different game parks, and eight collected from cattle from farms with suspected theileriosis, were investigated (Table 6.1).



Table 6.1 Geographical origin and source of blood samples (n=109) used for characterization of *T. parva* parasites

Geographical location	Province	Sample Name*	Host of blood sample	Date of collection/ References
Hluhluwe-iMfolozi Park (n=38)	KwaZulu-Natal	HIP 1, HIP 3, HIP 4, HIP 5 , HIP 6, HIP 7, HIP 8, HIP 9, HIP 10, HIP 11, HIP 12, HIP 13, HIP 14, HIP 15, HIP 16, HIP 18, HIP 19, HIP 20, HIP 21, HIP 22 , HIP 23, HIP 24, HIP 25, HIP 26, HIP 27, HIP 28, HIP 30, HIP 31, HIP 32 , HIP 33, HIP 34, HIP 35, HIP 36, HIP 37, HIP 38, HIP 39 , HIP 42, HIP 49	Buffalo	2004
Kruger National Park (KNP) (n=47)	Mpumalanga	KNP 39, KNP 42, KNP 43 , KNP 47, KNP 48, KNP 49, KNP 50, KNP 61, KNP 62, KNP 63, KNP 66, KNP 67, KNP 68, KNP 102, KNP AA5, KNP AB47, KNP AC10, KNP AD3, KNP B10, KNP B22, KNP D11, KNP D24, KNP E7, KNP E18, KNP F9, KNP G2, KNP G11, KNP H8, KNP J5, KNP L6, KNP L27, KNP M2, KNP M12, KNP M2706, KNP N1, KNP N8, KNP O1, KNP O11 , KNP S17, KNP U3, KNP U20, KNP V5 , KNP W8 , KNP X4, KNP Y4, KNP Y19, KNP Z4	Buffalo	2003
Ladysmith (n=6)	KwaZulu-Natal	Lad 2, Lad 06, Lad 10, Lad 17 Lad M119, Lad I438	Bovines	2003 Thompson <i>et al.</i> (2008)
Mabalingwe Game Reserve (n=6)	Limpopo	Mab A13, Mab A22, Mab B21, Mab BB37, Mab BB38, Mab BB43	Buffalo	2004
Ithala Game Reserve (n=10)	KwaZulu-Natal	Itha 1, Itha 2, Itha 3, Itha 4, Itha 5, Itha 6, Itha 7, Itha 8, Itha 9, Itha 10	Buffalo	2005/6
Schoonspruit (n=1)	Mpumalanga	Schoonspruit	Bovine	Neitz (1948)
Bloemfontein (n=1)	Free-State	Bloe B	Bovine	2004

*Samples in bold were selected for cloning to produce RFLP profiles from individual clones and only 27 of the 35 were used for sequencing (see Table 6.2).



6.3.3 Amplification of the PIM gene from *T. parva* samples

The variable region of the *T. parva* PIM gene was amplified from *T. parva* positive DNA samples using the semi-nested PCR described by De Deken *et al.* (2007). A nested PCR was performed using primers Pim1 [5' GTG AAT GTT GTG ATC TTA ATC C 3'] and PimR4 [5' CCC ACA ACC GTG GAA TGG CGT A 3'] for the primary PCR and primers PimFm [5' ATT CCA CTG GTT CTT CCG ATS TA 3', where S = C or G] and PimR4 for the secondary PCR. Briefly, 5 µl of total DNA was used in a 25 µl amplification reaction for the primary PCR and half a microlitre of the primary PCR product was used as a template for the secondary PCR, using the reaction and cycling conditions previously described (De Deken *et al.*, 2007).

6.3.4 Analysis of the PIM gene from *T. parva* samples using PCR-RFLP

Restriction fragment length polymorphism was performed as described by De Deken *et al.* (2007); briefly, PCR products were digested overnight with the restriction enzyme, *Bcl*I; the digested products were separated on a 10% polyacrylamide gel before DNA detection by SYBR[®] green (SIGMA-ALDRICH, USA). RFLP patterns were analysed by visual inspection and by using BioNumerics version 5.1 (Applied Maths, Kortrijk, Belgium). Normalisation of the RFLP profiles was done using the molecular weight marker 100 bp DNA Ladder (Fermentas Life Sciences, Germany), which was run in two lanes per gel. The software was used to calculate Dice coefficients of similarity, to cluster the RFLP profiles and to generate dendrograms by the unweighted-pair group method using average linkages (UPGMA). The most appropriate settings for optimization and tolerance, as determined by the software, were calculated. DNA fragments of less than 100 bp Were excluded from the analysis as these could not be accurately estimated using the 100 bp DNA ladder and in some instances had run out of the gel. Samples with similar RFLP profiles obtained from different animals were defined as clusters.

6.3.5 Cloning and sequencing of PIM PCR products

The PIM PCR products from 34 selected *T. parva* samples, including 27 buffalo and seven cattle samples (shown in bold in Table 6.1), were cloned into pCR[®]2.1-TOPO[®] cloning vector (Invitrogen, Carlsbad, USA); at least 24 clones were screened for each sample. The presence of inserts in the recombinants was confirmed by colony PCR following the secondary PCR



protocol used above. Amplicons produced from colony PCR were digested with *Bcl*I to produce RFLP profiles for individual clones. Only clones that produced amplicons which successfully digested with *Bcl*I were considered for further analysis. Consequently, clones from 20 *T. parva* samples from buffalo and seven from cattle were sequenced using the ABI Big Dye Terminator Cycle Sequencing kit version 3.1 (Applied Biosystems, Foster City, CA); 300 to 450 ng of plasmid DNA were used in the sequencing reactions. Sequencing was performed by INQABA Biotechnologies in South Africa, using a SpectruMedix model SCE 2410 automated sequencer (SpectruMedix, State College, PA).

6.3.6 Sequence analysis

The PIM gene sequences were assembled and edited using the GAP4 program of the Staden package (version 1.6.0 for Windows) (Bonfield *et al.*, 1995; Staden, 1996; Staden *et al.*, 2000). Sequences were aligned with previously published *T. parva* PIM sequences [Muguga (accession number: L06323), Marikebuni (accession number: L41148) and 7104 (accession number: L41833)] using MacClade v4.0 (Maddison and Maddison, 1992); the alignment was adjusted manually because of the highly polymorphic structure of the PIM gene. It was impossible to perform phylogenetic analysis for the PIM sequences because of the polymorphic nature of this gene.

6.4 Results

6.4.1 PIM PCR-RFLP profile analysis

The PIM gene PCR products obtained from *T. parva* positive samples analyzed in this study ranged in size from 0.7 to 1.2 kb (results not shown). Since PIM is a single copy gene (Toye *et al.*, 1995b), multiple infections were indicated by multiple PCR products in cases where the amplicon sizes obtained from a single sample varied.

From visual inspection of the PIM PCR-RFLP profiles, profiles from all 47 samples from KNP were heterogeneous (Figure 6.1a). However, the profiles obtained from 23/38 (61%) of the Hluhluwe-iMfolozi samples from buffalo were relatively homogeneous (Figure 1b). Similarly 4/6 (67%) samples from Mabalingwe and all 10 from Ithala produced relatively homogeneous profiles (Figures 6.1c and 6.1d). Further analysis of this result by cluster analysis using BioNumerics was not possible as the PCR-RFLP profiles were too complex, as



a result of mixed infections. The profiles were characterized by multiple bands from multiple PCR products; the PCR products were present at different concentrations resulting in multiple bands of different intensities. It was difficult to distinguish between bands from incompletely digested amplicons and authentic bands. Therefore, 27 samples representative of *T. parva* samples from buffalo that produced homogeneous and heterogeneous profiles as well as seven cattle samples (shown in bold in Table 6.1) were selected for cloning in order to produce RFLP profiles from individual clones.

Mixed infections were evident from 31/34 (91.2%) samples, as more than one profile was obtained from different clones of each of these samples. Three samples from Ladysmith (Lad 02, Lad 06 and Lad 10) were exceptions, as all clones from these samples produced only one profile and this profile was identical to that of *T. parva* Muguga (a *T. parva* stock causing ECF in Kenya) and *T. parva* Schoonspruit (an isolate obtained from a bovine infected during the ECF epidemic in the former Transvaal, now Gauteng Province, in South Africa) (Neitz, 1948) (Figure 6.2). When RFLP profiles obtained from clones produced from samples which had homogeneous overall profiles were visually analysed, it was observed that there were dominant profiles that were responsible for the apparently homogenous overall profile between different samples. For example, among other profiles, three profiles were found to be dominant in clones produced from the 10 samples from Ithala; the three profiles were obtained in, respectively, 20/61 (33%), 14/61 (23%) and 11/61 (18%) clones produced from four different samples.





(c)

(**d**)









Figure 6.2 PIM gene *Bcl*I PCR-RFLP profiles obtained from (a) cattle *T. parva* samples from Ladysmith and (b) clones produced from cattle sample, Lad 10.

Cluster analysis of PCR-RFLP profiles using BioNumerics identified five cluster groups, A, B, C, D and E, from 261 clones produced from both buffalo and cattle *T. parva* samples (Figures 6.3 and 6.4). Cluster A was the largest group with 105/261 (40%) clones followed by cluster D with 73/261 (28%), then C (42/261, 16%), B (26/261, 10%) and E (15/261, 6%). No correlation with geographic distribution could be established from the major cluster groups. PIM profiles from clones obtained from KNP, Hluhluwe-iMfolozi, Mabalingwe and Ithala buffalo *T. parva* samples were distributed in all five cluster groups on the dendrogram. Profiles from clones produced from cattle samples Lad 02, Lad 06 and Lad 10 grouped with *T. parva* Muguga and *T. parva* Schoonspruit profiles in cluster A (Figures 6.3 and 6.4). Although most PIM profiles in cluster A were obtained from buffalo samples, 42/48 (88%) of the PIM profiles from clones produced from the other cattle *T. parva* samples from Ladysmith and Bloemfontein grouped closely with profiles obtained from buffalo samples from Hluhluwe-iMfolozi and Ithala in different subgroups within cluster A (Figures 6.3 and 6.4).



Figure 6.3 A simplified similarity dendrogram generated by BioNumerics v5.1 cluster analysis of PCR-RFLP profiles from cloned PIM amplicons using the Dice Coefficient analysis.





Figure 6.4 A detailed similarity dendrogram generated by BioNumerics v5.1 cluster analysis of PCR-RFLP profiles from cloned PIM amplicons using the Dice Coefficient analysis showing actual profiles used to produce the dendrogram. Figure 6.4 continues on pages 136 to 139.





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Specific 'signatures' composed of several small fragments (less than 150 bp) were produced in the PIM PCR-RFLP profiles obtained from some *T. parva* field samples from KNP and Hluhluwe-iMfolozi. Two specific 'signatures' were associated with some RFLP profiles from clones from KNP samples. One of these was defined by five small DNA fragments of sizes ~50, 60, 80, 100 and 120 bp, and the other defined by six fragments of sizes ~50, 60, 80, 100, 120 and 130 bp (Figure 6.5a); both 'signatures' co-occurred with other bands of larger sizes. The Hluhluwe-iMfolozi 'signature' was characterized by four fragments of approximately 50, 80, 100 and 120 bp in size (Figure 6.5b). The KNP 'signatures' were observed in 26/52 (50%) clones from three samples, while the Hluhluwe-iMfolozi 'signature' was observed in almost all the clones (31/36, 86%) from the two Hluhluwe-iMfolozi *T. parva* samples that were analyzed. The Hluhluwe-iMfolozi 'signature' was also apparent in profiles from field samples obtained from buffalo from Ithala (Figure 6.1d), and from three bovines from Ladysmith (Lad 17, Lad I438 and Lad M119) and a bovine from Bloemfontein (results not shown). It was also found in profiles obtained from clones of PIM amplicons from these samples (results not shown).





Figure 6.5 Characteristic 'signatures', indicated by brackets ([), were identified in *T. parva* PIM RFLP profiles obtained from cloned PCR products from samples collected from buffalo from Kruger National Park and Hluhluwe-iMfolozi Game Park. (a): PIM RFLP profiles with specific band 'signatures' obtained from clones from sample KNP W8 from Kruger National Park, characterized by five (clones 1, 2, 3, 6, 7 and 11) and six (clones 13 and 14) small DNA fragments of sizes ranging from ~ 50 to 130 bp. (b): PIM RFLP profiles obtained from clones from sample HIP 5 from Hluhluwe-iMfolozi with the 'four band signature' consisting of ~ 50, 80, 100 and 120 bp DNA fragments.



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6.4.2 PIM gene sequence analysis

Clones from different cluster groups were selected for sequencing. A total of 97 PIM sequences were obtained from cloned amplicons produced from 27 selected *T. parva*-positive samples (Table 6.2). Analysis of the amino acid alignment of the PIM sequences revealed three groups of PIM sequences, cattle-type, buffalo-type and 'mixed'-type (Figure 6.6).

Cattle-type PIM sequences:

A tetrapeptide repeat, QPEP (position 428-447 shown in a solid-line block in Figure 6.6), in the variable region was previously identified by Toye *et al.* (1995b) as characteristic of cattlederived *T. parva* PIM sequences. The amino acid sequences between positions 29 and 259 and positions 493 and 497 were also identified as exclusive to cattle-type PIM alleles in this study. In this way, seven PIM sequences obtained from samples investigated in this study were identified as cattle-type *T. parva* PIM sequences (Table 6.2). Six of these were obtained from two *T. parva* cattle samples from a farm in Ladysmith (Lad 02 and Lad 10) and one from *T. parva* Schoonspruit. These sequences had 98% identity to the *T. parva* Muguga PIM sequence, with one to three amino acid differences; the major difference being a deletion of eight amino acids at position 420-427 in the South African sequences (Figure 6.6). Cattle-type PIM sequences were not identified from samples obtained from buffalo in this study.



Table 6.2	Number of different types of PIM sequences obtained from clones produced from 27	
	selected T. parva samples	

Origin of sample	Sample	Type and number of PIM sequence(s) obtained						
	designation	Cattle-type (7 sequences obtained from 3 samples)	Buffalo-type (53 sequences obtained from 20 samples)'	'Mixed' type (37 sequences obtained from 12 samples)				
Kruger National Park	KNP V5	-	1	-				
(n=4)	KNP W8	-	-	4				
	KNP 102	-	2	-				
	KNP O11	-	2	-				
Hluhluwe-iMfolozi Park	HIP 5	_	3	-				
(n=4)	HIP 19	-	3	2				
	HIP 22	-	1	4				
	HIP 32	-	6	4				
Ithala Game Reserve	Itha 2	_	1	2				
(n=9)	Itha 3	-	5	-				
	Itha 4	-	6	1				
	Itha 5	-	3	-				
	Itha 6	_	1	6				
	Itha 7	_	2	-				
	Itha 8	_	2	-				
	Itha 9	-	4	-				
	Itha 10	-	2	-				
Mabalingwe Game Reserve	Mab A13	-	5	-				
(n=3)	Mab BB38	_	2	-				
	Mab BB43	-	-	1				
Schoonspruit (n=1)	Schoonspruit	1	-	-				
Ladysmith	Lad 2	2	-	-				
(n=5)	Lad 10	4	-	-				
	Lad 17	-	-	7				
	Lad M119	-	-	1				
	Lad I438	-	1	2				
Bloemfontein (n=1)	Bloe B	-	1	3				

All samples in bold were obtained from cattle



	10	20	30	40	50	60	70 80	D	90	100		
T - 1 10 11						<u> </u>						
Lad 10-11 Lad 02-7	DSTGSSDLTHVDTES	NDTSSSSETSQ							SGQQG-I			
Lad 10-4/6	DSTGSSDVTOVDTES	NDTSSSSETSO	KPOPDOP				-ODOPDOHOOPT(DGDT	SGOOG-1	P-DTPOP		
Lad 10-4/2	DSTGSSDVTQVDTES	NDTSSSSETSQ	KPOPDOP				-ODOPDOHOOPT	DGDT	SGOOG-1	P-DTPOP		Cattle-type
Lad 02-3/9	DSTGSSDVTQVGTES	NDTSSSSETSQ	CKPQPDQP				-QDQPDQRQQPT(QGDT	SGQQG-1	P- D TPQP		
Lad 10-4/8	DSTGSSDVTQVDTES	NDTSSSSETSQ	QCKPQPDQP				-QDQPDQHQQPT(2GDT	S <mark>GQQG</mark> —I	P- D TPQP		
Schoonspruit	DSTGSSDVTQVDTES	NDTSSSSETSQ	QCKPQPDQP				-QDQPDQHQQPT(2 GD T	SGQQG-1	P- D TPQP		
T. parva Muguga	DSTGSSDVTQVDTES	NDTSSSSETSQ	QCKPQPDQP				-QDQPDQHQQPT(2GDT	SGQQG-1	P-DTPQP		
T. parva Marikebuni	DSTGSSDVTQVDTES	NDTSSSSETSQ						2GDT	SGQQG-9		4	
HTP 22 8-17	DSTGSSDVTQVDSES	ND SSSSSETSQ							SCOOC-1			
Bloe B 4.0.	DSTGSSDVTOADSES	NDSSSSSETSO	OCOPOPDOP				-ODOPDOHOOPT(DGDT	SGOOG-1	P-DTPOP		
Itha 6_c16-6	DSTGSSDVTQVDSES	NDTSSSSETSQ	OPOPDOP				QDOPDOHOOPT	DGDT	SGQQG-1	P-HTPOP		'Mixed'-type
HIP 32_1-18	DSTGSSDVTQVDSES	NDTSSSSETSQ	QCQPQPDQP				-QDQPDQHQQPT(QGDT	SGQQG-1	P-HTPQP		infined type
Lad M119_9	DSTGSSDVTQADSES	NDSSSSSETSQ	QPQPDQP				-QDQPDQHQQPT(2GDT	S <mark>GQQG</mark> —I	P- D TPQP		
Lad I438_10-10	DSTGSSDVTQADSES	NDSSSSSETSQ	QPQPQP D QP				-QDQPDQHQQPT(2GDT	SGQQG-1	P- D TPQP		
Lad 17_10	DSTGSSDVTQADSES	NDSSSSSETSQ	20QPQPDQP	'			-QDQPDQRQQPT(2GDT	SGQQG-1	P-DTPQP		
HIP 22_8-13	DSTGSSDVTQADSES	NDSSSSSETSQ	DROOPDRDOP	ZDOOOR-WOO				DODEDO		PTDORT-	4	
KNP 011 4/1	DSTGSSDVTOVDTES	NDDSSSSETSO	PPDOP	VD000P-V00	2	PSODOPSGPDS	SODOPVDHOOPT(DADSSG-0	0 <mark>G</mark> 00000	PLDOPT-		
HIP 32 1-8	DSTGSSDVTQVDSES	NDTSSSSETSQ	POOPPDOP	VDOOOP-VOO	2)	PSODOPSGPDS	SODOPVDHOOPT	ADSSG-Q	0 G 00000	PLDOPT-		
Itha3_c13-17	DSTGSSDLTHVDTEY	NDDSSSSETSQ	PQQPPDQP	VDQQQP-VQQ		PSQDQPSGPDS	SQDQPVDHQQPT(ADSSG-Q	QGQQQQI	PLDQPT-		
HIP 32_1-1	DSTGSSDVTHVDTEY	NDDSSSSETSQ	QPQQPP D QP	VDQQQP-VQQ	2	PSQDQPSGPDS	SODOPVDHOOPT(ADSSG-Q	Q <mark>G</mark> QQQQI	PLDQPT-		
KNP 102_05 5	DSTGSSDLTHVDTEY	NDDSSSSETSQ	QPQQPP D QP	VDQQQP-VQQ	2	PSQDQPSGPDS	SODOPVDHOOPT(QADSSG-Q	Q <mark>G</mark> QQQQQ	PLDQPT-		
Lad 1438_10-19	DSTGSSDLTHVDTEY	NDDSSSSETSQ	0PQQPP D QP	VDQQQP-VQQ	2	PSQDQPSGPDS	SODOPVDHOOPT(2ADSSG-Q	Q <mark>G</mark> QQQQI	PLDQPT-		
Mab A13_1/2	DSTGSSDLTHVDTEY	NDDSSSSETSQ)PQQPP D QP	VD000P-V00		PSODOPSGPDS	DOBADOBAD	DADSSG-Q		PLDQPT-		Buffalo-type
Ithas 4_C14-18	DSTGSSDVTQADSES	NDDSSSSETGQ	DACOCEDCE.	VDHQQP-VQD	DSSGQQGQQP	ETPSQDQPSGQGE	PVE-PADQQQPTQ	2GDSSG-Q	QGQQI	PPVQPV-		• 1
$101a \ 5_015 \ 14$ Ttha 9 cl9-5	DSTGSSDVTQADSES	NDDSSSSETCO		VDHOOP-VOD	DSSGOOGOOP	ETPSODOPSGOGE		CDSSG-0	0 <mark>6</mark> 001	PPVOPV-		
Itha 2 cl2-14	DSTGSSDLTHVDTEY	NDDSSSSETSQ	POOPPDOP	VDQQQP				202020 2	2022			
HIP 19_2/8	DSTGSSDVTQADSES	NDSSSSSETLQ	GOOPPVOP	VDQQQT								
Mab BB38_114	DSTGSSDVTQVDTES	NDNSTSSETSQ	PBAEB	VDQQQT								
Bloe B_5-2	DSTGSSDVTQADSES	NDSSSSSETSQ	2 <mark>G</mark> Q									
KNP 102_5 57	DSTGSSDVTQVDSES	NDTSSSSETSQ	PTQD-DQP	VDQQQPT		QDQPSGQGE	PVE-PVDQPQPE	2PPVQPVD	HQI	PPVQPV-		
KNP V5 16	DSTGSSDITHVDTEY	NDDSSSSETSO	DEUOBBDOB	VD000P		QDQF36Q6F	FVE-FVDQFQFE		nQi			
HIP 32 1-2	DSTGSSDVTQVDSES	NDTSSSSETSQ)PT			ODOPSGOPI	PVE-PVDQPQPE	PPVOPVD	OPOPEO	PPVQPV-		
HIP 19_2/1	DSTGSSDVTQVDSES	NDTSSSSETSQ	PT			QDQPSGQGI	PVE-PVDQ					
Lad 10-11 Lad 02-7	110 IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ	120 	130 200	140	150 PTQGDTSG	160 	170 180) 2TPDQSGQ 2TPDQSGQ	190 QPGPDAI QPGPDTI	200 . PDQPVYQ PDQPVYQ]	
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2	110 	120 GPVEPVDQG GPVEPVDQG GPVEPVDQG	130 200 200	140	150		170 180) 2TPDQSGQ 2TPDQSGQ 2TPDQSGQ 2TPDQSGQ	190 QPGPDAI QPGPDTI QPGPDTI	200 . PDQPVYQ PDQPVYQ PDQPVYQ]	
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9	110 IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ	120 GPVEPVDQG GPVEPVDQG GPVEPVDQG GPVEPVDQG	130 	140	150 PTQCDTSG PTQCDTSG PTQCDTSG PTQCDTSG PTQCDTSG	160 2000 2000 2000 2000 2000 2000 2000 2	170 180 20DGQDSQGTPE 20DGQDSQGTPE 20DGQDSQGTPE 20DGQDSQGTPE 20DGQDSQGTPE) TPDQSGQ TPDQSGQ TPDQSGQ TPDQSGQ TPDQSGQ TPDQSGQ	190 <u>PGPDA</u> <u>PGPDT</u> <u>PGPDT</u> <u>QPGPDT</u> <u>OPGPDT</u>	200 		Cattle-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8	110 IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPFGPVQPDQTGQ IQEPFGPVQPDQTGQ IQEPFGPVQPDQTGQ IQEPFGPVQPDQTGQ	120 GPVEPVDQ GPVEPVDQ GPVEPVDQ GPVEPVDQ GPVEPVDQ GPVEPVDQ	130 200 200 200 200	140	150 PTQGDTSG PTQGDTSG PTQGDTSG PTQGDTSG PTQGDTSG	160 2000 200 PV01 2000 200 PV01 2000 200 200 200 2000 200 200 200 2000 200 200 200 2000 200 200 200 200	170 180 20DGQDSQCTPE(20DGQDSQCTPE(20DGQDSQCTPE(20DGQDSQCTPE(20DGQDSQCTPE(20DGQDSQCTPE(20DGQDSQCTPE() TPDQSGQ TPDQSGQ TPDQSGQ TPDQSGQ TPDQSGQ TPDQSGQ TPDQSGQ	190 QPGPDAI QPGPDTI QPGPDTI QPGPDTI QPGPDTI QPGPDTI	200 PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ		Cattle-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 00-4/2 Lad 02-3/9 Lad 10-4/8 Schoonspruit	110 IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPFGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ	120 	130 200 200 200 200 200 200	140	150 PTQGDTSG PTQGDTSG PTQGDTSG PTQGDTSG PTQGDTSG PTQGDTSG	160 22602P0D0PV01 22602P0D0PV01 22602P0D0PV01 22602P0D0PV01 22602P0D0PV01 22602P0D0PV01 22602P0D0PV01	170 184 20DG0DSQGTPE 20DG0DSQGTPE 20DG0DSQGTPE 20DG0DSQGTPE 20DG0DSQGTPE 20DG0DSQGTPE 20DG0DSQGTPE	D DTPDQSGQ DTPDQSGQ DTPDQSGQ DTPDQSGQ DTPDQSGQ DTPDQSGQ DTPDQSGQ	190 	200 PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ		Cattle-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 Schoonspruit T. parva Muguga	110 IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPFGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ	120 	130 	140	150 PTQGDTSG PTQGDTSG PTQGDTSG 	160 20G02P0D2PV01 20G02P0D2PV01 20G02P0D2PV01 20G02P0D2PV01 20G02P0D2PV01 20G02P0D2PV01 20G02P0D2PV01 20G02P0D2PV01	170 188 20DGQDSQGTPE 20DGQDSQGTPE 20DGQDSQGTPE 20DGQDSQGTPE 20DGQDSQGTPE 20DGQDSQGTPE 20DGQDSQGTPE	D D D D D D D D D D D D D D D D D D D	190 QPGPDAI QPGPDTI QPGPDTI QPGPDTI QPGPDTI QPGPDAI QPGPDTI	200 PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ		Cattle-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 Schoonspruit T. parva Murgaga T. parva Marikebuni	110 102ESGP02P02T62 102ESGP02F02 102ESGP02F02 102ESGP02F02 102ESGP02F02 102ESGP02F02 102ESGP02F02 102ESGP02 102	120 	130 	140	150 	160 20G00P0D0PV01 20G00P0D0PV01 20G00P0D0PV01 20G00P0D0PV01 20G00P0D0PV01 20G00P0D0PV01 20G00P0D0PV01 0P0D0PV01	170 180 20DGQDSQGTPE 20DGQDSQGTPE 20DGQDSQGTPE 20DGQDSQGTPE 20DGQDSQGTPE 20DGQDSQGTPE 20DGQDSQGTPE 20DGQDSQGTPE 20DGQDSQGTPE	D TPDQSGQ TPDQSGQ TPDQSGQ TPDQSGQ TPDQSGQ TPDQSGQ TPDQSGQ TPDQSGQ TPDQSGQ	190 OPGPDAI OPGPDTI OPGPDTI OPGPDTI OPGPDTI OPGPDTI OPGPDAI OPGPDTI	200 PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ		Cattle-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 00-4/2 Lad 02-3/9 Lad 10-4/8 Schoonspruit T. parva Muguga T. parva Muguga T. parva Marikebuni Itha 2_c12-11 HTP 22 8-17	110 IQEPSGPVQPD076Q IQEPSGPVQPD076Q IQEPSGPVQPD076Q IQEPSGPVQPD076Q IQEPSGPVQPD076Q IQEPSGPVQPD076Q IQEPSGPVQPD076Q IQEPSGPVQPD076Q IQEPSGPVQPD076Q	120 	130 	140	150 	160 20CQOPODOPVOI 20CQOPODOPVOI 20CQOPODOPVOI 20CQOPODOPVOI 20CQOPODOPVOI 20CQOPODOPVOI 20CQOPODOPVOI 	170 180 20DeQDSQCTPE 20DeQDSQCTPE 20DeQDSQCTPE 20DeQDSQCTPE 20DeQDSQCTPE 20DeQDSQCTPE 20DeQDSQCTPE 20DeQDSQCTPE 20DeQDSQCTPE 20DeQDSQCTPE 20DeQDSQCTPE	2 2 2 2 2 2 2 2 2 2 2 2 2 2	190 	200 PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ		Cattle-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 Schoonspruit T. parva Muguga T. parva Muguga T. parva Muguga T. parva Muguga B. parva 4.0. HIP 22_8-17 Bloe B 4.0.	110 IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ	120 	130 	140 	150 	160	170 18(32DGQDSQGTPE; 32DGQDSQGTP; 32DGQDSQGTP; 32DGQDSQGTP; 32DGQDSQGTP; 32DGQDSQGTP; 32DGQDSQGTP; 32DGQDSQGTP; 32DGQDSQGTP; 32DGQDSQGTP; 32DGQDSQGTP; 32DGQDSQGTP; 32DGQDSQGTP; 32DGQDSQGTP; 32DGQDSQGTP; 32DGQD; 32DGQ	2 2 TPDQSGQ 2 TPDQSQ 2 TPDQSQ 2 TPDQSQ 2 TPDQSQ 2 TPDQSQ 2 TPDQSQ 2 TPDQSQ 2 TPDQSQ 2 TPDQ 2 TPDQ 2 TPDQ 2 TPDQSQQ 2 TPDQSQQ 2 TPDQS	190 	200 PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVQ PDQPVQ PDQPQQQ 		Cattle-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 02-3/9 Lad 10-4/2 Schoonspruit T. parva Muguga T. parva Marikebuni Itha 2_c12-11 HIP 22_8-17 Bloe B_4.0. Itha 6_c16-6	110 IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ IQEPSGPVQPDQTGQ	120 	130 	140	150 	160	170 18(2 2 2 2 2 2 2 2 2 2 2 2 2 2	190 	200 PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPYYQ 		Cattle-type
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Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 Schoonspruit T. parva Muguga T. parva Muguga T. parva Marikebuni Itha 2_c12-11 HIP 22_8-17 Bloe B_4.0. Itha 6_c16-6 HIP 32_1-18 Lad M119_9	110 	120 	130 	140 	150 	160 :: 2000/PDDPV01 2000/PDDPV01 2000/PDDPV01 2000/PDDPV01 2000/PDDPV01 2000/PDDPV01 2000/PDDPV01 	170 18:	27PDQSGQ 27PQ 27PQ 27PQ 27PQ 27PQ 27PQ 27PQ 27P	190 OPGPDAI OPGPDAI OPGPDTI OPGPDTI OPGPDTI OPGPDTI OPGPDTI OPGPG- DOPTG- DOPTG- DOPTG- DOPTG-	200 PDQPYQ PDQPYQ PDQPYQ PDQPYQ PDQPYQ PDQPYQ PDQPYQ PDQPYQ 		Cattle-type 'Mixed'-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 02-3/9 Lad 10-4/2 Schoonspruit T. parva Muguga T. parva Muguga T. parva Muguga T. parva Muguga T. parva Marikebuni Itha 2_c12-11 HIP 22_8-17 Bloe B.4.0. Itha 6_c16-6 HIP 32_1-18 Lad M19_9 Lad 1438_10-10 Lad 1438_10-10	110 IQEPSGPVQPDQTGQ IQEPSGPVQPDQ IQEPSGPVQPDQ IQEPSGPVQPDQ IQEPSGPVQPDQ IQEPSGPVQPDQ IQEPSGPVQP IQEP	120 	130 	140 	150 	160	170 18 	2 2 2 2 2 2 2 2 2 2 2 2 2 2	190 OPGPDAI OPGPDAI OPGPDTI OPGPDTI OPGPDTI OPGPDAI OPGPDAI OPGPTG	200 PDQPVQ PDQPVQ PDQPVQ PDQPVQ PDQPVQ PDQPVQ PDQPVQ PDQPVQ PDQPVQ 		Cattle-type 'Mixed'-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 Schoonspruit T. parva Muguga T. parva Muguga T. parva Muguga T. parva Auguga T. parva Auguga T. parva Auguga T. parva Auguga T. parva Auguga T. parva Muguga T.	110 	120 	130 	140	150 	160	170 18(2DGQDSQCTPE 2DGQDSQCTPE 2DGQDSQCTPE 2DQQDQCQCTPE 2PSGQPI))))))))))))))	190 	200 PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVQ 		Cattle-type 'Mixed'-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 Schoonspruit T. parva Muguga T. parva Muguga T. parva Muguga Itha 2_c12-11 HIP 22_8-17 Bloe B_4.0. Itha 6_c16-6 HIP 32_1-18 Lad M119_9 Lad 1438_10-10 Lad 17_10 HIP 22_8-13 T. parva 7014	110 	120 	130 	140 	150 	160	170 18: SDG0DSQCTPE SDG0DSQCTPE SDG0DSQCTPE SDG0DQQC SDSD0DQQCDQQC SDG0DQQQC SDSD0DQQQQC SDG0DQQQC SDSD0DQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQ))))))))))))))	190 	200 PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVQ PDQPVQ PDQPVQ PDQPVQ PDQPQQ PDQPQQ PDQPQQ PDQPQQ PDQPQQ PDQPQQ PDQPQQ PDQPQQ PDQPQQ PDQPQQ PDQPQQ PDQPQQ PDQPQQ PDQPQQ PDQPQQ PDQPQQ PDQPQQ PDQPVQ PDQ PDQ PDQ PDQ PDQ PDQ PDQ PDQ PDQ PD		Cattle-type 'Mixed'-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 Schoonspruit T. parva Muguga T. parva Muguga T. parva Muguga T. parva Auguga T. parva Auguga T. parva Auguga T. parva Auguga HIP 22_8-17 Bloe B.4.0. Itha 6_c16-6 HIP 32_1-18 Lad M19_9 Lad 1438_10-10 Lad 17_10 HIP 22_8-13 T. parva 7014 KNP 011_4/1	110 IQEPSGPVQPDQTGQ IQEPSGPVQPDQTQ IQEPSGPVQPDQ IQEPSGPVQPDQTGQ IQEPS	120 	130 	140 POPEPOPDOP POPEPOPOPDOP	150 	160	170 181))))))))))))))	190 	200 PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVQQ PDQPQQQQ		Cattle-type 'Mixed'-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 02-3/9 Lad 10-4/8 Schoonspruit T. parva Muguga T. parva Marikebuni Itha 2_c12-11 HIP 22.8-17 Bloe B_4.0. Itha 6_c16-6 HIP 32_1-18 Lad M19_9 Lad I438_10-10 Lad 17_10 HIP 22_8-13 T. parva 7014 KNP 011_4/1 HIP 32_1-8	110 	120 	130 	140 	150 	160	170 18())))))))))))))	190 	200 pDQPVYQ pDQPVYQ pDQPVYQ pDQPVYQ pDQPVYQ pDQPVYQ pDQPVYQ		Cattle-type 'Mixed'-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 Schoonspruit T. parva Muguga T. parva Muguga T. parva Muguga T. parva Marikebuni Itha 2 cl2-11 HIP 22_8-17 Bloe B_4.0. Itha 6_cl6-6 HIP 32_1-18 Lad M119_9 Lad 1438_10-10 Lad 17_10 HIP 22_8-13 T. parva 7014 KNP 011_4/1 HIP 32_1-8 Itha3_cl3-17	110 	120 	130 	140 	150 	160	170 18: 	Image: Constraint of the second sec	190 	200		Cattle-type 'Mixed'-type
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Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 02-3/9 Lad 10-4/2 Schoonspruit T. parva Muguga T. parva Muguga T. parva Muguga T. parva Marikebuni Itha 2_cl2-11 HIP 22_8-17 Eloe B.4.0. Itha 6_cl6-6 HIP 32_1-18 Lad M19_9 Lad 1438_10-10 Lad 119_9 Lad 1438_10-10 Lad 119_9 Lad 1438_10-10 Lad 11_10 HIP 32_1-8 Itha3_cl3-17 HIP 32_1-1 KNP 102_05 5 Lad 1438_10-19	110 IQEPSGPVQPDQTGQ IQEPSGPVQPDTGQ IQEPSGP	120 	130 	140 POPEPOPODO POPEPOPODO PEPOP- PEPOP- PEPOP- PEPOP- PEPOP- PEPOP- PEPOP- PEPOP- PEPOP- PEPOP- PEPOP- PEPOP- PEPOP- PEPOPOPOPOPOPOPOPOPOPOPOPOPOPOPOPOPOPOP	150 	160	170 181))))))))))))))	190 	200 PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVQ PDQPVQ PDQPVQ PDQPVQ PDQPVQ PDQPVQ PDQPVQ PDQPVQ PQQQQ PQQQQQ PQQQQQQ		Cattle-type 'Mixed'-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 00-4/2 Lad 02-3/9 Lad 10-4/8 Schoonspruit T. parva Muguga T. parva Muguga T. parva Marikebuni Itha 2_c12-11 Bloe B_4.0. Itha 6_c16-6 HIP 32_1-18 Lad M19_9 Lad 1438_10-10 Lad 17_10 HIP 22_8-13 T. parva 7014 KNP 011_4/1 HIP 32_1-8 Itha3_c13-17 HIP 32_1-1 KNP 102_05 5 Lad 1438_10-19 Mab A13 1/2	110 	120 	130 	140 	150 	160	170 18: 20Dc0D5QCTPE; 20Dc0D5QCTPE	2 2 2 7 7 7 7 7 7 7 7 7 7 7 7 7	190 	200 PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVQQ PQQQQ -QQQQQ		Cattle-type 'Mixed'-type
Lad 10-11 Lad 02-7 Lad 10-4/2 Lad 00-4/2 Lad 00-4/2 Lad 02-3/9 Lad 10-4/8 Schoonspruit T. parva Muguga T. parva Muguga T. parva Marikebuni Itha 2_cl2-11 HIP 22_8-17 Eloe B_4.0. Itha 6_cl6-6 HIP 32_1-18 Lad M119_9 Lad 1438_10-10 Lad 17_10 HIP 32_1-18 HIP 32_1-8 Itha3_cl3-17 HIP 32_1-1 KNP 011_4/1 HIP 32_1-1 KNP 102_05_5 Lad 1438_10-19 Mab A13_1/2 Htha3_cl3-18	110 	120 	130 	140 	150 	160	170 18:))))))))))))))	190			Cattle-type 'Mixed'-type Buffalo-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 02-3/9 Lad 10-4/2 Schoonspruit T. parva Muguga T. parva Muguga T. parva Muguga T. parva Auguga T. parva Auguga T. parva Auguga T. parva Auguga HIP 22.8-17 Bloe B.4.0. Itha 6_c16-6 HIP 32_1-18 Lad M19_9 Lad 17.10 HIP 32_1-18 Lad 119_9 Lad 1438_10-10 HIP 32_1-8 Itha3_c13-17 HIP 32_1-1 KNP 012_05_5 Lad 1438_10-19 Mab A13_1/2 Itha3_c13-14	110 IQEPSGPVQPQTGQ IQEPSGPVQPQTQQ IQEPSGPVQPQ IQEPSGPVQP IQEPSGPV IQEPSGPV IQEPSGPV IQEPSGPV IQEPSGPV IQEPSGPV IQEPSGPV IQEPSGPV IQEPSGPV IQEPSGPV IQEPSGPV IQEPSGPV	120 	130 	140 	150	160	170 181))))))))))))))	190	200 PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVQQ PDQPQQQQQ QQQQQQ QQQQQQQ QQQQQQQQQQ		Cattle-type 'Mixed'-type Buffalo-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 Schoonspruit T. parva Muguga T. parva Marikebuni Itha 2_cl2-11 Bloe B_4.0. Itha 6_cl6-6 HIP 32_1-18 Lad H19_9 Lad H438_10-10 Lad 17.10 HIP 22_8-13 T. parva 7014 KNP 011_4/1 HIP 32_1-8 Itha3_cl3-17 HIP 32_1-1 KNP 102_05 5 Lad H38_10-19 Mab A13_1/2 Ithas 4_cl4-18 Itha 3_cl3-14 Itha 3_cl3-14	110 	120 	130 	140 	150 	160	170 18: 	2 2 2 2 2 2 2 2 2 2 2 2 2 2	190	200 PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ		Cattle-type 'Mixed'-type Buffalo-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 00-4/2 Lad 02-3/9 Lad 10-4/8 Schoonspruit T. parva Muguga T. parva Muguga T. parva Marikebuni Itha 2 cl2-11 HIP 22_8-17 Bloe B.4.0. Itha 6_cl6-6 HIP 32_1-18 Lad M19_9 Lad I438_10-10 Lad 17_10 HIP 32_1-8 Itha3_cl3-17 HIP 32_1-8 Itha3_cl3-17 HIP 32_1-1 KNP 001_4/1 HIP 32_1-8 Itha3_cl3-17 HIP 32_1-1 KNP 102_05 5 Lad 1438_10-19 Mab A13_1/2 Itha3_cl3-14 Itha3_cl3-14 Itha3_cl9-5 Itha3_cl9-5 Itha3_cl9-5 Itha3_cl9-5 Itha3_cl9-5	110 	120 	130 	140 	150 	160	170 18: 	2 2 2 7 7 7 7 7 7 7 7 7 7 7 7 7	190			Cattle-type 'Mixed'-type Buffalo-type
Lad 10-11 Lad 02-7 Lad 10-4/2 Lad 00-4/2 Lad 00-4/2 Lad 00-4/8 Schoonspruit T. parva Muguga T. parva Muguga T. parva Muguga T. parva Muguga Dieb B.4.0. Itha 2_c12-11 HIP 22_8-17 Bloe B.4.0. Itha 6_c16-6 HIP 32_1-18 Lad M19_9 Lad 1438_10-10 Lad 17_10 HIP 32_1-18 Lad 1438_10-10 HIP 32_1-8 Itha3_c13-17 HIP 32_1-8 Itha3_c13-17 HIP 32_1-1 KNP 102_05 5 Lad 1438_10-19 Mab A13_12 Itha3_c13-14 Itha3_c13-14 HIP 3_2.2-5 Itha2_c12-14 HIP 19_2/8 Mab B38_114	110 1029526902050 10205526902050 10205526902050 102055290 102055290 1020550	120 	130 	140 POPEPOPDOP POPEPOPOPO PEPOP- POPOPOPOPOPOPOPOPOPOPOPOPOPOPOPOPOP	150 	160 :: QCCQPPDDPV01 QCCQPPDDPV01 QCCQPPDDPV01 QCCQPPDDPV01 QCCQPPDDPV01 QCCQPPDDPV01 QCCQPPDDPV01 QCCQPPDDPV01 QCPVEPVDQPCG QPVEPVDQPCG QPVEPVDQPCG QPVEPVDQPCG QPVPDVDPTG QPV2PVDQPCG QPVDQPVDQPCG Q	170 181))))))))))))))	190			Cattle-type 'Mixed'-type Buffalo-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 00-4/2 Lad 02-3/9 Lad 10-4/8 Schoonspruit T. parva Muguga T. parva Muguga T. parva Marikebuni Itha 2_cl2-11 Bloe B_4.0. Itha 6_cl6-6 HIP 32_1-18 Lad M19_9 Lad 1438_10-10 Lad 17_10 HIP 22_8-13 T. parva 7014 KNP 011_4/1 HIP 32_1-8 Itha3_cl3-17 HIP 32_1-8 Itha3_cl3-17 HIP 32_1-8 Itha3_cl3-17 HIP 32_1-1 KNP 102_05 5 Lad 1438_10-19 Mab A13_1/2 Ithas 4_cl4-18 Itha 3_cl3-14 Itha 2_cl2-5 Itha 2_cl2-14 HIP 19_2/8 Mab BB38_114 Bloe B 5-2	110 	120 	130 	140 	150 	160	170 18: 20DG0D5QCTPE; 20DG0D5QCTPE	2 2 2 7 7 7 7 7 7 7 7 7 7 7 7 7	190	200 PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ PDQPVYQ		Cattle-type 'Mixed'-type Buffalo-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 00-4/2 Lad 00-4/2 Lad 02-3/9 Lad 10-4/8 Schoonspruit T. parva Muguga T. parva Muguga T. parva Muguga Itha 2_c12-11 HIP 22_8-13 T. parva Muguga Lad 1438_10-10 Lad 17_10 HIP 32_1-18 Lad M19_9 Lad 1438_10-10 Lad 17_10 HIP 32_1-8 Itha3_c13-17 HIP 32_1-1 KNP 101_4/1 HIP 32_1-1 KNP 101_4/1 HIP 32_1-1 KNP 102_05_5 Lad 1438_10-19 Mab A13_1/2 Itha3_c13-14 HIP 32_19-5 Itha3_c19-5 Itha3_c19-5 Itha3_c14 HIP 19_2/8 Mab B836_114 Bloe B_5-2 KNP 102_5_57	110 	120 	130 	140 	150 	160	170 18: 	2 2 2 2 2 2 2 2 2 2 2 2 2 2	190			Cattle-type 'Mixed'-type Buffalo-type
Lad 10-11 Lad 02-7 Lad 10-4/2 Lad 00-4/2 Lad 00-4/2 Schoonspruit T. parva Muguga T. parva Muguga T. parva Muguga Itha 2_c12-11 HIP 22_8-17 Bloe B_4.0. Itha 6_c16-6 HIP 32_1-18 Lad M19_9 Lad 1438_10-10 Lad 17_10 HIP 32_1-18 Lad M19_9 Lad 1438_10-10 HIP 32_1-18 Itha3_c13-17 HIP 32_1-8 Itha3_c13-17 HIP 32_1-1 KNP 102_05 5 Lad 1438_10-19 Mab A13_1/2 Ithas 4_c14-18 Itha3_c13-14 HIP 3_2.12-14 HIP 19_2/8 Mab BB38_114 Bloe B_5-2 KNP 102_5 57 Mab A13_4/2	110 10295269020760 10295269020760 10295269020760 10295269020760 10295269020760 10295269020760 10295269020760 102952690200760 102952690200760 102952690200760 1029526902000760 1029526902000760 1029526902000760 1029526902000760 1029526902000760 1029526902000760 1029526902000760 1029526902000760 1029526902000760 1029526902000760 1029526902000760 1029526902000760 102952690200000 102952690200000 102952690200000 1029526902000000 1029526902000000 1029526902000000 10295269020000000000000000000000000000000	120 	130 		150 	160 :: QCCQPPDDPVD QCCQPPDDPVD QCCQPPDDPVD QCCQPPDDPVD QCCQPPDDPVD QCCQPPDDPVD QCCQPPDDPVD QCCQPPDDPVD QCCQPPDDPVD QCCQPPDDPVD QCQPPDDPVD QPVEPVDQPCG QPVEPVDQPCG QPVEPVDQPCG QPVEPVDQPCG QPVEPVDQPCG QPVDPVDQPCG QPVDPVDQPCG QPVDPVDQPCG QPVDPVDQPCG QPVDPVDQPCG QPVDPVDQPCG QPVDPVDQPCG QPQDQPQPQPDPDPEP(QPQQQQQQPQDQPDDPDPD QPQDQDQDQDQDQD QPVDPVDQPCG QPQQQQQQQDQD QPVDPVDQPCG QPQQQQQQQDQD QPVDPVDQPCG QPVDPVDQPCG QPQQQQQQQQDQD QPVDPVDQPCG QPVDPVDQPCG QPVDPVDQPCG QPVDPVDQPCG QPQQQQQQDQD QPVDPVDQPCG QPVDPVDQPCG QPVDPVDQPCG QPVDPVDQPCG QPVDPVDQPCG QPVDPVDQPCG QPVDPVDQPCG QPVDPVDQPCG QPVDPVDQPCG QPVDPVDQPCG QPVDQPVDQPCG QPVDPVDQPCG QPVDQPQDQPQDQDQ QPVDQPVDQPCG QPVDQPVDQPCG QPVDQPQDQDQ QPVDQPQDQDQ QPVDQPQDQDQ QPVDQPQDQDQ QPVDQPQDQDQ QPVDQPQDQ QPVDQPQDQ QPVDQPQDQ QPVDQ QPVDQ QPVDQ QPVDQ QPVDQ QPVDQ QPVDQ QPVDQ QPVDQ QPVDQ QPQDQ QPQDQ QPQDQ QPVDQ QPVDQ QPVDQ QPVDQ QPVDQ QPVDQ QPQDQ QPQDQ QPVDQ	170 18:))))))))))))))	190			Cattle-type 'Mixed'-type Buffalo-type
Lad 10-11 Lad 02-7 Lad 10-4/2 Lad 02-3/9 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 Schoonspruit T. parva Muguga T. parva Muguga T. parva Marikebuni Itha 2_c12-11 Bloe B_4.0. Itha 6_c16-6 HIP 32_1-18 Lad H13.9 Lad H13.9 Lad H13.10-10 Lad 17.10 HIP 22_8-13 T. parva 7014 KNP 011_4/1 HIP 32_1-8 Itha3_c13-17 HIP 32_1-8 Itha3_c13-17 HIP 32_1-1 KNP 102_05 5 Lad 1438_10-19 Mab A13_1/2 Ithas 4_c14-18 Itha3_c13-14 HIP 3_2/8 Mab BE38_114 Bloe B_5-2 KNP 102_5 57 Mab A13_4/2 KNP 102_5 57	110 	120 	130 	140 	150 	160	170 18: 	Image: Constraint of the second sec	190	200		Cattle-type 'Mixed'-type Buffalo-type
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 00-4/2 Lad 02-3/9 Lad 10-4/8 Schoonspruit T. parva Muguga T. parva Muguga T. parva Marikebuni Itha 2_c12-11 HIP 22_8-17 Bloe B_4.0. Itha 6_c16-6 HIP 32_1-18 Lad M119_9 Lad 1648_10-10 Lad 17_10 HIP 22_8-13 T. parva 7014 KNP 001_4/1 HIP 32_1-8 Itha3_c13-17 HIP 32_1-8 Itha3_c13-17 HIP 32_1-8 Itha3_c13-17 HIP 32_1-8 Itha3_c13-17 HIP 32_1-18 Itha3_c13-14 Itha3_c13-14 Itha3_c13-14 Itha3_c13-14 Itha3_c12-5 Itha3_c12-5 Itha2_c12-5 Itha2_6 JA3_12 KNP 102_c5_57 Mab A13_4/2 KNP 102_5_57 Mab A13_4/2 KNP 102_5_57 Mab A13_4/2 KNP 102_5_16 HIP 32_1-2	110 1028526V02P076Q 1028526V02P076Q 1028526V02P076Q 1028526V02P076Q 1028526V02P076Q 1028526V02P076Q 1028526V02P076Q 1028526V02P076Q 1028526V02P076Q 1028526V02P076Q 1028526V02P076Q 1028526V02P076Q 1028526V02P076Q 1028526V02P076Q 1028526V02P076Q 1028526V02P076Q 1028527V02P076Q 1028527V02P076Q 1028527V02P076Q 1028527V02P076Q 1028527V02P076Q 1028527V02P076Q 1028527V02P076Q 1028527V02P076Q 1028527V02P076Q 1028527V02P076Q 1028527V02P076Q 1028527V02P076Q 1028527V02P076Q 1028527V02P076Q 1028527V02P076Q 1028527V02P076Q 1027527 102757 1027577 1027577 1027577 1027577 10275777 102	120 	130 	140 	150 	160	170 18: SQDGQDSQCTPE; SQDGQDSQCTPE; SQDGQDSQCTP; SQDGQDSQCTPE; SQDGQDSQCTP; SQDGQDSQCTP; SQDGQDSQCTP; SQDGQDSQCTP; SQDGQDSQC; SQDGQDSQCTP; SQDGQDSQC; SQDGQDSQC; SQDGQD; SQDGQDSQC; SQD; SQDQQQ; SQD; SQDQQQ; SQD; SQDQQQ; SQD; SQDQQQ; SQD; <td< th=""><th>) </th><th>190</th><th>200</th><th></th><th>Cattle-type 'Mixed'-type Buffalo-type</th></td<>) 	190	200		Cattle-type 'Mixed'-type Buffalo-type

Figure 6.6 Multiple sequence alignment of the inferred PIM amino acid sequences obtained from buffalo and cattle *T. parva* samples collected from different geographical areas in South Africa (Table 6.1). PIM sequences were aligned with previously published *T. parva* PIM sequences, Muguga (accession number: L06323), Marikebuni (accession number: L41148) and 7104 (accession number: L41833). The sequence alignment was constructed manually because of the extreme polymorphism in the central region of the PIM gene. Amino acid motifs characteristic of cattle-type PIM sequence are shown in solid-line blocks including the tetrapeptide repeat characteristic of the central variable region of cattle-derived PIM sequences at positions 428-447. All amino acid motifs characteristic of buffalo-type PIM sequence are shown in broken-line blocks. Figure 6.6 continues on pages 145 and 146.





	210	220	230	240	250	260	270	280	290	300	
Lad 10-11	QQP V QQPS <mark>G</mark> QQQQP	QPRPQPQPDQP	VDQQQEPPT	PEDQPSGPDS	PDQPDQHH	QPTPAAQ				ר	
Lad 02-7	QQPVQQPSGQQQQP	QP R PQPQP D QP	VDQQQEPPT	PEGQPSGPDS	PDQPDQHH	QPTPAAQ					
Lad 10-4/6 Lad 10-4/2		QP R PQPQP D QP QP R PQPQP D QP	VDQQQEPPT VDQQQEPPT	PEDQPSGPDS	PDQPDQHH	QPTPAAQ QPTPAAQ					Cattle tons
Lad 02-3/9	<u>QQ</u> P V QQPS <mark>G</mark> QQQQP	QP R PQPQP D QP	VDQQQEPPT	PEDQPPGPDS	PDQPDQHH	QPTPAAQ					Cattle-type
Lad 10-4/8 Schoonspruit	QQPVQQPSGQQQQP OOPVOOPSGOOOOP	OPRPOPOPDOP OPRPOPOPDOP	VDQQQEPPT VD000EPPT	PEDQPSGPDS PEDOPSGPDS	PDQPDQHH PDOPDOHH	QPTPAAQ OPTPAAO					
T. parva Muguga	QQP V QQPS <mark>G</mark> QQQQP	QP R PQPQP D QP	VDQQQEPPT	PEDQPSGPDS	PDQPDQHH	QPTPAAQ					
T. parva Marikebuni Itha 2 cl2-11	PVOE	PAKDDPTGOOO	0	POPEPEPEOT	PETPPOOK	OPTPDDHPSC	OO-PODEPVOOE	OGAODSPTPD			
HIP 22_8-17	PPVQPVDQQQPVQE	PAKDDPTGQQQ	Q	PEPEPEQT	PETPPQQE	QPTPDDHPS(QQ-GQQPPV-QG	QGAQDSPTPD	DOPVDHOO		
Bloe B_4.0. Itha 6 cl6-6	PPIOPVDHOOPVOE PPVOPVDOOOTPKE	PAKDDPTGQQQ PAKHDPTGQQQ	QPQ	PEPEPEPEQT	PETPPQQE	OPTPDDHPS(OPTPDDHPS(QQ-PQGEPV-QG	OGAODSPTPD			
HIP 32_1-18	PPVQPVDQQQTPKE	PAKDDPTGQQQ	QPQ	POPOPEPEOT	PETPPQQE	QPTPDGHPS0	QQ-PQDEPV-QE	QRAQDSPTPD	DOPVDOOD		'Mixed'-type
Lad M119_9 Lad T438 10-10	PPVQPVDQQQ	PSKDDPTCOOO	0P0	DEDEDEDEOT							
Lad 17_10	PPVQPVDQQQTPKE	PSKDDPTGQQQ	QPQ	PEPEPEPEQT	PETPPQQE	QPTPDDHPS(QQ-PQDEPV-QG	QGAQDSPTPD	DOPVDOOQ		
HIP 22_8-13 T parwa 7014	PRIORVDOOOPVOR	PAKDDPTCOOO	0P0	DEDEDEDEOT			PV-QEP	GAQDSPTPDD			
KNP 011_4/1	PPVQPVDQQQTPKQ	PAKDDPTGQQQ	QPQPK	PEPEPEPEQT	PETPPQQE	QPTPDDQPVI	DOODPODEPVOOE	QGPQDSPTPD	DOPVDOOQ		
HIP 32_1-8	PPIQPVDQQQPVQE	PAKHDPTGQQQ	Q	POPOPEPEOT	PETPPQQE	OPTPDDHPS(QQ-GQQPPA-QG	QGAQDSPTPD			
HIP 32_1-1	PPIQPVDQQQPVQE	PAKHDPTGQQQ	ι <u>α</u> ΙQ	POPOPEPEOT	PETPPQQE	QPTPDDHPS(QQ-GQQPPV-QG	QGAQDSPTPD	DOPVDHOQ		
KNP 102_05 5	PPIOPVDOOOPVOE	PAKHDPTGQQQ	Q	POPOPEPEOT	PETPPQQE	OPTPDDHPS(QQ-GQQPPV-QG	OCAODSPTPD	DOPVDHOQ		
Mab A13_1/2			. <u>v</u>			QF IF DDHF 30					
Ithas 4_c14-18	PPVQPVDQQQTPQE	QTKDDPTGLQQ	QPEPPAA-Q	POPOPEPEOI	PETPAQQQ	IPTPDDHQS	QQ-PQDEPVQQE	QGAQDSPTPD	DOPVDOOD		Buffalo-type
Itha 9_c19-5	I PPVQPVDQQQTPQE	QTKDDPTGLQQ	QPEPPAA-Q	POPOPEPEOI	PETPAQQQ	IPTPDDHQS(QQ-PQDEPV-QQ	QGAQDSPTPD			
Itha 2_cl2-14	IVQE	PAKDDPAGQQQ	QP	QPEPEPEQTP	ETPPQQKQ	P-TPDDHPS	QQ-PQDEPVQQE	QGAQDSPTPD	DOPVDOOQ		
Mab BB38_114	PKQ	PAKDDPTGQQQ PAKDDPTGQQQ	QPQP K	PEPEPEPEQT	PETPPQQEQ	QPTPDDQPVI	QQQPQDEPVQQE	QGPQDSPTPD QGPQDSPTPD	DQPVDQQQ		
Bloe B_5-2									!		
Mab A13_4/2							PV-QEP PV-QEP	VGQDSPTPDD	QPDQHQQPVQI QPDQHQQPVQI	DDASGK	
KNP V5_16							PV-QEP	VGQDSPTPDD	QPDQHQQPVQI	DASGK	
HIP 32_1-2 HIP 19_2/1							PV-QEP PV-QEP	VGQDSPTPDD	QPDQHQQPVQI QPDQHQQPVQI	DDASGK	
Lad 10-11	310 	320 	330	340	350	360 	370	380	390	400 	
Lad 02-7											
Lad 10-4/8 Lad 10-4/2											~ -
Lad 02-3/9											Cattle-type
T. parva Schoonspruit											
T. parva Muguga											
T. parva Marikebuni Itha 2_cl2-11					P	VQEPEPSEE	PQPQPQ	PEPEPEQPPV	EPVDQQQQP+		
HIP 22_8-17					P	VQEPVQQEE-	PQPEPQP	QPEPEPEQPQ			
Itha 6_c16-6					P	PQQE	EPOPOOOPOPEP	QPEPEPQQPR	DOPVDOOOP		
HIP 32_1-18					P	VQEPVQQ	GQPEPQPQPEP				'Mixed'-type
Lad I438_10-10					P	VQE	PQPEP	QPEPEPEQPP	VQPVDQQQP		
Lad 17_10 HTP 22 8-13					P	VOEPEPSEE	PQPEP		VOPVDQQQP+-		
T. parva 7014					P	VHEPVQDQT	PQQ-PQPQP E PQ	PEPEPGQQPP	VQPVDQQQP		
KNP 011_4/1 HTP 32 1-8					P	VQEPVQQEQ-	PQPEPEPEP	OPEPEPEOPC	DOPVDHOOP-		
Itha3_c13-17						F	EPQPQQQPQPEP	QPEPEPQQPQ	DOPVDOOOP		
HIP 32_1-1 KNP 102 05 5					P	VQEPVQQEE	PQPEPQP	OPEPEPEQPC	DOPVDQQQP		
Lad 1438_10-19						F	EPQPQQQPQPEP	QPEPEPQQPQ	DOPVDOOOP		
Mab A13_1/2 Tthas 4 cl4-18					p	VOEPEPSEE)	PEPEPEOPP	EPVDQQQQP		Dff-1 4
Itha 3_c13-14					P	VQEPVQQEQ-	PEPQPQPEP	QPQPEPEQPQ	DOBADOOOD		Burrato-type
Itha 9_c19-5					P	VQEPVRQEQ-	PQP	EPEPOPEOPO			
HIP 19_2/8					P	VQEPVQQEQ-	P E PQPQP E P	QPQP E P EL PQ	DOBADOOOD-+-		
Mab BB38_114					P	VQEPVQQEQ-	PQPEPEPEP	QPQPEPEQPQ			
KNP 102_5 57	QETPVQPVDQQQPT	QDDQPVDHQQ-			P	VOEPVOOEE-	POPEPOP	OPEPEPEOPC	DOPVDOOP+		
Mab A13_4/2	QETPVQPVDQQQPT		PSGQE	DOPTPDDQPQ	PEQTPEPP	VEPVDQKQ					
HIP 32_1-2	QETPVQPVDQQQPT QETPVQPVDQQQPT	2 D 0	PSGQE	DOPTPDDOPO	PEQTPEPP	VEPVDQKQ					
HIP 19_2/1	QETPVQPVDQQQPT	Q <mark>D</mark> Q	PS <mark>G</mark> QE	DQPTPDDQPQ	PEQTPEPP	VEPVDQKQ					



	410	420	430 440		450 460	470	480	490	500	
		· · · · · · · · ·				<u> </u> _	-
Lad 10-11 Lad 02-7			POPEPOPEPOPEP	OPEPVQ	E		PPEQTPER	TPSEDDASGE		
Lad 10-4/6			POPEPOPEPOPEP	OPEPVOI	E		PPEQTPER	TSSKDDASGE	VPVK	
Lad 10-4/2			PQPEPQPEPQPEP	QPEPVQ	E		PPEQTPER	TPSKDDASGE	VPVK	
Lad 02-3/9			PQPEPQPEPQPEP	QPEPVQ	E		PPEQTPER	TPSKDDASGE	VPVK	Cattle-type
Lad 10-4/8			POPEPOPEPOPEP	QPEPVQ	E		PPEQTPER	TPSKDDASGE	VPVK	
T. parva Schoonspruit		POTOPOP	POPEPOPEPOPEP	OPEPVOI	E		PPEOTPEH	TPSKDDASGE	VPVK	
T. parva Marikebuni	OPEPOPEPOPEPOPEP	OPEPOPEPOPE	POPEPOPEPOPEP	OPEPVO	OTPETPAPOEOPOTPD	DUTPEORPDC	PVOE-PPEOTPER	TPSPDDLSC	PVK _	
Itha 2_cl2-11					VQDQPSGKETPQPTQGD	QPVQDPSGQE	QPEPEQTPER	TPSKDDPTGE	EPVK	1
HIP 22_8-17				1	VQDQPSGQETPQPIPDG	QP V Q E PP E Q K	PEPEPEQTPER	TPSKDDLSGE	EPVK	
Bloe $B_4.0.$					VQDQPSGQETPQPIPED	OPVOEPTEOK OPVODRCOK	PEPEQTPER	TPSKGDTSGE	EPVQ	
HTP 32 1-18					TODOPSCOETPOPTOCO	OPVOEPTEOK	PEPEQIPER	TPSKDDLSGE		(Miyod) type
Lad M119 9					VODOPSGOETPOPIPED	OPVOEPPEOK	PEPEPEQTPER	TPSKDDPTGE	EPVK	wiixeu -type
Lad 1438_10-10					VQDQPSGQETPQPIPED	QP V Q E PP E Q K	PEPEPEQTPER	TPSKDDPTGE	EPVK	
Lad 17_10					VQDQPSGQETPQPIPED	QP V Q E PP E Q K	PEPEPEQTPER	TPSKDDPTGE	EPVK	
HIP 22_8-13					HE-PSGQETPQPIPDD	QPVREPTE-K	-EPEPEQKPDH	TPSKDDTSGE	EPVQ	4
KNP 011 4/1					SODOPSGOETPOPIPED	OPVOEPTEOK	PEPEOTPEH	APSKDDPTGE	EPVK	
HIP 32_1-8					QDQPSGQETPQPIPDG	<u><u>O</u>PVO</u> EPPEOK	PEPEPEQTPER	TPSKDDLSGE	E PVK	
Itha3_cl3-17				1	VQDQPSGKETPQPIPED	QQ V Q E PT E Q K	PEPEQTPER	SPSKDDLSGE	EPVK	
HIP 32_1-1					VQDQPSGQETPQPIPDG	QPVQEPPEQK	PEPEPEQTPKH	TPSKDDLSGE	EPVK	
KNP 102_05 5					VODOPSGOETPOPIPDG VODOPSCKETPOPIPDG	OVOEPPEOR	PEPEPEQTPER	SPSEDDLSGE	PVK	
Mab A13 1/2					VODOPSGKETPOPTOGD	OPVODPSGOE	OPEPEOTPEH	TPSKDDPTGE	EPVK	
Ithas 4_c14-18					VQDQPSGKETPQPTQGD	QP V Q D PSGQE	QPEPEQTPER	TPSKDDPTGE	EPVK	Buffelo type
Itha 3_cl3-14					TQDQPSGQETPQPTQGD	QP V Q D PSGQ E	QPEPEQTPEH	TP SKDDP TGE	EPVK	Dullalo-type
Itha 9_c19-5					SQDQPSGQETPQPIPED	QPAREPTEQK	PEPEQTPEH	TPSKDDPTGE	EPVK	
Itha 2_c12-14 HTP 19 2/8					VQDQPSGKETPQPTQGD TODOPSCOFTPOPTPED	OPVODPSGOE OPVOEPTEOK	QPEPEQTPER	TPSKDDPTGE	FPVK	
Mab BB38 114					SODOPSGOETPOPIPED	OPVOEPTEOK	PEPEOTPEH	APSKDDPTGE	EPVK	
Bloe B_5-2					VQDQPSGQETPQPIPED	<u><u><u></u></u> <u>O</u>PV<u>O</u>EPTEOR</u>	PEPEQTPEH	TPSKDDTSGE	EPVQ	
KNP 102_5 57					VQDQPSGQETPQPIPDG	QP V Q E PP E Q K	PEPEPEQTPER	TPSKDDLSGE	EPVK	
Mab A13_4/2				-PVQEP	SQDQPSGQETPQPIPDG	QPVQEPPEQK	PEPEQTPEH	TPSKDDPSGE	EPVK	
HTD 32 1-2					SODOPSCOLTPOPIPDC	OPVOEPPEOR	PEPEQIPER	TPSEDDPICE		
HIP 19_2/1				-PVQEP	SQDQPSGQETPQPIPDG	QP V Q E PP E Q K	PEPEQTPEH	TPSKDDPSGE	EPVK	
	510 52 .	0 530 	540 	· _						
Lad 10-11	510 52	0 530	540	K						
Lad 10-11 Lad 02-7 Lad 10-4/6	510 52	0 530	540 	K						
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2	510 52 PSEGHMTGAAADGSGQ PSEGHMTGAAADGSGQ PSEGHMTGAAADGSGQ PSDGHMTGAAADGSGQ	0 530 PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK	540 	K K K	Cottle trace					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9	510 52 PSEGHMTGAADGSGQ PSEGHMTGAAADGSGQ PSEGHMTGAAADGSGQ PSDGHMTGAAADGSGQ PSEGHMTGAAADGSGQ	0 530 PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK	540 GKDGSKSGSGTPS GKDGSKSGSGTPG GKDGSKSGSGTPS GKDGSKSGSGTPS	K K K K	Cattle-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8	510 52 PSEGHMTGAAADGSGQ PSEGHMTGAAADGSGQ PSDGHMTGAAADGSGQ PSDGHMTGAAADGSGQ PSDGHMTGAAADGSGQ PSDGHMTGAAADGSGQ	0 530 PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDSK PPDKKTDDSK PPDKKTDDSSK	540 GKDGSKSGSGTPS GKDGSKSGSGTPG GKDGSKSGSGTPG GKDGSKSGSGTPS GKDGSKSGSGTPS		Cattle-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 T. parva Schoonspruit T. parva Muquqa	510 52 PSEGIMTGAAADGSGQ PSEGIMTGAAADGSGQ PSEGIMTGAAADGSGQ PSEGIMTGAAADGSGQ PSEGIMTGAAADGSGQ PSEGIMTGAAADGSGQ PSEGIMTGAAADGSGQ	0 530 PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK	540 		Cattle-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 T. parva Schoonspruit T. parva Muguga T. parva Marikebuni	510 52 PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ	0 530 PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDSK PPDKKTDDSK PPDKKTDDSK PPDKKTDDSSK PPDKKTDDSSK	540 		Cattle-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 T. parva Schoonspruit T. parva Muguga T. parva Muguga T. parva Marikebuni Itha 2_cl2-11	510 52 PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSDGIMTGAADGSGQ PSDGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ	0 530 PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDSSK PPDKKPGDDSK	540 		Cattle-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/6 Lad 02-3/9 Lad 10-4/8 T. parva Schoonspruit T. parva Muguga T. parva Muguga T. parva Marikebuni Itha 2.cl2-11 HIP 22.8-17	510 52 PSEGIMTGAADGSQ PSEGIMTGAADGSQ PSEGIMTGAADGSQ PSEGIMTGAADGSQ PSEGIMTGAADGSQ PSEGIMTGAADGSQ PSEGIMTGAADGSQ PSEGIMTGAADGSQ PSEGIMTGAADGSQ PSEGIMTGAADGSQ PSEGIMTGAADGSQ	0 530 S30 PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSS PPDKKPDDSS PPDKKPDDSS	540 		Cattle-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/6 Lad 02-3/9 Lad 10-4/8 T. parva Schoonspruit T. parva Muguga T. parva Marikebuni Itha 2_cl2-11 HIP 22_8-17 Bloe B_4.0. Itha 6_cl6-6	510 52 PSEGIMTGAADGSCQ PSEGIMTGAADGSCQ PSEGIMTGAADGSCQ PSEGIMTGAADGSCQ PSEGIMTGAADGSCQ PSEGIMTGAADGSCQ PSEGIMTGAADGSCQ PSEGIMTGAADGSCQ PSEGIMTGAADGSCQ PSEGIMTGAADGSCQ PSEGIMTGAADGSCQ PSEGIMTGAADGSCQ	0 530 	540 GKDCSKSCGTPS GKDCSKSCGTPS GKDCSKSCGTPS GKDCSKSCGTPS GKDCSKSCGTPS GKDCSKSCGTPS GKDCSKSCGTPS GKDCSKSCGTPS GKDCSKSDSCTPS GKDCSKSDSCTPS GKDCSKSCGTPS		Cattle-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 T. parva Muguga T. parva Muguga T. parva Marikebuni Itha 2_c12-11 HIP 22_8-17 Bloe B_4.0. Itha 6_c16-6 HIP 32 1-18	510 52 PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ	0 530 	540 GRD GSKSGSGTPS GRDGSKSGSGTPS GRDGSKSGSGTPS GRDGSKSGSGTPS GRDGSKSGSGTPS GRDGSKSGSGTPS GRDGSKSGSGTPS GRDGSKSGSGTPS GRDGSKSGSGTPS GRDGSKSGSGTPS GRDGSKSGSGTPS		Cattle-type 'Mixed'-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 T. parva Schoonspruit T. parva Muguga T. parva Marikebuni Itha 2_c12-11 HIP 22_8-17 Bloe B_4.0. Itha 6_c16-6 HIP 32_1-18 Lad M119_9	510 52 PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSDGIMTGAADGSGQ PSDGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ	0 530 	540 GRDGSKSGSGTP GRDGSKSGSGTP GRDGSKSGSGTP GRDGSKGSGTP GRDGSKGSGTP GRDGSKGSGTP GRDGSKGSGTP GRDGSKGSGTP GRDGSKGSGTP GRDGSKSGSGTP GRDGSKSGSGTP GRDGSKSGSGTP GRDGSKSGSGTP GRDGSKSGSGTP	. K K K K K K K K K K K K K K K K K K K	Cattle-type 'Mixed'-type					
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Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/6 Lad 10-4/8 T. parva Schoonspruit T. parva Muguga T. parva Marikebuni Itha 2_c12-11 Hip 22_8-17 Bloe B_4.0. Itha 6_c16-6 HiP 32_1-18 Lad M119_9 Lad 1438_10-10 Lad 17_10 HiP 22_8-13 T. parva 7014 KNP 011_4/1 HiP 32_1-8	510 52 PSEGIMTGAADGSGQ	0 530 PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKPDDSK PPDKKPDDSK PPDKKPDDSSK PPDKF PDSKPDSK PDSKPDDSSK PPDKF PDSKPDSK PDSKPDSK PDSKPDSK PPDKF PDSKPDSK PDSKPDSK PDSKPDSK PDSKPDSK PDSK PDSKPDSK PDSK	540 GRNOSKSGSTPS GRNOSKSGSTPS GRNOSKSGSTPG GRNOSKSGSTPG GRNOSKSGSTPG GRNOSKSGSTPS GRNOSKSGSTPS GRNOSKSGSTPS GRNOSKSSSTPS GRNOSKSDSGTPS GRNOSKSDSGTPS GRNOSKSDSGTPS GRNOSKSDSGTPS GRNOSKSDSGTPS GRNOSKSDSGTPS GRNOSKSDSGTPS		Cattle-type 'Mixed'-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 T. parva Schoonspruit T. parva Marikebuni Itha 2_c12-11 HIP 22_8-17 Bloe B_4.0. Itha 6_c16-6 HIP 32_1-18 Lad H38_10-10 Lad 17.10 HIP 22_8-13 T. parva 7014 KNP 011_4/1 HIP 32_1-8 Itha3_c13-17 UP 20_1_1	510 52 PSEGINTGAADGSGQ	0 530 , 530 PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDSK PPDKKTDDSK PPDKKPDDSK	540 GRD GSK 65GTP 5 GRD GSK 65GTP 1 GRD GSK 65GTP 1 GRD GSK 65GTP 5 GRD GSK 65		Cattle-type 'Mixed'-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 T. parva Muguga T. parva Marikebuni Itha 2 c.2-11 HIP 22.8-17 Bloe B.4.0. Itha 6 c.26-6 HIP 32.1-18 Lad M19.9 Lad 1438.10-10 Lad 17.10 HIP 22.8-13 T. parva 7014 KNP 011.4/1 HIP 32.1-8 Itha3_c.13-17 HIP 32.1-1 KNP 00.5 5	510 52 PSECIMITGAADGSCQ PSEC	0 530 PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKPDDSK PPDKKPDDSSK	540 GRD GSK SGGTPS GRD GSK SGGTPS GRD GSK SGGTPS GRD GSK SGGTPS GRD GSK GGTPS GRD GSK GGTPS GRD GSK GGTPS GRD GSK GGTPS GRD GSK GGTPS GRD GSK SGGTPS GRD GSK SGGTPS GFD GSK		Cattle-type 'Mixed'-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 T. parva Schoonspruit T. parva Marikebuni Itha 2_02-11 HIP 22_8-17 Bloe B_4.0. Itha 6_02-01 HIP 32_1-18 Lad M19_9 Lad 1438_10-10 Lad 17_10 HIP 32_8-13 T. parva 7014 KNP 011_4/1 HIP 32_1-8 Itha3_013-17 HIP 32_1-1 KNP 102_05 5 Lad 1438_10-19	510 52 PSEGIMTGAADGSQ	0 530 PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKPDDSK PPDKKPDDSSK PPDKKPDSK PPDKKPDSK PPDKKPDSK PPDKKPDSK PPDKKPDSK PPDKKPDSK PPDKKPDSK PPDKKPDSK PPDKKPDSK PPDKKPDSK PPDKKPDSK PPDKKPDSK PPDKKPDSK PPDKKPDSK PPDKKPDSK PPDKKPDSK PPDKKPDSK PPDK P	540 GRD GSK 65GTP S GRD GSK 65GTP S	· KKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKK	Cattle-type 'Mixed'-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/6 Lad 10-4/8 T. parva Schoonspruit T. parva Muguga T. parva Marikebuni Itha 2_cl2-11 Hip 22_8-17 Bloe B_4.0. Itha 6_cl6-6 HIP 32_1-18 Lad M119_9 Lad 1438_10-10 Lad 1438_10-10 Lad 17_10 HIP 22_8-13 T. parva 7014 KNP 011_4/1 HIP 32_1-8 Itha3_cl3-17 HIP 32_1-1 KNP 102_05 5 Lad 1438_10-19 Mab A13_1/2	510 52 PSEGIMTGAADGSQ	0 530 , 530 PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKPDDSK PPDKKPDDSSK PPDKFPDDSSK PPDKFPDSSK PPDKFPDSSK PPDKFPDSSK PPDKFPDSSK PPDKFPDSSK PPDKFPDSSK PPDKFPDSSK PPDKFPDSSK PPDKFPDSSK PPDKFPDSSK PPDKFPDSSK PPDKFPDSSK	540 GRD os Kásós Grave GRD os Kásós Grave	· K K K K K K K K K K K K K K K K K K K	Cattle-type 'Mixed'-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 T. parva Schoonspruit T. parva Marikebuni Itha 2_c12-11 Hirp 22_8-17 Bloe B_4.0. Itha 6_c16-6 HiP 32_1-18 Lad H38_10-10 Lad I438_10-10 Lad I438_10-10 Lad I438_10-10 Hirp 22_8-13 T. parva 7014 KNP 011_4/1 Hirp 32_1-8 Itha3_c13-17 Hirp 32_1-1 KNP 102_05 5 Lad I438_10-19 Mab A13_1/2 Ithas 4_c14-18	510 52 PSEGINTGAADGSGQ	0 530 , 530 , 530 PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKPDSK PPDKKPDSK PPDK PPDK PPDK P	540 GRD OS KSGSGTPS GRD OS KSGSGTPS G	· K K K K K K K K K K K K K K K K K K K	Cattle-type 'Mixed'-type Buffalo-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/6 Lad 10-4/2 Lad 02-3/9 Lad 10-4/8 T. parva Schoonspruit T. parva Marikebuni Itha 2.02-11 HIP 22.8-17 Bloe B_4.0. Itha 6_016-6 HIP 32.1-18 Lad M19.9 Lad 1438.10-10 Lad 17_10 HIP 22.8-13 T. parva 7014 KNP 011_4/1 HIP 32.1-8 Itha3_013-17 HIP 32.1-1 KNP 102_05 5 Lad 1438_10-19 Mab A13_1/2 Itha3_013-14 Itha3_013-14	510 52 PSEGIMTGAADGSGQ	0 530 PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDSK PPDKKTDDSK PPDKKPDDSK PPDKKPDDSK PPDKKPDDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKF PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSK PPDKF PPDKF PP	540 GRN OS KSGSTPS GRN OS KS	· K K K K K K K K K K K K K K K K K K K	Cattle-type 'Mixed'-type Buffalo-type					
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Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/6 Lad 10-4/8 T. parva Schoonspruit T. parva Maguga T. parva Marikebuni Itha 2_cl2-11 Hip 22_8-17 Bloe B_4.0. Itha 6_cl6-6 HiP 32_1-18 Lad M19_9 Lad M19_9 Lad M19_10 HIP 22_8-13 T. parva 7014 KNP 011_4/1 HIP 32_1-8 Itha3_cl3-17 HIP 32_1-8 Itha3_cl3-17 HIP 32_1-1 KNP 102_05 5 Lad I438_10-19 Mab A13_1/2 Ithas 4_cl4-18 Itha 3_cl3-14 Itha 2_cl2-5 Itha 2_cl2-14 HIP 19_2/8	510 52 PSEGIMTGAADGSGQ PSEGIMTGAADGSG PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTGAADGSGQ PSEGIMTG	0 530 , 530 PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDSSK	540 GRD os Kascs or Ps GRD os Kascs or Ps	· K K K K K K K K K K K K K K K K K K K	Cattle-type 'Mixed'-type Buffalo-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/6 Lad 10-4/8 T. parva Schoonspruit T. parva Muguga T. parva Marikebuni Itha 2 c.2-11 HIP 22.8-17 Bloe B.4.0. Itha 6 c.26-6 HIP 32.1-18 Lad M19.9 Lad 17.10 HIP 22.8-13 T. parva 7014 KNP 011.4/1 HIP 32.1-8 Itha3.c.13-17 HIP 32.1-1 KNP 102.05 5 Lad 1438.10-19 Mab A13.1/2 Itha3.c.13-14 Itha3.c.13-14 Itha3.c.13-14 HIP 32.5 Itha 4.2.4-18 Itha3.c.13-5 Itha 2.c.12-14 HIP 19.2/8 Mab B338_114	510 52 PSEGIMTGAADGSQ	0 530 PPDKKTDD05K PPDKKTDD05K PPDKKTDD05K PPDKKTDD05K PPDKKTDD05K PPDKKTDD05K PPDKKTDD05K PPDKKPD05K PPDKF P	540 GKD GSK SG GTP S GKD GSK	· K.K.K.K.K.K.K.K.K.K.K.K.K.K.K.K.K.K.K.	Cattle-type 'Mixed'-type Buffalo-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/6 Lad 02-3/9 Lad 10-4/8 T. parva Schoonspruit T. parva Marikebuni Itha 2_c12-11 Hir 22_8-17 Bloe B_4.0. Itha 6_c16-6 HIP 32_1-18 Lad M19_9 Lad 1438_10-10 Lad 17_10 HIP 22_8-13 T. parva 7014 KNP 011_4/1 HIP 32_1-8 Itha3_c13-17 HIP 32_1-1 KNP 012_05 5 Lad 1438_10-19 Mab A13_1/2 Ithas 4_c14-18 Itha 3_c13-14 Itha 9_c19-5 Itha 2_c12-14 HIP 19_2/8 Mab BB38_114 Bloe B_5-2 VNP 012_6 5 7	510 52 PSEGIMTGAADGSQ PSECIMTGAADGSQ	0 530 PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKPDDSK PPDKKPDDSK PPDKKPDDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDKKPDSSK PPDSK PPDKF PPDSKKPDSSK PPDSK PPDSK PPDSK PPDSK	540 GRN OSK SGSTPS GRN OSK S	· K K K K K K K K K K K K K K K K K K K	Cattle-type 'Mixed'-type Buffalo-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/6 Lad 10-4/8 T. parva Schoonspruit T. parva Muguga T. parva Marikebuni Itha 2_c12-11 Hip 22_8-17 Bloe B_4.0. Itha 6_c16-6 HIP 32_1-18 Lad 1438_10-10 Lad 1438_10-10 Lad 1438_10-10 Lad 1438_10-10 Lad 1438_10-10 Lad 1438_10-10 Lad 1438_10-10 HIP 22_8-13 T. parva 7014 KNP 011_4/1 HIP 32_1-8 Itha3_c13-17 HIP 32_1-8 Itha3_c13-17 HIP 32_1-8 Itha3_c13-17 HIP 32_1-8 Itha3_c13-14 HIP 3_2/8 Mab BB38_114 Bloe B_5-2 KNP 102_5 57 Mab A13_4/2	510 52 PSEGINTGAADGSGQ	0 530 	540 GRN OS KSGS GTP 9 GRN OS K	· K K K K K K K K K K K K K K K K K K K	Cattle-type 'Mixed'-type Buffalo-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/6 Lad 10-4/8 T. parva Schoonspruit T. parva Maguga T. parva Marikebuni Itha 2_cl2-11 Hin 2_2.8-17 Bloe B_4.0. Itha 6_cl6-6 HiP 32_1-18 Lad H38_10-10 Lad I438_10-10 Lad I438_10-10 Lad I438_10-10 Lad I438_10-10 HiP 22_8-13 T. parva 7014 KNP 011_4/1 HiP 32_1-8 Itha3_cl3-17 HiP 32_1-8 Itha3_cl3-17 HiP 32_1-1 KNP 102_05 5 Lad I438_10-19 Mab A13_1/2 Ithas 4_cl4-18 Itha 3_cl3-14 Itha 2_cl2-5 Itha 2_cl2-14 HiP 19_2/8 Mab BB38_114 Bloe B_5-2 KNP 102_5 57 Mab A13_4/2 KNP 102_5 57 Mab A13_4/2 KNP 102_16	510 52 PSEGMTGAADGSGG PSGMTGAADGSG PSGMTGAAD	0 530 PPDKKTDD05K PPDKKTDD05K PPDKKTDD05K PPDKKTDD05K PPDKKTDD05K PPDKKTDD05K PPDKKTDD05K PPDKKTDD05K PPDKKPD05K PPDKKPD	540 GRD os Káss Garps GRD os Káss Garps	· K K K K K K K K K K K K K K K K K K K	Cattle-type 'Mixed'-type Buffalo-type					
Lad 10-11 Lad 02-7 Lad 10-4/6 Lad 10-4/6 Lad 10-4/8 T. parva Schoonspruit T. parva Muguga T. parva Marikebuni Itha 2.cl2-11 HIP 22.8-17 Bloe B_4.0. Itha 6.cl6-6 HIP 32.1-18 Lad M19.9 Lad 1438.10-10 Lad 17_10 HIP 22.8-13 T. parva 7014 KNP 011_4/1 HIP 32.1-8 Itha3.cl3-17 HIP 32.1-1 KNP 102_05 5 Lad 1438.10-19 Mab A13_1/2 Itha3.cl3-14 Itha3.cl3-14 Itha3.cl3-14 Itha 9.cl2-14 HIP 19.2/8 Mab B38.114 Bloe B_5-2 KNP 102_5 57 Mab A13_4/2 KNP 05_16 HIP 32.1-2	510 52 PSEGMTGAADGSGQ	0 530 PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDDSK PPDKKTDDSK PPDKKTDDSK PPDKKTDDSK PPDKKPDDSK PDKKPDSK PPDKKPDSK PPDK PPDKF	540 GRN os Káss Gerre GRN os Káss Gerre	· K K K K K K K K K K K K K K K K K K K	Cattle-type 'Mixed'-type Buffalo-type					



Buffalo-type PIM sequences:

Toye *et al.* (1995b) identified a 20-amino-acid insert (VDQQQPVQQPSQDQPSGPDS shown in broken-line block at position 36-68 in Figure 6.6) as characteristic to buffalo-type PIM amino acid sequences. In addition to this, two other buffalo-type amino acid motifs occurring at positions 352-393 and 447-473, were identified in this study (shown in broken-line blocks in Figure 6.6). However, some of the PIM sequences obtained from *T. parva* field samples collected from buffalo from KNP, Hluhluwe-iMfolozi, Mabalingwe and Ithala game parks lacked the 352-393 amino acid motif; instead, these sequences contained unique inserts of variable sizes between position 292-360 (underlined in Figure 6.6). The 20-amino-acid insert identified by Toye *et al.* (1995b) from PIM sequences from buffalo-derived *T. parva* 7014 and Hluhluwe stocks PIM sequences, was missing from 9/53 (17%) of the buffalo-type PIM sequences obtained in this study. Furthermore, a different insert between positions 36 and 68 was identified from buffalo-type sequences obtained from Ithala buffalo samples.

Using these motifs, 54.6% (53/97) of the PIM sequences obtained in this study were identified as buffalo-type PIM sequences and were obtained from *T. parva* samples collected from both cattle and buffalo (Table 6.2). One sequence obtained from KNP V5 had a large insert in the central region of the gene which was very different in sequence from that of other PIM sequences (results not shown). Very short PIM sequences, missing most of the variable central region, were obtained from some clones of PIM amplicons obtained from samples originating from buffalo (results not shown). However, the sequences flanking the central region from these alleles were characteristic of buffalo-type PIM sequences as defined in this study.

'Mixed'-type PIM sequences:

The use of the amino acid motifs identified in this study allowed identification of 37 'mixed' PIM sequences (Figure 6.6). It is possible that hybrid PCR products could arise during PCR amplification in samples containing mixed infections, as a result of template switching. These PCR artifacts are identifiable by sequence identities in hybrid sequences when compared with sequences of other amplicons produced in the same amplification reaction. To confirm whether the 'mixed' sequences obtained in this study were authentic, the PIM nucleic acid sequences in the more conserved regions, i.e. sequences flanking the central variable region, were compared to those of other sequences obtained from the same sample. None of the 'mixed' sequences were identical to any of the other PIM sequences obtained from the same



sample in these conserved regions, suggesting that these sequences were therefore genuine and could not have resulted from PCR artifacts.

Two subtypes of 'mixed' PIM sequences were identified, M-I (32/37) and M-II (5/37) (Figure 6.7). Subtype M-I consisted of sequences characteristic of cattle-type *T. parva* PIM sequences at the amino-terminus and buffalo-type sequences at the carboxy-terminus (Figure 6.7). Subtype M-II comprised sequences characteristic of buffalo-type *T. parva* PIM sequences at the amino-terminus and cattle-type sequences at the carboxy-terminus (Figure 6.7).

The 37 'mixed' PIM sequences were obtained from samples collected from both cattle and buffalo (Table 6.2). Of the 22 *T. parva* PIM sequences obtained from seven cattle samples in this study, 31.8% (7/22) were cattle-type, 59.1% (13/22) were 'mixed'-type, and only 9.1% (2/22) were buffalo-type (Table 2). In comparison, no cattle-type PIM sequences were identified from 20 buffalo samples, 32% (24/75) of the PIM sequences obtained from buffalo samples were mixed-type, and 68% (51/75) were buffalo-type.



Chapter 6

	10	20 30	40	50	60	70	80	90	100	
				.						
Itha2_cl2-11	DSTGSSDVTQVDSESNDT	SSSSETSQQGQPQP	DQP		<u>2D</u> 9	QP D QHQQPTQG	TSGQQGPHT	PQPIQ <mark>E</mark> PSGE	PVQPDQ	
HIP22_8-9	DSTGSSDVTQADSESNDS	SSSSETSQQGQPQP	D QP		ID 9	QP D QHQQPTQ <mark>G</mark>	TSGQQGPHT	PQP IQE PSGE	P V QP D Q	
HIP19_2/4	DSTGSSDVAQADSESNDS	SSSSETSQQGQPQP	D QP		ID 9	QPDQHQQ	+			
BloeB_4.0.2	DSTGSSDVTQADSESNDS	SSSSETSQQGQPQP	DQP		DD	<u><u>O</u>PD<u>OHOOPTOG</u></u>	TSGQQGPDT	PQPIQEPSGE	PVQPDQ	
BloeB_12-2	DSTGSSDVTQADSESNDS	SSSSETSQQGQPQP	DQP		DD	<u><u>O</u>PD<u>OHOOPTOG</u></u>	TSGQQGPDT	PQPIQEPSGE	PVQPDQ	
HIP32_24-0-1	DSSGSSDVTQVDSESNDT	SSSSETSQQGQPQP	DQP		<u>p</u> p	<u><u>O</u>PD<u>O</u>H<u>O</u>OPT<u>O</u>G</u>	TSGQQGPHT	PQPIQEPSGE	V QP D Q	M-I
Itha2_cl2-24	DSTGSSDVTQVDSEPNDT	SSSSETSQQGQPQP	DQP		<u>p</u> p	<u><u>O</u>PD<u>O</u>H<u>O</u>OPT<u>O</u>G</u>	DTSGQQGPHA	PQPIQEPSGE	V QP D Q	
Itha6_c16-22	DSTGSSDVTQVDSESNDT	SSSSETSQQGQPQP	DQP		<u>p</u> p	2PDQHQQPTQG	TSGQQGPHT	POPIQEPSGE	PVQPDQ	
Lad MI19_9	DSTGSSDVTQADSESNDS	SSSSETSQQGQPQP	DQP		00	<u>DOHOOLLOG</u>	TSGQQGPDT.	POPIQEPSGE	VQPDQ	
Lad 1438_10-15	DSTGSSDVTQADSESNDS	SSSSETSQQGQPQP	DQP		00	<u>J</u> PDQHQQPTQG	TSGQQGPDT.	POPIQEPSGE	VQPDQ	
Lad 1/_10	DSTGSSDVTQADSESNDS	SSSSETSQQGQPQP	DQP		00	<u>J</u> P D Q R QQPTQG	TSGQQGPDT	POPIQEPSGE	VOPDR	
HIP 22_0-13	DSIGSSDVIQADSESNDS	SSSSEISQQGQFQR		DEODO				ODI DODICOL		
KNP WO_17	DSIGSSDLIQVDIESNDG	SSSSEISUUPP			COCDVE		Daagoogoooo	QF L <mark>D</mark> QF I GQF	DIOD	
KNP WO_0-2	DSIGSSDVIQVDIESNDI	SSSSE1SQQGRPQP		DUCADOS	COCDVE	PVDQQQQPVQE				М П
KNP WO_/	DSTGSSDLINVDIESNDI	SSSSEISQQGRPQP		IQADQS			096			IVI-II
Mab BB/3 1210	DSTGSSDVINVDIESNDI							PDOSCONPD-	VOR	
MaD 6645_1210	DSIGSSDVIQVDIESNDN	SISSEISQUPP	VERVD000			QPDQS	DISGQQGPII	PDQSGQRFF-		
	310	320 330	340	350	360	370	380	390	400	
	1	.+			+					
Itha2_cl2-11	PEQPPVEPVDQQQQPVQD	QPS <mark>GKE</mark> TPQPTQ <mark>GD</mark>	QPVQDPSGQEQ	PEPEQTPEHTP	SKDDPTGE	EBVKPSEGHMT	GAAADGSGQP	PDKKPGDDS	GKDGS	
HIP22_8-9	EPEQPPVQPVDQQQPVQD	QPSGQETPQPIPED	QP V Q E PP E Q K P E -	PEPEQTPEHTP	SKDDPTGE	EFVKPSEGHMT	GAAADGSGQP	PDKKPDDDSE	GKDGS	
HIP19_2/4	QPVQD	QPSGQETPQPIPDG	QP V Q E PP E Q K P E -	PEPEQTPEHTP	SKDDLSGE	ELVKPSEGHMT	GAAADGSGQP	PDKKPDDDSE	GKDGS	
BloeB_4.0.2	[EPEQPQDQPVDQQQPVQD	QPSGQETPQPIPED	QPVQEPTEQK	PEPEQTPEHTP	SKGDTSGE	EPVQPSEGHMT	GAAADGSGQP	PDKKPDDDSE	GKDDS	
BloeB_12-2	EPEQPQDQPVDQQQPVQD	QPSGQETPQPIPED	QPVQEPTEQK	PEPEQTPEHTP	SKDDTSGE	EFVQPSEGHMT	GAAADGSGQP	PDKKPDDDSE	GKDDS	
HIP32_24-0-1	EPEQPQDQPVDQQQPTQD	QPSGQETPQPIPED	QPVQEPTEQK	PEPEQTPEHTP	SKDDLSGE	EPVKPPEGLMT	GAAADGSGQP	PDKKPGDDSE	GKDGS	M_I
Itha2_c12-24	PEQTPVEPVDQQQQPVQD	QPSGKETPQPTQGD	QP V Q D PSGQ E Q	PEPEQTPEHTP	SKDDPTGE	EPVKPSEGHMT	GAAADGSGQP	PDKKPDDDSE	GKDGS	141-1
Itha6_cl6-22	EPEQPQDQPVDQQQPTQD	QPSGQETPQPIPED	QPVQEPTEQK	PEPEQTPEHTP	SKDDLSGE	ERVKPSEGLMT	GAAADGSGQP	PDKKPGDDSE	GKDGS	
Lad M119_9	EPEQPPVQPVDQQQPVQD	QPSGQETPQPIPED	QP V Q E PP E Q K P E -	PEPEQTPEHTP	SKDDPTGE	EF VKP SEGHMT	GAAADGSGQP	PDKKPDDDSE	GKDGS	
Lad I438_10-15	EPDQPPVQPVDQQQPVQD	QPSGQETPQPIPED	QP V Q E PP E Q K P E -	PEPEQTPEHTP	SKDDPTGE	EPVKPSEGHMT	GAAADGSGQP	PDKKPDDDSE	GKDGS	
Lad 17_10	EPEQPPVQPVDQQQPVQD	QPSGQETPQPIPED	QP V Q E PP E Q K P E -	PEPEQTPEHTP	SKDDPTGE	EEVKPSEGHMT	GAAADGSGQP	PDKKPDDDSI	GKDGS	
HIP 22_8-13	QTPEPPVEPVDQQQPVHE	-PSGQETPQPIPDD	QPVREPTE-K-E-	PEPEQKPDHTP	SKDDTSGE	EF VQP SEGHMT	GAAPDGSGQP	PEKKPGDDSE		
KNP W8_17	EPEQPQDQPVDQQQPVQD	QPSGQETPQPIPDD	QP V Q E PP E Q K P E -	PEPEQTPEHTP	SKDDASGE	VP VKP SEGHMT	GAAADGSGQP	PDKKPDDDSI	GKDGS	
KNP W8_8-2	DQPQDQPVDQQQPTQD	QPSGQETPQPIPDD	QP V Q E PP E Q K P E -	PEPEQTPEHTP	SKDDASGE	VP VKP SEGHMT	GAAADGSGQP	PDKKPDDDSE	GKDGS	
KNP W8_7	DQPQDQPVDQQQPTQD	QPSGQETPQPIPDD	QPVQEPPEQKPE-	PEPEQTPEHTP	SKDDASGE	VPVKPSEGHMT	GAAADGSGQP	PDKKPDDDSE	GKDGS	M-II
KNP W8_1	E PDQPQDQPVDQQQPTQD	QPSGQETPQPIPDD	QP V Q E PP E Q K P E -	PEPEQTPEHTP	SKDDASGE	VPVKPSEGHMT	GAAADGSGQP	PDKKPDDDSI	GKDGS	
Mab BB43_1210	Į <u>O</u> PEOPPVOPVDOOOPVOD	QPSGQETPQPIPED	<u>QPAKDEPTGQQD</u>	PEPEQTPEHTP	SKDDASCE	VVKPSEGLMT	GAAADGSGQP	PDQPSDDDS	GKDGS	
	410									
	410									
Itha2 c12-11	KSDSGTPSKDKKDSK									
HIP22 8-9	KSDSGSPIKDKKDSK									
HIP19 2/4	KSDSGTPSKDKKHSK									
BloeB 4.0.2	KSGSGTPSKDKKDSK									
BloeB 12-2	KSGSGTPSKDKKDSK									
HIP32 24-0-1	KSDSGTPSKDKKHSK									
Itha2 c12-24	KSDSGTPIKDKKHYK	M-I								
Itha6_c16-22	KSDSGTPSKDKKDSK									
Lad M119_9	KSDSGSPIKDKKHSK									
Lad I438_10-15	KSDSGTPIKDKKDSK									
Lad 17_10	KSDSGSPIKDKKHSK									
HIP 22_8-13	KSDSGTPSKDKKHSK									
KNP W8_17	KSDSGSPIKDKKHSK									
KNP W8_8-2	KSDSGSPIKDKNIPN									
KNP W8_7	KSDSGSPIKDKKHSK	мп								
KNP W8_1	KSDSGSPIKDKKHSK	171-11								
Mab BB43_1210	KSDSGSPIKDRKHPK									

Figure 6.7 Multiple sequence alignment of representative amino acid sequences of 'mixed'-type *T. parva* PIM sequences, showing subtypes M-I and M-II. Regions typical of buffalo-type and cattle-type PIM sequences are indicated in broken-line and solid-line blocks, respectively. This alignment excludes the region between positions 101 and 300, which has buffalo-type PIM sequences in all subtypes.



6.5 Discussion

Previous studies on characterization of South African *T. parva* field samples revealed the presence of cattle-type p67 and p104 alleles (Chapter 4; Chapter 5; Sibeko *et al.*, 2010), suggesting the presence of parasites with characteristics of cattle-derived *T. parva* in South Africa. Although ECF has not been reported in South Africa since its eradication in the early 1950s, these findings are a concern. In this chapter, a third gene was investigated to further characterize *T. parva* samples obtained from cattle and buffalo in South Africa to confirm results obtained by analysis of p67 and p104 genes.

Polymorphic immunodominant molecule PCR-RFLP profiles for the majority of *T. parva* field samples obtained from buffalo in this study were complex. Buffalo-derived *T. parva* isolates are expected to be heterogeneous and have previously been shown to display widely variable RFLP profiles because of multiplicity of infections in buffalo compared to single infections in cattle (Geysen, 2000; Geysen *et al.*, 2004). Five PIM RFLP cluster groups were identified from BioNumerics cluster analysis of RFLP profiles obtained from cloned PIM amplicons but these showed no correlation to geographic origin of the samples within each group, as was shown for p104.

Surprisingly, relatively homogeneous PIM profiles were observed from samples originating from buffalo from Hluhluwe-iMfolozi, Mabalingwe and Ithala game parks. These findings could suggest that there is limited genetic diversity in *T. parva* parasites in these relatively small buffalo populations. However, RFLP profiles obtained from clones of PIM amplicons indicated that genetic diversity does exist in the Hluhluwe-iMfolozi, Mabalingwe and Ithala *T. parva* populations, as profiles from clones produced from these samples clustered in different groups. Although multiple PIM profiles were identified in cloned PIM amplicons from samples with homogeneous profiles, some profiles occurred more frequently than others, explaining the apparently homogeneous profiles the presence of a common 'signature' contributed to the apparent homogeneity of the profiles.

The extensively characterized *T. parva* PIM (Baylis *et al.*, 1993; Toye *et al.*, 1996) is encoded by a single copy gene and its structure consists of a central variable region, flanked by conserved 5' and 3' termini (Toye *et al.*, 1995a; 1995b; Geysen *et al.*, 2004). Previous studies have identified two groups of PIM sequences, and a number of characteristics could be used



to distinguish PIM sequences from cattle-derived T. parva isolates from those from buffaloderived isolates (Toye et al., 1995b; Geysen et al., 2004). None of the PIM gene sequences obtained from T. parva field samples characterized in this study were identical, providing further evidence that the PIM gene evolves at an extremely high rate (Toye et al., 1995b). Extensive sequence heterogeneity among PIM sequences was demonstrated in the South African T. parva samples from buffalo examined in this study, confirming the extensive genetic diversity reported previously in T. parva parasites in buffalo (Chapter 4; Chapter 5; Collins and Allsopp, 1999; Sibeko et al., 2010). Furthermore, sequence variants of buffalotype PIM sequences were identified which have never been reported before. The extensive polymorphism found in the coding region of the PIM gene is associated with selective pressure from the protective immune response and may confer selective advantage to the parasite (Toye et al., 1995a). Geysen et al. (2004) reported a high Ns/S (non-synonymous / synonymous substitutions) ratio in the PIM sequence which is an indication of the influence of selective forces on the sequence and the evasion of the host immune responses. Mechanisms responsible for the extensive diversity of the PIM gene and which influence its mosaic structure are not known, although X-like recombination motifs responsible for initiation of gene conversion events have been identified in PIM sequences (Geysen et al., 2004) and it is thought that this mechanism is likely to be responsible for the mosaic nature of the PIM gene. Novel alleles have been reported to arise from re-shuffling of important epitopes as a result of gene conversion and reciprocal intergenic exchanges (Dormoy et al., 1997). The discriminative characteristics of the PIM gene render it a good candidate for exploitation in discriminatory assays used for T. parva isolates (Bishop et al., 2001; De Deken et al., 2007). Nevertheless, it is advisable that assays based on this gene should be continuously evaluated because of the rapid evolution of the PIM gene.

In this study, for the first time, 'mixed' PIM sequences with characteristics of both PIM sequence types were identified. The combinations of different "blocks" of sequence observed in the PIM gene are reminiscent of the structure that has previously been shown for the precursor to the major merozoite surface antigens (PMMSA) in *Plasmodium falciparum* (Peterson *et al.*, 1988). Recombination within the conserved blocks in the PMMSA is thought to result in reassembling of the variable blocks and accounts for much of the antigenic variation in this molecule. Studies of the population structure of *T. parva* parasites in Uganda showed that genetic exchange occurs frequently between isolates of *T. parva*, confirming the existence of a sexual cycle (Oura *et al.*, 2005). Evidence for recombination between *T. parva* parasites, in a form of mosaic segments, has previously been observed in the internal



transcribed spacer (ITS) region (Collins and Allsopp, 1999) and sexual recombination between different *T. parva* stocks has been demonstrated in the laboratory (Morzaria *et al.*, 1993; Bishop *et al.*, 2002). It seems likely that the 'mixed' PIM sequences have arisen through recombination between cattle-type and buffalo-type PIM sequences.

While the 'mixed' PIM sequences identified in this study may well represent recombination events, we do not know the full extent of the recombination in the rest of the genome in these parasites. Recombination between buffalo-type *T. parva* parasites and cattle-type parasites can only occur where there has been contact between buffalo and cattle, and it has been reported that it is unlikely for recombinant parasites to become established in the cattle population (Geysen, 2000; Geysen *et al.*, 2004). Nonetheless, 'mixed' PIM sequences were obtained from samples originating from both buffalo and cattle in this study, and the results indicate that 'mixed' PIM sequences are more prevalent in *T. parva* samples from cattle than in *T. parva* samples from buffalo. This could suggest that *T. parva* parasites with the 'mixed' PIM allele might be more likely to establish in cattle. In fact, very few buffalo-type PIM sequences were identified in cattle, suggesting that there may have been selection for both 'mixed'-type and cattle-type PIM sequences in cattle

Interestingly, no cattle-type PIM sequences were obtained from T. parva samples collected from buffalo. Buffalo are believed to carry a heterogeneous population of parasites and, as original hosts of the parasite, cattle-derived T. parva parasites are thought to have originated in buffalo, so we might have expected to see cattle-type PIM sequences in T. parva samples from buffalo. However, given the rapid nature of the evolution of the PIM gene, it is probable that cattle-type alleles are present at a low frequency in T. parva parasites in buffalo. It would therefore be necessary to clone the PIM genes from a larger number of buffalo samples in order to identify cattle-type PIM sequences. Findings obtained in a study on characterization of another T. parva antigenic gene, p67, revealed the presence of variants of the p67 allele similar to those of cattle-derived parasites in *T. parva* samples collected from buffalo in South Africa (Collins, 1997; Chapter 4; Sibeko et al., 2010), although no p67 sequence identical to the cattle-type allele was identified in buffalo samples. The PIM sequences obtained from samples with variants of cattle-type p67 were either buffalo-type (6/25, 24%) or 'mixed'-type (19/25, 76%) showing that other genes in parasites with variants of the cattle-type p67 allele are not necessarily cattle-type alleles. There is therefore a need to establish the significance of these alleles in the epidemiology of theileriosis and the risk they pose to the naïve cattle population in South Africa.



PCR-RFLP profiles similar to that of the *T. parva* Muguga stock were obtained from three of the six cattle samples from the Ladysmith farm and the inferred amino acid sequences of the PIM gene from two of these samples (Lad 02 and Lad 10) were almost identical to the *T. parva* Muguga PIM sequence. This finding supports recent studies in which p67 and p104 alleles similar to those of the *T. parva* Muguga stock were identified from the same Ladysmith samples (Chapter 4; Chapter 5; Sibeko *et al.*, 2010). While it is not known if the Muguga-like RFLP profiles or sequences can be associated with the pathogenicity of *T. parva* isolates, findings in these studies strengthen the evidence for the presence of a subpopulation of *T. parva* parasites similar to ECF-causing East African strains in South Africa, at least on one farm. Our results might provide evidence for the selection of a subpopulation of *T. parva* parasites through cattle-to-cattle transmission of *T. parva* on the Ladysmith farm (resulting in parasites with cattle-type p67, p104 and PIM alleles). However, similarity between sequences is not necessarily an indicator of the association of the cattle-type PIM sequence with the disease syndrome, as ECF was not diagnosed on the Ladysmith farm.

Polymorphic immunodominant molecule PCR-RFLP profiles obtained from the remaining three samples from cattle from the Ladysmith farm occurred in three cluster groups, A, C and D. All three had the specific 'signature' which was observed from most of the *T. parva* samples collected from buffalo from Hluhluwe-iMfolozi Park, suggesting that some of the *T. parva* parasites on this farm may have originated from the Hluhluwe-iMfolozi buffalo. Furthermore, buffalo-type p67, p104 and PIM sequences were obtained from these three Ladysmith samples, further supporting the possibility of buffalo-to-cattle transmission on this farm. Unfortunately it was not possible to confirm whether the cattle on this farm had contact with infected buffalo (Thompson *et al.*, 2008), but circumstantial evidence and our results indicate that this might have occurred.

The PIM profile obtained from a bovine sample from a farm in Bloemfontein had the 'signature' characteristic of most Hluhluwe-iMfolozi profiles also observed in some of the Ladysmith samples. The RFLP profiles from this sample occurred in cluster A where 28% and 18% of Ladysmith and Hluhluwe-iMfolozi profiles, respectively, also grouped. Moreover, buffalo-type and 'mixed'-type PIM sequences were obtained from the Bloemfontein sample. These results suggest that the *T. parva* parasite characterized from the Bloemfontein bovine sample was similar to *T. parva* parasites from Hluhluwe-iMfolozi, and in fact, the infected buffalo breeding herd on the Bloemfontein farm originated from Hluhluwe-iMfolozi. Therefore, it might be possible to use PIM RFLP profiles to track the



origin of *T. parva* infections, especially when the profiles have a specific signature that characterizes a particular parasite population. This case presents something of a puzzle: it is not known how the bovine on the Bloemfontein farm was infected as the tick vector, *R. appendiculatus*, has not been known to occur in the Bloemfontein area. It is possible that the vector tick was introduced onto the property along with the infected buffalo but did not become established, as intensive tick surveys failed to identify the presence of vector ticks (FT Potgieter, unpublished results).

6.6 Summary

In summary, the findings in this study confirm the presence of a cattle-type PIM allele in the same cattle T. parva samples from which p67 and p104 alleles similar to that of T. parva Muguga were obtained. Results presented here suggest that there may have been both cattleto-cattle and buffalo-to-cattle transmission of T. parva on the Ladysmith farm. No cattle-type PIM alleles were identified from samples obtained from buffalo in this study. However, if the cattle-type alleles are present at a low frequency in T. parva parasites in buffalo, it is possible that insufficient samples were examined in this study and a larger number of samples would have to be investigated before any conclusions could be made. The extensive genetic diversity of T. parva parasite populations in South Africa was demonstrated in the identification of novel and 'mixed' PIM sequences. The significance of T. parva parasites carrying 'mixed' PIM alleles will have to be established and their risk to cattle evaluated. With the extent of genetic diversity that has been demonstrated by the three characterization studies presented in this thesis, it is clear that the population genetic structure of T. parva parasites in South Africa needs to be studied in detail, possibly by using other molecular tools such as mini- and microsatellite markers (Oura et al., 2003; 2005), to fully establish the parasite populations circulating in the country, and their threat and significance in the epidemiology of theileriosis in South Africa.

Chapter 6



6.7 References

- Anonymous, 1981. The eradication of East Coast fever in South Africa. *Journal of the South African Veterinary Association*, **52**, 71-73.
- Baylis, H.A., Allsopp, B.A., Hall, R. and Carrington, M., 1993. Characterisation of a glutamine-rich and proline-rich protein (QP protein) from *Theileria parva*. *Molecular and Biochemical Parasitology*, **61**, 171-78.
- Bishop, R.P., Geysen, D., Spooner, P., Skilton, R., Nene, V., Dolan, T. and Morzaria, S., 2001. Molecular and immunological characterization of *Theileria parva* stocks which are components of 'Muguga cocktail' used for vaccination against East Coast fever in cattle. *Veterinary Parasitology*, **94**, 227-37.
- Bishop, R., Geysen, D., Skilton, R., Odongo, D., Nene, V., Allsopp, B., Mbogo, S., Spooner,
 P. and Morzaria, S., 2002. Genomic polymorphism, sexual recombination and
 molecular epidemiology of *Theileria parva*. In: *Theileria*, Mckeever, D. and
 Dobbelaere, D. (Eds.). Kluwer Academic, Dordrecht, pp. 23-40.
- Bonfield, J. K., Smith, K.F. and Staden, R., 1995. A new DNA sequence assembly program. *Nucleic Acids Research*, **23**, 4992-99.
- Collins, N.E., 1997. The relationship between *Theileria parva parva* and *T. parva lawrencei* as shown by sporozoite antigen and ribosomal RNA gene sequences. Ph.D. Thesis, University of the Witwatersrand, South Africa.
- Collins, N.E. and Allsopp, B.A., 1999. *Theileria parva* ribosomal internal transcribed spacer sequences exhibit extensive polymorphism and mosaic evolution: application to the characterisation of parasites from cattle and buffalo. *Parasitology*, **83**, 541-51.
- De Deken, R., Martin, V., Saido, A., Madder, M., Bradt, J. and Geysen, D., 2007. An outbreak of East Coast Fever on the Comoros: A consequence of the import of immunised cattle from Tanzania? *Veterinary Parasitology*, 143, 245-53.
- Dormoy, A., Reviron, D.V., Froelich, N., Weiller, P.J., Mercier, P.J. and Tongio, M.M., 1997. Birth of a new allele in a sibling: *cis* or *trans* gene conversion during meiosis? *Immunogenetics*, 46, 520-23.



- Geysen, D., 2000. The application of Molecular Biology techniques to analyse diversity in *Theileria parva* populations in Zambia. Ph.D. Thesis. Brunel University, UK.
- Geysen, D., Bishop, R., Skilton, R., Dolan, T.T. and Morzaria, S., 1999. Molecular epidemiology of *Theileria parva* in the field. *Tropical Medicine and International Health*, **4**, A21-27.
- Geysen, D., Bazarusanga, T., Brandt, J. and Dolan, T.T., 2004. An unusual mosaic structure of the PIM gene of *Theileria parva* and its relationship to allelic diversity. *Molecular and Biochemical Parasitology*, **133**, 163-74.
- Graham, S.P., Saya, R., Awino, E., Ngugi, D., Nyanjui, J.K., Hecker, R., Taracha, E.L.N. and Nene, V., 2007. Immunostimulatory CpG oligodeoxynucleotides enhance the induction of bovine CD4⁺ cytotoxic T-lymphocyte responses against the polymorphic immunodominant molecule of the protozoan parasite *Theileria parva*. *Veterinary Immunology and Immunopathology*, **115**, 383-89.
- Katende, J., Morzaria, S., Toye, P., Skilton, R., Nene, V., Nkonge, C. and Musoke, A., 1998.
 An enzyme-linked immunosorbent assay for detection of *Theileria parva* antibodies in cattle using a recombinant polymorphic immunodominant molecule. *Parasitology Research*, 84, 408-16.
- Lawrence, J.A., 1992. History of bovine theileriosis in southern Africa. In: *The Epidemiology* of *Theileriosis in Africa*, R.A.I. Norval, B.D. Perry and A.S. Young (Eds.), Academic Press, London, pp. 1-39.
- Maddison, W.P. and Maddison, D.R., 1992. MacClade: Analysis of phylogeny and character evolution. Version 3. Sinauer Associates, Sunderland, Massachusetts.
- Morzaria, S.P., Young, J.R., Spooner, P.R., Dolan, T.T. and Bishop, R.P., 1993. Theileria parva: a restriction map and genetic recombination. In: Genome Analysis of Protozoan Parasites, Morzaria, S.P. (Ed.). ILRAD, Nairobi, pp. 67-73.
- Mukhebi, A.W., Perry, B.D. and Kruska, R., 1992. Estimated economics of theileriosis in Africa. *Preventative Veterinary Medicine*, **12**, 73-85.

Neitz, W.O., 1948. Studies on East Coast fever. South African Journal of Science, 1, 133-35.



- Neitz, W.O., 1955. Corridor disease: a fatal form of bovine theileriosis encountered in Zululand. *Bulletin of Epizootic Diseases of Africa*, **3**, 121-23.
- Oura, C.A., Odongo, D.O., Lubega, G.W., Spooner, P.R., Tait, A. and Bishop, R.P., 2003. A panel of microsatellite and minisatellite markers for the characterisation of field isolates of *Theileria parva*. *International Journal for Parasitology*, **33**, 1641-53.
- Oura, C.A., Asiimwe, B.B., Weir, W., Lubega, G.W. and Tait, A., 2005. Population genetic analysis and sub-structuring of *Theileria parva* in Uganda. *Molecular and Biochemical Parasitology*, **140**, 229–39.
- Perry, B. D. and Young, A. S., 1993. The naming game: the changing fortunes of East Coast fever and *Theileria parva*. *Veterinary Records*, **133**, 613-16.
- Perry, B. D. and Young, A. S., 1995. The past and future roles of epidemiology and economics in the control of tick-borne diseases of livestock in Africa: The case of theileriosis. *Preventative Veterinary Medicine*, 25, 107-20.
- Peterson, M.G., Coppel, R.L., Moloney, M.B. and Kemp, D.J. 1988. Third form of the precursor to the major merozoite surface antigens of *Plasmodium falciparum*. *Molecular and Cell Biology*, 8, 2664-67.
- Shapiro, S.Z., Fujisaki, K. and Mozaria, S.P., 1987. A life cycle stage-specific antigen of *Theileria parva* recognised anti-microschizont monoclonal antibodies. *Parasitology*, 94, 29-37.
- Shaw, M.K., 2003. Cell invasion by Theileria sporozoites. Trends in Parasitology, 19, 2-6.
- Sibeko, K.P., Oosthuizen, M.C., Collins, N.E., Geysen, D., Rambritch, N.E., Latif, A.A., Groeneveld, H.T., Potgieter, F.T. and Coetzer, J.A.W., 2008. Development and evaluation of a real-time polymerase chain reaction test for the detection of *Theileria parva* infections in Cape buffalo (*Syncerus caffer*) and cattle. *Veterinary Parasitology*, 155, 37-48.
- Sibeko, K.P., Geysen, D., Oosthuizen, M.C., Matthee, C.A., Troskie, M., Potgieter, F.T., Coetzer, J.A.W. and Collins, N.E., 2010. Four p67 alleles identified in South African *Theileria parva* field samples. *Veterinary Parasitology*, **167**, 244-254.



- Staden, R. 1996. The Staden Sequence Analysis Package. *Molecular Biotechnology*, **5**, 233-41.
- Staden, R., Beal, K.F. and Bonfield, J.K., 2000. The Staden package, 1998. Methods in Molecular Biology, 132, 115-130.
- Theiler, A., 1904. East Coast fever. Transvaal Agriculture Journal, 2, 421-38.
- Thompson, B.E., Latif, A.A., Oosthuizen, M.C., Troskie, M. and Penzhorn, B.L., 2008.
 Occurrence of *Theileria parva* infection in cattle on a farm in the Ladysmith district, Kwa-Zulu-Natal, South Africa. *Journal of the South African Veterinary Association*, 79, 31-35.
- Toye, P.G., Goodeeris, B.M., Iams, K., Musoke, A.J. and Morrison, W.I., 1991. Characterization of a polymorphic immunodominant molecule in sporozoites and schizonts of *Theileria parva*. *Parasite Immunology*, **13**, 49-62.
- Toye, P.G., Metzelaar, M.J., Wijngaard, P.J.L., Nene, V., Iams, K., Roose, J., Nyanjui, J.K., Gobright, E., Musoke, A.J. and Clevers, H.C., 1995a. Characterization of the gene encoding the polymorphic immunodominant molecule, a neutralizing antigen of *Theileria parva. Journal of Immunology*, 155, 1370-81.
- Toye, P.G., Gobright, E., Nyanjui, J., Nene, V. and Bishop, R., 1995b. Structure and sequence variation of the genes encoding the polymorphic immunodominant molecule (PIM), an antigen of *Theileria parva* recognized by inhibitory monoclonal antibodies. *Molecular and Biochemical Parasitology*, **73**, 165-77.
- Toye, P.G., Nyanjui, J., Goddeeris, B. and Musoke, A.J., 1996. Identification of neutralization and diagnostic epitopes on PIM, the polymorphic immunodominant molecule of *Theileria parva. Infection and Immunity*, **64**, 1832-38.