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Molecular diagnosis of *Theileria* infections in wildlife from Southern Africa ~ implications for accurate diagnosis.

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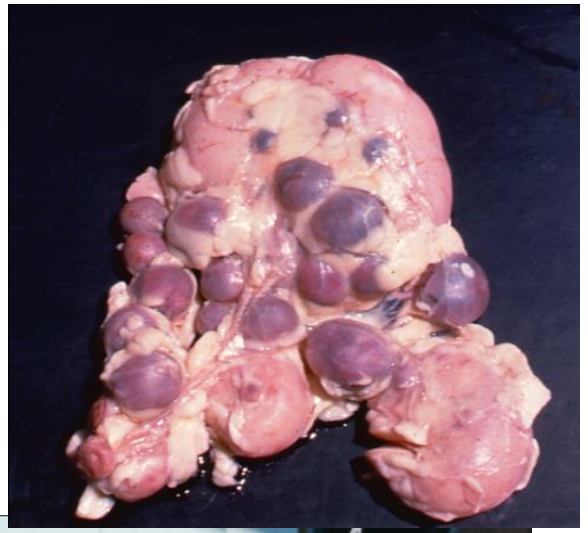
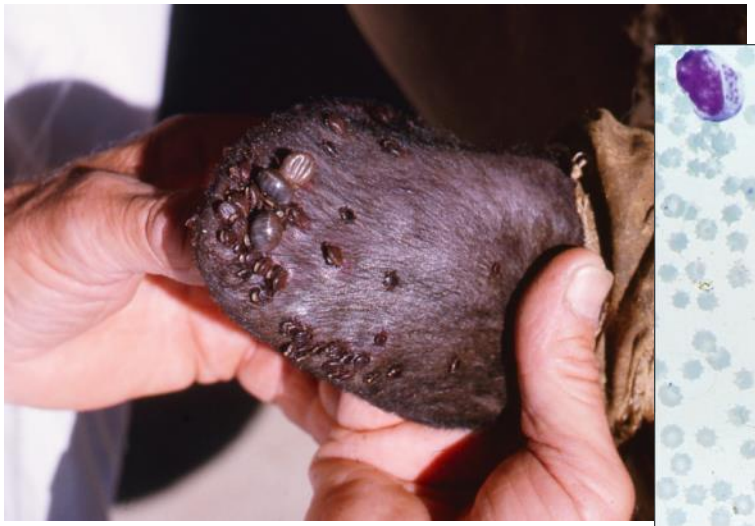
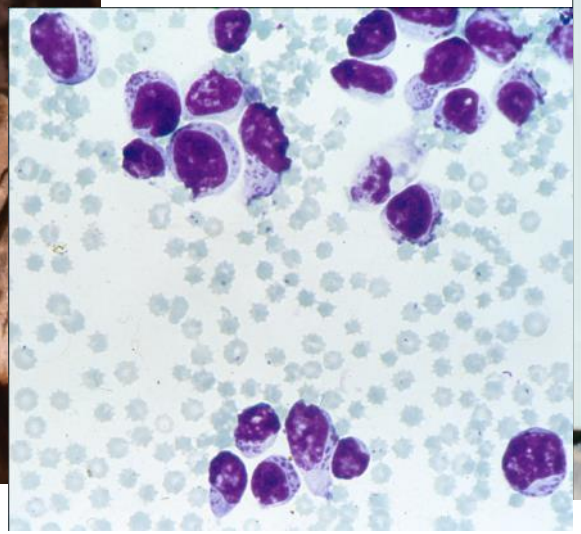
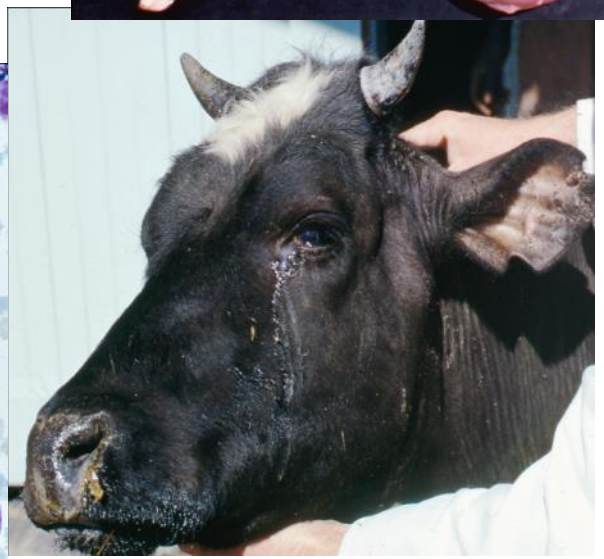
Corridor disease

- Cause: buffalo derived *Theileria parva*
  (Also cause of ECF – cattle derived *T. parva*)
- OIE List B reportable and controlled disease
- Fatal lymphoproliferating disorder in cattle
- Primary mammalian host: Buffalo
- Vectors: *Rhipicephalus appendiculatus*, *R. zambeziensis* & *R. duttoni* (Lessard, 1990)



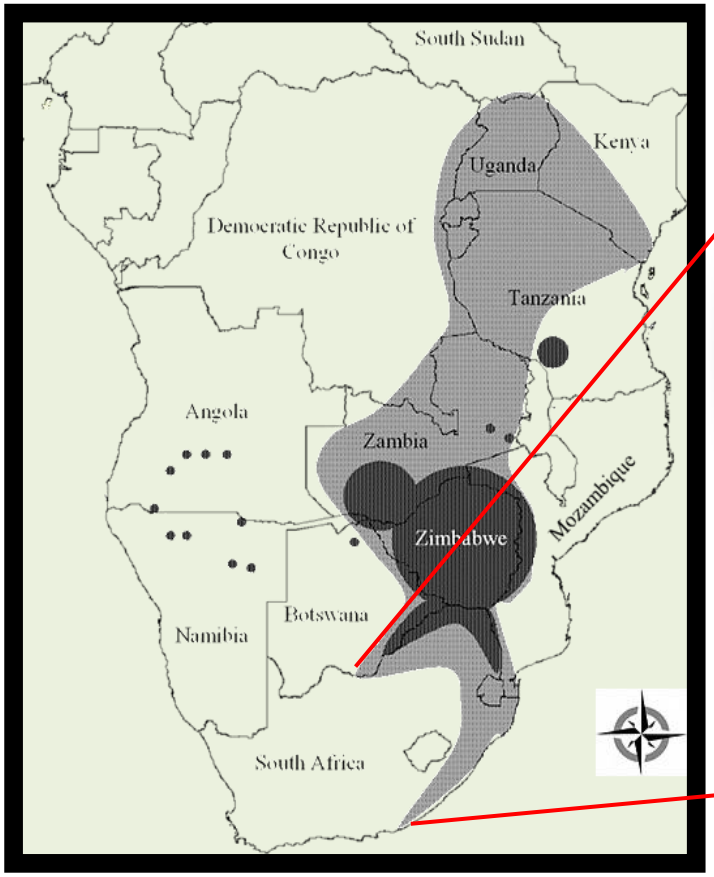






Epidemiology in South Africa

* ECF introduced ~1902, eradicated ~ 1956
* Kruger National Park, Hluhluwe-Umfolozi Park, regions between and bordering (Potgieter *et al.* 1988)



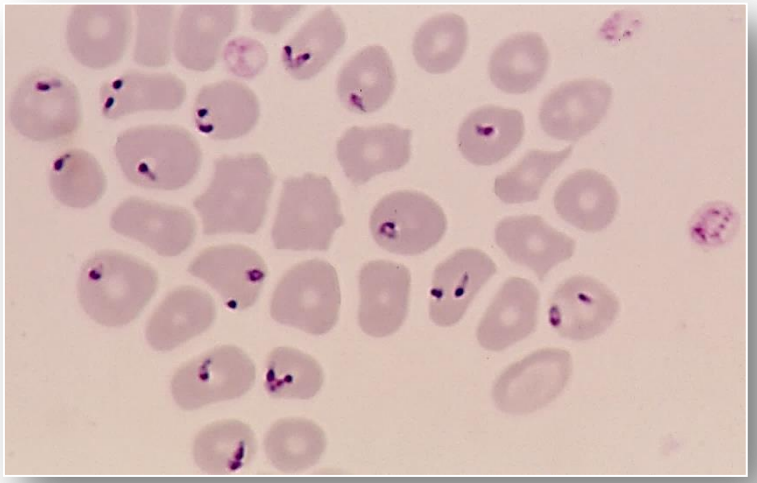
R. appendiculatus
R. zambesiensis
R. duttoni

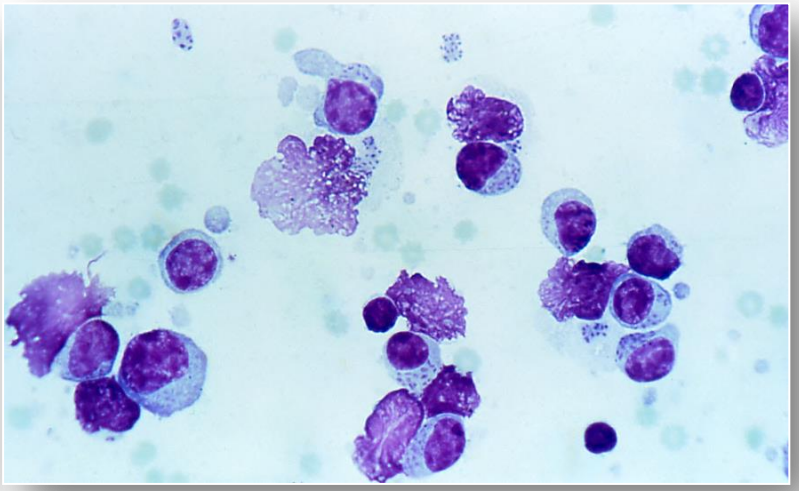
Diagnosis

Historically:

- Clinical disease manifestation in cattle (incubation period 8-12 days, Death after occurs after 7-10 days)

- Demonstration of two stages of parasite on blood smear (Limited use – morphologically indistinguishable)





- Xenodiagnoses through tick transmission (phased out – Animal ethics)

Amplifies ~
* *T. parva*,
* *T.* sp. (buffalo)
* *T.* sp. (bougasvlei)

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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

Kgomotso P. Sibeko [a,*], Marinda C. Oosthuizen [a], Nicola E. Collins [a], Dirk Geysen [b], Natasha E. Rambritch [c], Abdalla A. Latif [c], Hennie T. Groeneveld [d], Frederick T. Potgieter [c], Jacobus A.W. Coetzer [a]

So what was/is the problem?

The Hybrid II assay: a sensitive and specific real-time hybridization assay for the diagnosis of *Theileria parva* infection in Cape buffalo (*Syncerus caffer*) and cattle

RONEL PIENAAR[1,2], FRED T. POTGIETER[1], ABDALLA A. LATIF[1,3], ORIEL M. M. THEKISOE[2] *and* BEN J. MANS[1,3]*

Parasitology (2011), **138**, 1935–1944.
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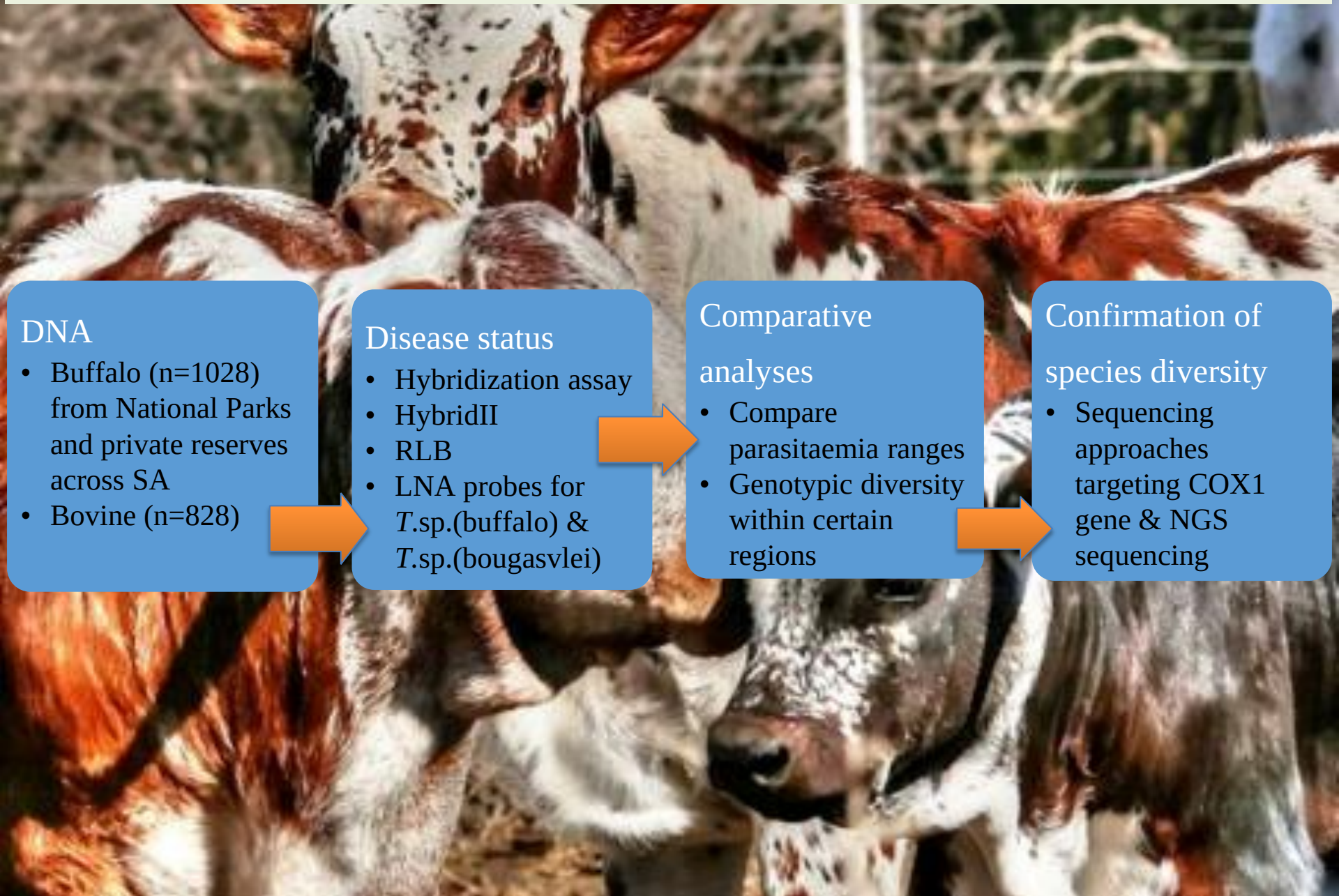
Unaffected by mixed infections

Problems with molecular diagnosis

1. What is the extend of diversity in the *Theileria* genus?
2. What are the parasitaemia ranges for the different genotypes affecting accurate diagnosis?
3. Do both genotypes suppress PCR signal?
4. What is the geographic distribution of *T.* sp. (buffalo) & *T.* sp. (bougasvlei)?

How to go about addressing the issues?

Materials & Methods



DNA
- Buffalo (n=1028) from National Parks and private reserves across SA
- Bovine (n=828)

Disease status
- Hybridization assay
- HybridII
- RLB
- LNA probes for *T*.sp.(buffalo) & *T*.sp.(bougasvlei)

Comparative analyses
- Compare parasitaemia ranges
- Genotypic diversity within certain regions

Confirmation of species diversity
- Sequencing approaches targeting COX1 gene & NGS sequencing

Sample localities:

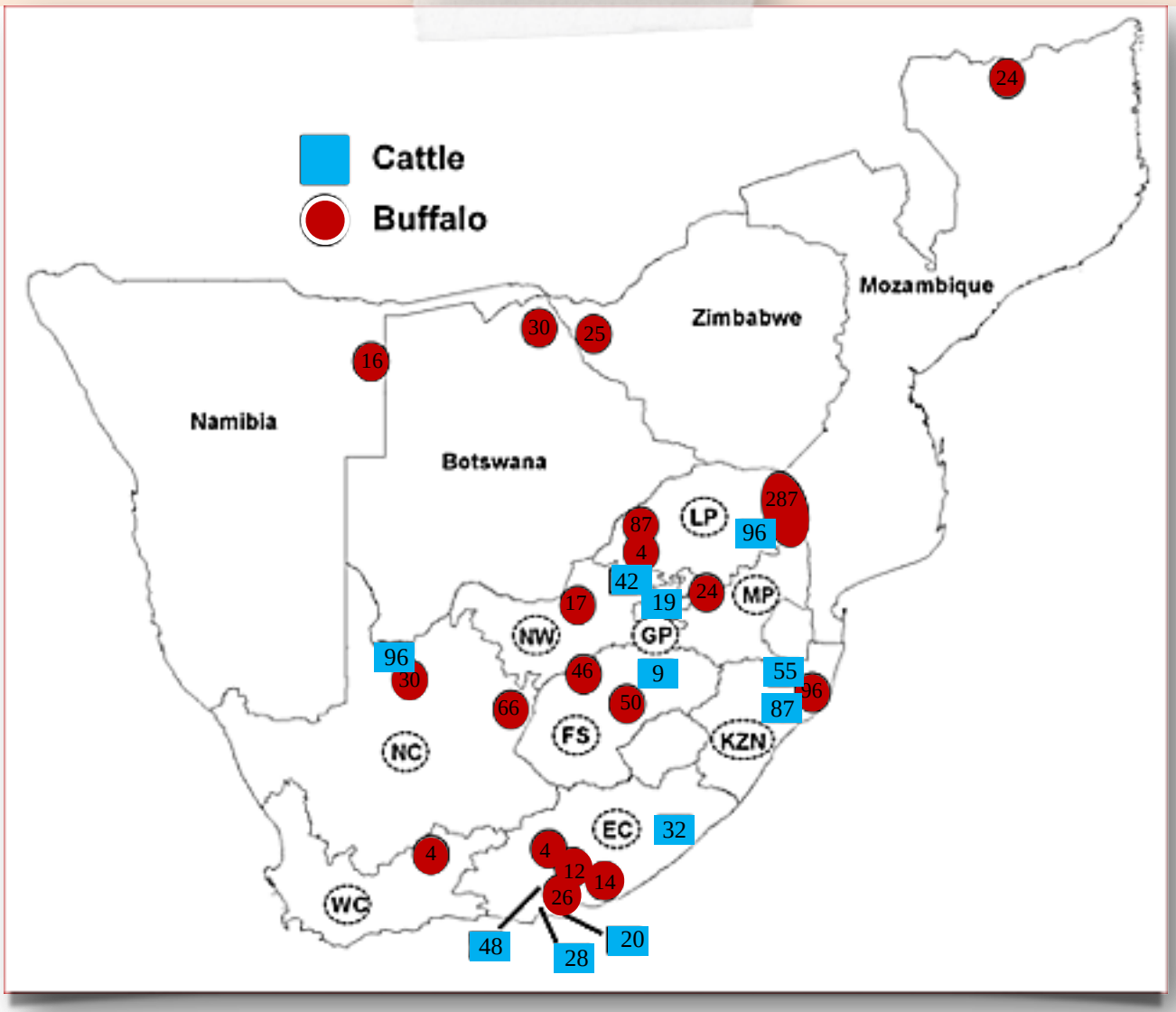
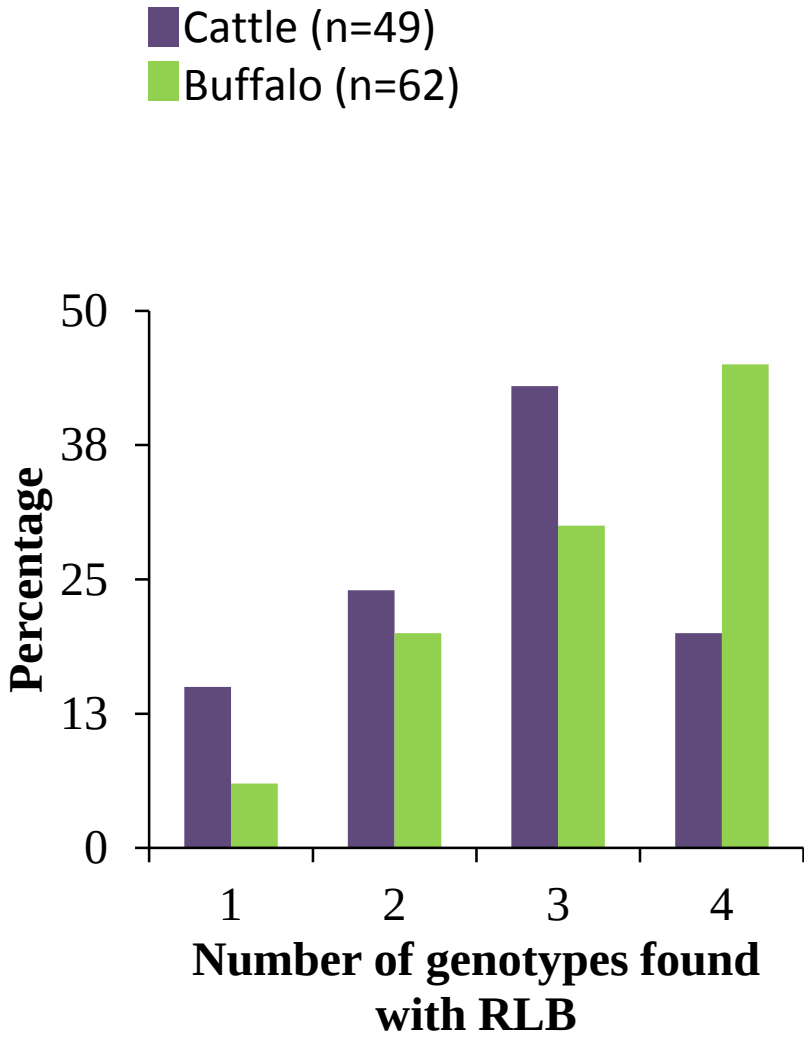
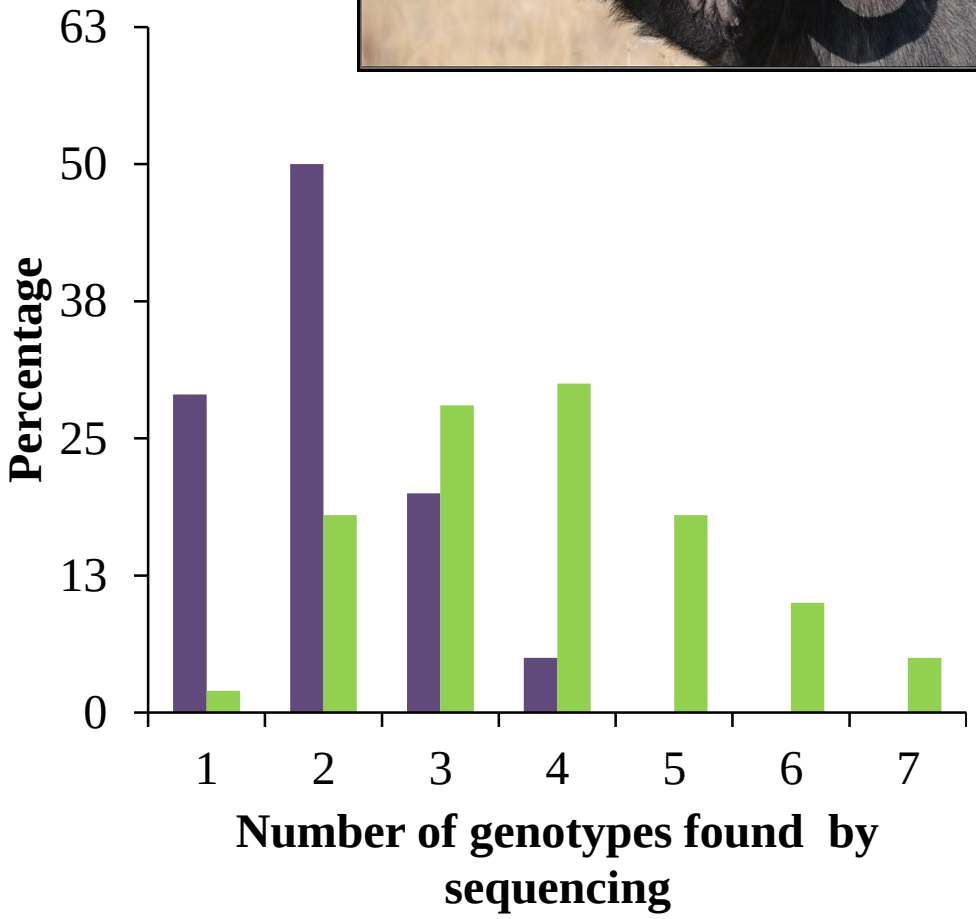


Fig 1: The number of animals sampled per site are indicated in circles (buffalo) or rectangles (cattle). Provinces in South Africa is indicated in dotted circles and include Western Cape (WC), Eastern Cape (EC), Northern Cape (NC), Free State (FS), Kwa-Zulu Natal (KZN), North-West (NW), Gauteng (GP), Limpopo (LP) and Mpumalanga (MP).

Results:

RLB *vs.* Conventional sequencing





Results: NGS

* ~10 fold increase in sample coverage
* GS Junior data correlate 97% with real-time data

Lane #	Species
1	*T.* sp (buffalo)
2	*T.* sp. (bougasvlei)
3-5	*T. mutans* (1, 2 &3)
6	*T. buffeli* C
7	*T.* sinensis-like
8	*T. velifera* A
9-13	*T. parva*
14	*T. mutans*
15	*T. mutans* (MSD)
16 &17	*T. velifera* & *T. velifera* B
18 & 19	*T. buffeli* & *T. buffeli* B
20	*T. taurotragi*
21	*B. bovis*
22	*B. bigemina*

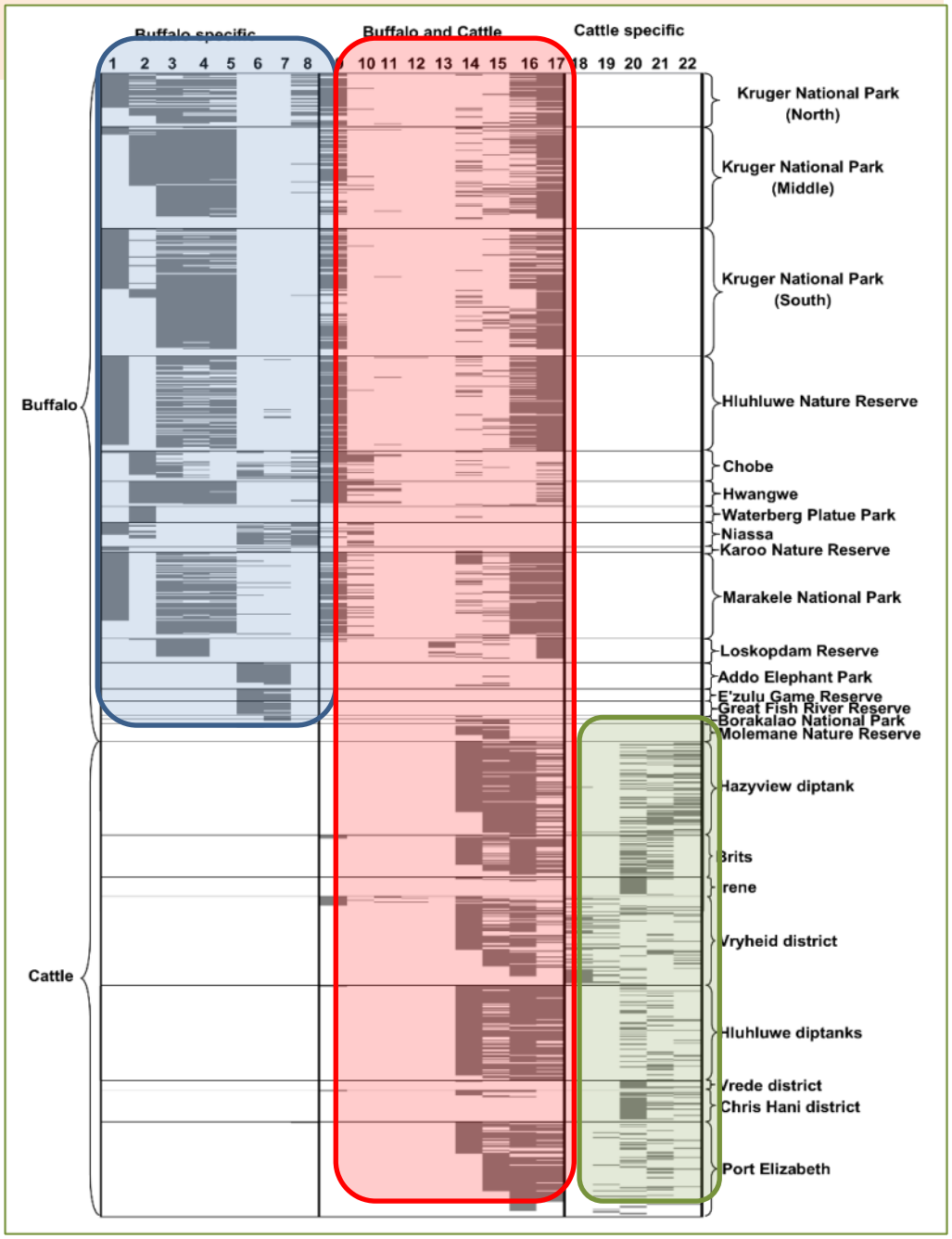


Figure 5: A presence-absence heat map of different *Theileria* and *Babesia* genotypes. Vertical axes: Buffalo and cattle samples and their origins. Horizontal axes: Genotypes (1-22) divided into buffalo specific, buffalo and cattle genotypes and cattle specific genotypes.
Grey = presence
White = abscence

Results:

Parasitaemia levels in National Parks

* Overlapping ranges for both parasites as expected
* Confirmed *T.* sp. (buffalo) contribute more to PCR suppression (Pienaar et al. 2011)

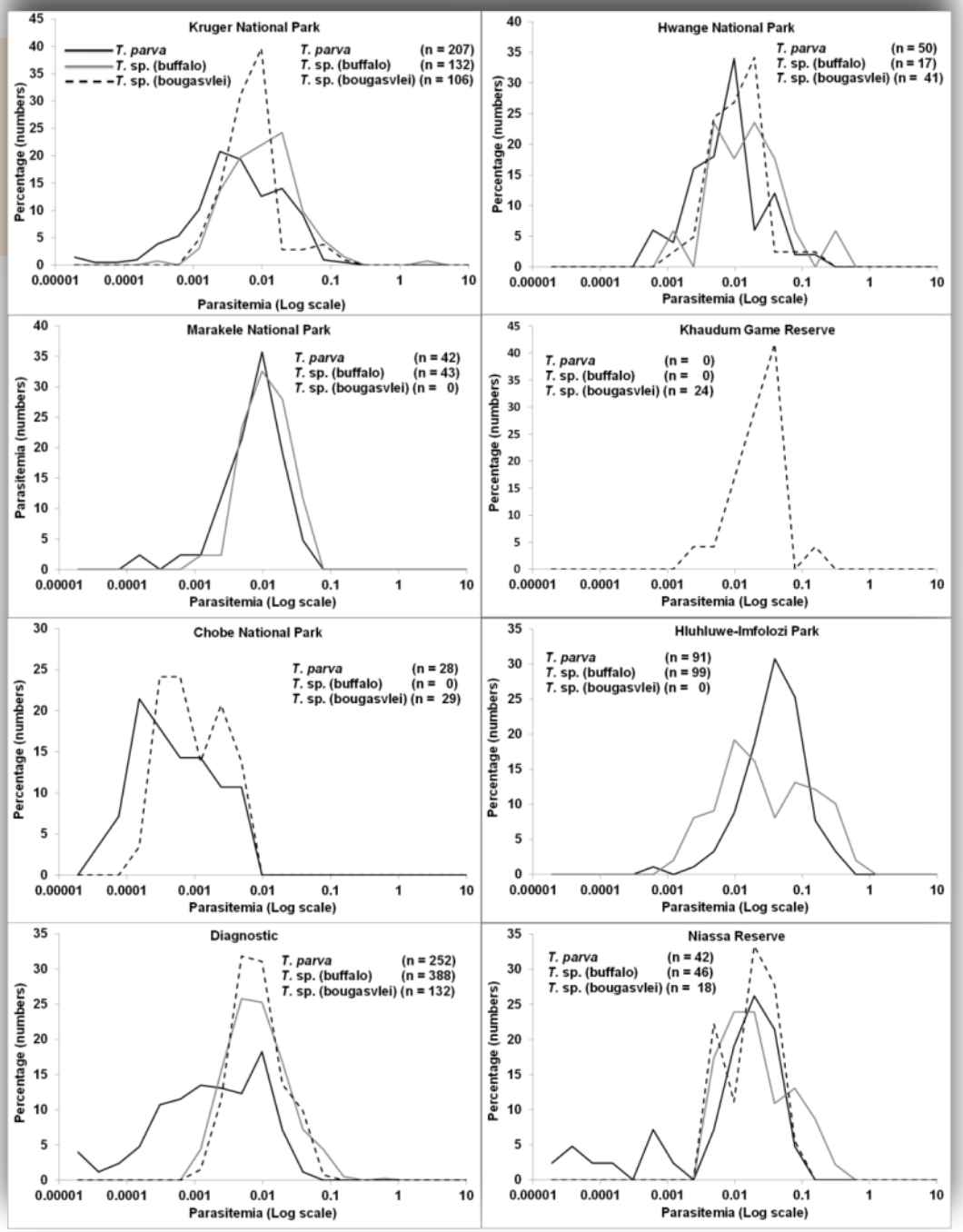
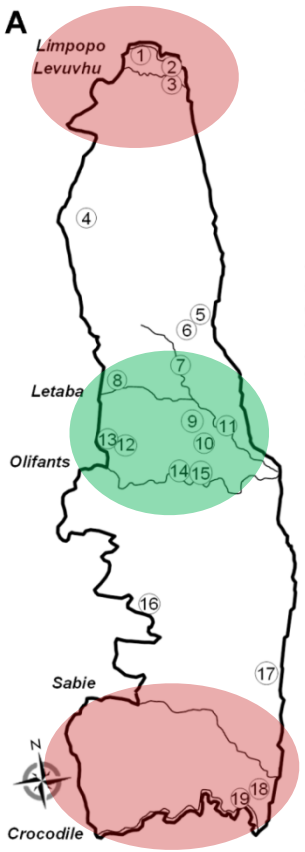


Fig 2: Parasitaemia ranges for *T parva*, *T.*sp. (buff) and *T.*sp. (bgvl) in different sample sets.

Distribution of *T. parva*, *T.* sp. (buffalo) & *T.* sp. (bougasvlei) in the KNP.



Place	Tpar	TsBuff	TsBgvl	Tmut	Tvel	Total
1. Banyini	2	16	9	16	16	16
2. Crook's Corner	3	6	3	6	6	6
3. Pafuri	3	4	2	4	4	4
4. Mhalamhala	2	0	2	3	1	3
5. Makhadzi	5	0	4	7	4	7
6. Nshawu	6	0	6	11	10	12
7. Middelvlei	7	0	5	10	9	10
8. Black Heron dam	18	3	21	17	16	22
9. Letaba air strip	11	2	9	14	8	14
10. Letaba shooting range	6	1	5	4	3	6
11. Longwe	1					
12. Masorini	4					
13. Phalaborwa	1					
14. Ndlotini	6					
15. Wilderness Windmill	10					
16. Manyeleti	11	0	10	33	30	35
17. Shishangeni	18	18	0	12	11	18
18. Mpanamane	43	53	5	44	28	53
19. Dzuweni	23	22	1	22	22	23

Are they different species?

Fig 3: A) Sampling sites are indicated with numbered circles with corresponding names and the number of positive samples per site found for *T. parva* (Tpar), *T.* sp. (buffalo) (TsBuff), *T.* sp. (bougasvlei)(TsBgvl), *T. mutans* (Tmut) and *T. velifera* (Tvel).

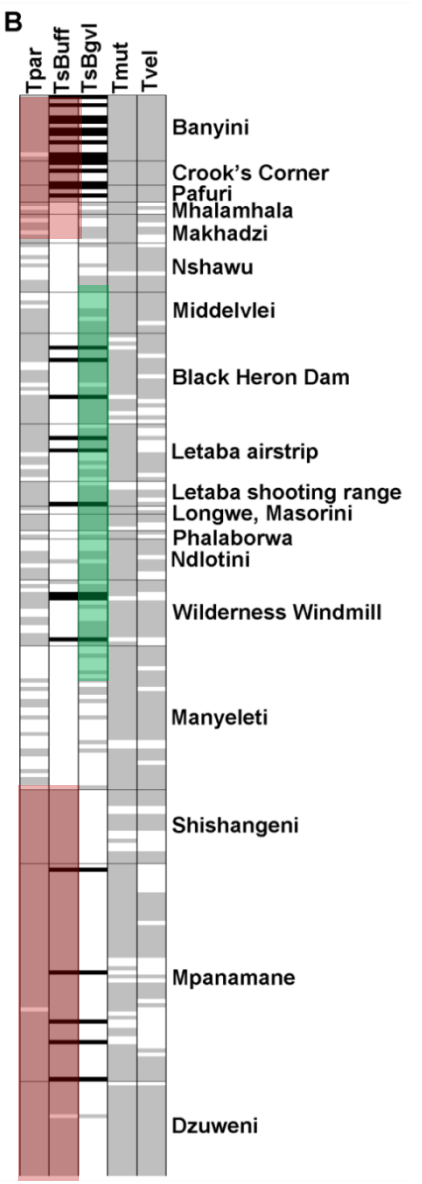


Fig 4: B) Heat map distribution indicates absence (white), presence (grey) or mixed-infections for *T.* sp. (buffalo) and *T.* sp. (bougasvlei) (black).

Competitive exclusion between different *Theileria* parasites

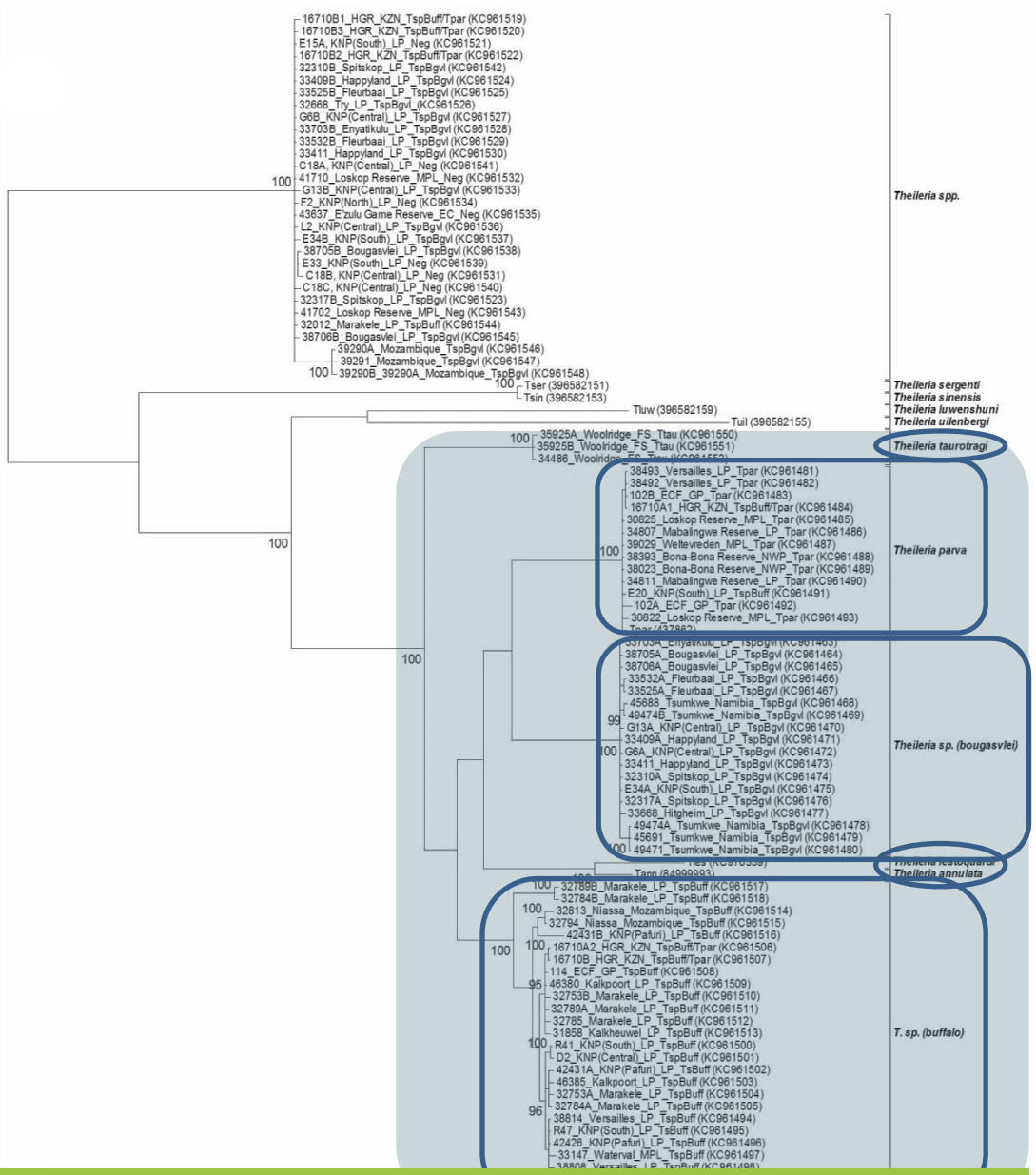
$$Rij = \frac{\frac{n}{n-1}\left[\frac{n11}{n} - \frac{(n11+n12)}{n}\frac{(n11+n21)}{n}\right]}{\sqrt{\frac{(n11+n12)}{n}\frac{(n22+n21)}{n}\frac{(n11+n21)}{n}\frac{(n22+n12)}{n}}}.$$

(Dib et al. 2008)

	Tpar/TspBuff	Tpar/Bgvl	TspBuff/Bgvl
KNP	0.031	-0.220	-0.737
KNP (South)	0.221	-0.713	-1.005
KNP (Mid)	-0.457	0.137	-0.573
KNP (North)	0.069	-0.163	-0.323
HGR	-0.057	-1.172	-0.736
CNP (Botswana)	-0.780	0.070	-0.771
MNP	0.127	-0.758	-0.770
HNP (Zimbabwe)	-0.469	0.102	-0.490
GNP (Zimbabwe)	-0.651	0.158	-0.651
GLTP (Sengwe corridor, Zimbabwe)	0.076	-0.226	-0.206
NNR (Mozambique)	-0.108	-1.09	-0.327
GLTP (Manguana Powerline, Mozambique)	0.072	-0.316	-0.435
KGR (Namibia)	-13.299	-0.787	-0.787
Diagnostic	-0.855	-1.57	-1.232

Table 1: Correlation of co-occurrence of *Theileria* parasites. Indicated are the *Rij* values.

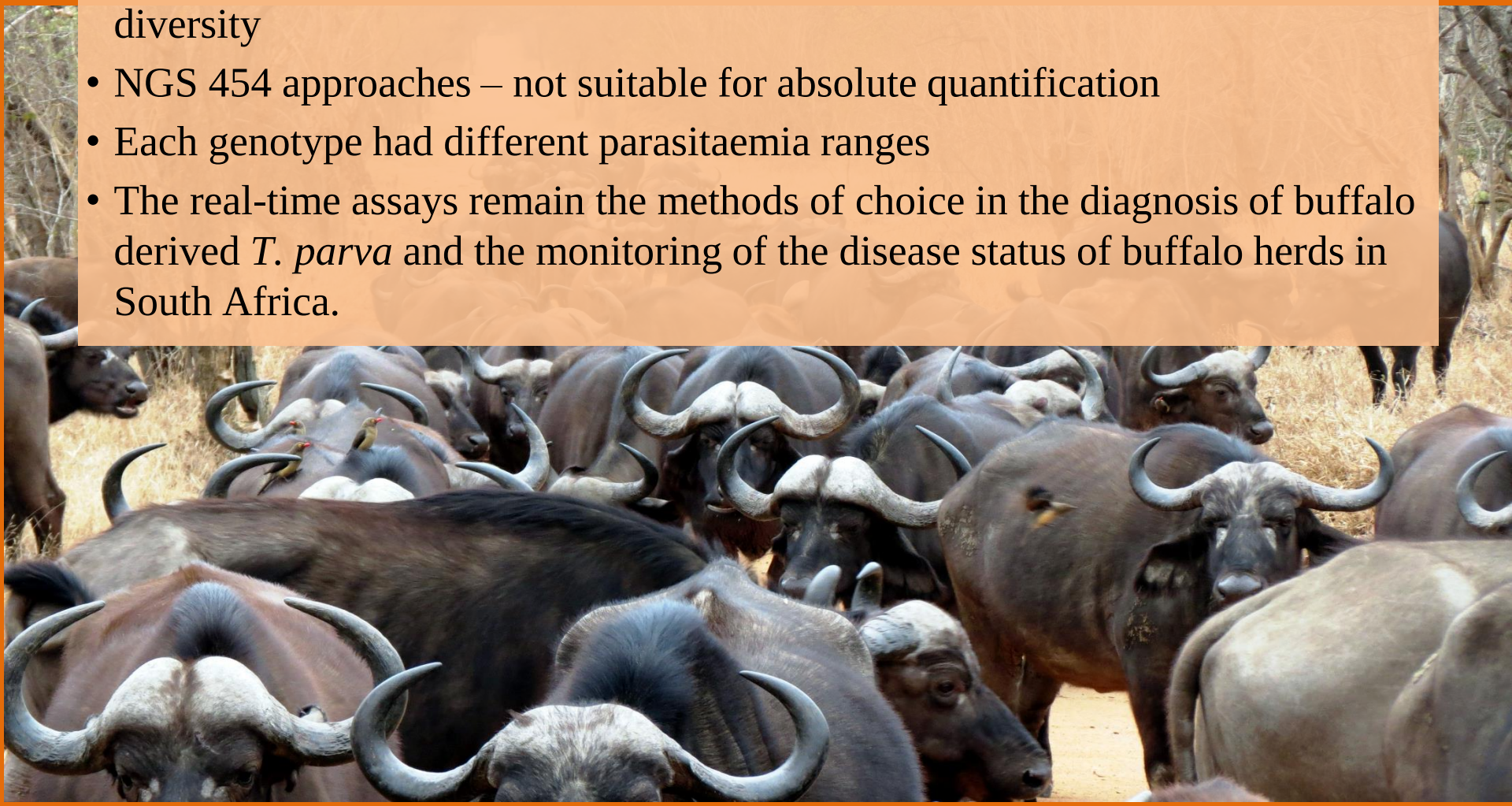
T. sp. (buffalo) and *T.* sp. (bougasvlei):different species



Automatic Barcode Gap Discovery (ABGD) Server (http://wwwabi.snv.jussieu.fr/public/abgd/)

Conclusions:

- Buffalo harbor more *Theileria* parasites than cattle
- No assay is 100% sensitive or specific
- Conventional sequencing give enough sequencing depth to cover sequence diversity
- NGS 454 approaches – not suitable for absolute quantification
- Each genotype had different parasitaemia ranges
- The real-time assays remain the methods of choice in the diagnosis of buffalo derived *T. parva* and the monitoring of the disease status of buffalo herds in South Africa.



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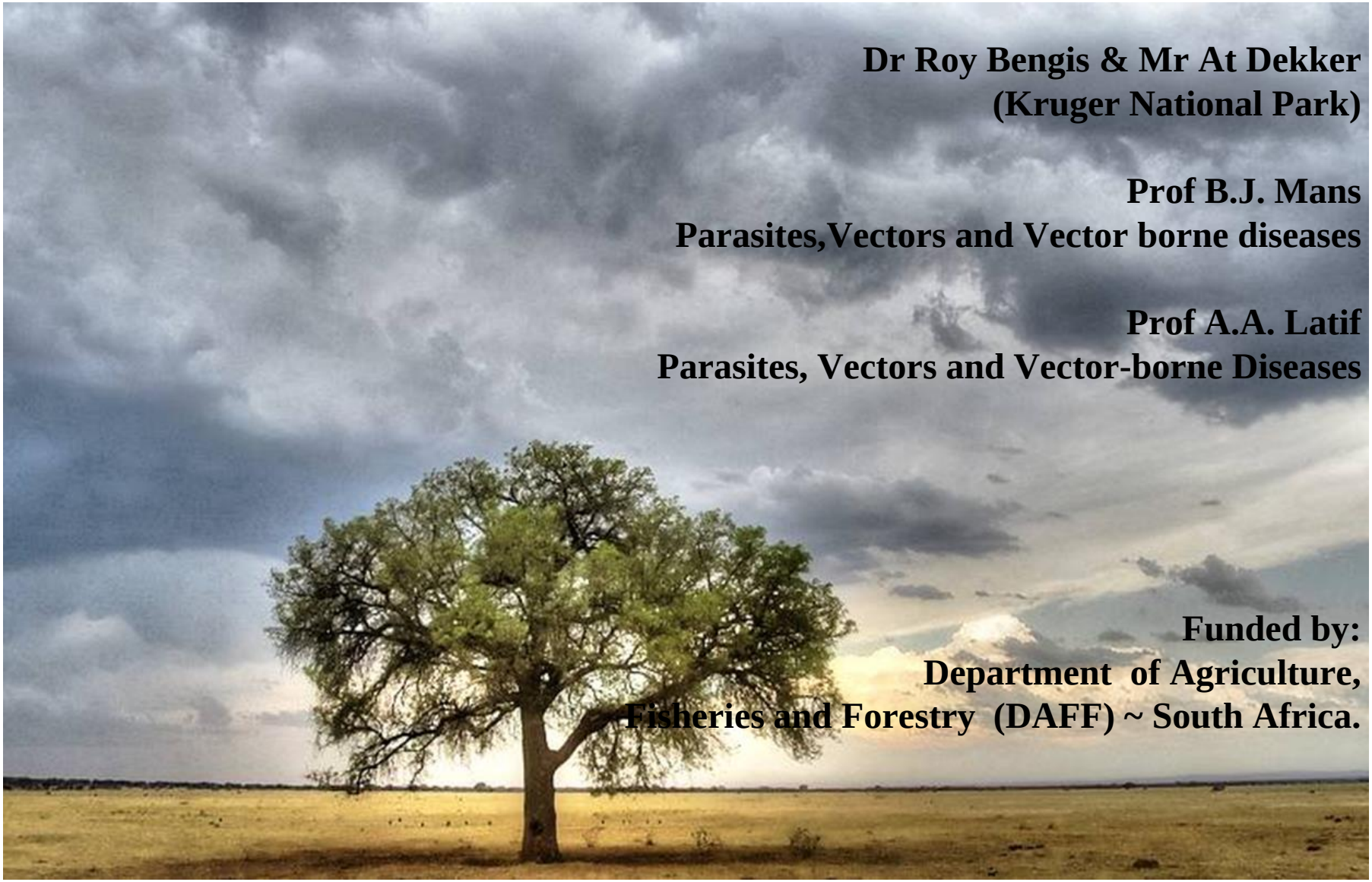

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Thank you!