Assessment of alternative diagnostic techniques for detection of *Trypanosoma equiperdum* infections in horses and donkeys in South Africa

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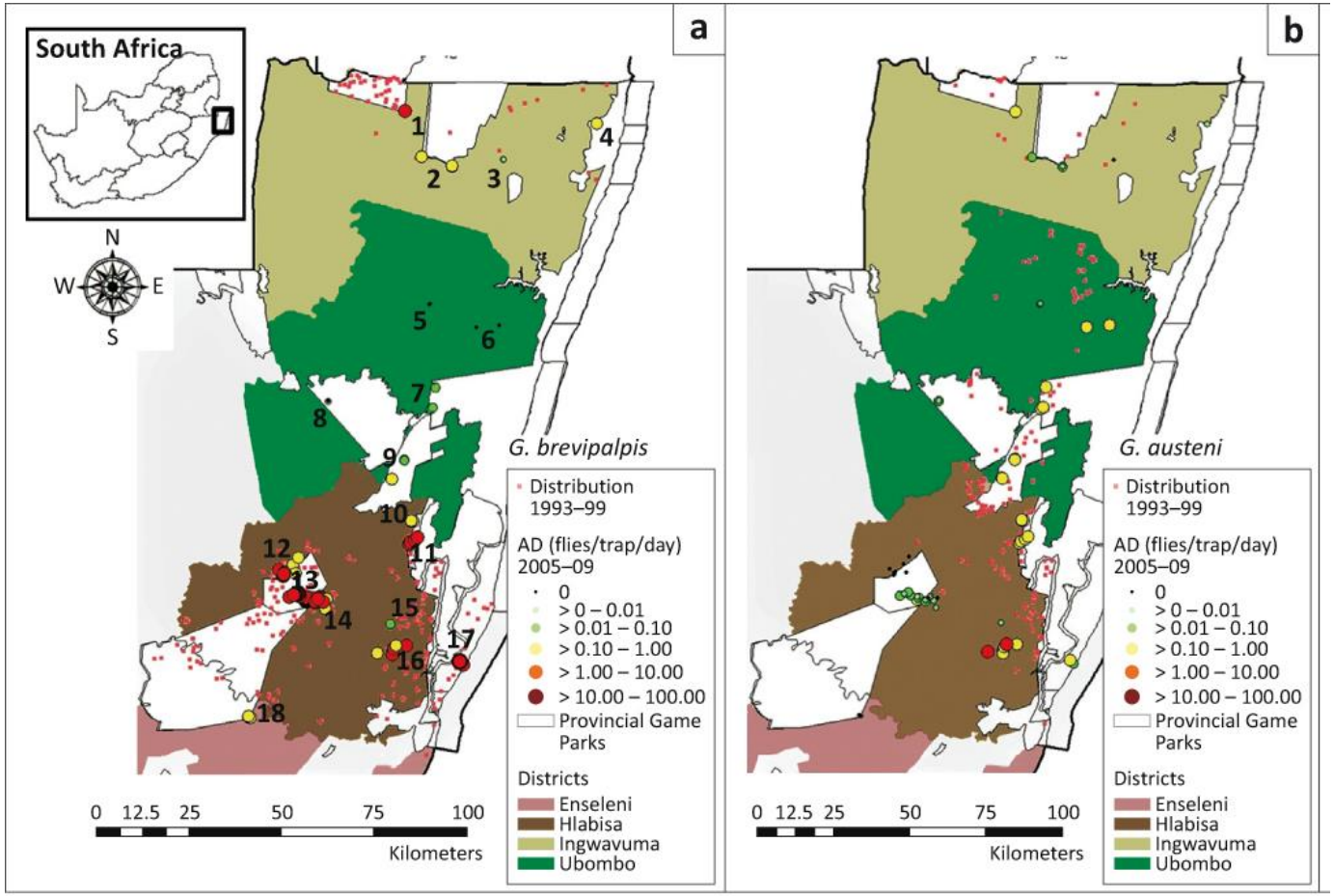
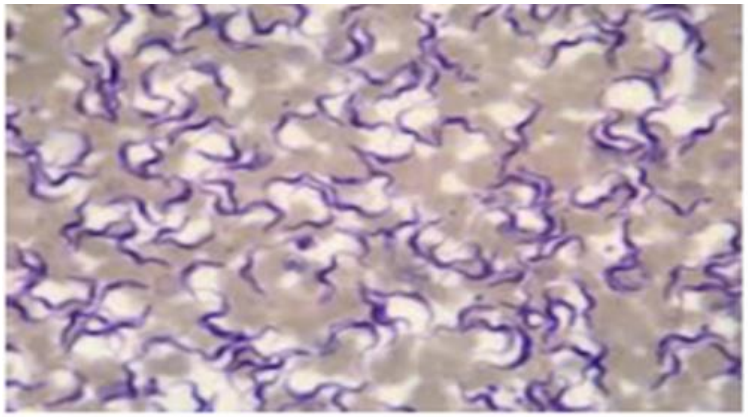
Tsetse flies in South Africa



Source: Created by C.J. de Beer, ArcGIS Desktop 10.1

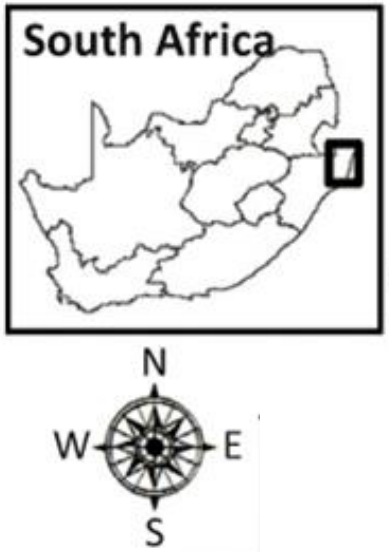
De Beer et al 2016, *Onderstepoort Journal of Veterinary Research*

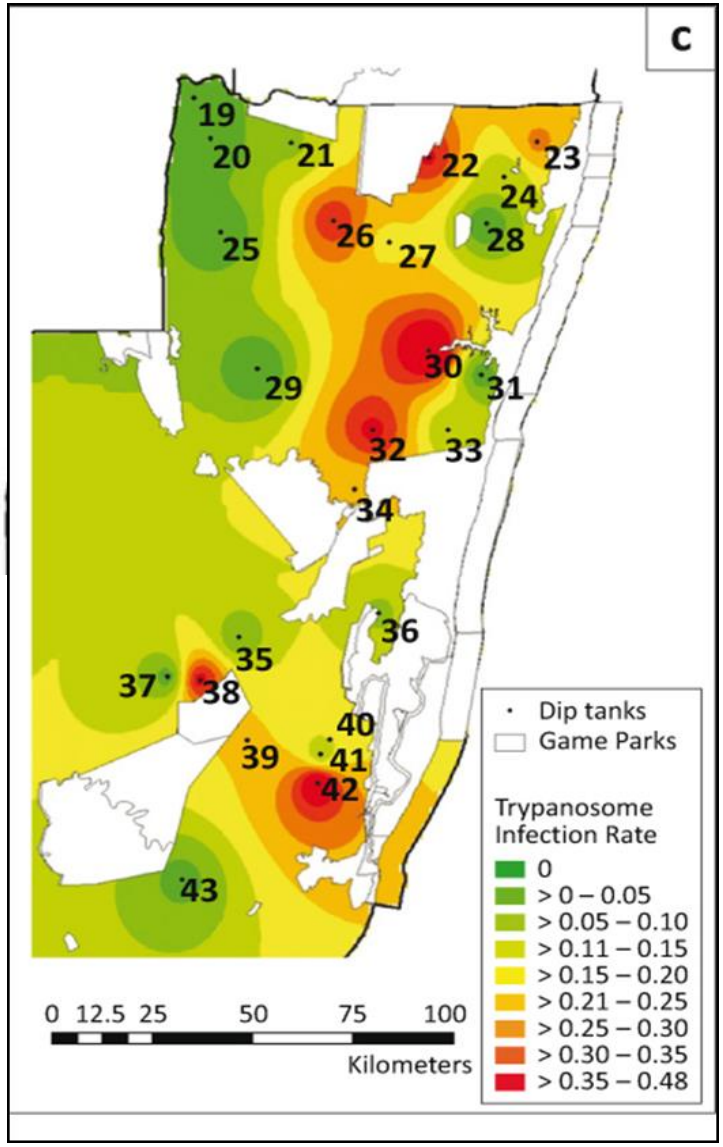
Nagana in South Africa



Trypanosoma congolense

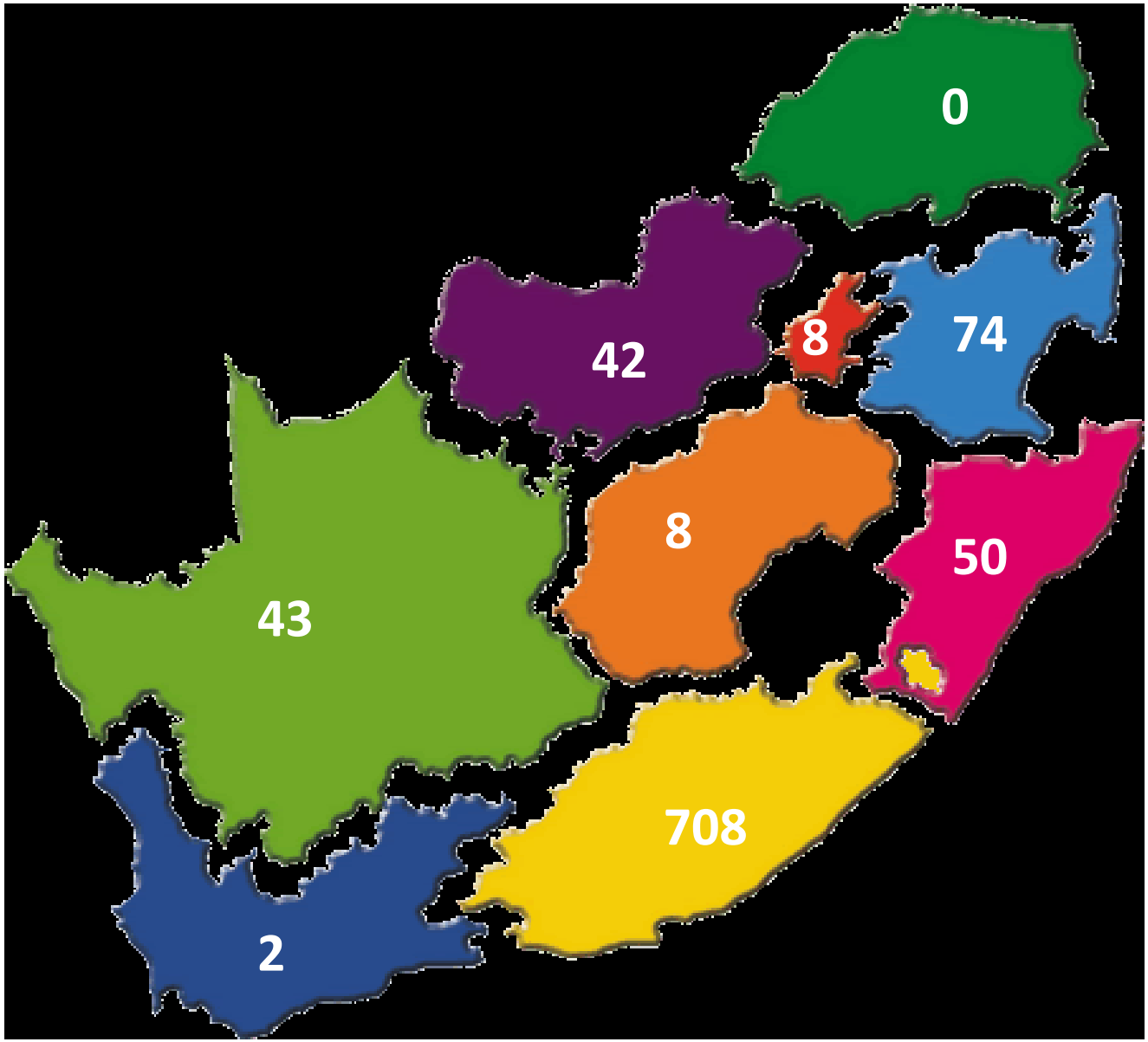
Trypanosoma vivax





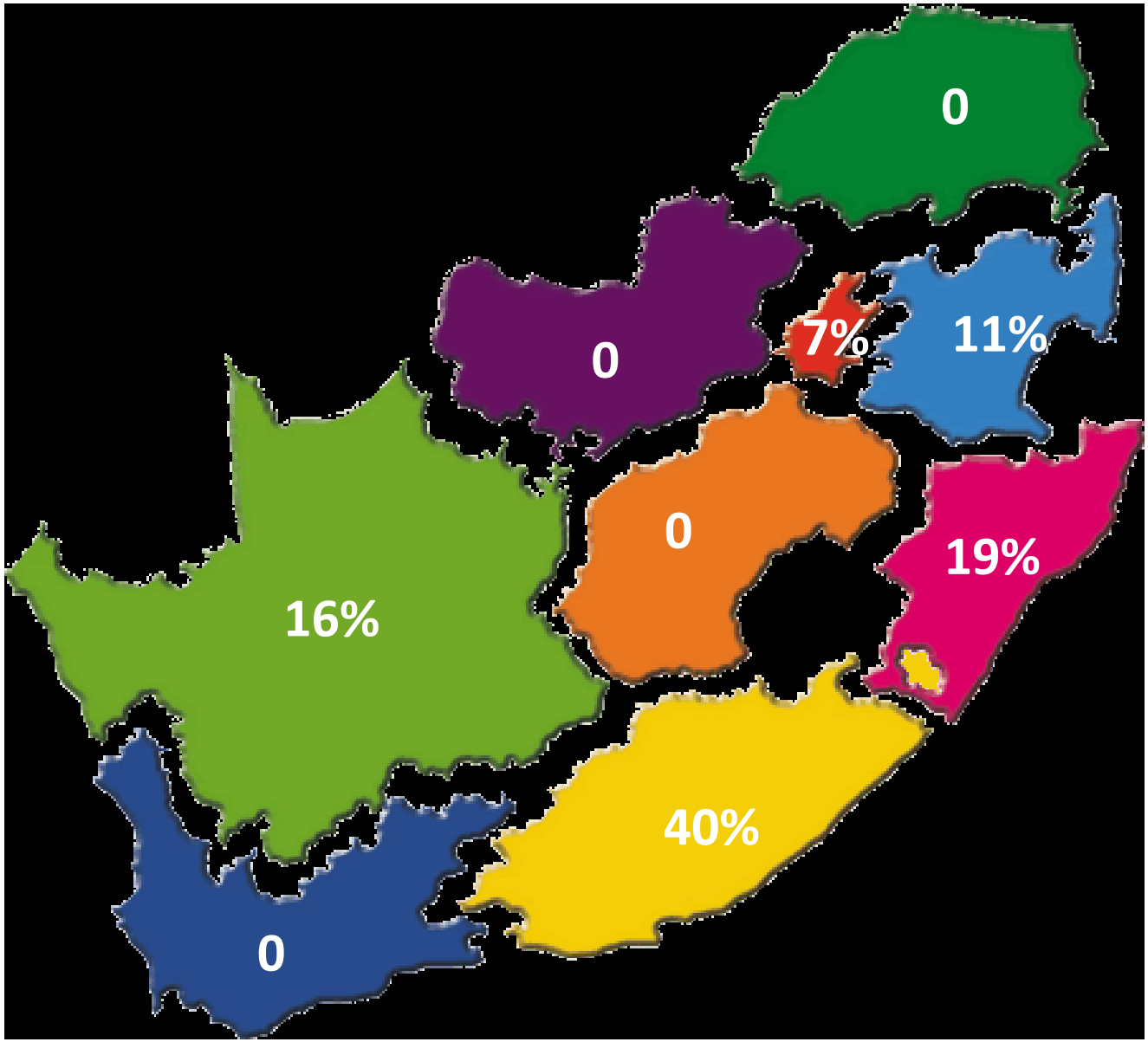
De Beer et al 2016, *Onderstepoort Journal of Veterinary Research*

Dourine cases per province: 2000 - 2010



908

Dourine cases per province: 2005 - 2015



105

Common clinical signs

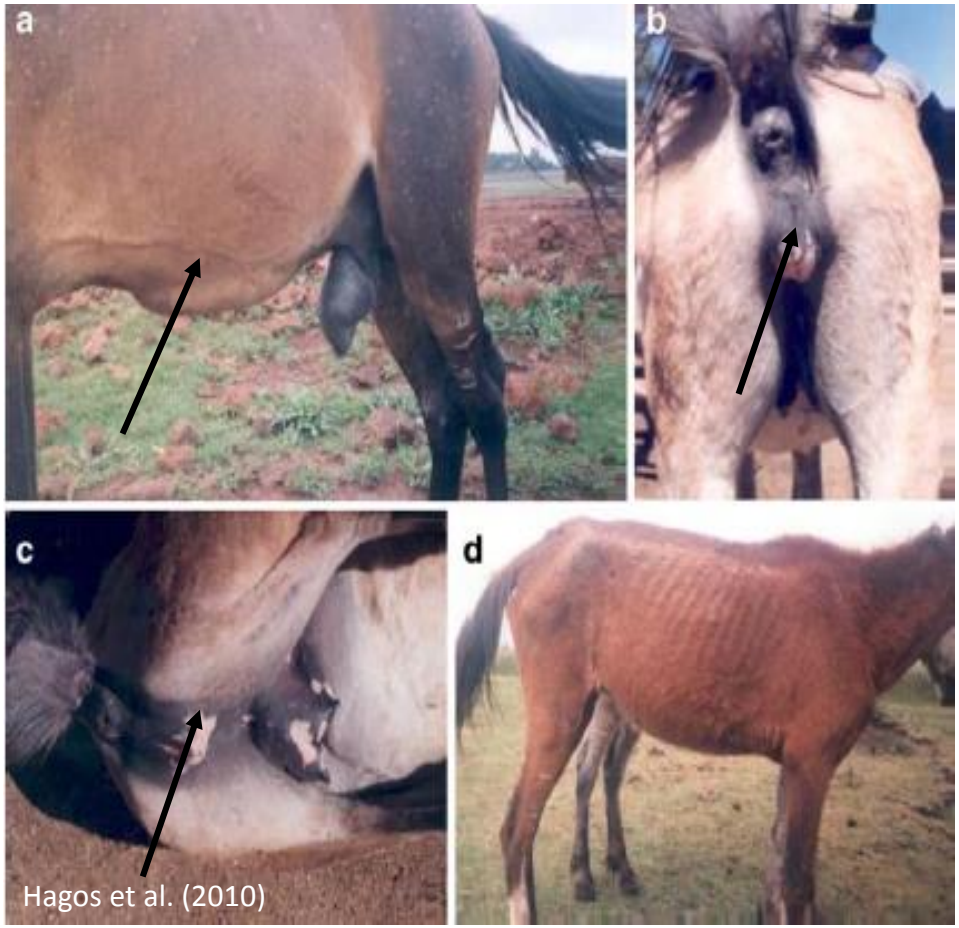
- Ataxia & stiffness/paralysis

- Oedematous swelling over the ventral abdomen and penis

- Depigmentation over the external genitalia

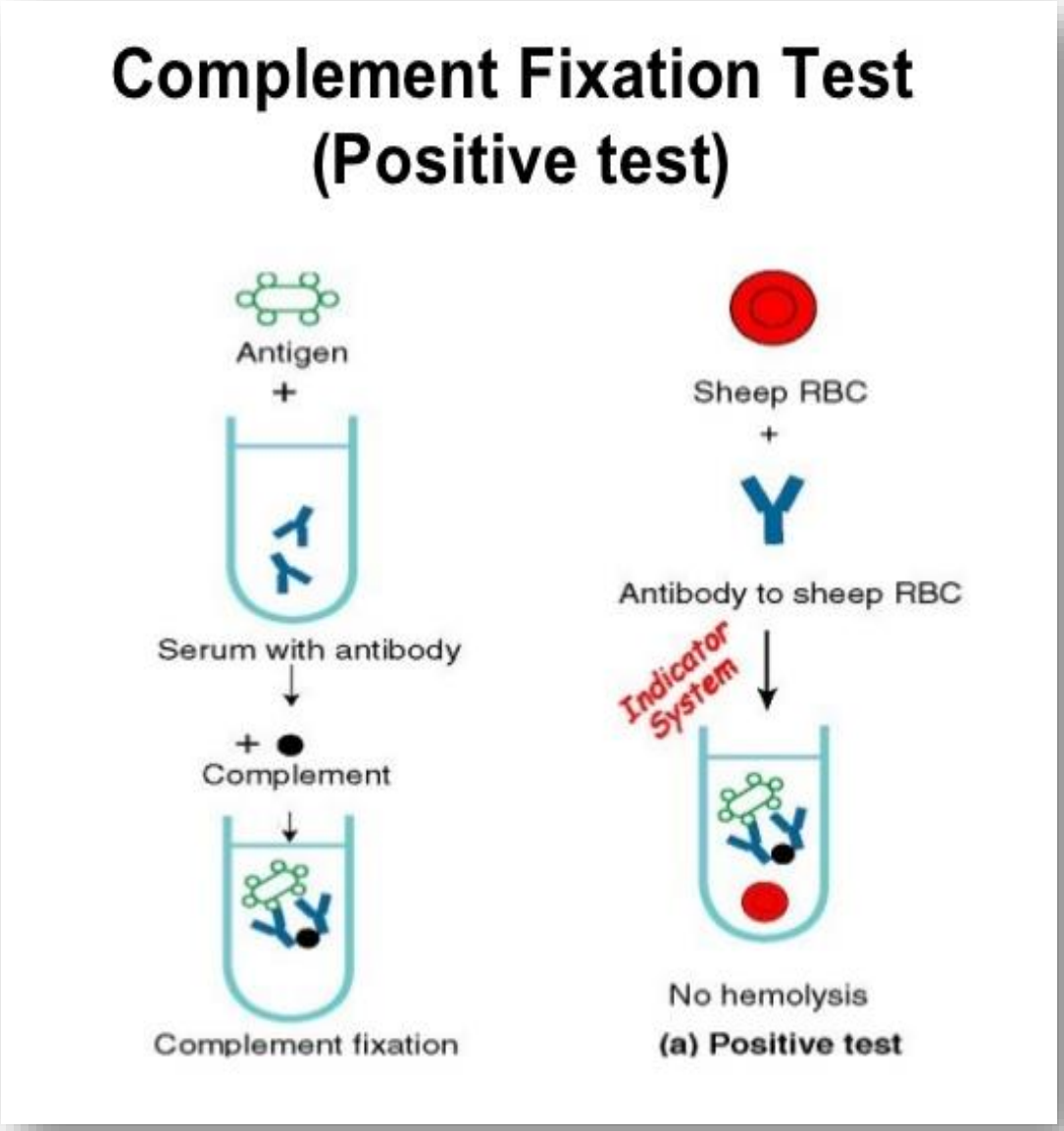
- Showing depigmentation over the external genitalia and udder

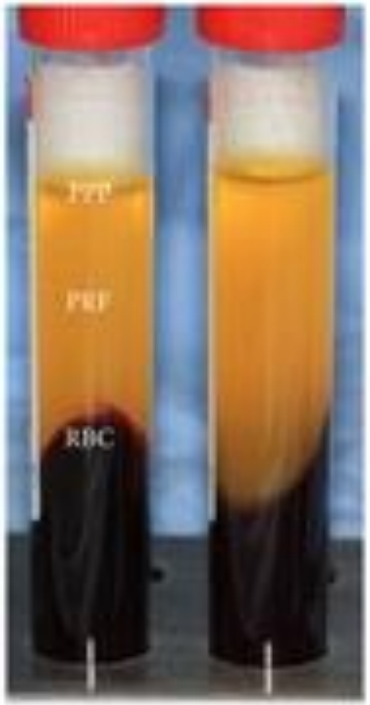
- Showing poor body condition



Hagos et al. (2010)

Laboratory diagnosis





Control

- New introduced animals must be tested serologically by Complement fixation test (CFT)
- Slaughter of seropositive horses
- Assisted mating is also recommended

Problem statement

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 23, Numbers 1 and 2, March, 1948.

Printed in the Union of South Africa by the Government Printer, Pretoria.

Transmission of the South African Strain of Dourine to Laboratory Animals.

By D. A. HAIG and (the late) A. S. LUND, Section of Bacteriology, Onderstepoort.

Dourine in South Africa.

23. **Faul A.**

J S Afr Vet Assoc. 1988 Mar;59(1):7. No abstract available.

PMID: 3361566

Trypanosoma equiperdum **OVI strain 1977**

No active scientific studies conducted on *T. equiperdum* and dourine in South Africa

Objective of the study

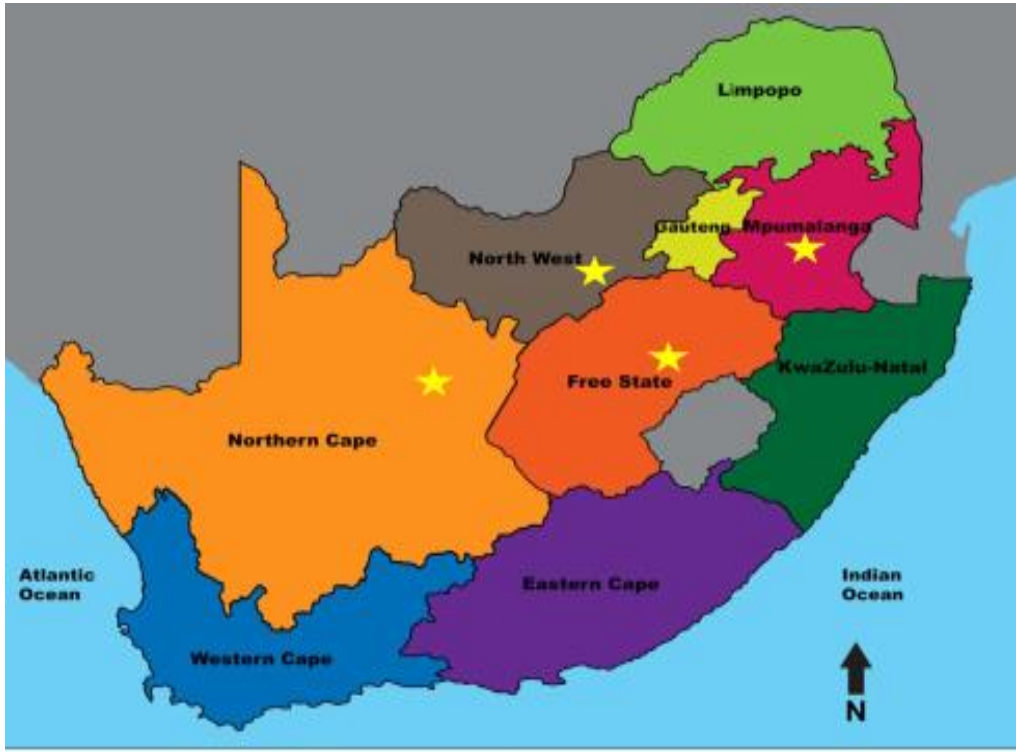
To assess the use of other diagnostic techniques (DNA based and serological) for detection of *T. equiperdum* infections in horses and donkeys in South Africa

Sample collection

- **A total of 294 Blood and Serum samples**

- **FS**: n = 40 (Horses =38; Donkeys = 2)
- **FS**: n = **38 genital secretions of horses**
- **MP**: n = 94 horses
- **NC**: n = 54 (Horses = 42; Donkeys = 12)
- **NW**: n = 68 (Horses = 48; Donkeys = 20)

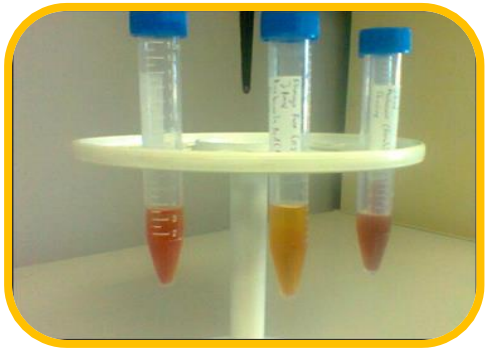
Samples were collected at the abattoir except NW & FS



Materials & Methods

- **Serology**

  - Serum was harvested from silicone coated vacutainers



(i) ELISA (Nguyen et al 2015, *J. Vet. Med. Sci*)

- Crude antigen ELISA [Crude (TeCA)]

- Recombinant antigen ELISA [rTeGM6-4r]

(ii) ICT (Nguyen et al 2015, *Parasitol Research***)**

- Recombinant antigen (rTeGM6-4r)

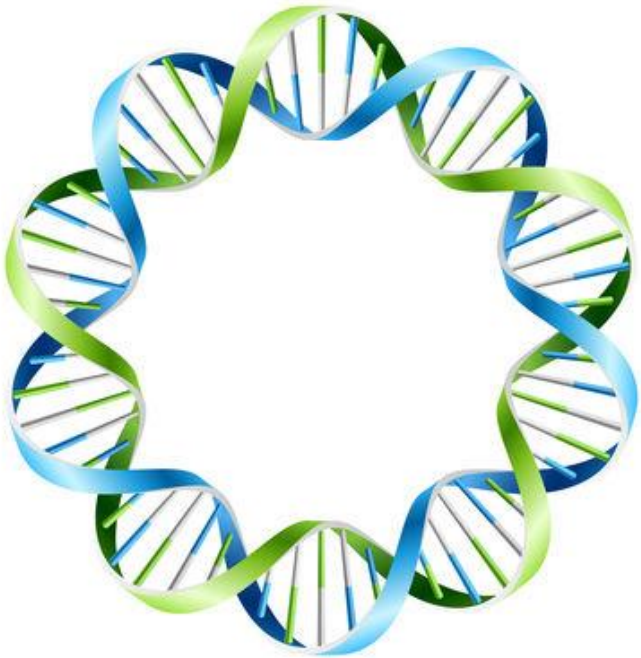
Positive

Negative

PCR

Universal Kinetoplast PCR

(i) Njiru et al 1995 - PCR
KIN1: GCGTTCAAAGATTGGGCAAT
KIN2: CGCCCGAAAGTTCACC



- **Nested PCR :18S rRNA** - Mamabolo *et al*., (2009)

(ii) 18ST nF2 CAA CGA TGA CAC CCA TGA ATT GGG GA
18ST nR3 TGC GCG ACC AAT AAT TGC AAT AC
18ST nR2 GTG TCT TGT TCT CAC TGA CAT TGT AGT G

(iiI) gGAPDH F: CTY MTC GGA MKG AGA TYG AYG - Hamilton *et al*., (2004)
gGAPDH R: GRT KSG ART ADC CCC ACT CG
G4a: GTT YTG CAG SGT CGC CTT GG

- PCR amplicons were sequenced

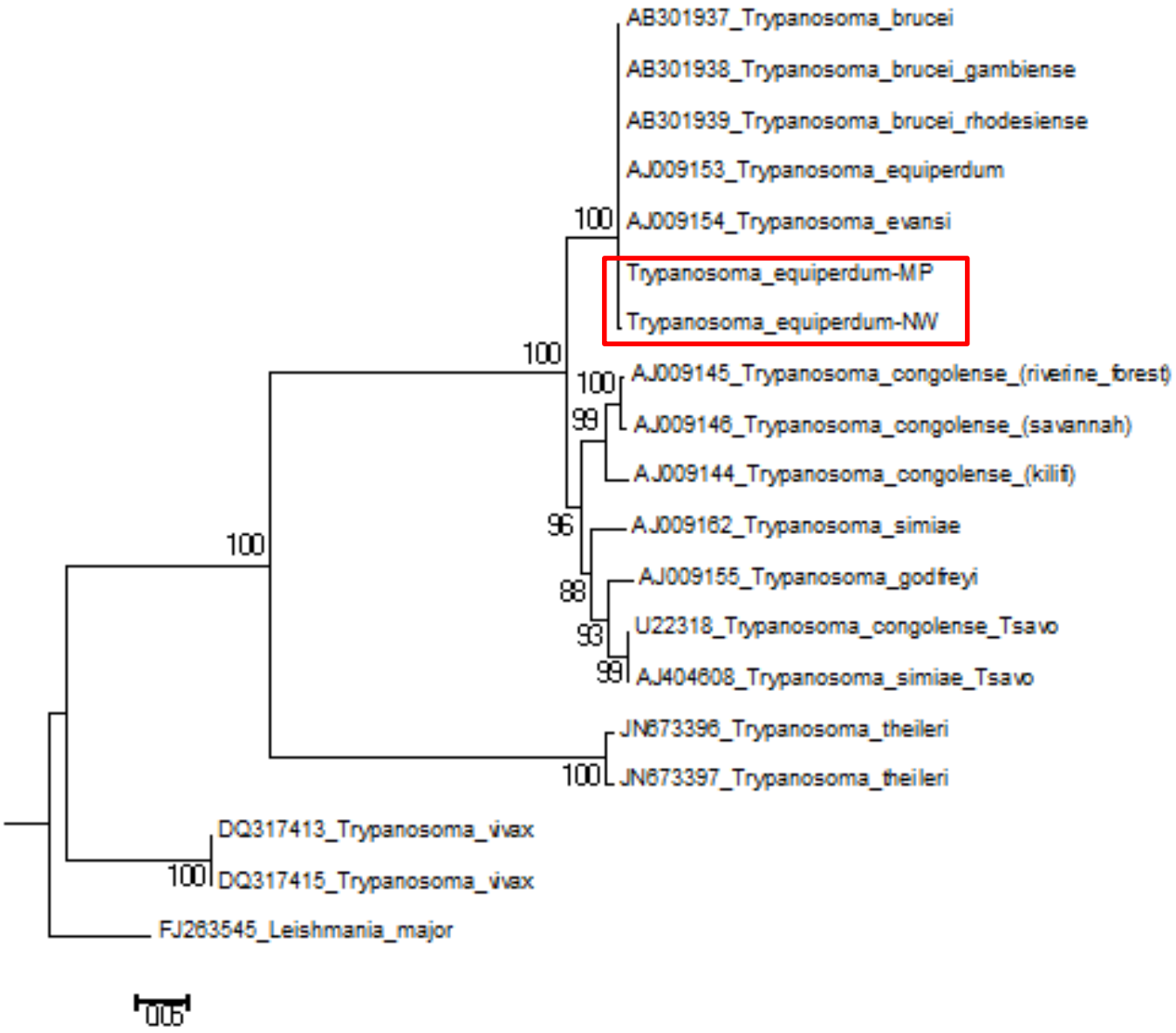
Serology Results

	Donkeys				Horses			
Province	No. of samples	TeGM6-4r	TeCA	ICT	No. of samples	TeGM6-4r	TeCA	ICT
FS	2	0	0	0	35	5	3	5
MP	-	-	-	-	94	12	19	0
NC	12	7	3	0	39	7	3	1
NW	20	1	0	0	48	14	11	0
Total	34	8	3	0	216	38	36	6
Percentage		23.5%	8.8%	0%		17.6%	16.6%	2.8%

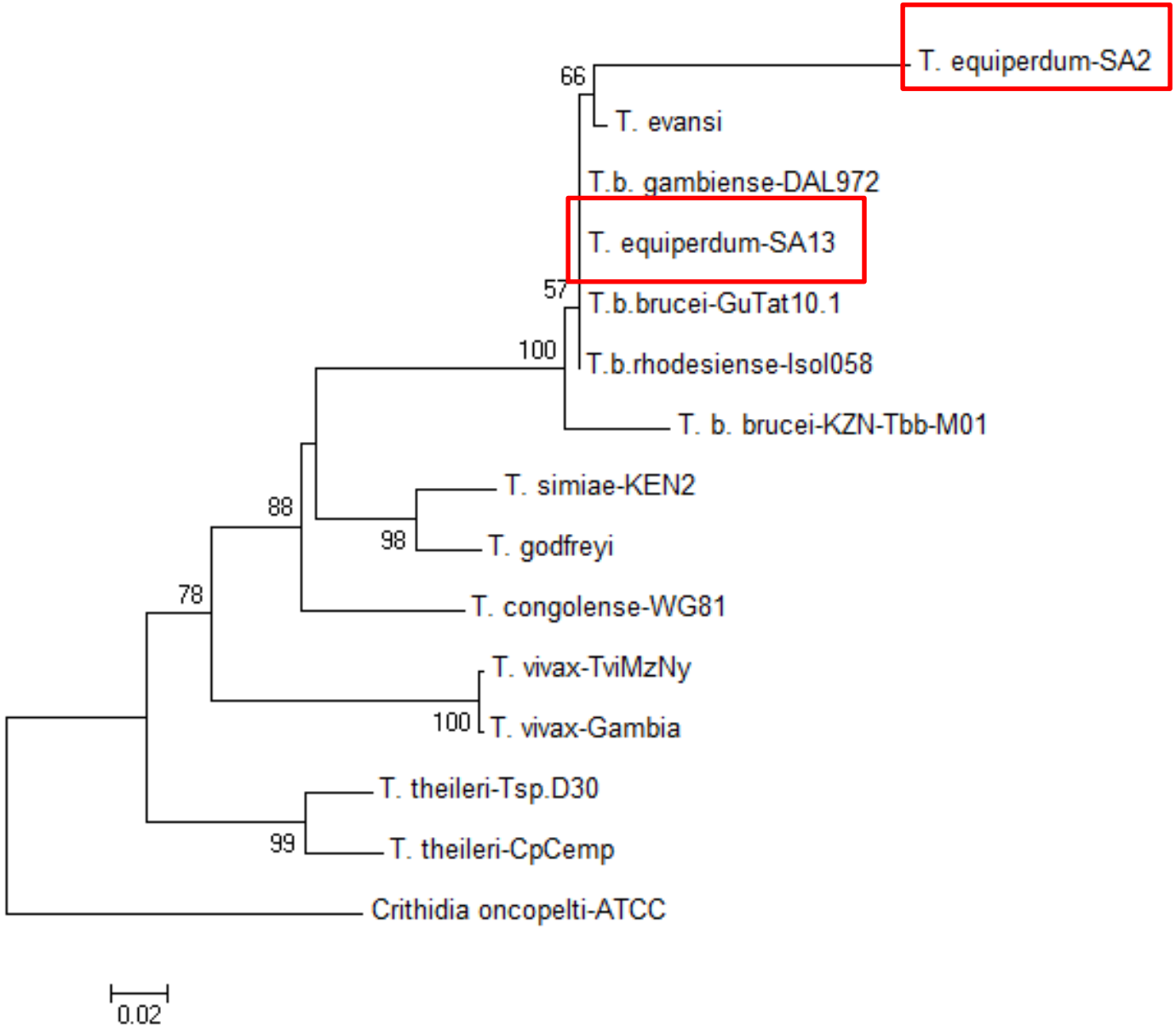
PCR results

Province	Total number of samples	PCR +ve (%)	Number of horses	PCR +ve (%)	Number of donkeys	PCR +ve (%)
FS	40	6 (15)	38	6 (15.8)	2	0 (0)
MP	94	16 (17)	94	16 (17.0)	–	–
NC	54	6 (11)	42	5 (11.9)	12	1 (8.3)
NW	68	8 (12)	48	6 (12.5)	20	2 (10)
Total	256	36	**222**	33	**34**	3
Percentage (%)		13.8		14.3		8.8

T. equiperdum (18S rRNA)



T. equiperdum (gGAPDH)



Discussion

- ELISA with rTeGM6-4r and crude TeCA detects *T. equiperdum* in horses and donkeys
- The detection efficiency of ICT for dourine was poor, and needs further improvements
- PCR efficiently detected *T. equiperdum* infections as confirmed by sequencing
- A phylogenetic tree constructed with 18S rRNA and gGADPH gene, the *T. equiperdum* correctly clustered with other trypanosomes of subgenus *Trypanozoon*
- Donkeys appear to be less susceptible to *T. equiperdum* as compared to horses
- ELISA and PCR can be used to supplement the currently recommended CFT for detection of *T. equiperdum* infections in horses and donkeys in South Africa

Future research

- Screening of all provinces as well as neighbouring Lesotho
- Isolation of *T. equiperdum* strains in South Africa

Acknowledgements

- State Vet - Gauteng department of Agriculture & Rural development
- State Vet and animal health technicians - Free State Province
- Horse and donkey owners
- Middelvlei abattoir
- Mr. Jacob Mabena (UFS Veterinary Technician)

NRF National Research Foundation