Human African Trypanosomiasis
The missing link?

Brice ROTUREAU
Trypanosome Transmission Group
Trypanosome Cell Biology Unit

FOR RESEARCH, FOR HEALTH,
FOR OUR FUTURE

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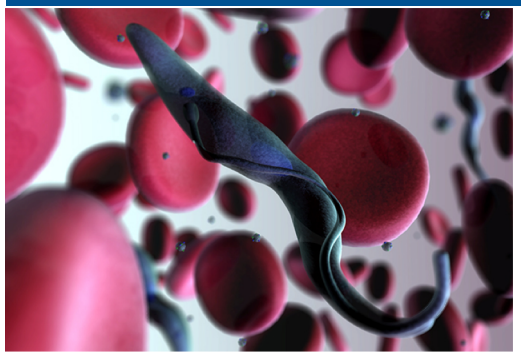
Human African Trypanosomiasis



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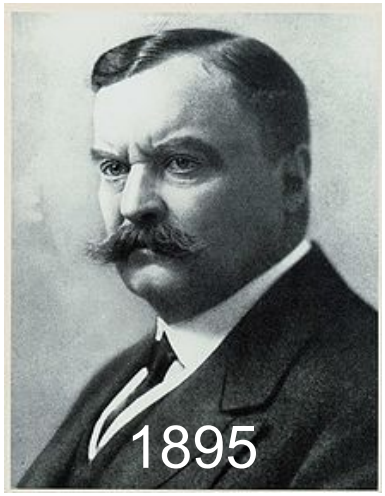
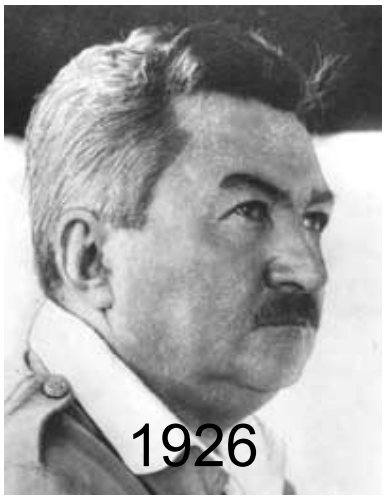
Epidemiology



David BRUCE

(1855-1931)



Eugène JAMOT

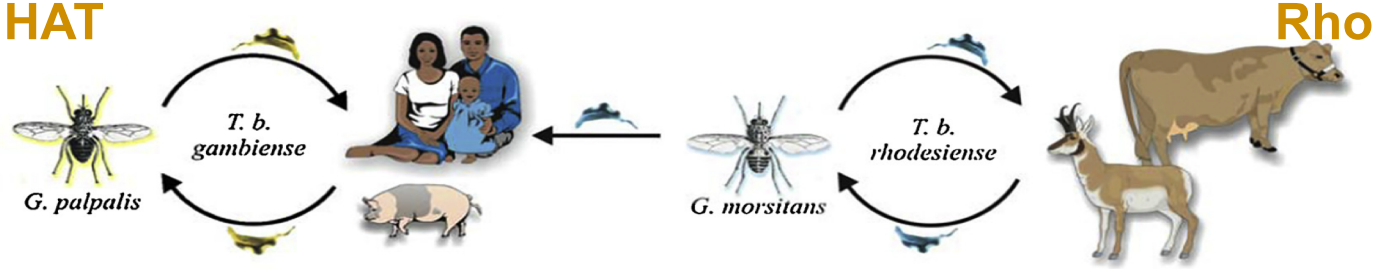
(1879-1939)

Welcome Trust, Institut Pasteur and WHO

Parasite cycles

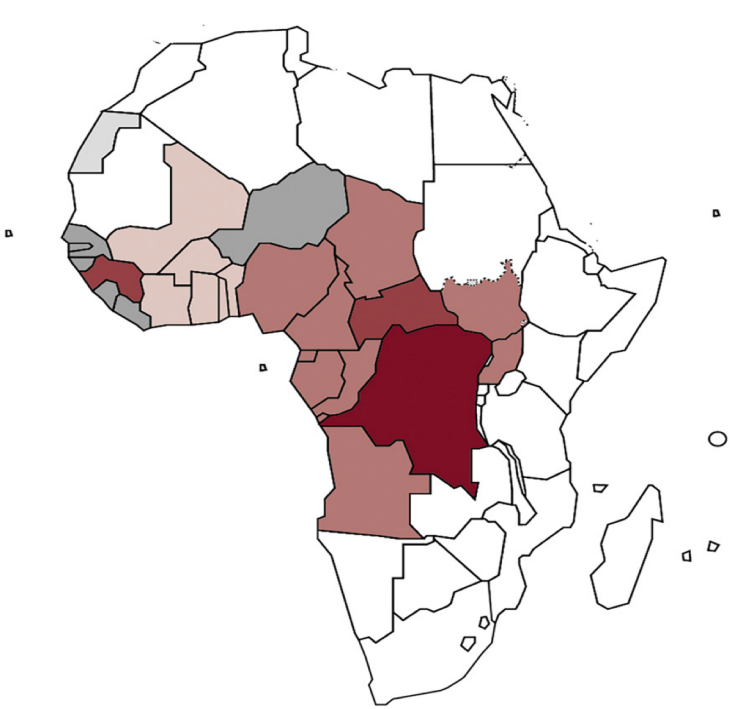
Gambiense HAT

Rhodesiense HAT



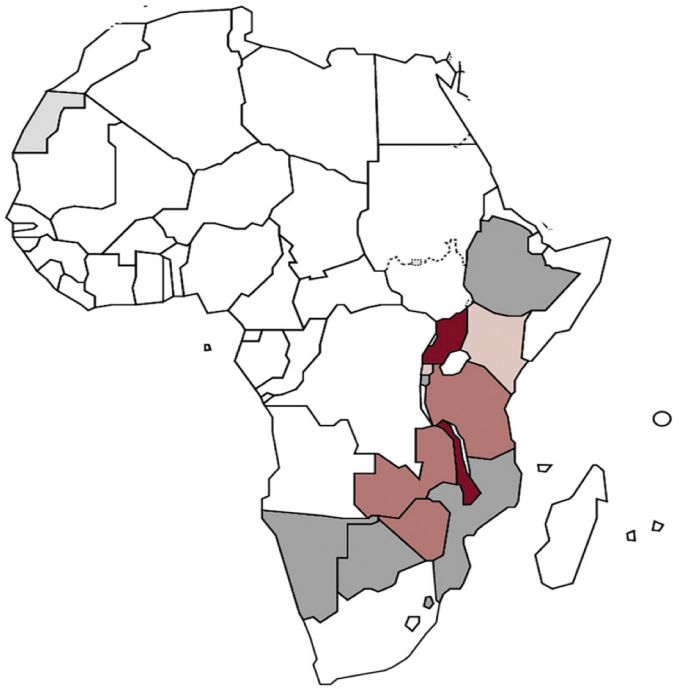
Chronic form (months to years)
98% cases

Acute form (weeks to months)
2% cases



Number of reported cases, 2016

- >1 000
- 100–1 000
- <100
- 0 cases reported
- Endemic countries (no data available)
- Non *T.b.gambiense* endemic countries
- Not applicable



Number of reported cases, 2016

- ≥10
- <10
- 0 cases reported
- Endemic countries (no data available)
- Non *T.b.rhodesiense* endemic countries
- Not applicable

MASOCHA W brain res bul 2018

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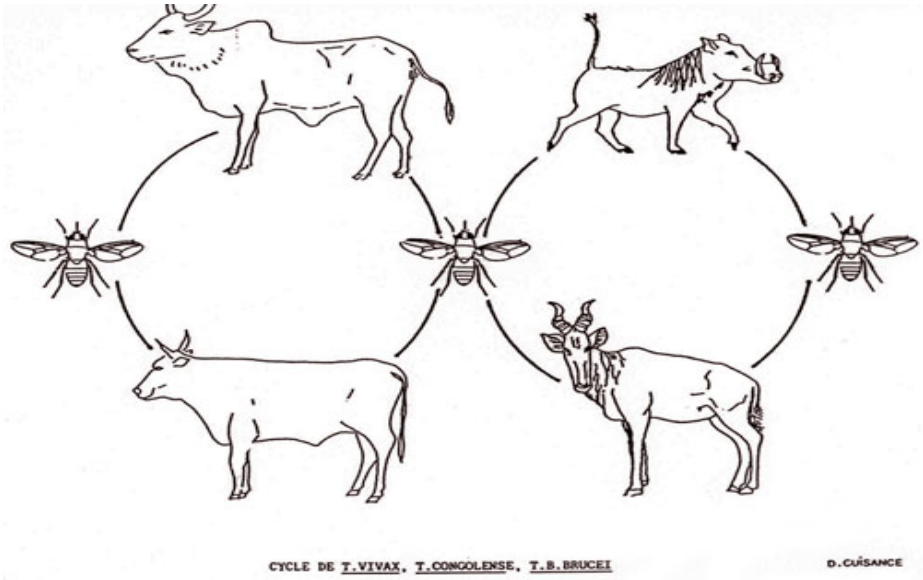
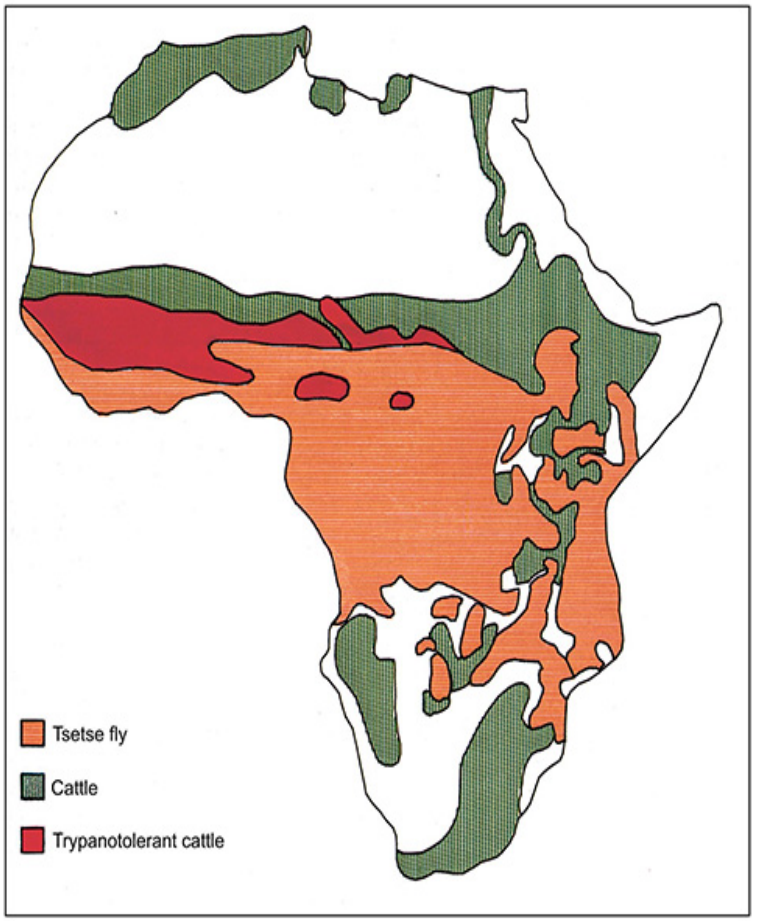
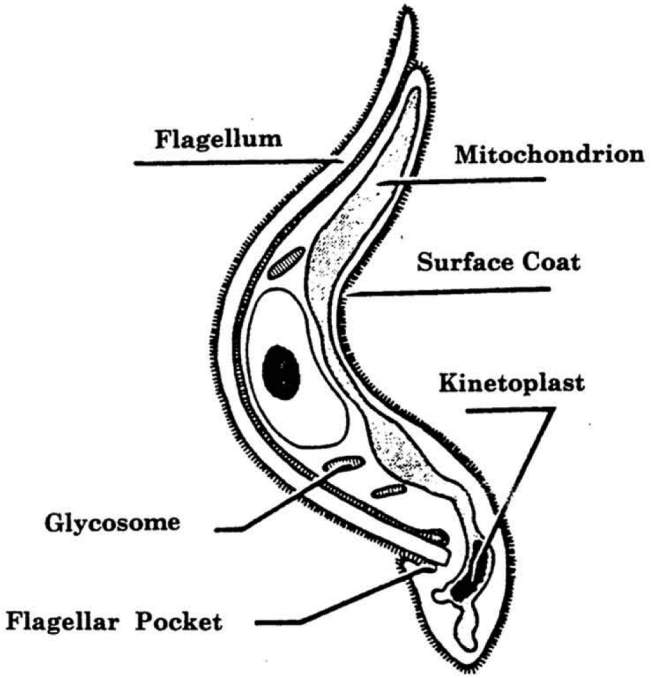
Nagana

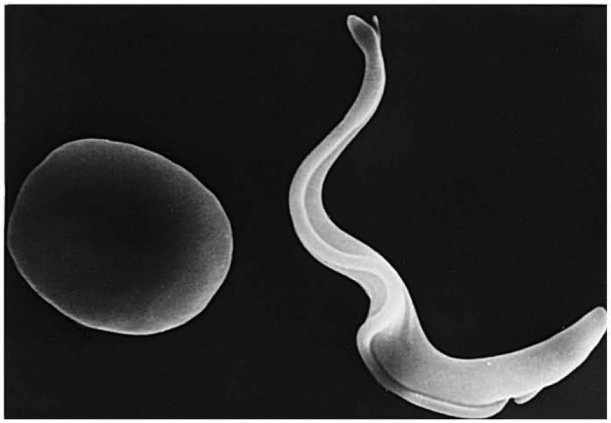
- *T. vivax, T. congolense & T. b. brucei*
- Abortion, decrease in milk / meat
- Cattle deaths: 3 M/y
- Annual losses: 4 B$

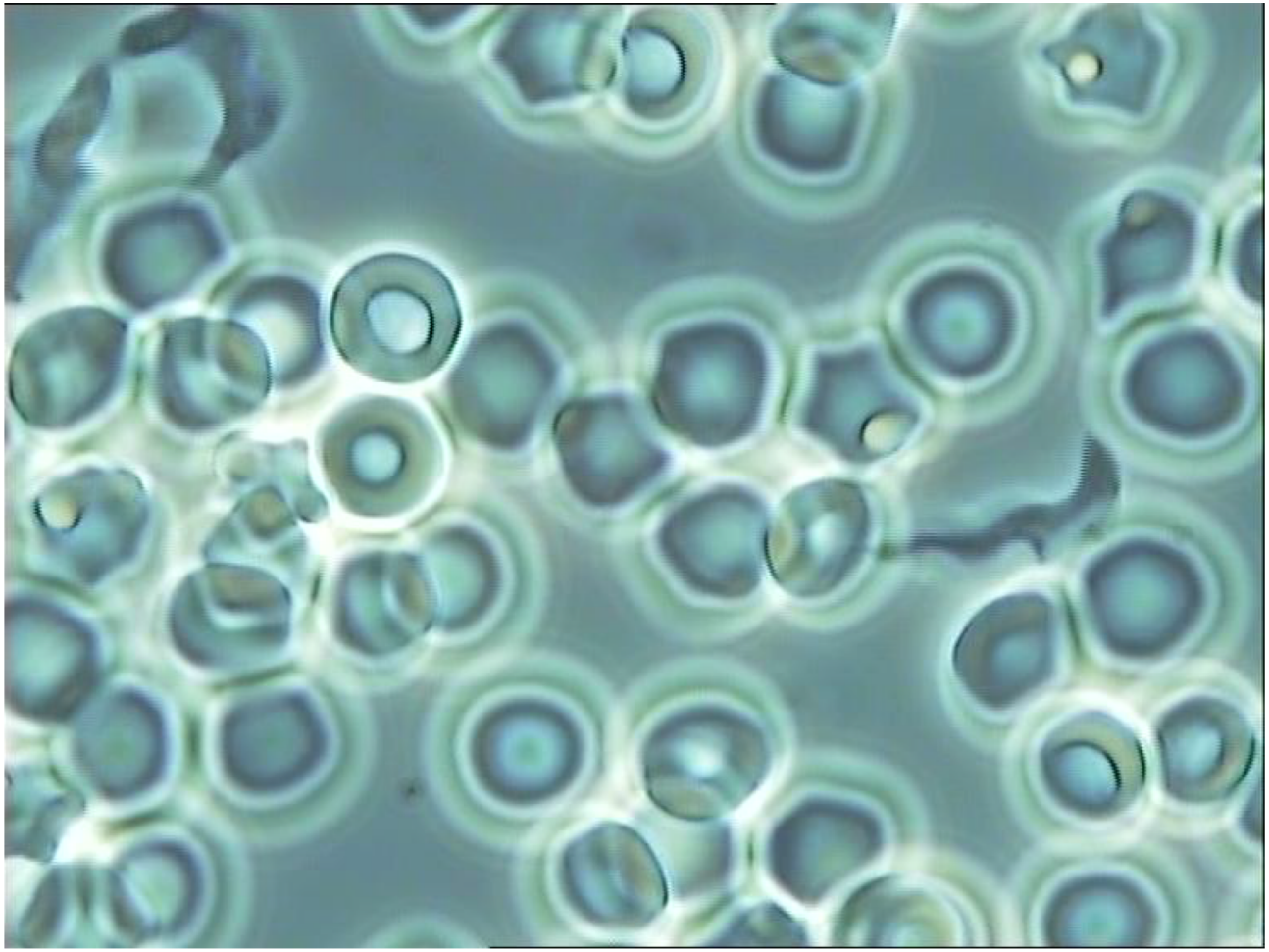




Stage 1: blood infection



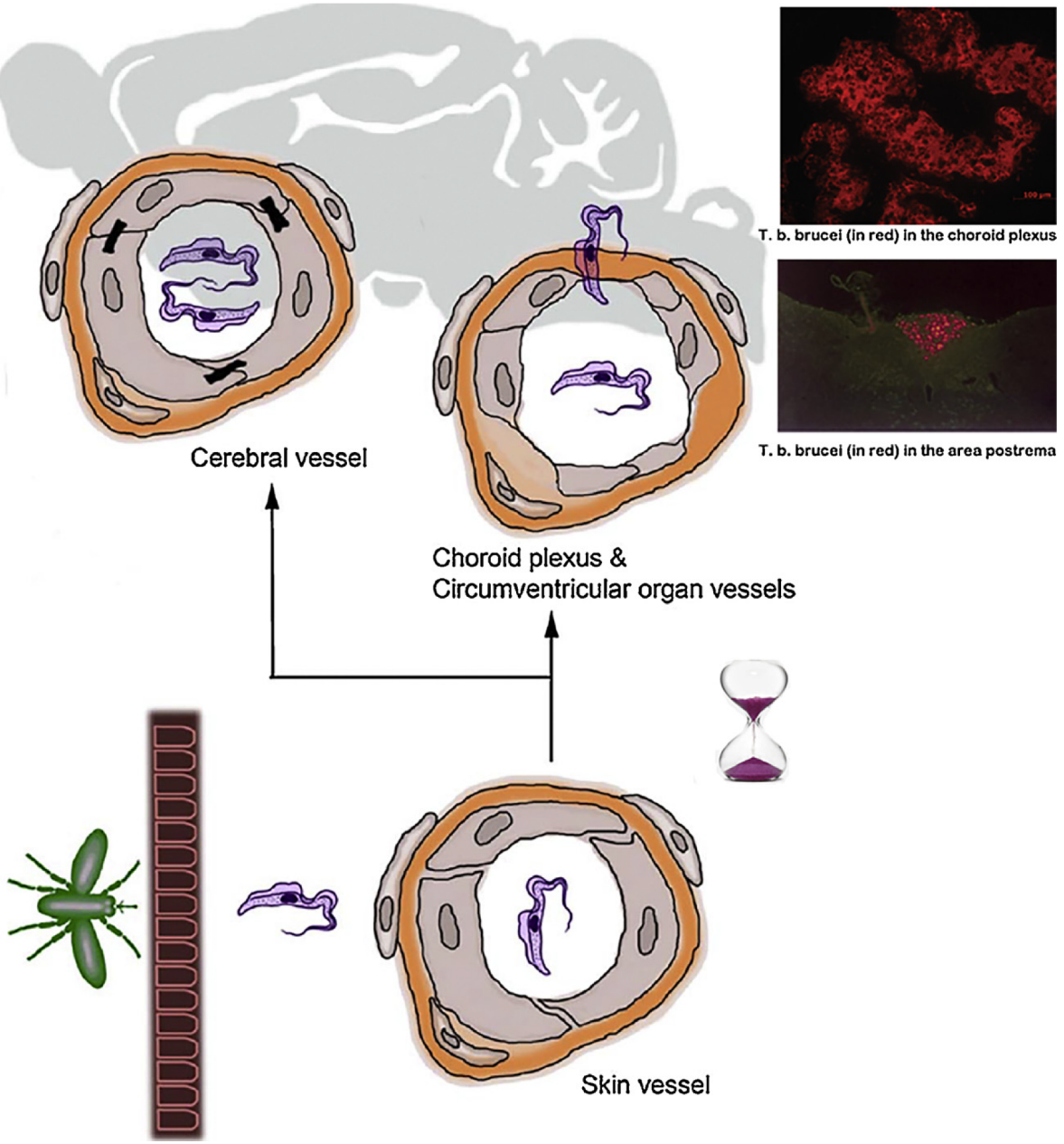




Ines Subota

Institut Pasteur

Stage 2: CNS infection



MASOCHA W brain res bul 2018

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Symptoms

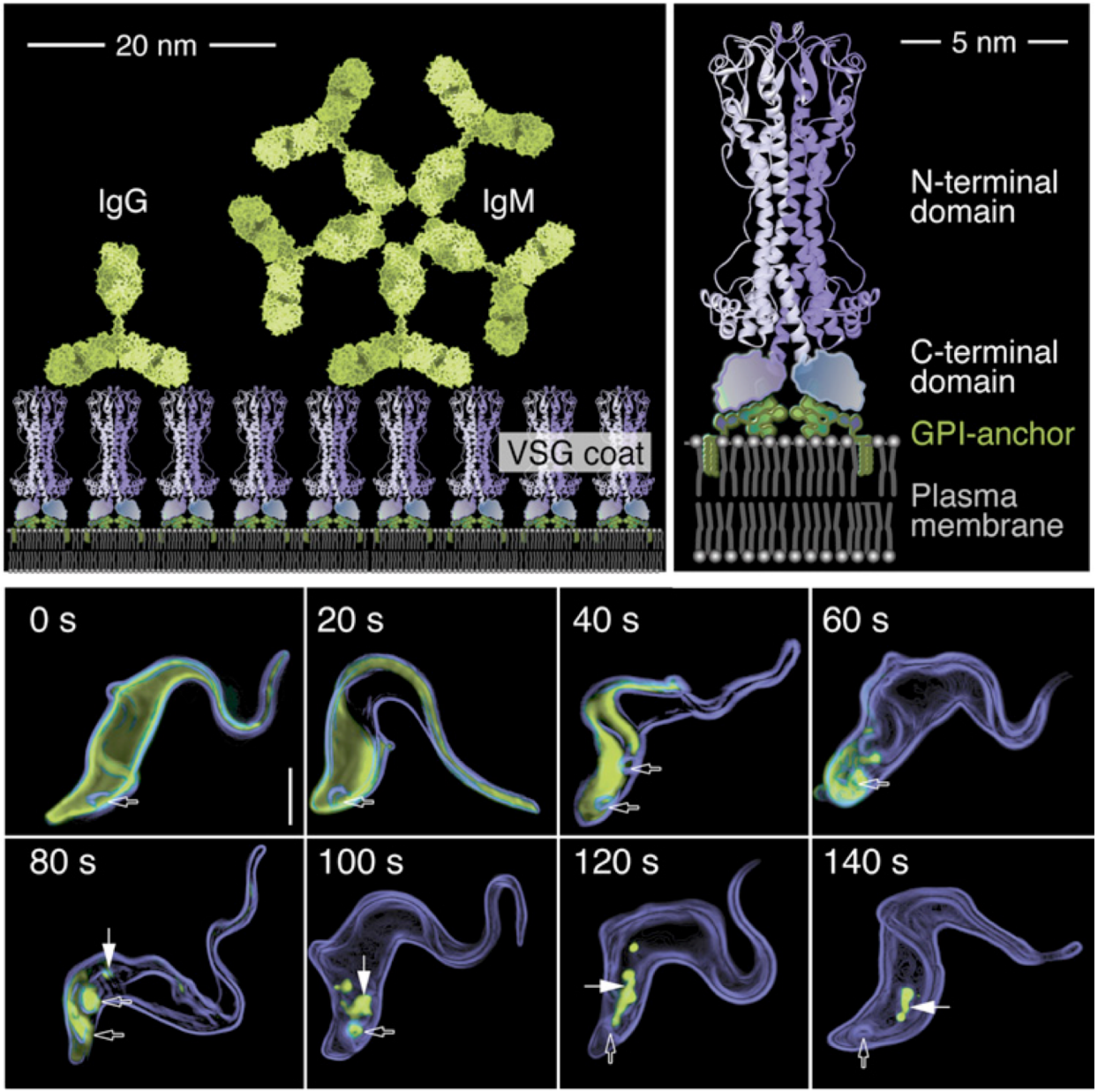
Blood → Lymphatics → CNS → DEATH

- ± chancre
- • intermittent fever
  • headache
- • continued febrile episodes
  • lymphadenopathy
  • deteriorating health
- • anorexia
  • minor neurological symptoms
  • apathy, lassitude
- • severe sleep disturbances
  • severe neurological symptoms
- • convulsions
  • coma
  • concurrent infections

months to years — *Tg*

weeks to months — *Tr*

Antigenic variation



ENGSTLER M cell 2007

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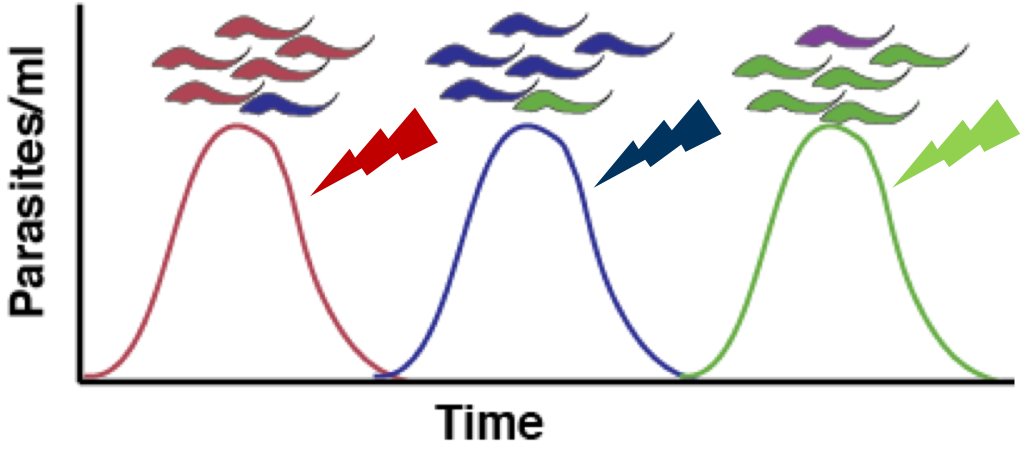
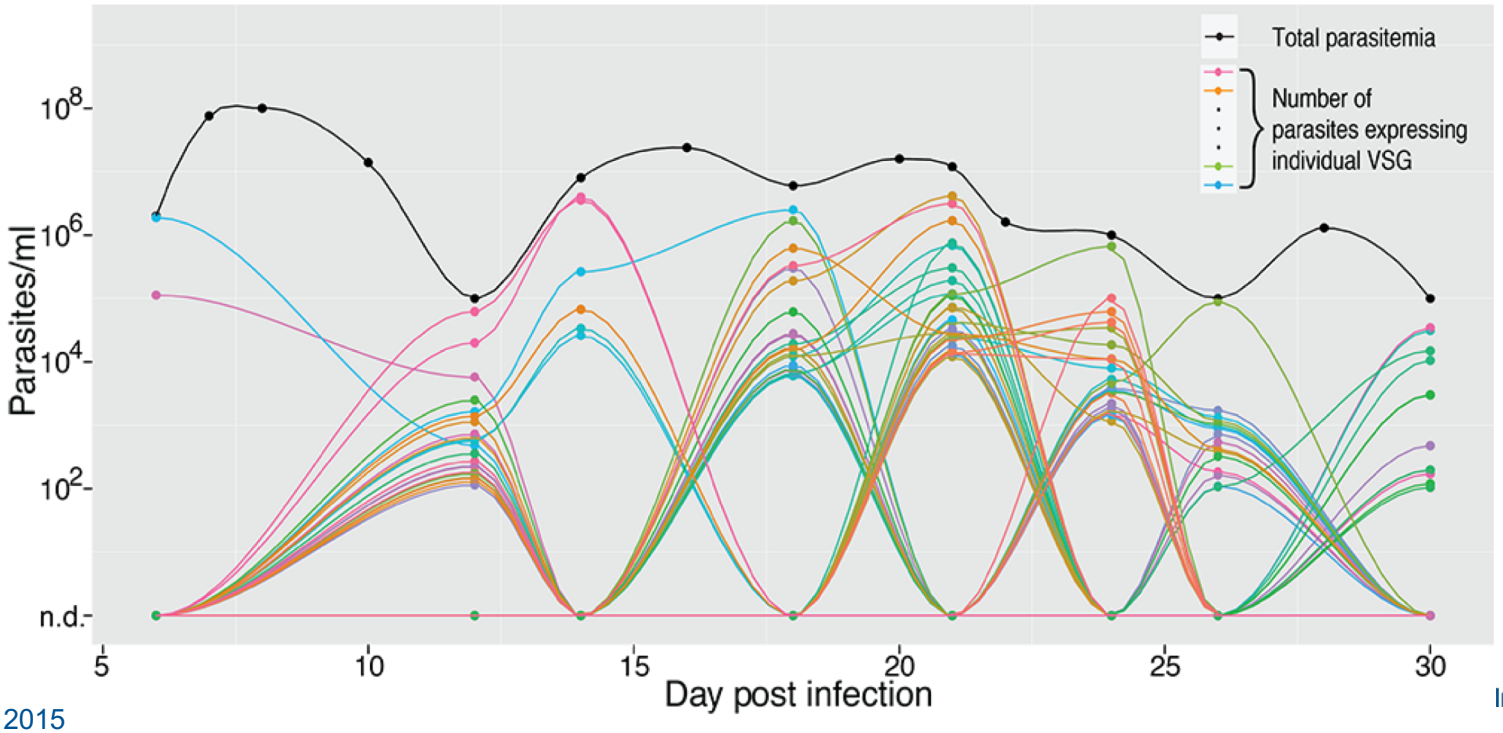
Antigenic variation





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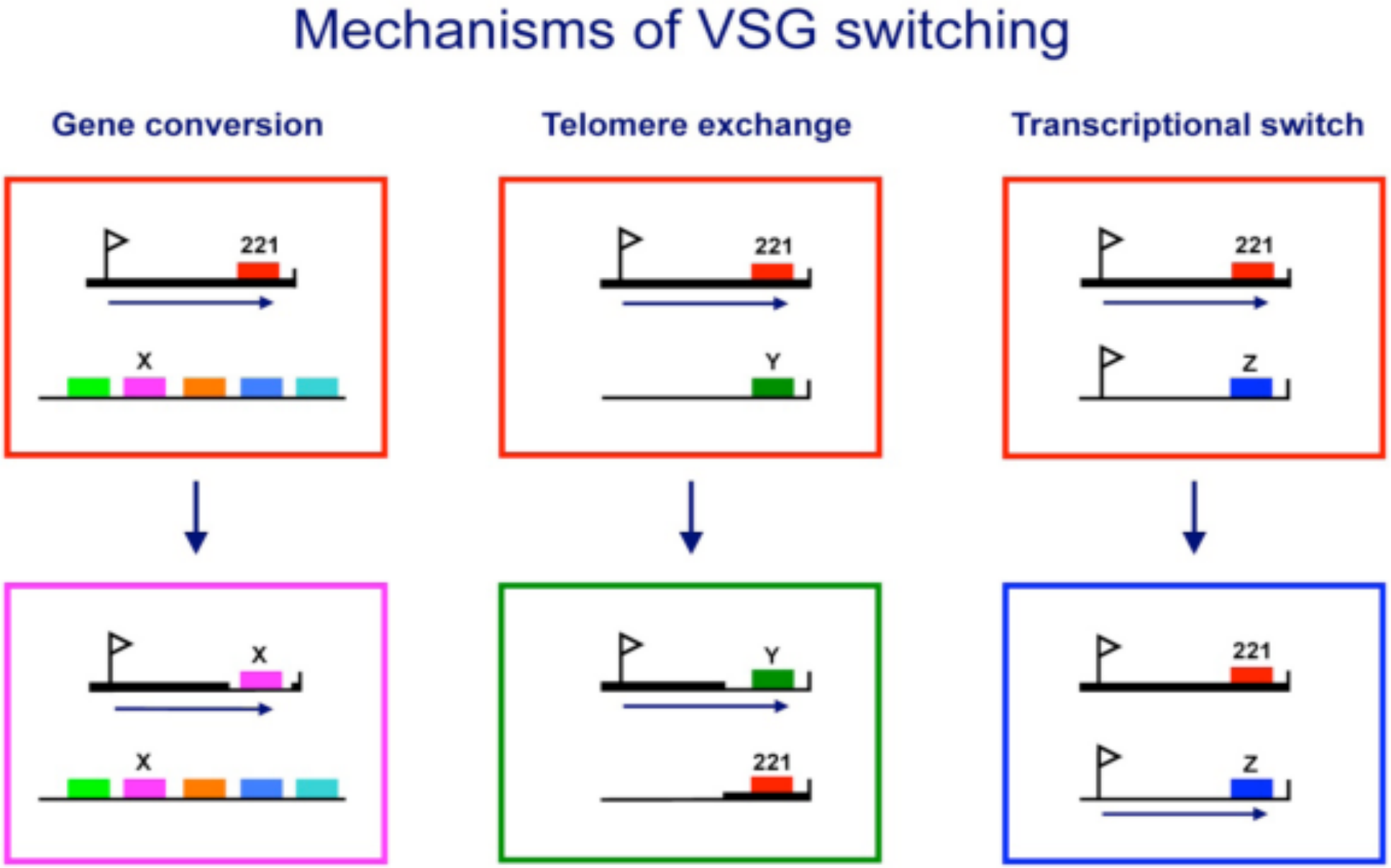
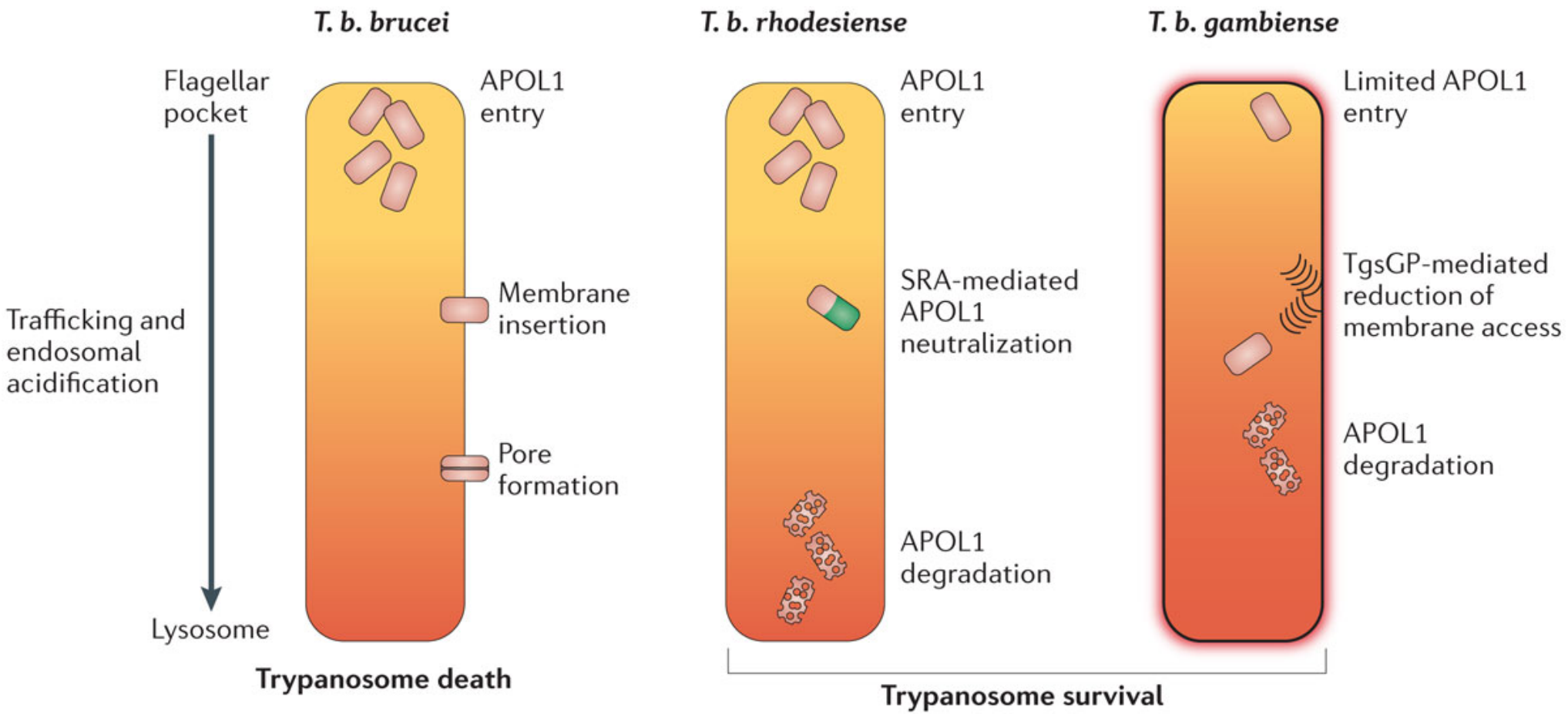
Antigenic variation



Resistance to human serum



Nature Reviews | Microbiology

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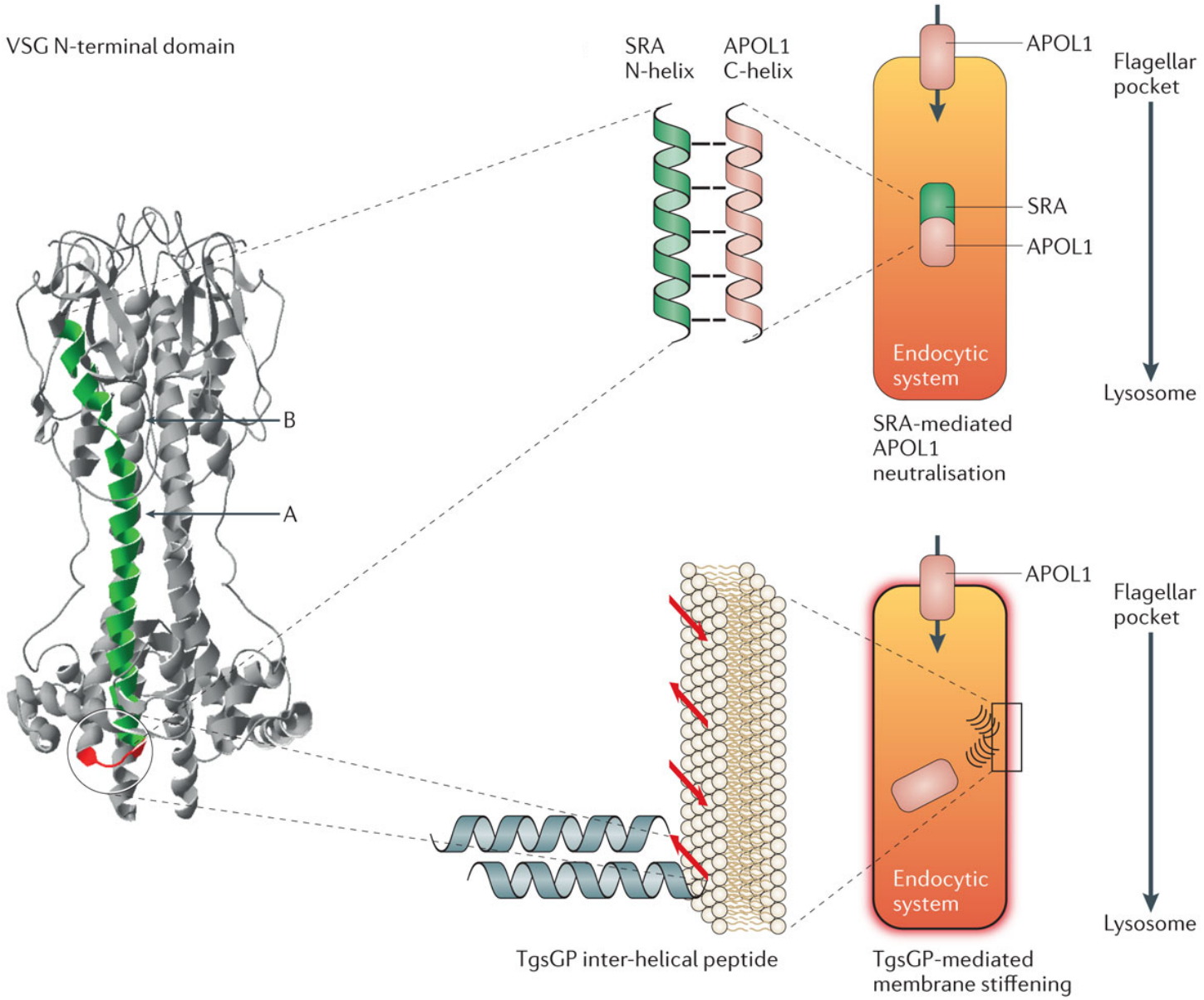
PAYS E nat rev microbiol 2014

Resistance to human serum



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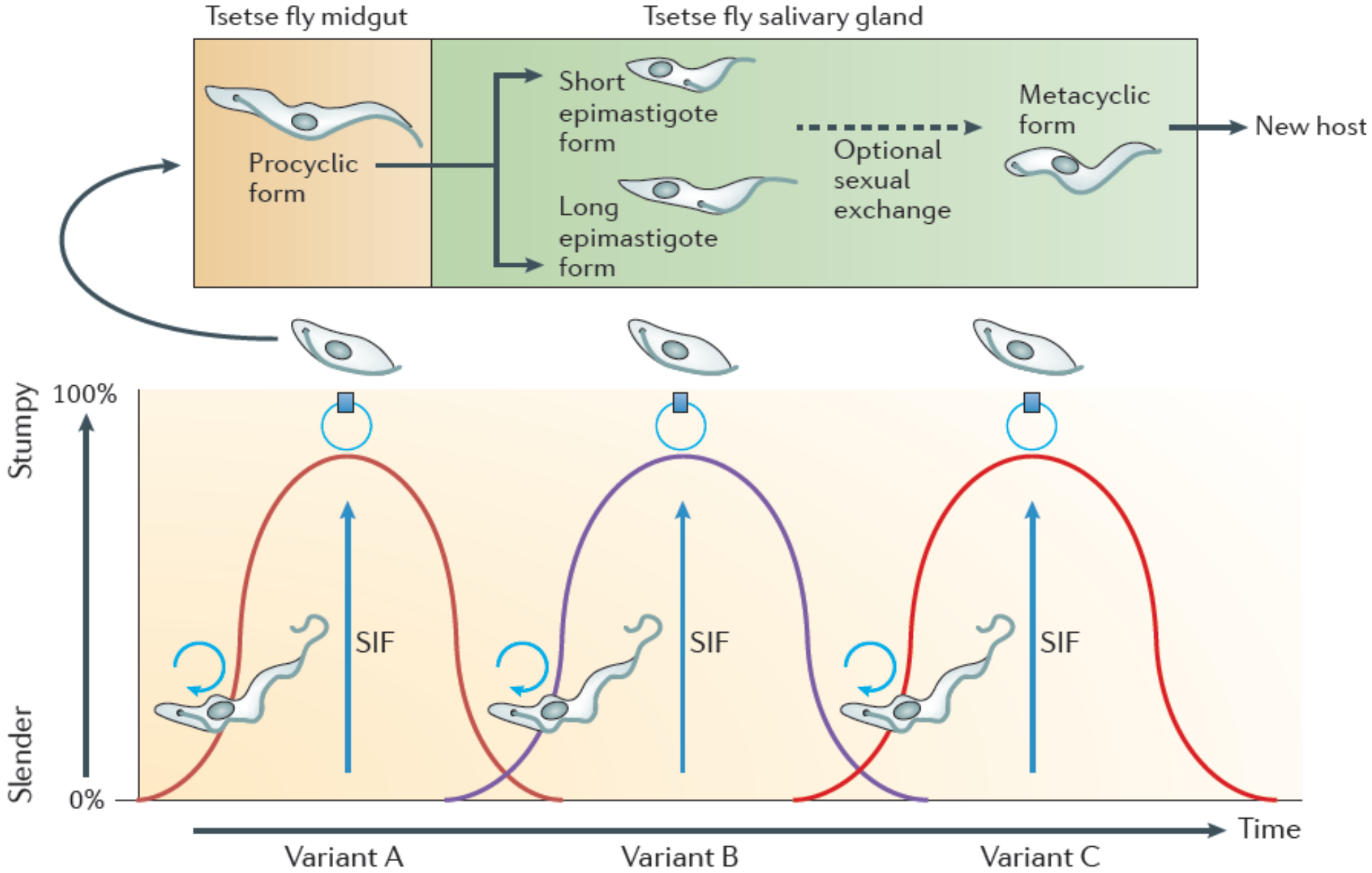
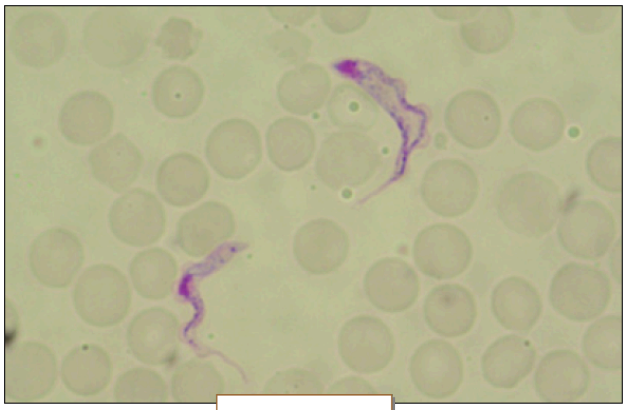
Differentiation for transmission



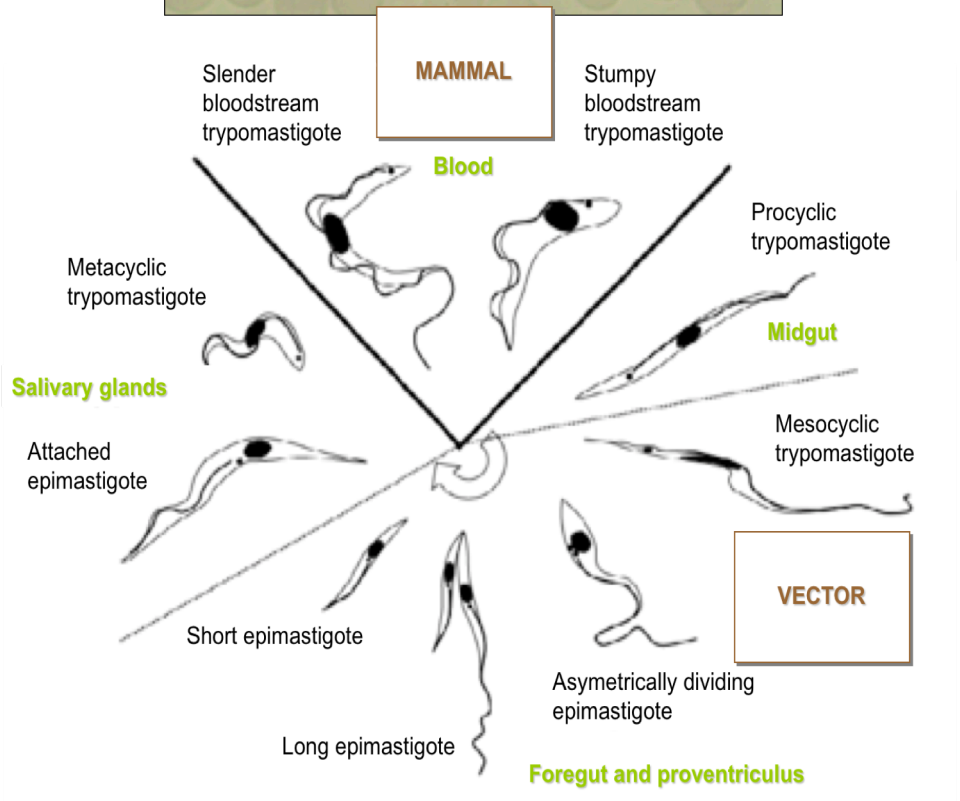
MACGREGOR P nat rev microbiol 2012

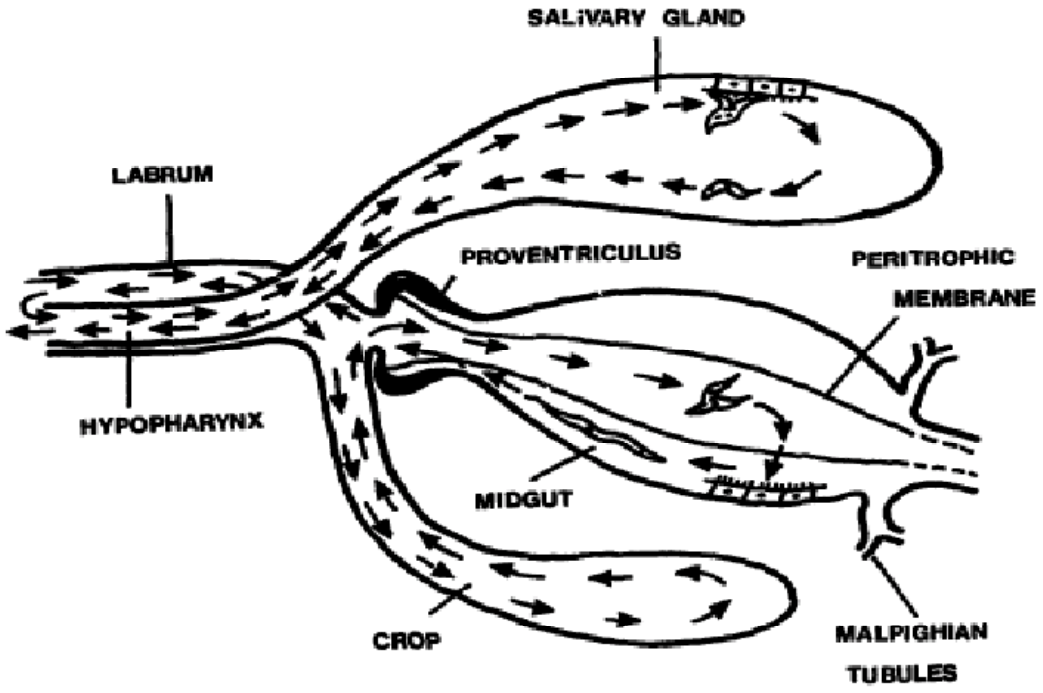
Cyclical development in the tsetse



BUSCHER P lancet 2017

Brice Rotureau

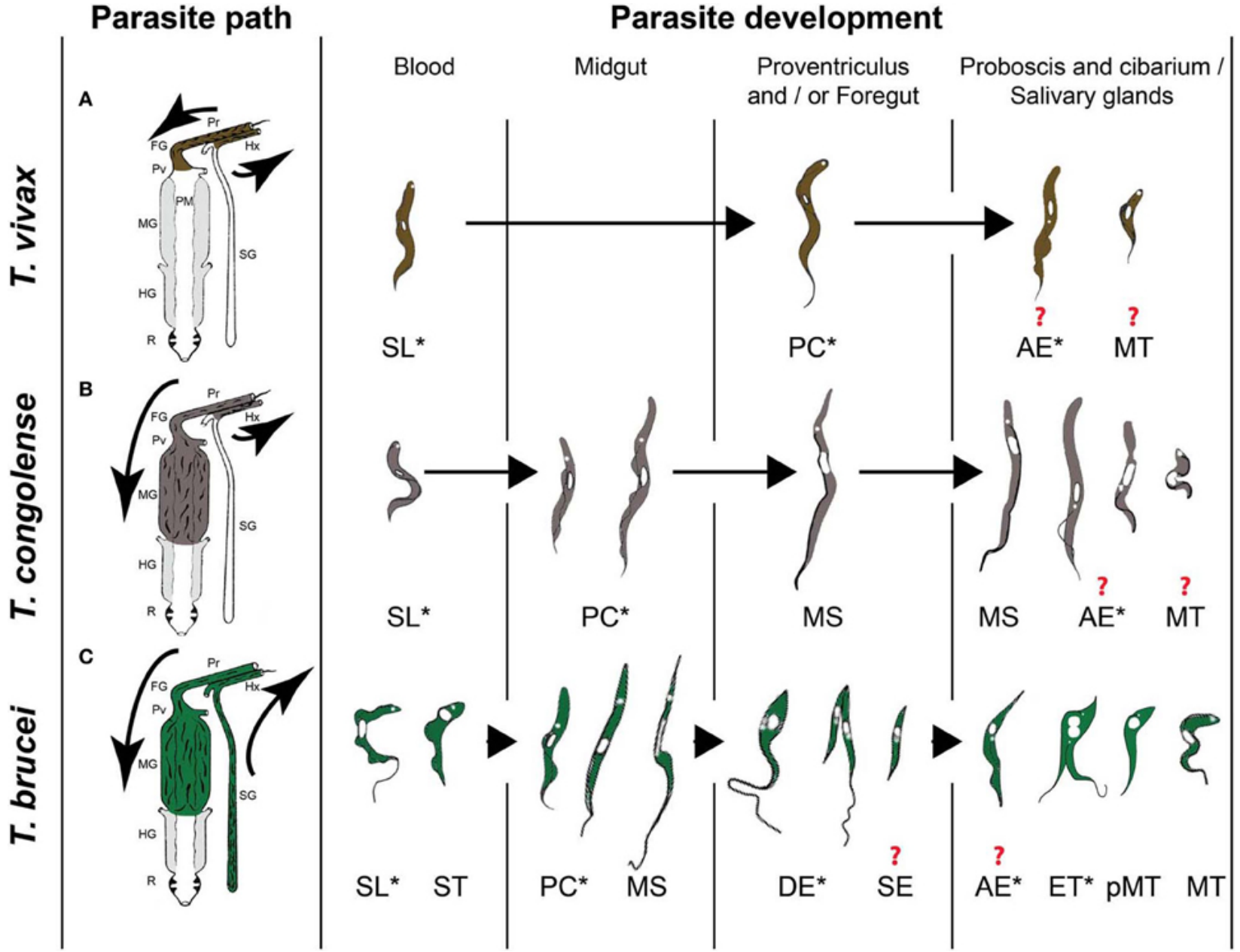




PEACOCK L kineto biol dis 2007

VICKERMAN K biol cell 1988

Cyclical development in the tsetse



ROTUREAU B front cell infect microbiol 2013

Diagnosis

Diagnosis of HAT

FIND foundation for innovative new diagnostics

Screening

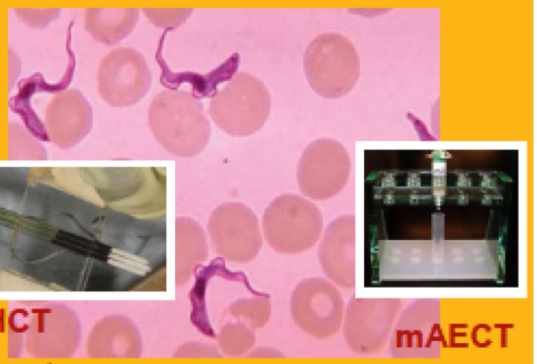
A finger prick!

Ab detection test:
CATT

Suspect

Confirmation

Microscopy



HCT

mAECT

Patient

Staging

Lumbar puncture

Choice of treatment and follow-up

Treatment

	First-line treatment	Dosage	Alternative treatment and dosage
Trypanosoma brucei gambiense			
First stage	Pentamidine	4 mg/kg per day intramuscularly or intravenously (diluted in saline, in 2-h infusions) x 7 days	..
Second stage	Nifurtimox–eflornithine combination therapy	Nifurtimox 15 mg/kg per day orally in three doses x 10 days; eflornithine 400 mg/kg per day intravenously in two 2-h infusions (each dose diluted in 250 mL of water for injection)* x 7 days	Eflornithine 400 mg/kg per day intravenously in four 2-h infusions (each dose diluted in 100 mL of water for injection)* x 14 days; third-line (eg, treatment for relapse) is melarsoprol 2·2 mg/kg per day intravenously x 10 days
Trypanosoma brucei rhodesiense			
First stage	Suramin	Test dose of 4–5 mg/kg intravenously (day 1), then 20 mg/kg intravenously once per week x 5 weeks (maximum 1 g/injection—eg, days 3, 10, 17, 24, and 31)	Pentamidine 4 mg/kg per day intramuscular or intravenously (diluted in normal saline, in 2-h infusions) x 7 days
Second stage	Melarsoprol	2·2 mg/kg per day intravenously x 10 days	..

*Children weighing <10 kg: dilute in 50 mL of water for injection. Children weighing 10–25 kg: dilute in 100 mL of water for injection. If water for injection is unavailable, eflornithine can be diluted in 5% dextrose or saline.

***Table*: Standard treatment for human African trypanosomiasis by form and stage**

BUSCHER P lancet 2017

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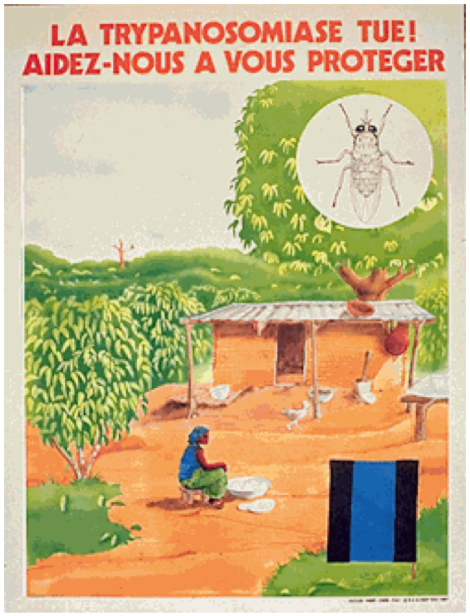
Vector control


Impregnated Screens




SIT


Traps

The missing link?

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Epidemiological context

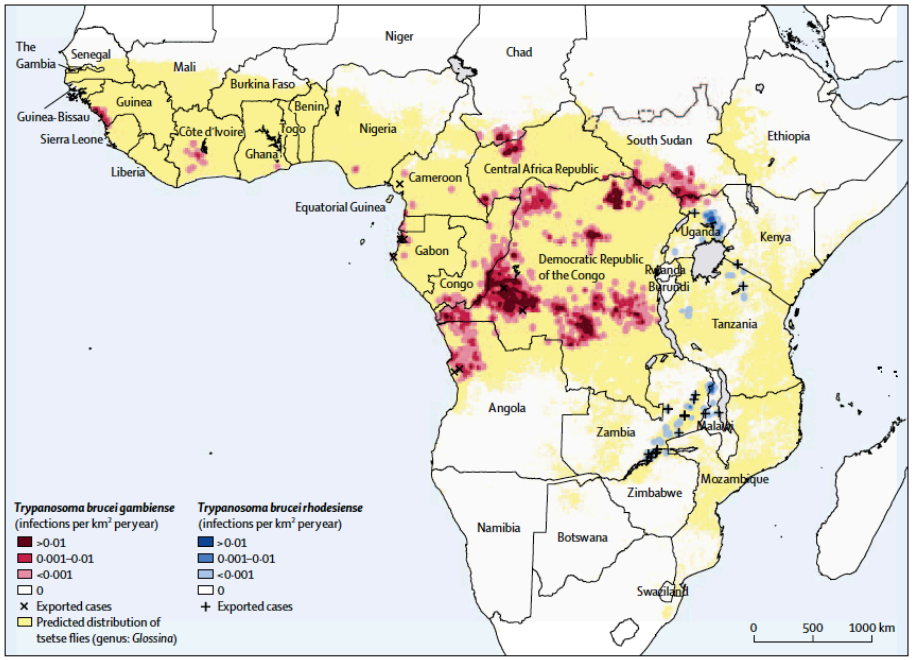
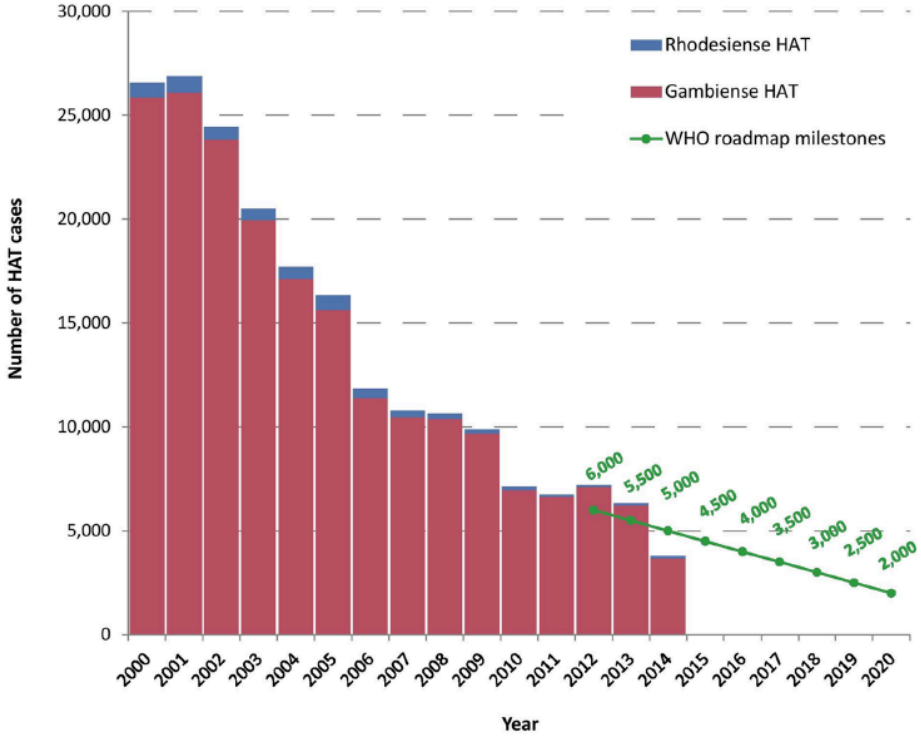


Figure 1: Geographical distribution of reported infections of human African trypanosomiasis (reporting period 2010–14)

BUSCHER *et al.* lancet 2017



FRANCO *et al.* plos ntd 2017

< 1,500 cases in 2017

→ **HAT targeted for elimination as public health problem by 2020**

= Zero transmission by 2030

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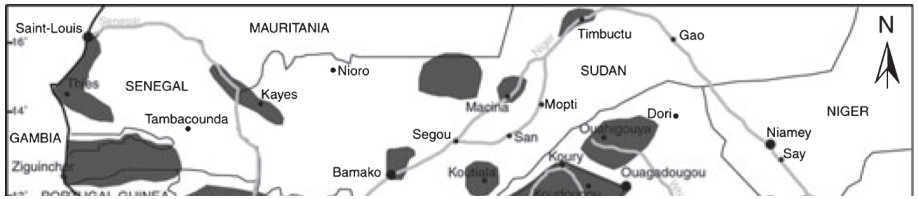
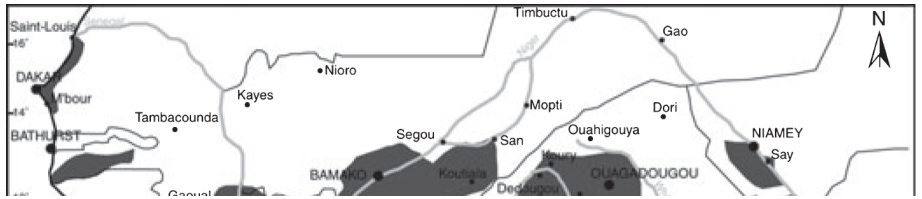
The transmission paradox

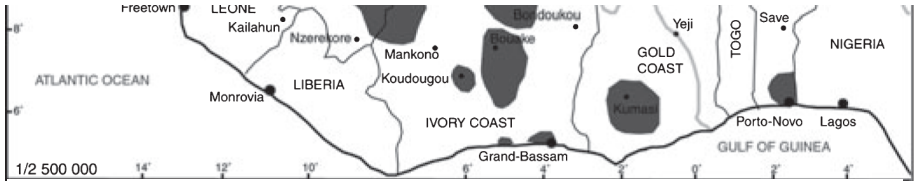
Persistence of historical HAT foci

- Despite multiple eradication campaigns
- Despite multiple vector control programs
- Lack of knowledge about animal reservoirs
- Anecdotal vertical and horizontal inter-human transmission

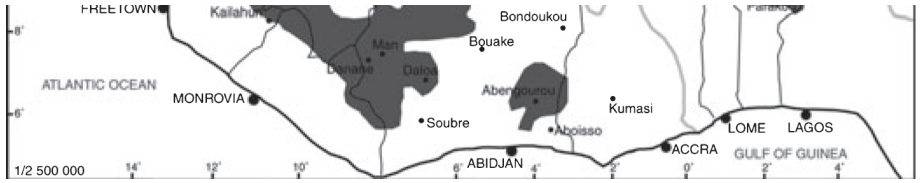




TRANSMISSION?



HAT foci 1900-1930



HAT foci 1930-1950

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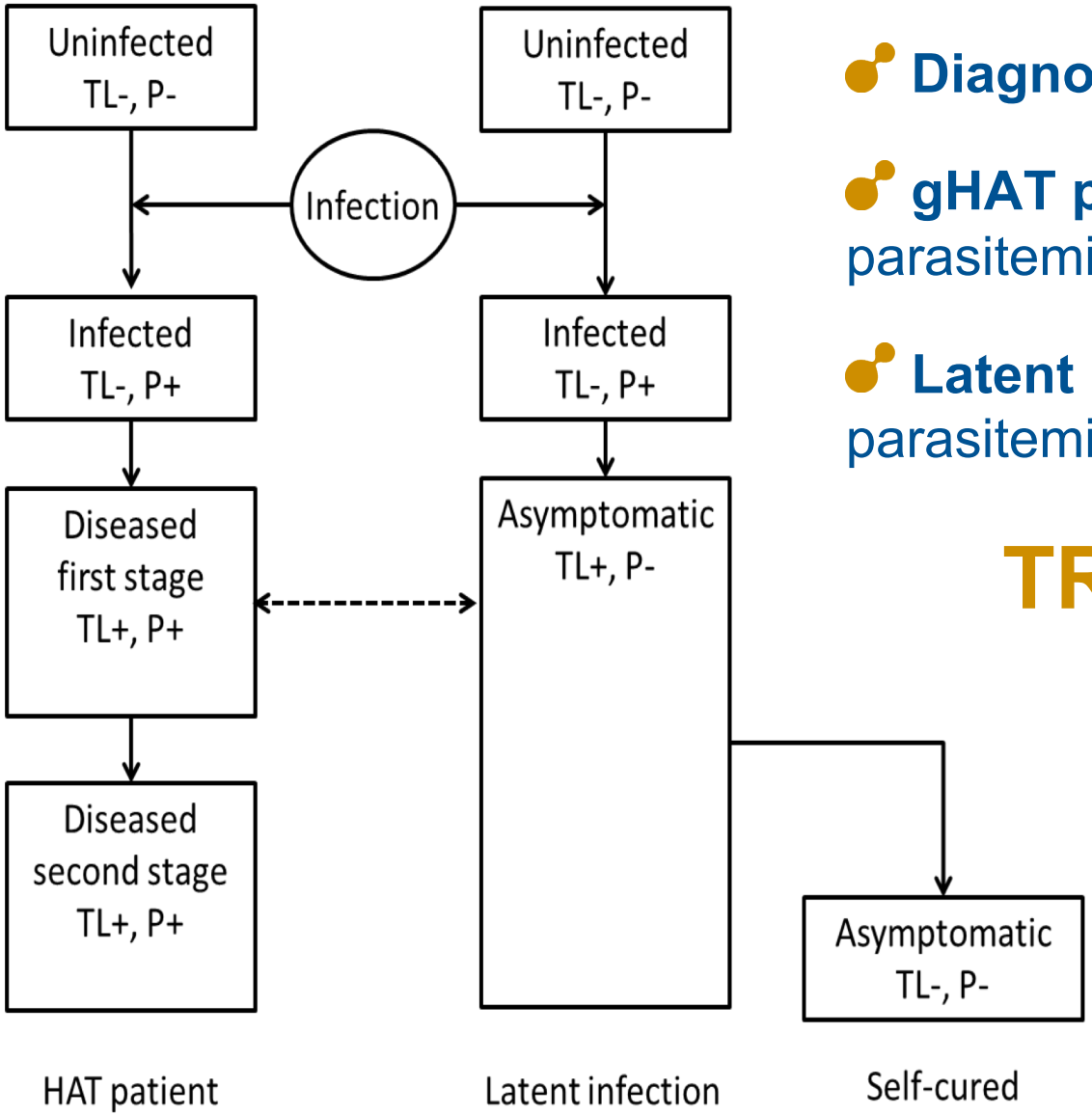
The transmission paradox



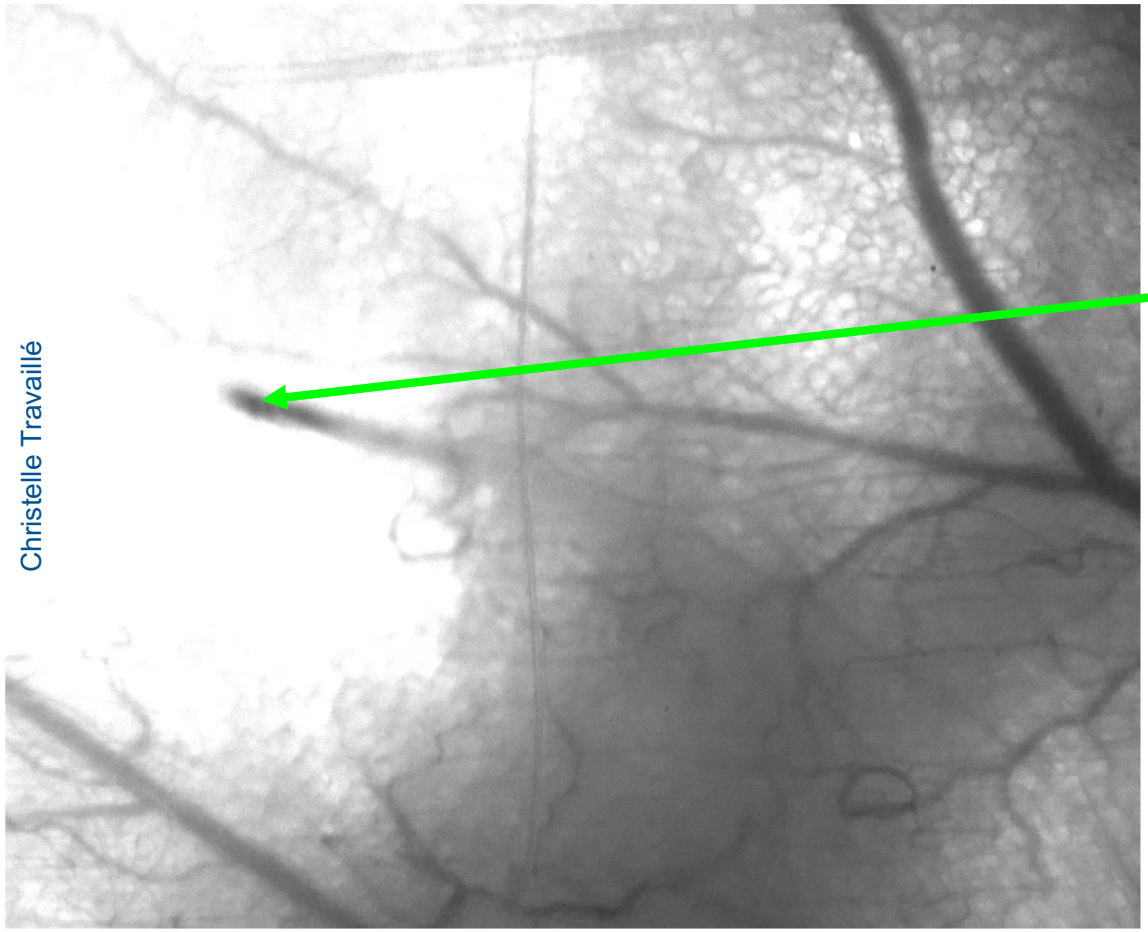
- **Diagnosis: serology + parasitology**
- **gHAT patients** usually with very low parasitemia
- **Latent infections** with no detectable parasitemia (Jammoneau *et al.* 2012, Berthier *et al.* 2015)

TRANSMISSION?

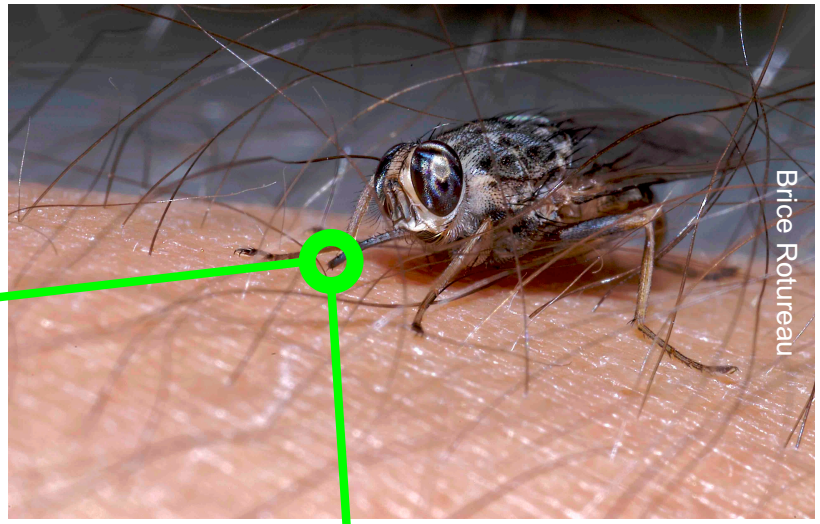
BUSCHER *et al.* TiP 2018

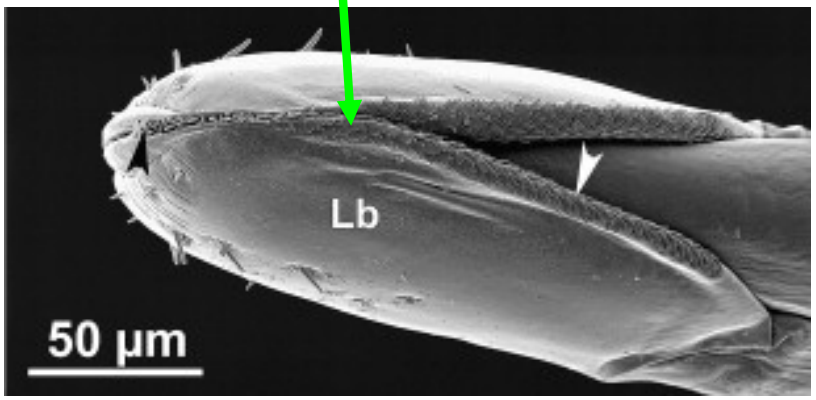
Institut Pasteur

The transmission paradox



Internal view of the skin





- Patients with low parasitemia: <0.1 parasites/µl
- Tsetse bloodmeal: 10-20µl

TRANSMISSION?

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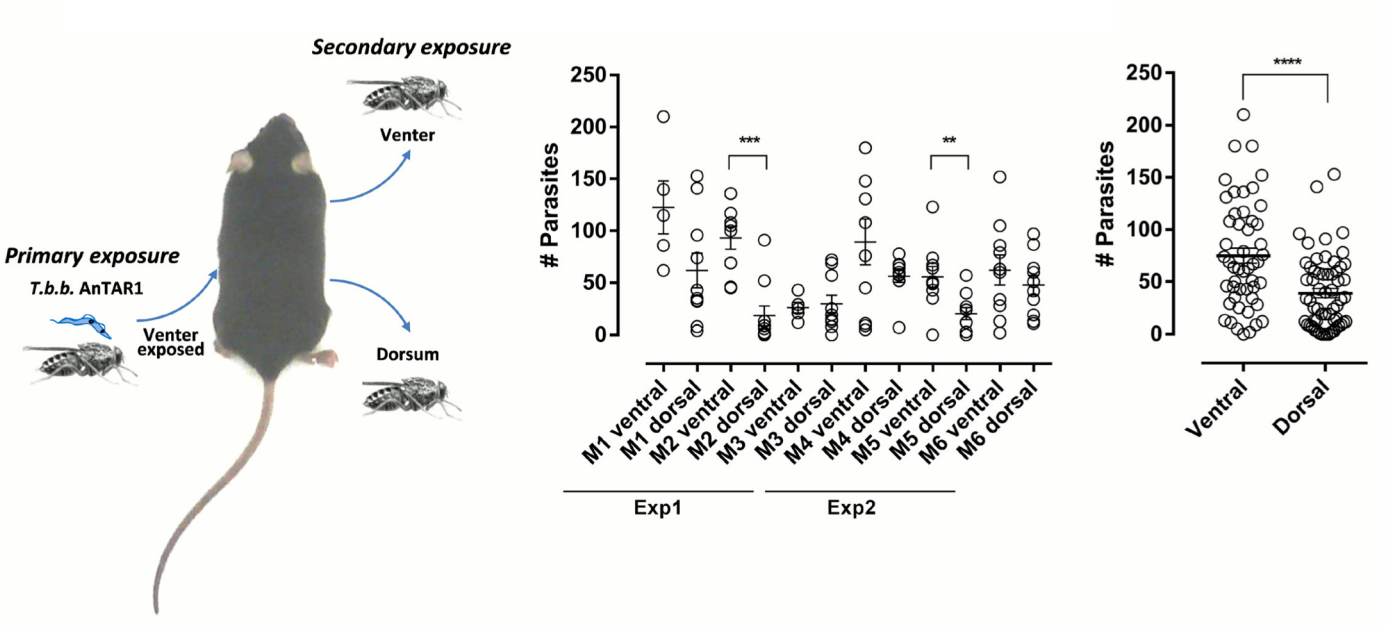
Extravascular parasites in the skin?

WOMBOU TCM parasite 2011



CALJON G plos path 2016

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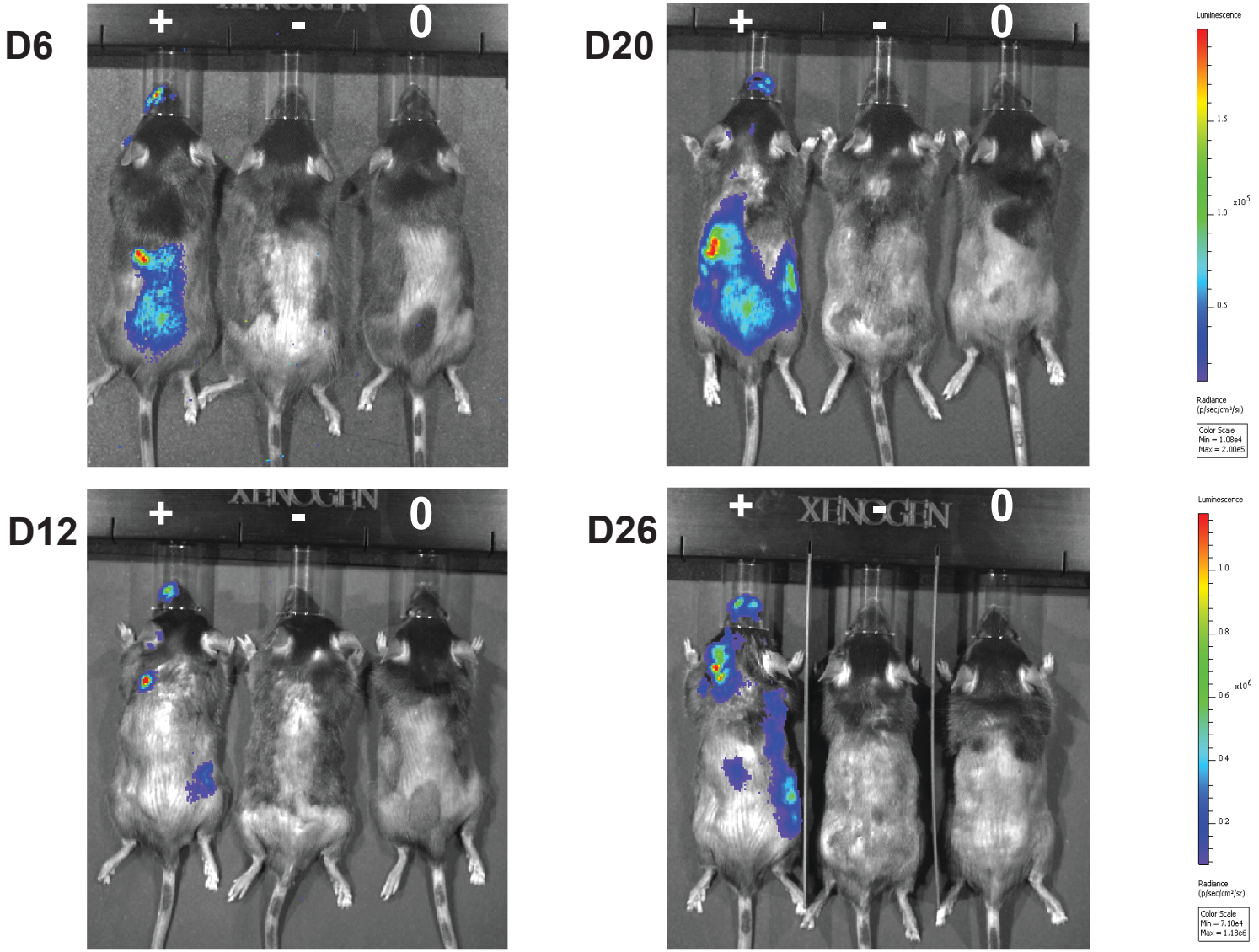
Kinetics of natural infections



Capewell *et al.* eLife 2016

T. b. brucei AnTat1.1E AMLuc/TY1/tdTomato

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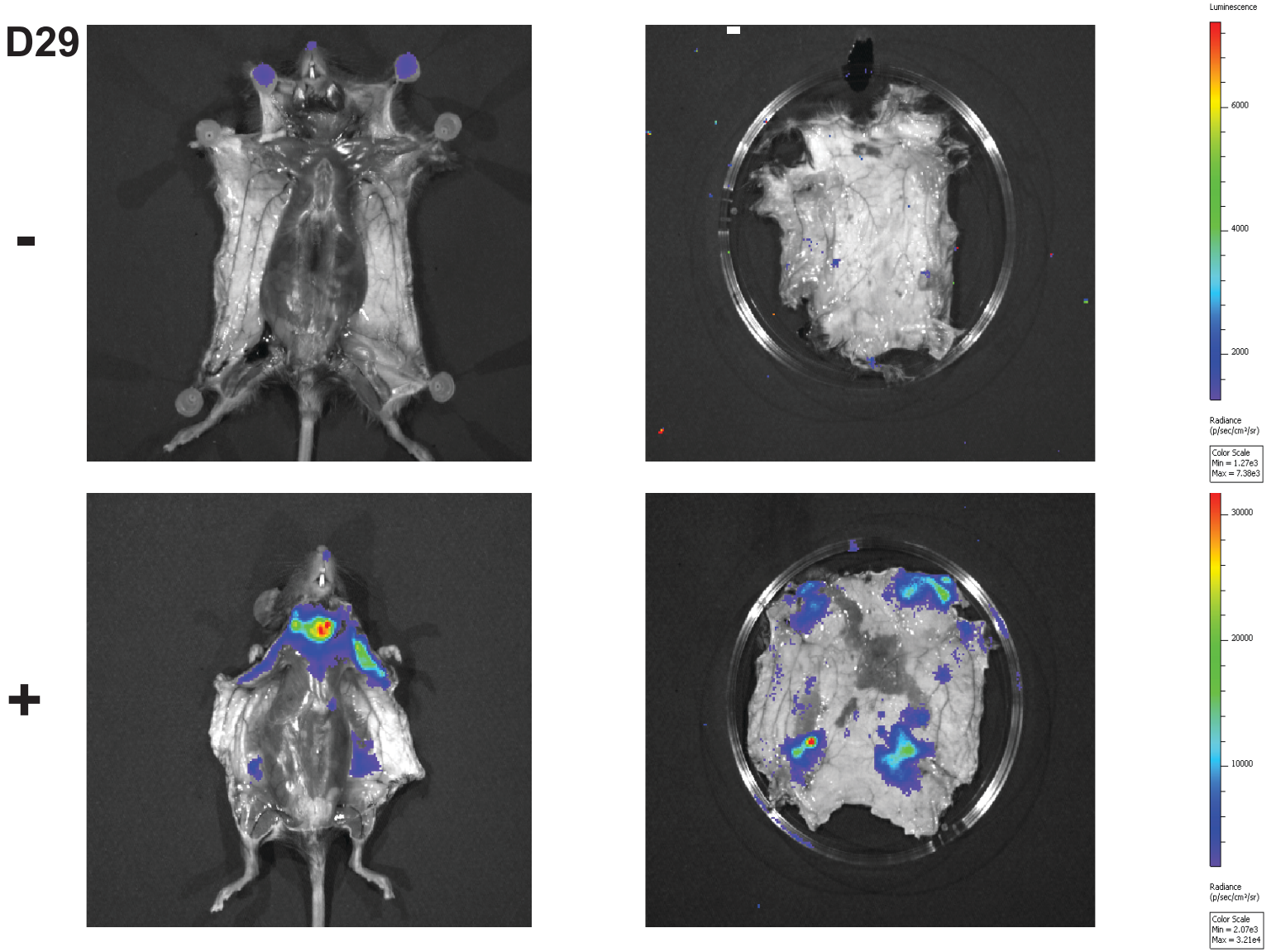
Trypanosomes are found in the dermis



Capewell *et al.* eLife 2016

T. b. brucei AnTat1.1E AMLuc/TY1/tdTomato

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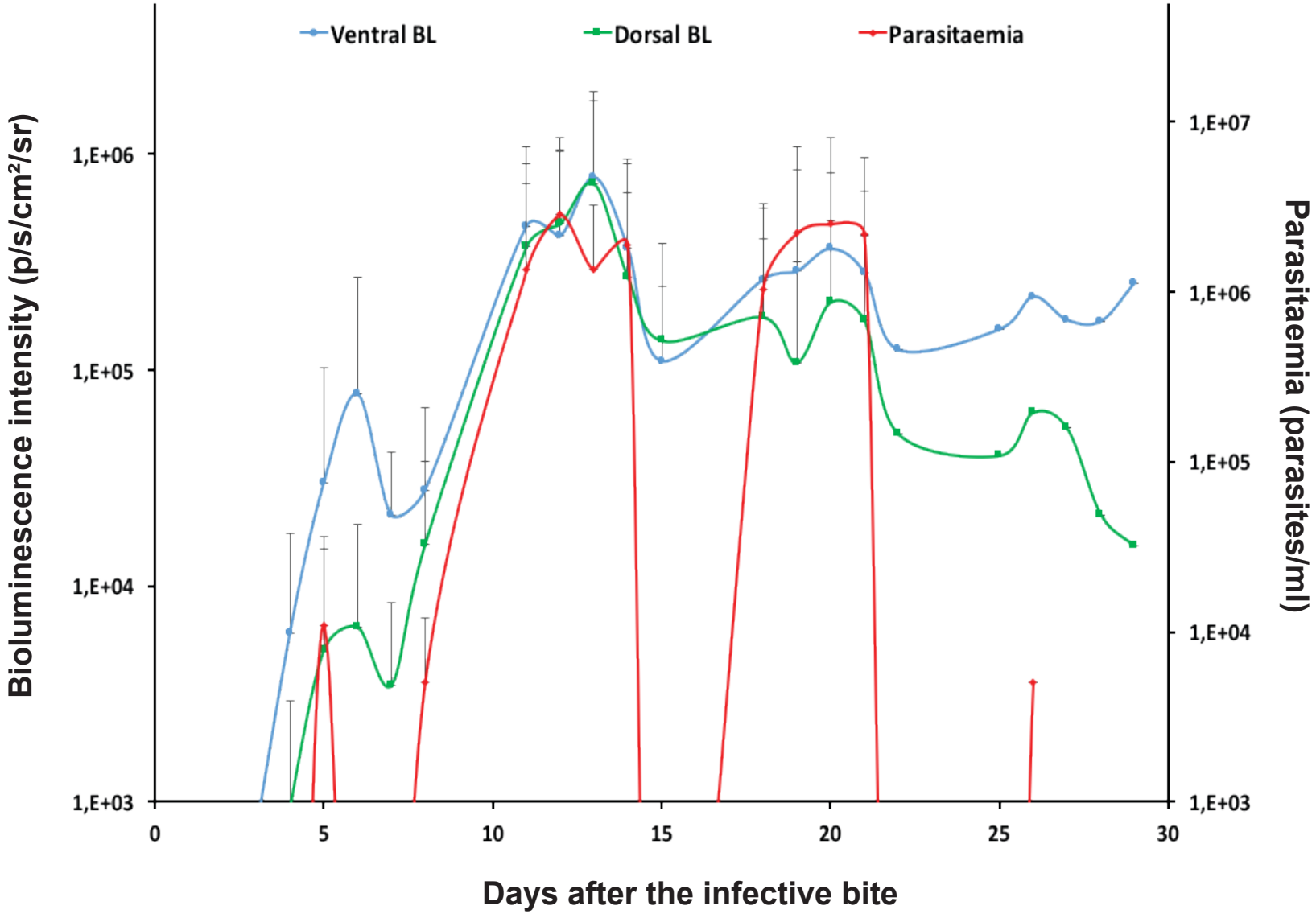
Trypanosomes remain in the skin



Capewell *et al.* eLife 2016

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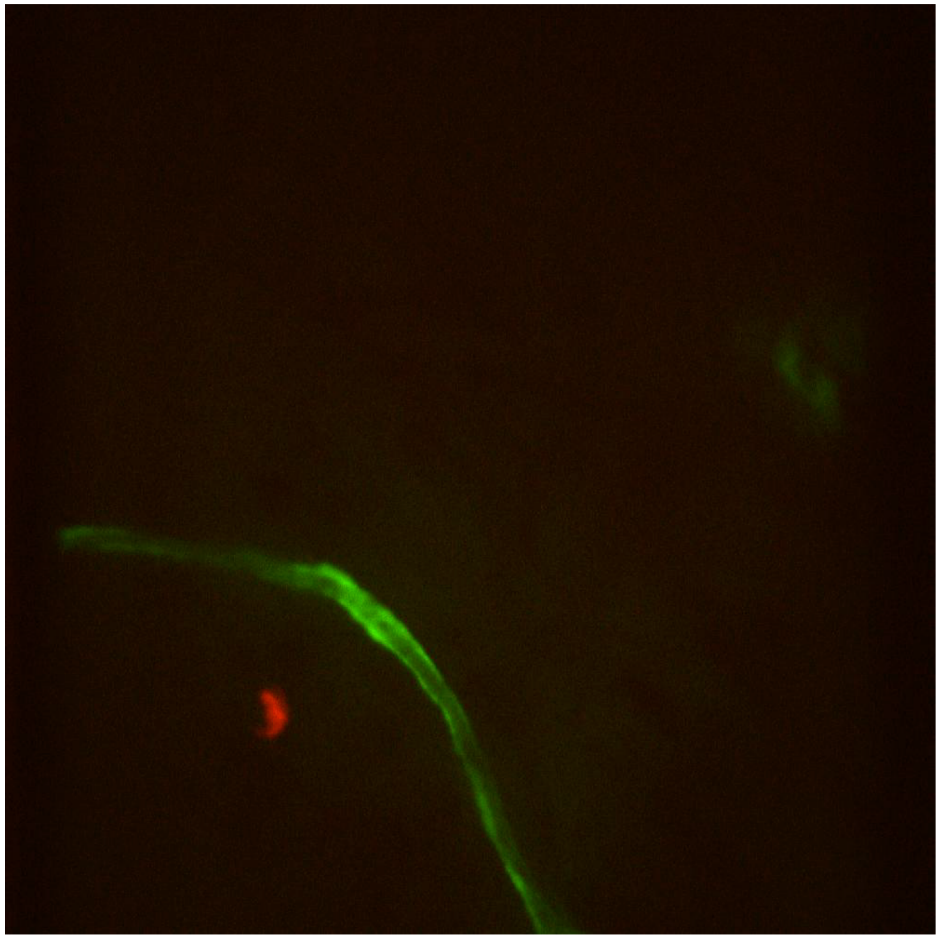
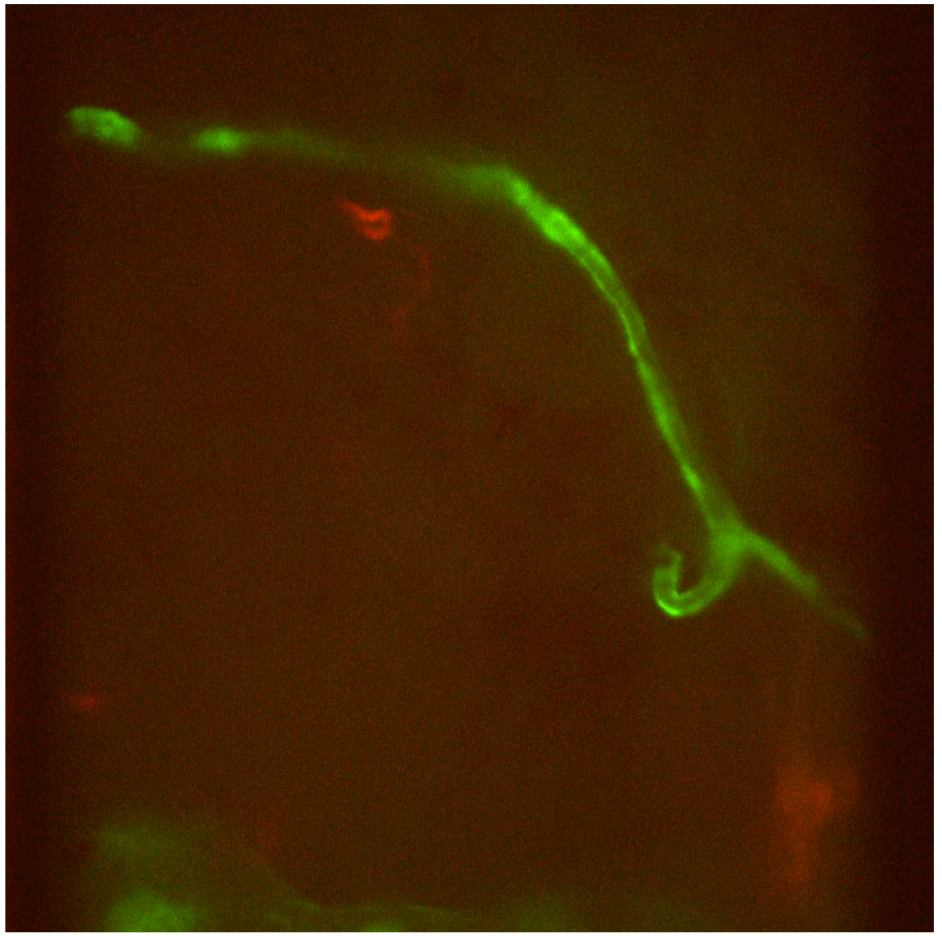
Skin-dwelling trypanosomes are motile





Vascular / lymphatic vessels **Parasites**

T. b. brucei AnTat1.1E AMLuc/TY1/tdTomato, 25 days after natural infection, ear of a C57BL/6 Kdr (FLK1)

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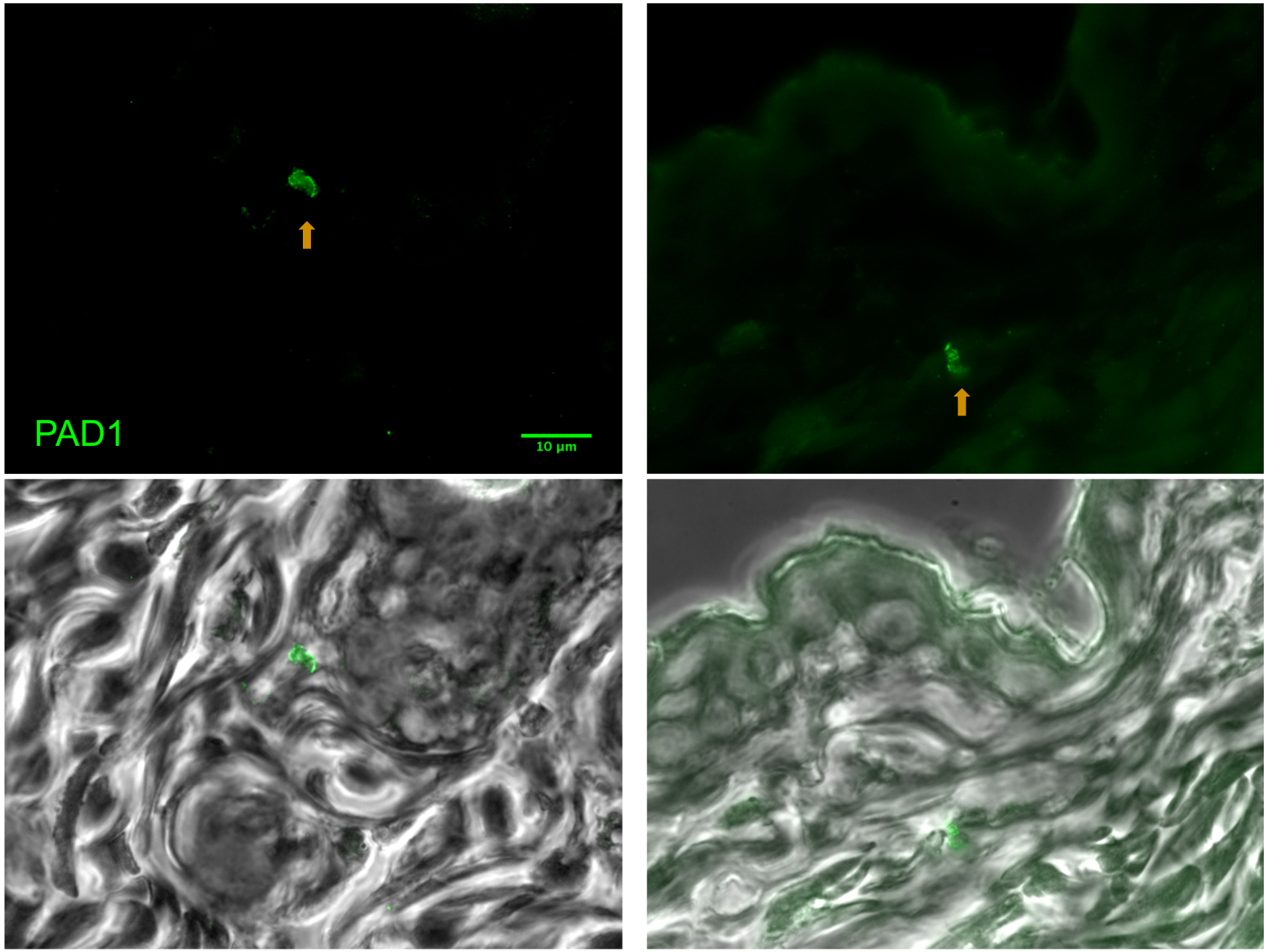
Capewell *et al.* eLife 2016

Skin-dwelling trypanosomes are transmissible



Capewell *et al.* eLife 2016

T. b. brucei AnTat1.1E AMLuc/TY1/tdTomato, 29 days

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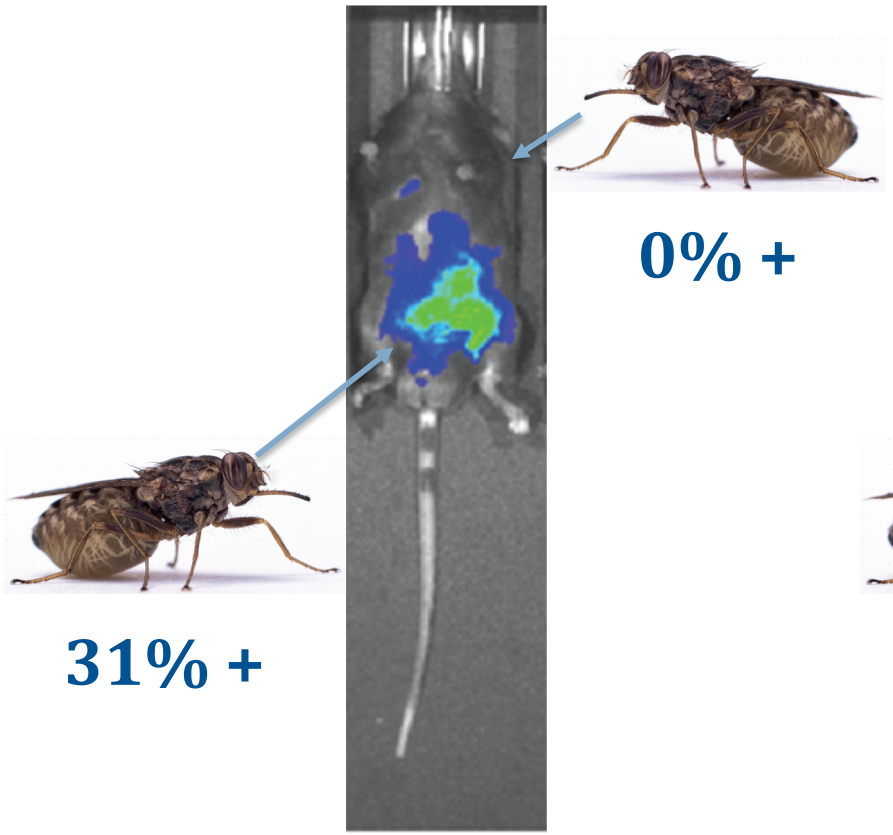
Skin-dwelling trypanosomes are transmitted

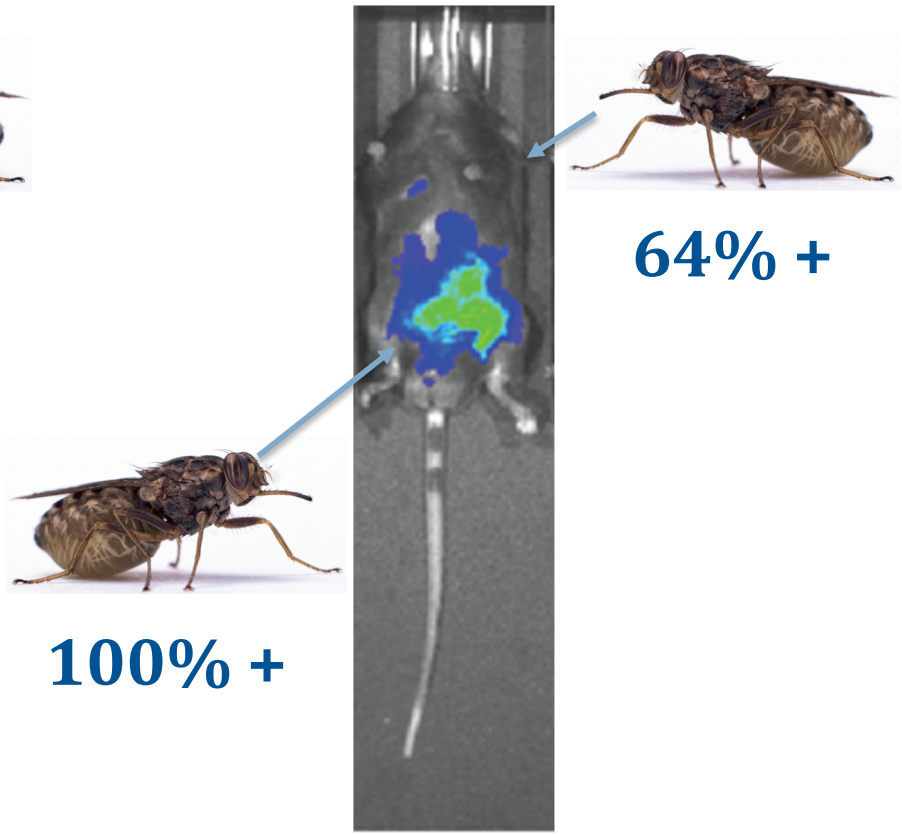
No blood parasites
Skin parasites



0% +

31% +

Blood parasites
Skin parasites



64% +

100% +

Capewell *et al.* eLife 2016

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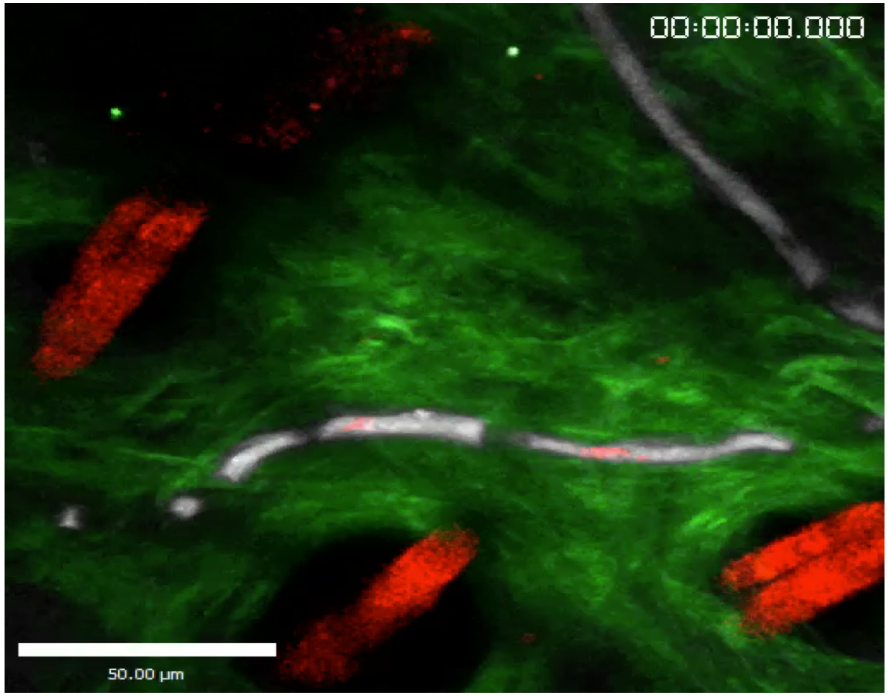
Importance of trypanosomes in the skin?

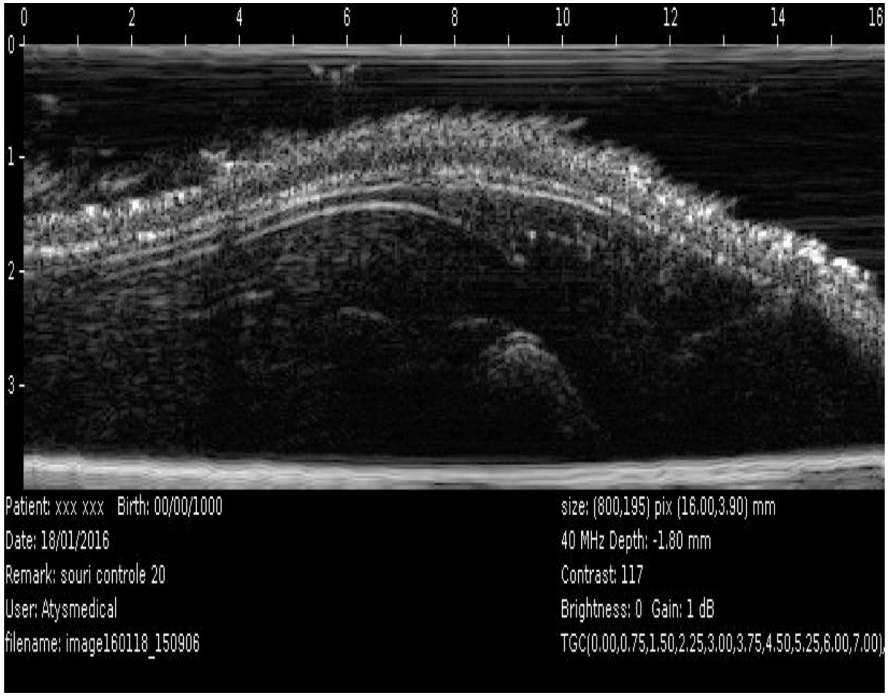
Fundamental questions:

- Tropism?
- Immune response?
- Differentiation?
- Proliferation?
- Metabolism?
- Exchanges?…

Applied issues:

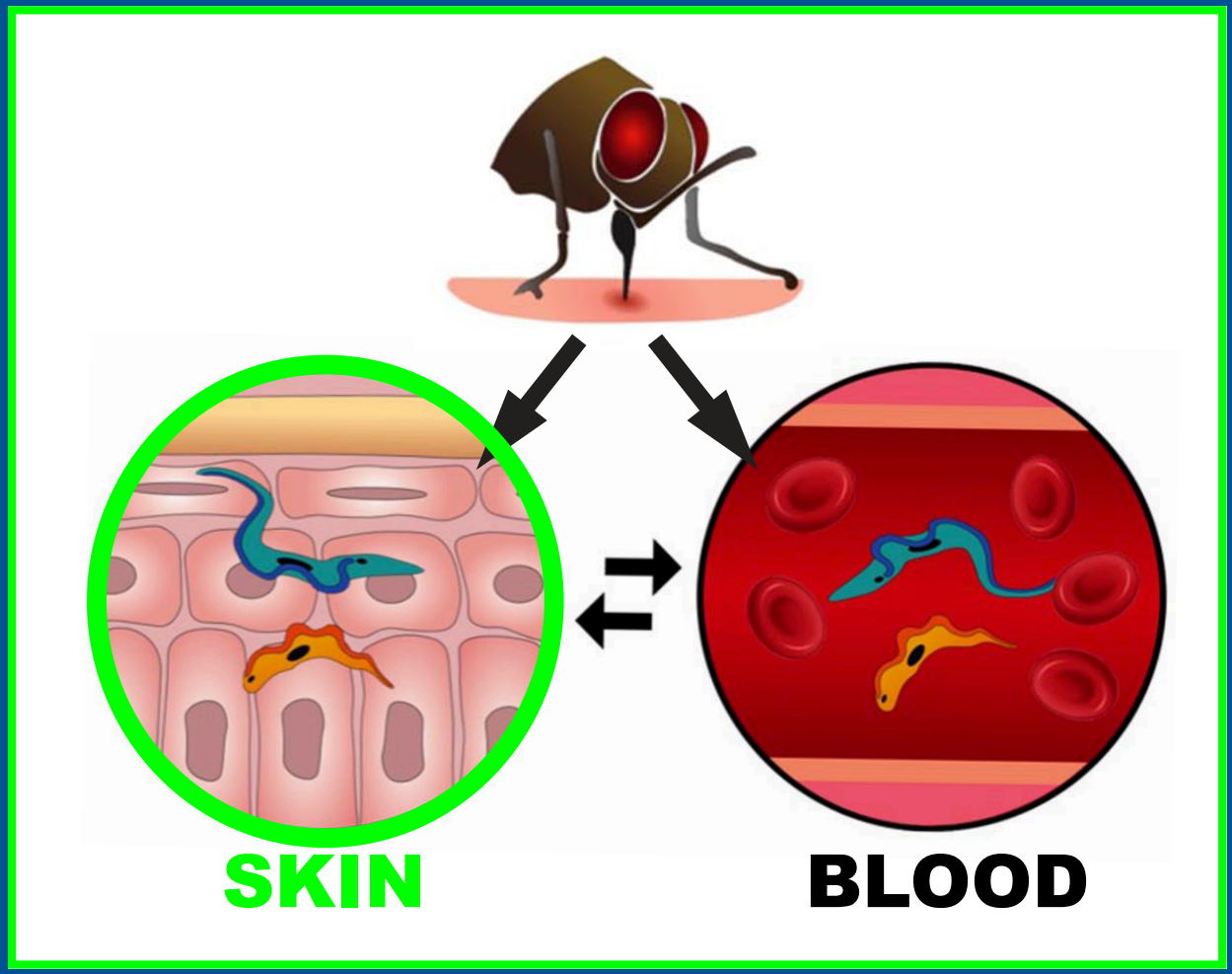
- Prevalence?
- Reservoirs?
- Diagnosis?
- Treatment?...





Prevalence of skin-dwelling trypanosomes?

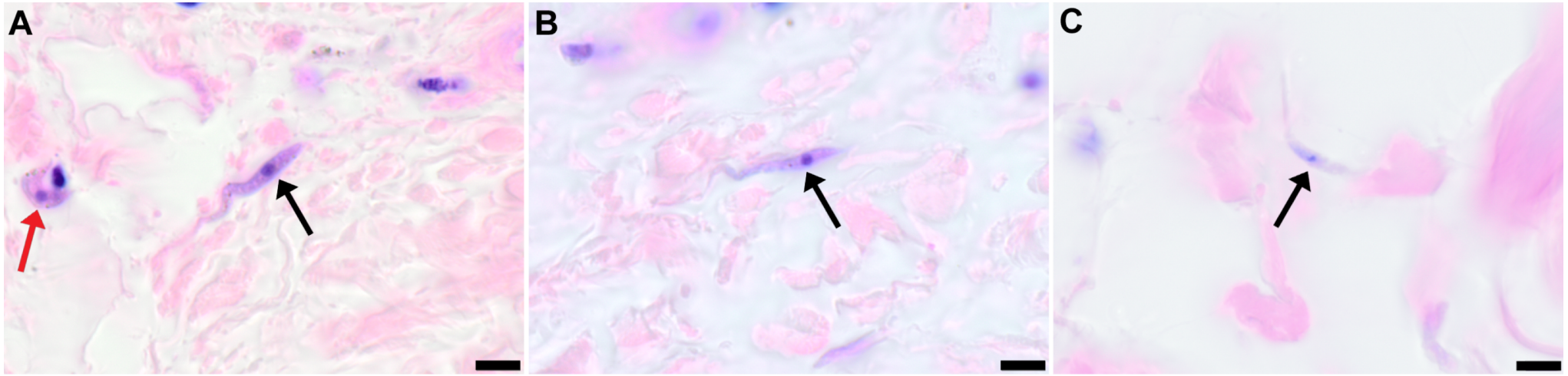
?



Adapted from Casas-Sanchez *et al.* elife 2016

Trypanosomes found in human skin

Retrospective analysis of 1,121 archived skin biopsies from an *Onchocerca* screening in an active HAT focus in DRC (2% prevalence)



Extravascular trypanosomes in the skin of at least 6 individuals

- **Human skin = anatomical reservoir?**
- **Trypanosomes in the skin of confirmed and latent cases?**

Capewell *et al.* eLife 2016

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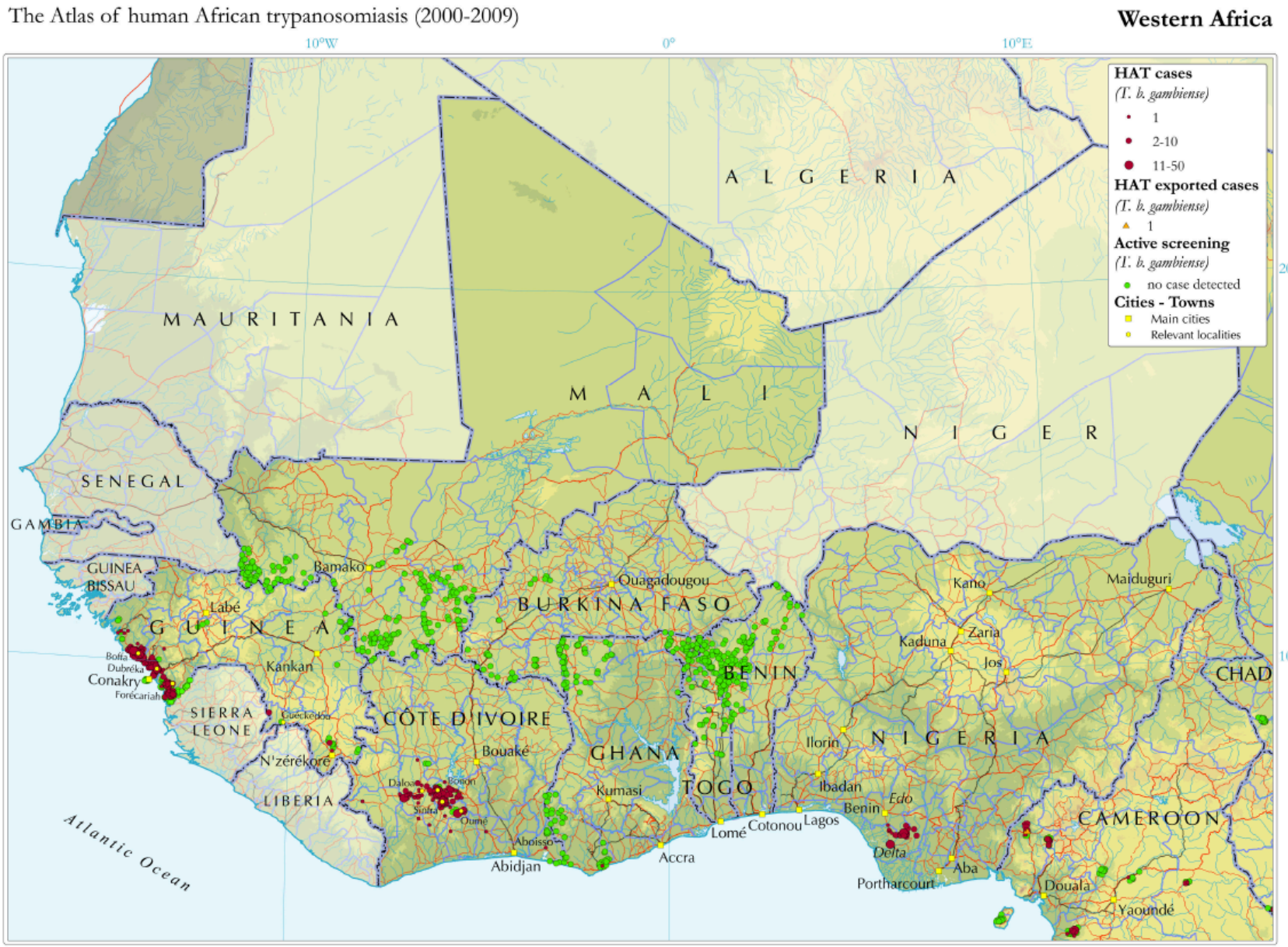
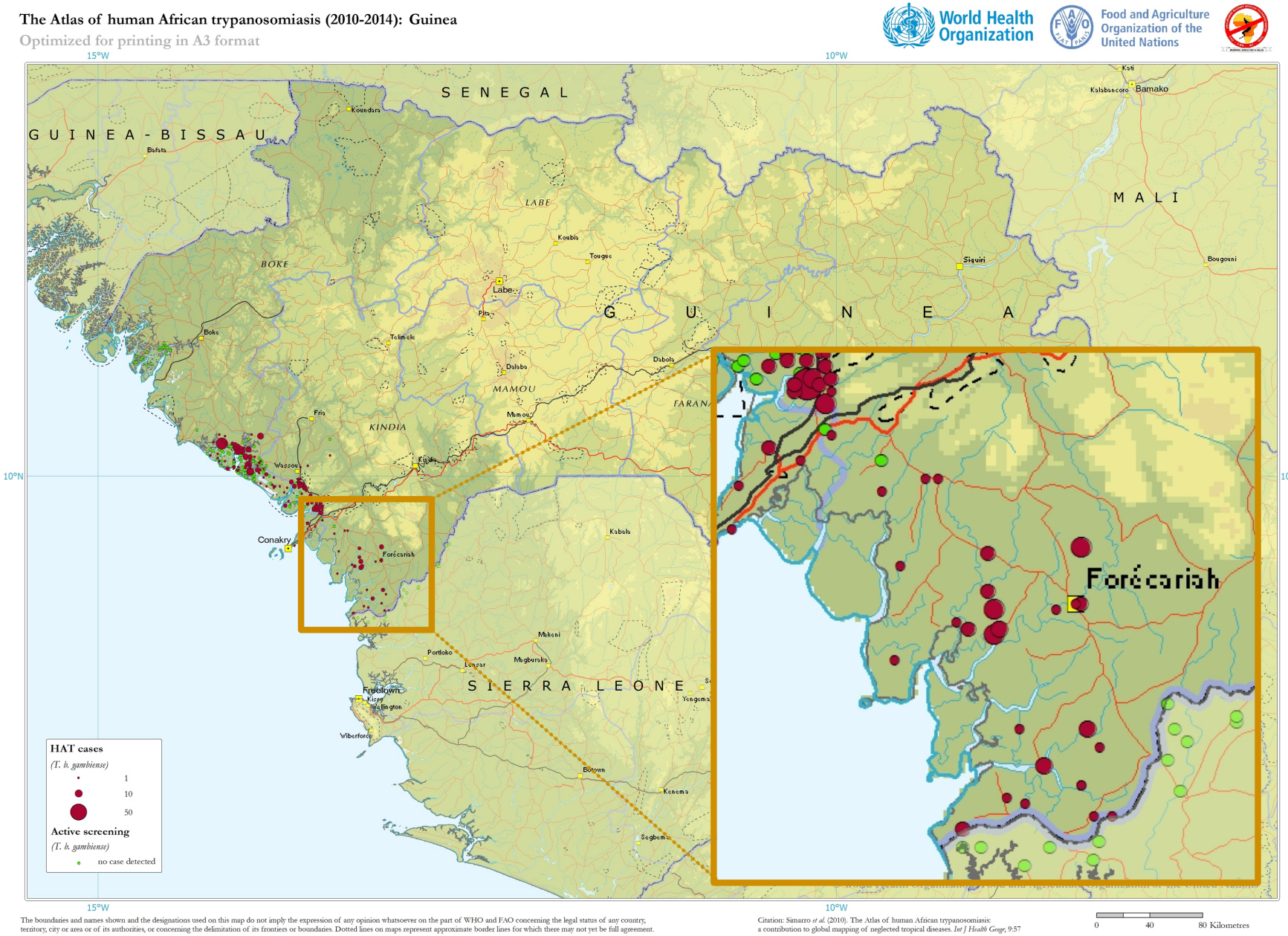
Study: HAT focus



WHO

Figure 2

Study: HAT focus



Study: HAT focus

Ecotopes: mangroves, swamps and tropical forest

Fauna: no reservoir identified so far… but no prospective studies

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Study: population and ethics

Period:

- April-May 2017

Population:

- Screening in 23 villages of the Forecaryah district
- Inclusion of informed and consenting, non-pregnant adults without medical cons-indications

Ethics:

- Protocol THA-Diag-Cut from the HAT-NCP approved by the Guinean MoH (13-05-2017)
- Involvement of a dermatologist and an anatomopathologist recruited for the study
- Signed informed consent for all patients included
- Free follow-up and treatment
- Data-base in progress

Study: diagnostic process and inclusion

1- Serological screening: CATTwb / RDT

Study: diagnostic process and inclusion

2- Parasitological confirmation:
buffy coat / LN aspirate

3- Staging: cytorachia on CSF

Study: diagnostic process and inclusion

Groups		Diagnostic process				Total No. patients	HAT prevalence	No. patients included
		1- Serological screening	2- Screening confirmation	3- Parasitological confirmation	4- Staging			
		CATTwb / RDT	CATTp	BC / LN aspirate	No. cells in CSF			
Negative		-	ND	ND	ND	5 313	-	29
		+	< 1/4	ND	ND	76	-	-
Seropositive		+	≧ 1/4	-	ND	12	-	8
HAT	**ND**	+	≧ 1/4	+	ND	2	0.04%	-
	Stage 1	+	≧ 1/4	+	0-5	8	0.15%	4
	Stage 2	+	≧ 1/4	+	≧6	18	0.33%	14
Total						**5 429**	**0.52%**	**55**

NB: 33 gHAT cases reported to WHO in 2014 in Guinea…

Study: protocol

Patients

1. Signature of the informed consent form
2. Epidemiological interview
3. General clinical examination and interview
4. Dermatological examination and interview
5. Single skin punch biopsy (2mm) on the right shoulder for CATT+ only
6. Blood sampling (5ml) for trypanolysis test
7. Treatment (Pentamidine for S1 and NECT for S2) and follow-up
8. Last clinical examination and interview 12 days after inclusion

Samples

1. Giemsa staining of touch-preps from fresh biopsies (in the field)
2. Giemsa staining and immuno-histology on paraffin-embedded biopsies (in the lab)
3. Trypanolysis test (in the lab)
4. Data analyses

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Study: protocol

Skin punch biopsy

Giemsa on touch-preps

Results: epidemiological and clinical data

Parameters n/total (%) or mean (SD)		**Groups** (n=55)						
		Negative (n=29)	**Latent** (n=8)		**Confirmed** (n=18)			
				p value*	Stage 1 (n=4)	Stage 2 (n=14)	All (n=18)	p value*
Epidemiological	HAT case(s) in the family since 2010 (n=54)	7/29 (24%)	2/7 (29%)	0.341	2/4 (50%)	5/14 (36%)	7/18 (39%)	0.336
	Age (n=54)	37.1 (14)	36.6 (18)	**0.796	31.0 (17)	34.3 (15)	33.6 (15)	**0.368
	Male sex (n=55)	15/29 (52%)	3/8 (37%)	0.693	2/4 (50%)	5/14 (36%)	7/18 (39%)	0.549
	Occupationnal risk (n=55)	11/29 (38%)	4/8 (50%)	0.690	2/4 (50%)	5/14 (36%)	7/18 (39%)	1.000
Clinical	Swollen LN (n=54)	0/28 (0%)	6/8 (75%)	**<0.0001**	4/4 (100%)	13/14 (93%)	17/18 (94%)	**<0.0001**
	Weight loss (n=50)	2/28 (7%)	3/8 (38%)	0.101	2/4 (50%)	6/10 (60%)	8/14 (57%)	**0.0001**
	Asthenia (n=55)	15/29 (54%)	4/8 (50%)	1.0	4/4 (100%)	14/14 (100%)	18/18 (100%)	**0.0003**
	Fever (n=52)	3/27 (11%)	1/7 (14%)	0.789	2/4 (50%)	9/14 (64%)	11/18 (61%)	**0.001**
	Eating disorders (n=55)	1/29 (3%)	1/8 (13%)	0.390	0/4 (0%)	7/14 (50%)	7/18 (39%)	**0.003**
	Circadian rythm disruptions (n=55)	0/29 (0%)	1/8 (13%)	0.216	0/4 (0%)	5/14 (36%)	5/18 (28%)	**0.006**
	Sexual dysfunctions (n=54)	2/28 (7%)	1/8 (13%)	0.640	0/4 (0%)	5/14 (36%)	5/18 (28%)	0.089
	Headache (n=54)	18/28 (64%)	6/8 (75%)	0.758	3/4 (75%)	13/14 (93%)	16/18 (89%)	0.118
	Behaviour changes (n=52)	2/28 (7%)	0/7 (0%)	0.640	0/4 (0%)	3/13 (23%)	3/17 (18%)	0.549
	Dermatitis (n=55)	6/29 (21%)	5/8 (63%)	**0.035**	4/4 (100%)	11/14 (79%)	15/18 (83%)	**<0.0001**
	Pruritus (n=55)	2/29 (7%)	2/8 (25%)	0.198	0/4 (0%)	11/14 (79%)	11/18 (61%)	**<0.0001**

Clinical factors associated to gHAT:
Swollen lymph nodes and **dermatological signs**

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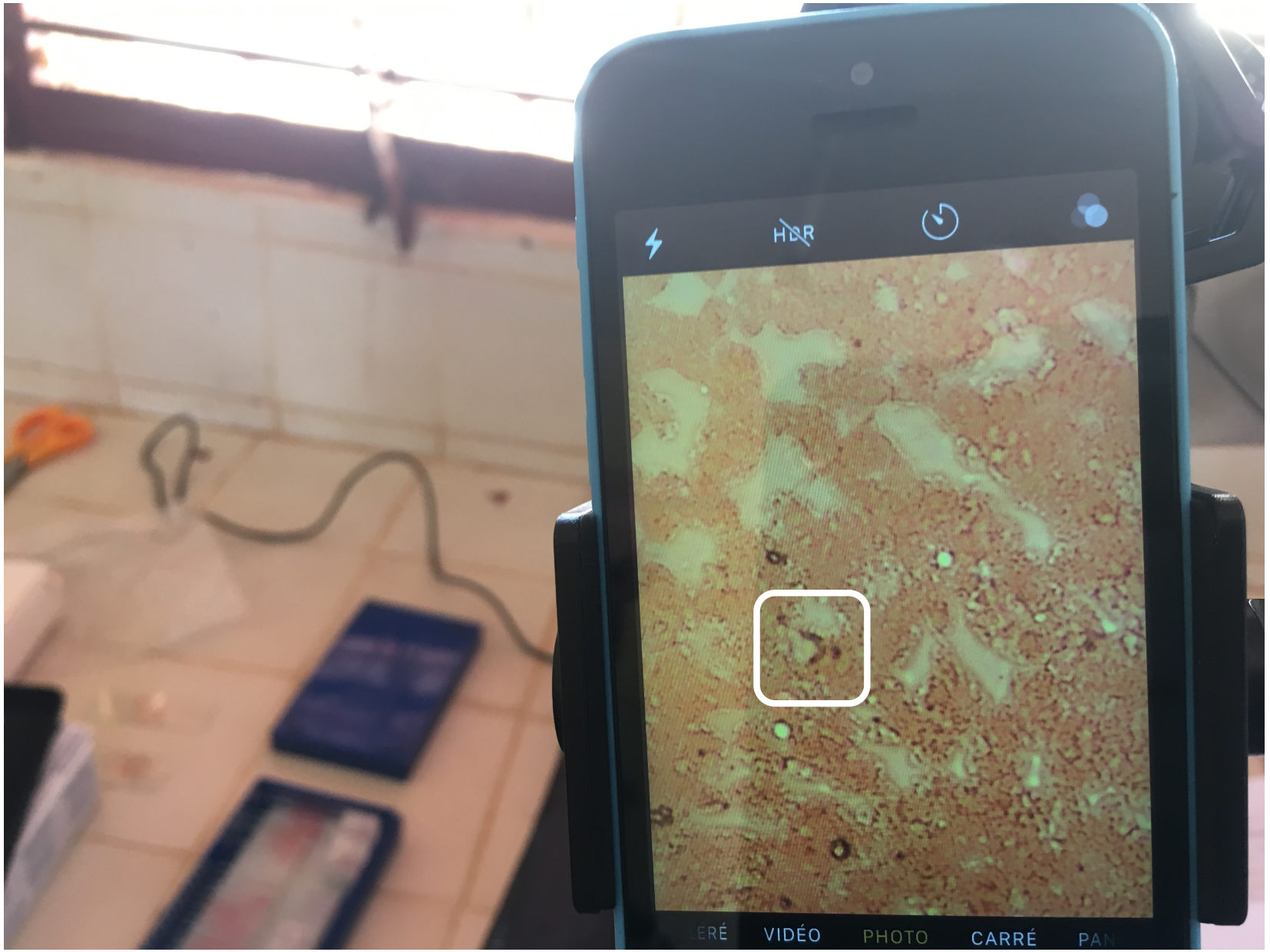
Results: serology and molecular biology

Analyses n/total (%)		**Groups** (n=26)			
		Latent (n=8)	**Confirmed** (n=18)		
			Stage 1 (n=4)	Stage 2 (n=14)	All (n=18)
Trypanolyses	LiTat 1.3 positive	2/8 (25%)	4/4 (100%)	14/14 (100%)	18/18 (100%)
	LiTat 1.5 positive	2/8 (25%)	4/4 (100%)	12/14 (86%)	16/18 (89%)
	LiTat 1.6 positive	2/8 (25%)	4/4 (100%)	12/14 (86%)	16/18 (89%)
	Negative for all	6/8 (75%)	0/4 (0%)	0/14 (0%)	0/18 (0%)
PCRs on blood	TBR positive	0/8 (0%)	4/4 (100%)	12/14 (86%)	16/18 (89%)
	TgsGP positive	0/8 (0%)	3/4 (75%)	9/14 (64%)	12/18 (67%)
	Negative for all	8/8 (100%)	0/4 (0%)	2/14 (14%)	2/18 (11%)
PCRs on skin	TBR positive	5/8 (63%)	0/4 (0%)	9/14 (64%)	9/18 (50%)
	TgsGP positive	0/8 (0%)	0/4 (0%)	0/14 (0%)	0/18 (0%)
	Negative for all	3/8 (37%)	4/4 (100%)	5/14 (36%)	9/18 (50%)

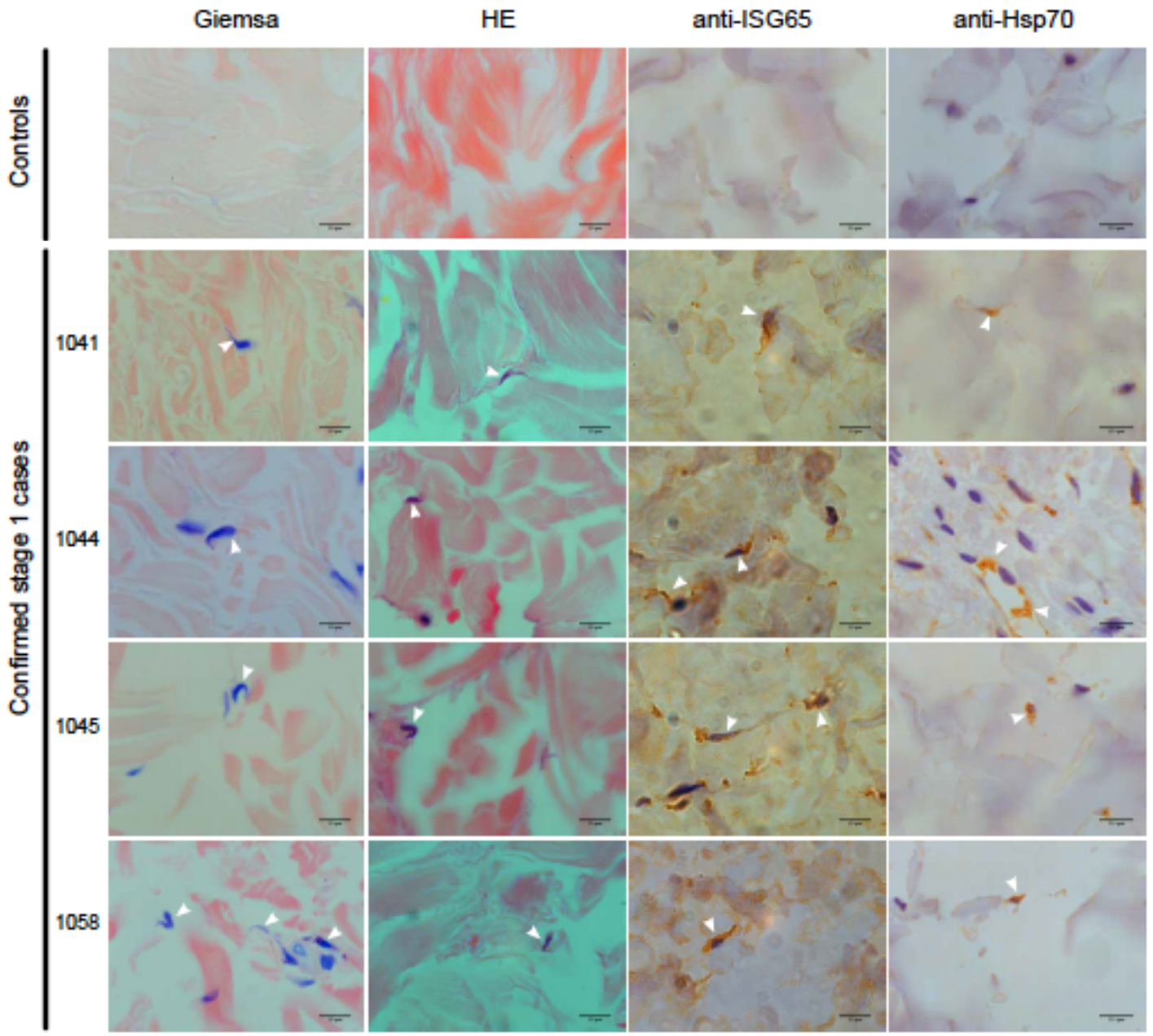
Presence of *T. brucei* DNA in the skin

Results: skin biopsies



Annette MacLeod

Results: skin biopsies



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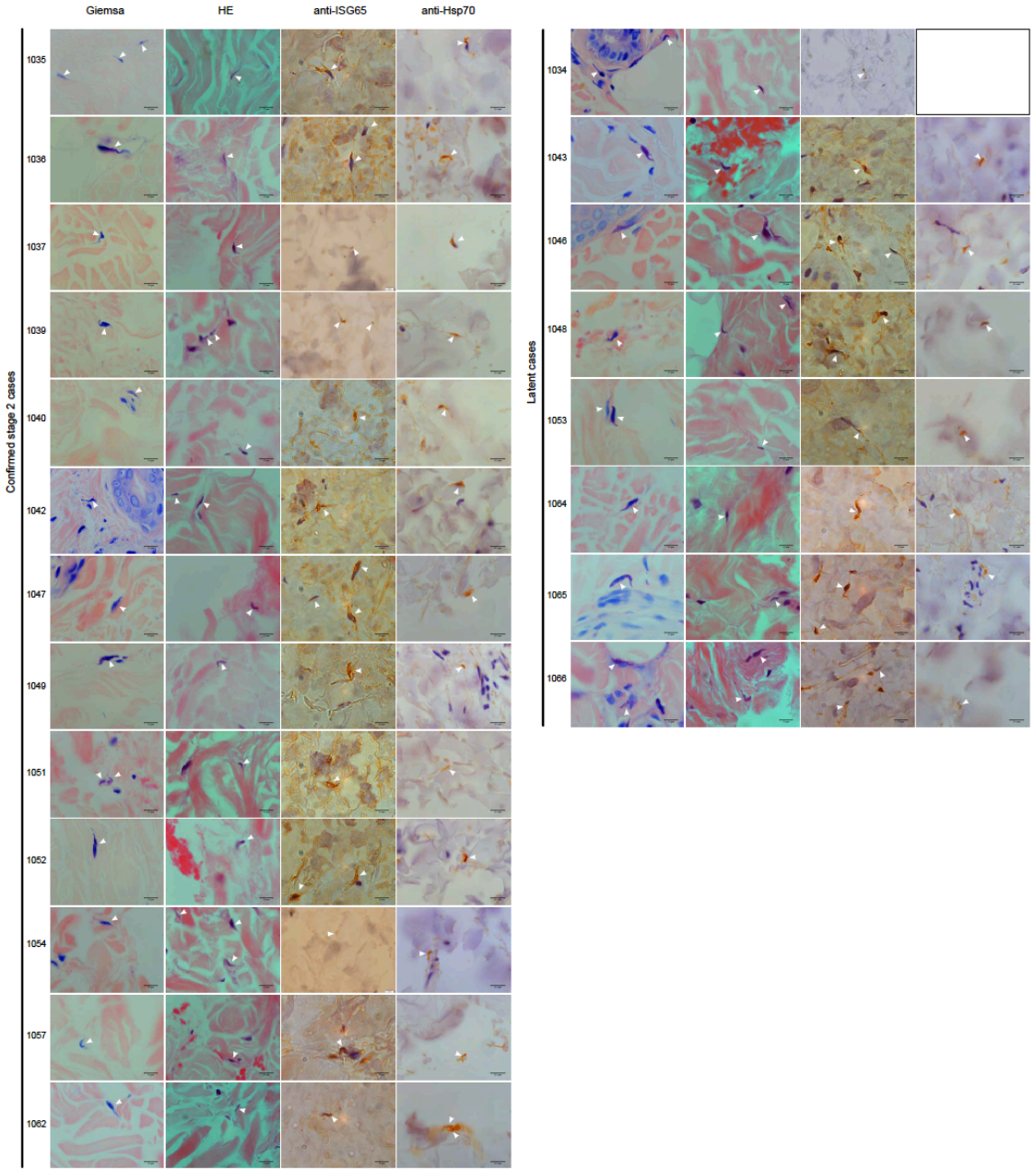
Results: skin biopsies



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Results: skin biopsies

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	TgsGP positive	0/8 (0%)	3/4 (75%)	9/14 (64%)	12/18 (67%)
	Negative for all	8/8 (100%)	0/4 (0%)	2/14 (14%)	2/18 (11%)
PCRs on skin	TBR positive	5/8 (63%)	0/4 (0%)	9/14 (64%)	9/18 (50%)
	TgsGP positive	0/8 (0%)	0/4 (0%)	0/14 (0%)	0/18 (0%)
	Negative for all	3/8 (37%)	4/4 (100%)	5/14 (36%)	9/18 (50%)
Histology on skin	Dermal touchprep positive	2/4 (50%)	2/3 (66%)	13/13 (100%)	15/16 (94%)
	Giemsa positive	8/8 (100%)	4/4 (100%)	14/14 (100%)	18/18 (100%)
	HE positive	8/8 (100%)	4/4 (100%)	13/13 (100%)	17/17 (100%)
	Hsp70 positive	7/8 (87%)	4/4 (100%)	14/14 (100%)	18/18 (100%)
	ISG65 positive	8/8 (100%)	4/4 (100%)	14/14 (100%)	18/18 (100%)
	Negative for all	0/8 (0%)	0/4 (0%)	0/14 (0%)	0/18 (0%)

Conclusions

- *T. brucei* in the dermis of 100% HAT cases
- All suspected latent infections with parasites in the skin

Skin-dwelling trypanosomes in aparasitemic confirmed cases and in suspected latent infections!

= Missing link in the transmission maintenance?

Next: studies on larger cohorts, seropositive follow-up, mathematical modelling, …

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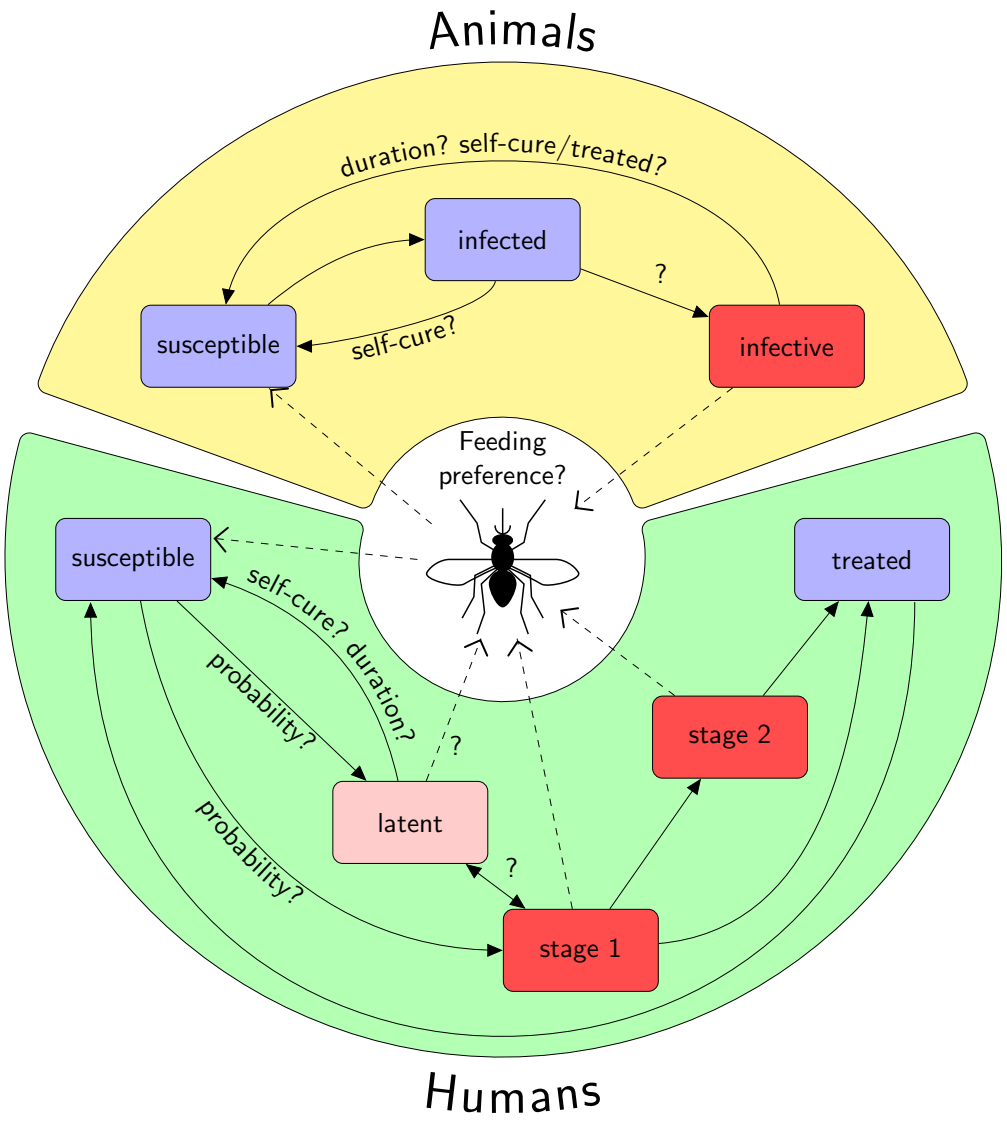
Modelling the transmission



BUSCHER *et al.* TiP 2018

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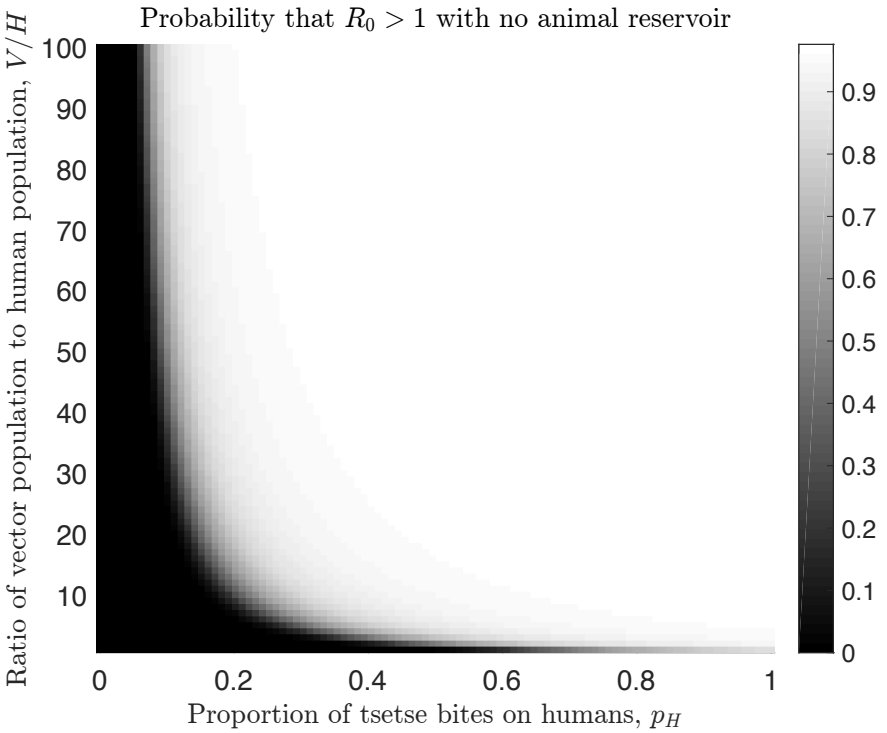
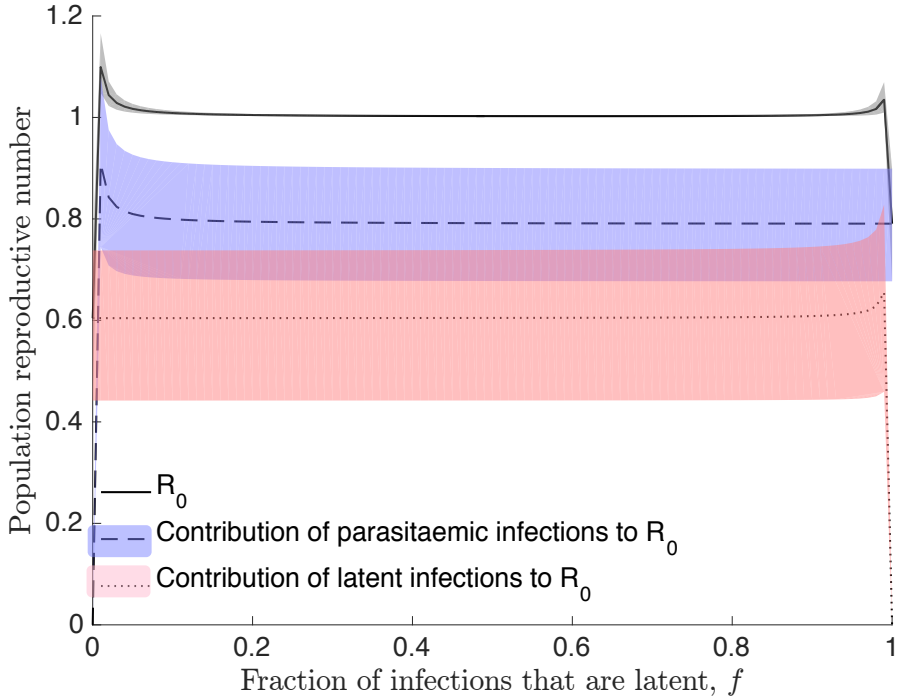
Modelling transmission

Mathematical modelling of HAT foci without an animal reservoir





- Humans sustain transmission alone with >30% bites on humans and tsetse / humans ratio >10:1
- Importance of latent infections to perpetuate transmission

Atkins *et al.* PLoS Biol 2018

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Perspectives

- What is the real prevalence of latent infections?
- Is it the norm in all *T. b. gambiense* transmission foci?
- Are current trypanocidal treatments efficient against skin-dwelling parasites?
- Should the WHO policy be changed to include the treatment of individuals with latent infection?
- How could skin-dwelling trypanosomes be easily detected?

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Partners, fundings and aknowledgements

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