

Use of mobile genetic element PCR
(MGE-PCR) as a tool to investigate
the distribution and spread of
parasitic diseases

Professor Geoff Hide
University of Salford, UK

Overview

MGE-PCR: What is Mobile Genetic Element PCR?

Examples:

Spread of human sleeping sickness in Uganda

Strain typing in *Toxoplasma*

Genetic markers for identifying parasites

- Examples
- RAPDs
- RFLP
- Microsatellite genotyping (MS)

Genetic markers for identifying parasites

- Examples
- RAPDs
- RFLP
- Microsatellite genotyping (MS)
- All have specific uses

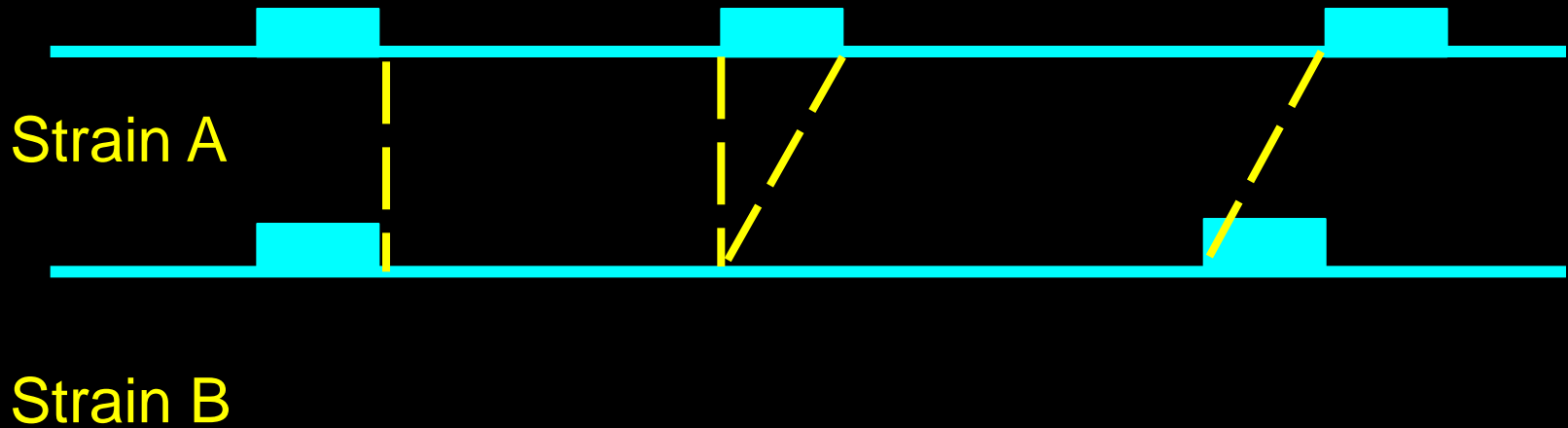
Genetic markers for identifying parasites

- limitations
- Most techniques require several stages

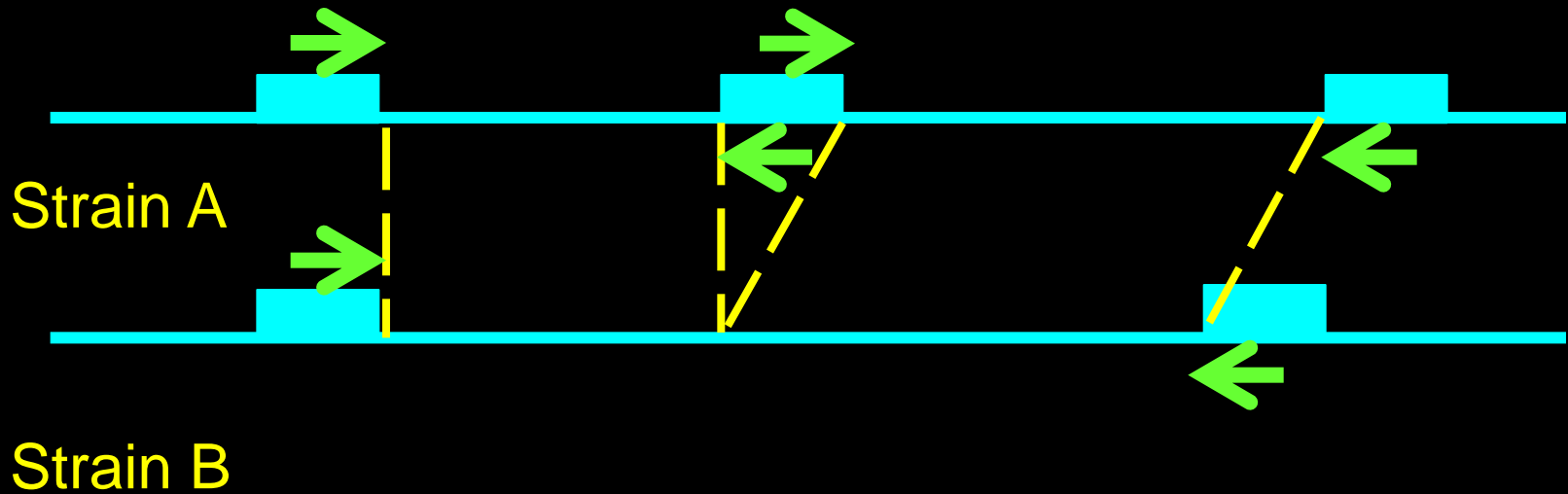
Genetic markers for identifying parasites

- Aim
- To develop a single stage analytical tool that provides good discrimination between parasite strains

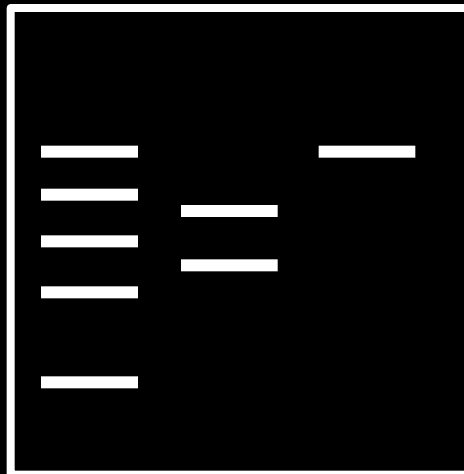
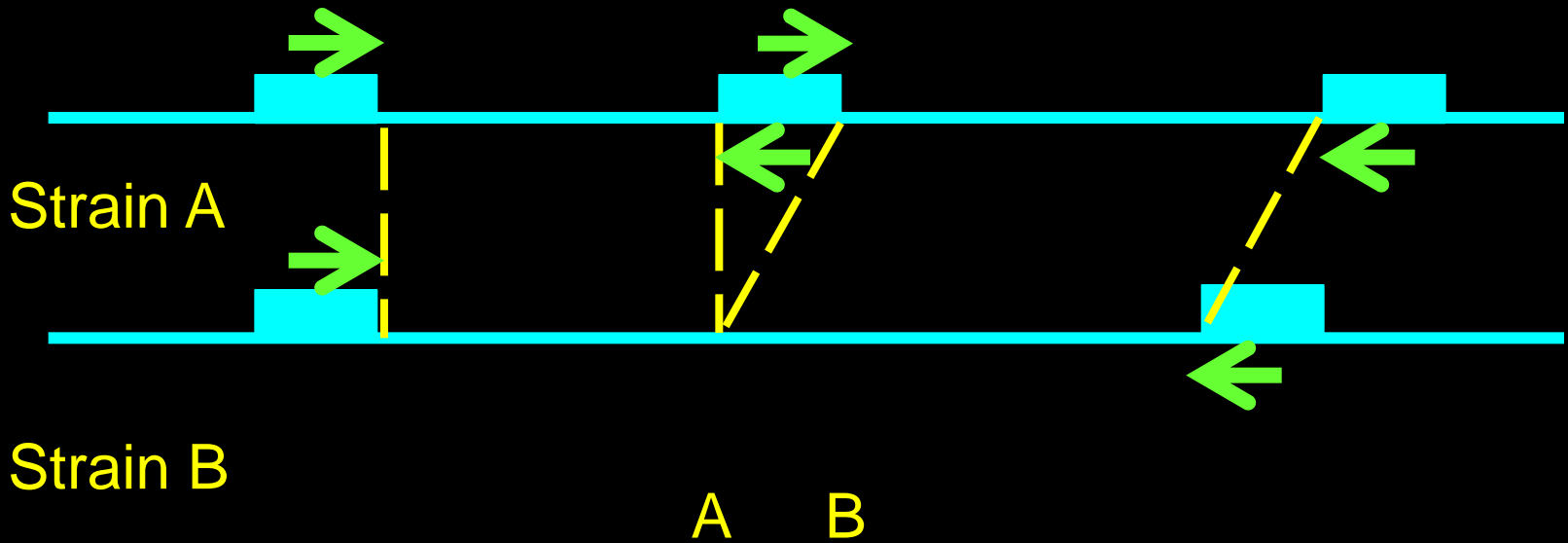
Principles of MGE-PCR



Principles of MGE-PCR



Principles of MGE-PCR



Structure of a typical MGE

Inverted
repeat



Inverted
repeat



Use of a single PCR primer for MGE-PCR



A single PCR primer is complementary to both inverted repeats

Examples

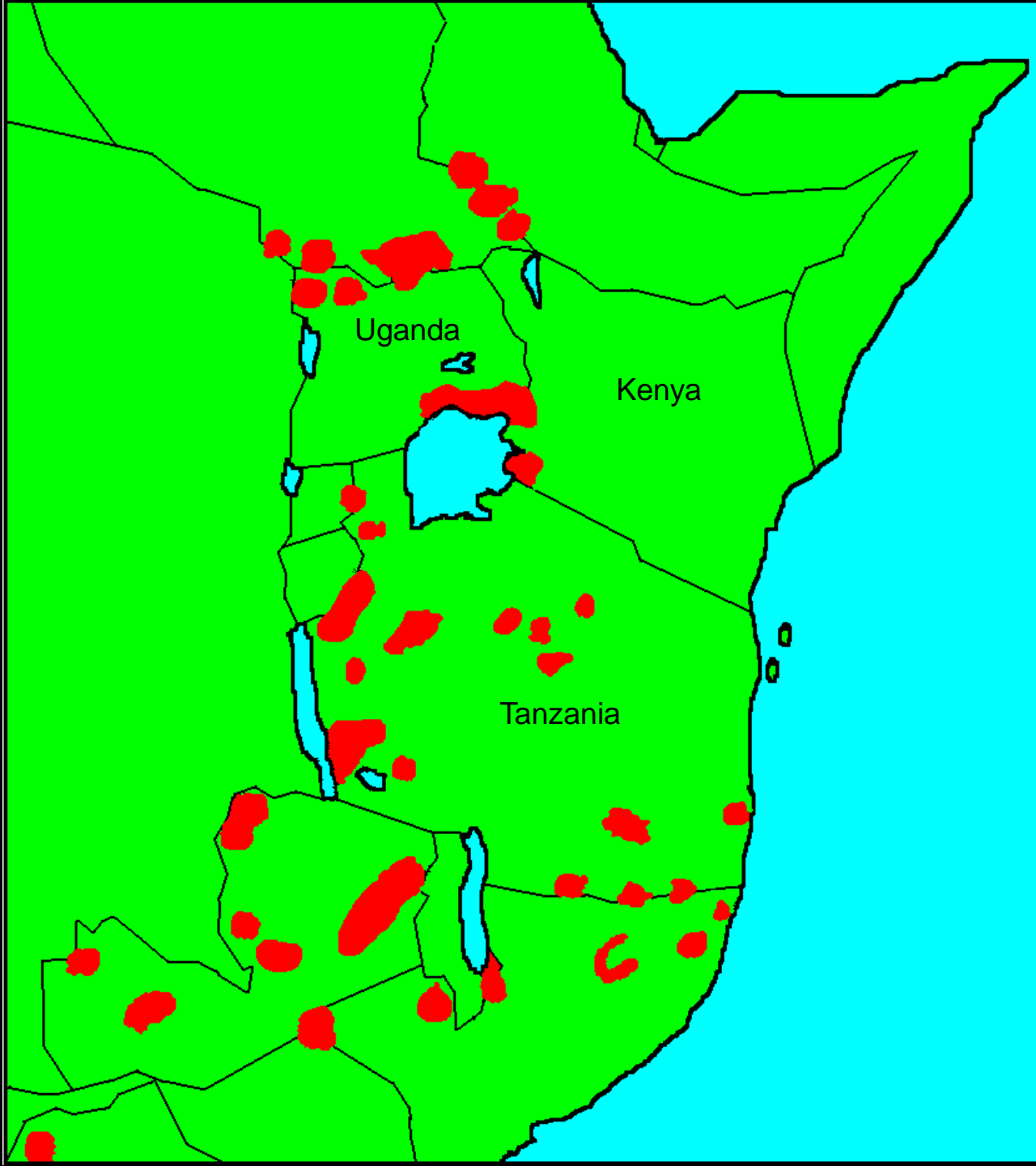
Examples

The origins of Human sleeping
sickness epidemics

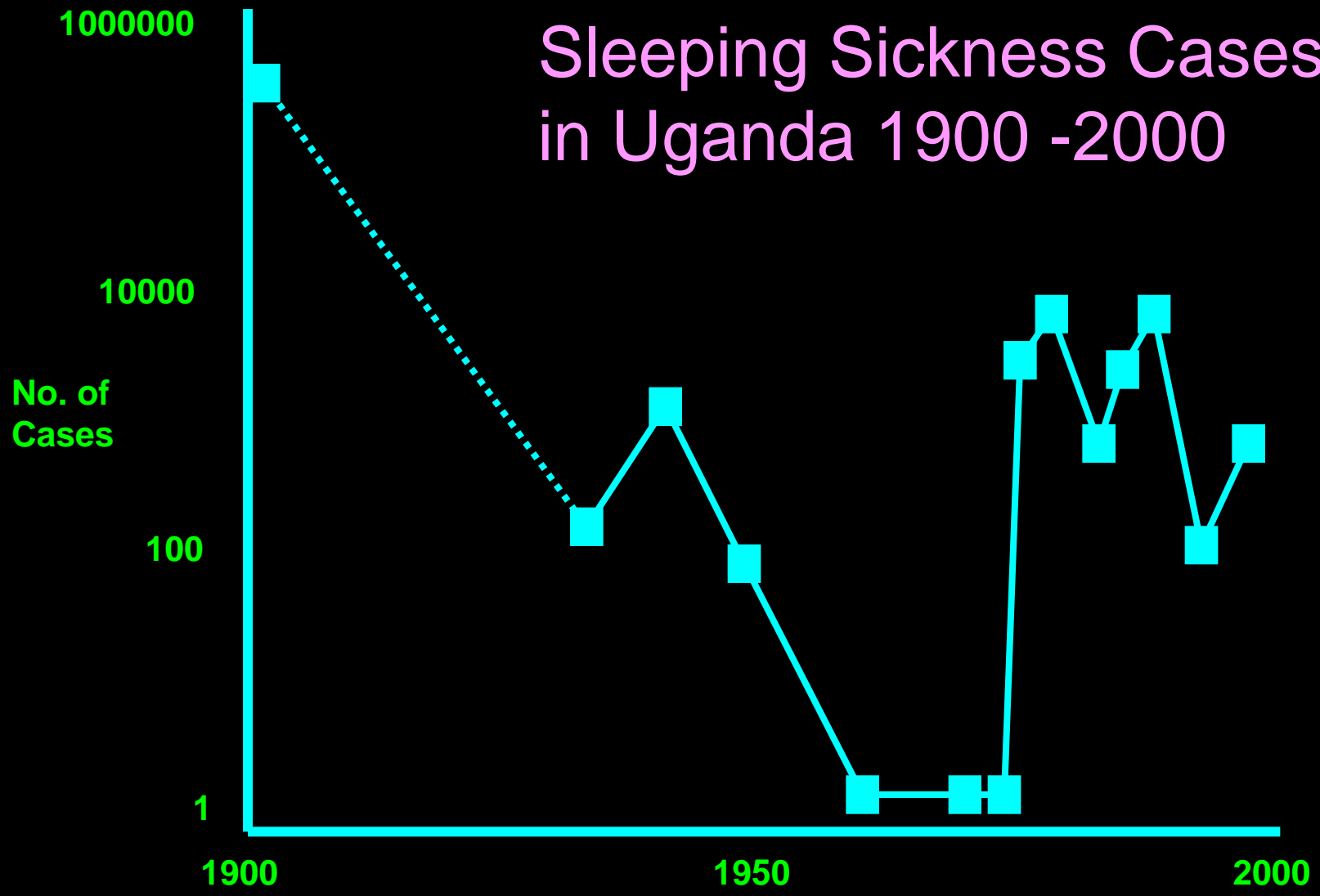
Human sleeping sickness in Uganda

Why are sleeping sickness
foci where they are?

What causes epidemics?



Sleeping Sickness Cases in Uganda 1900 -2000

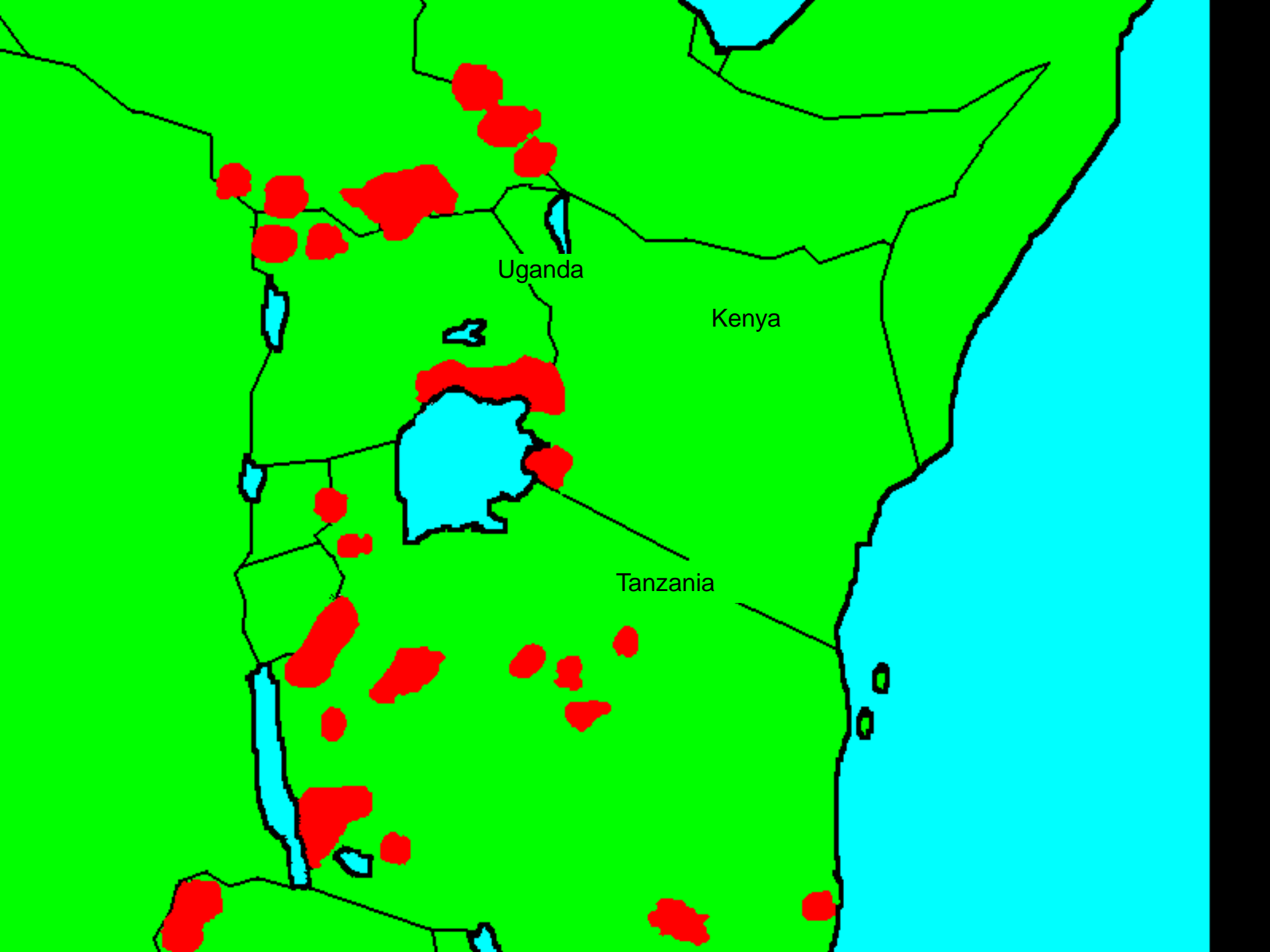


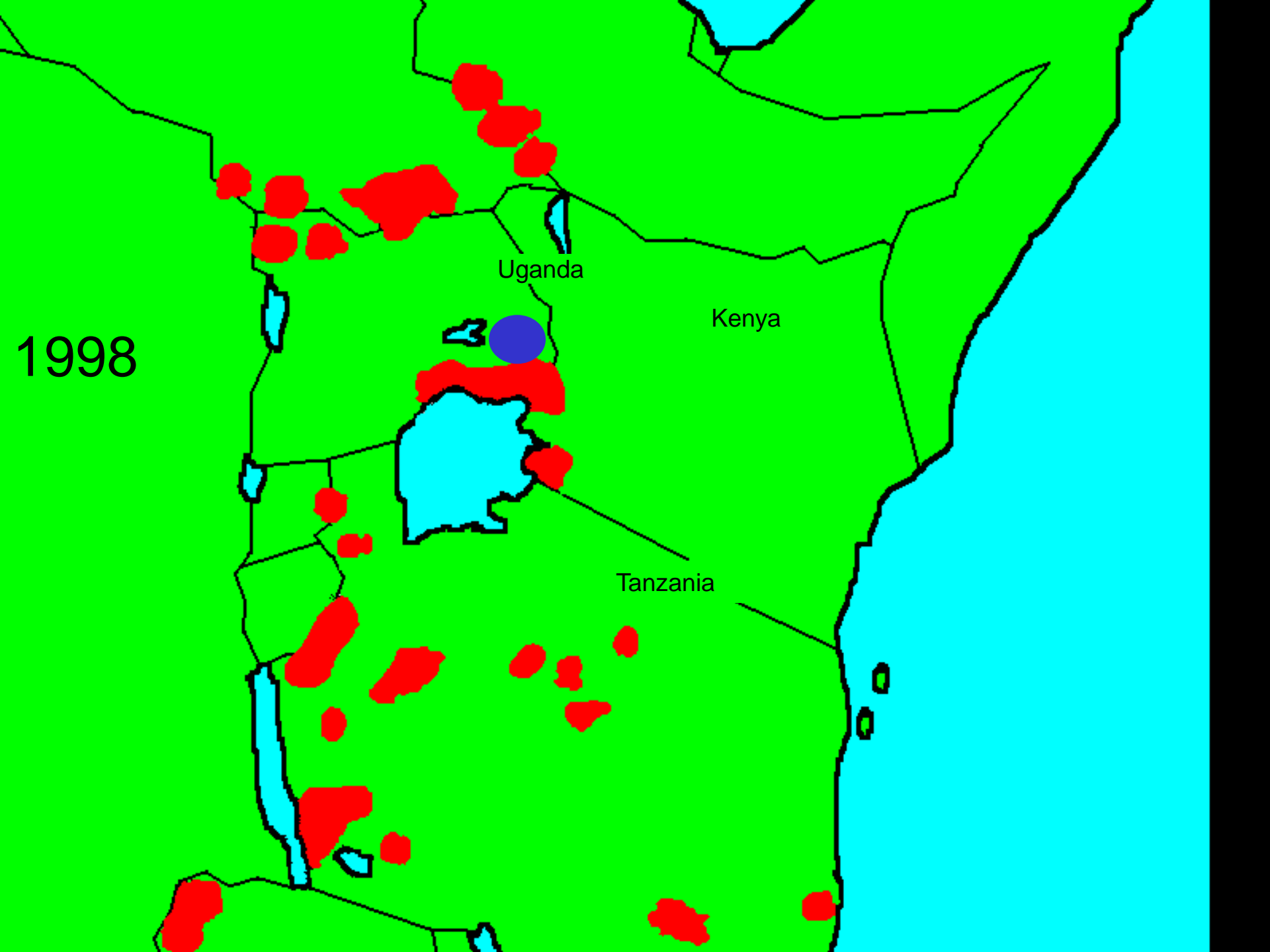
Patterns of Human Disease

- Animal disease throughout
- Human disease localised to specific foci
- Human disease occurs as sporadic epidemics

Patterns of Human Disease in Uganda

- Human focus is spreading
- North and West
- Merge with a *T.b.gambiense* focus??



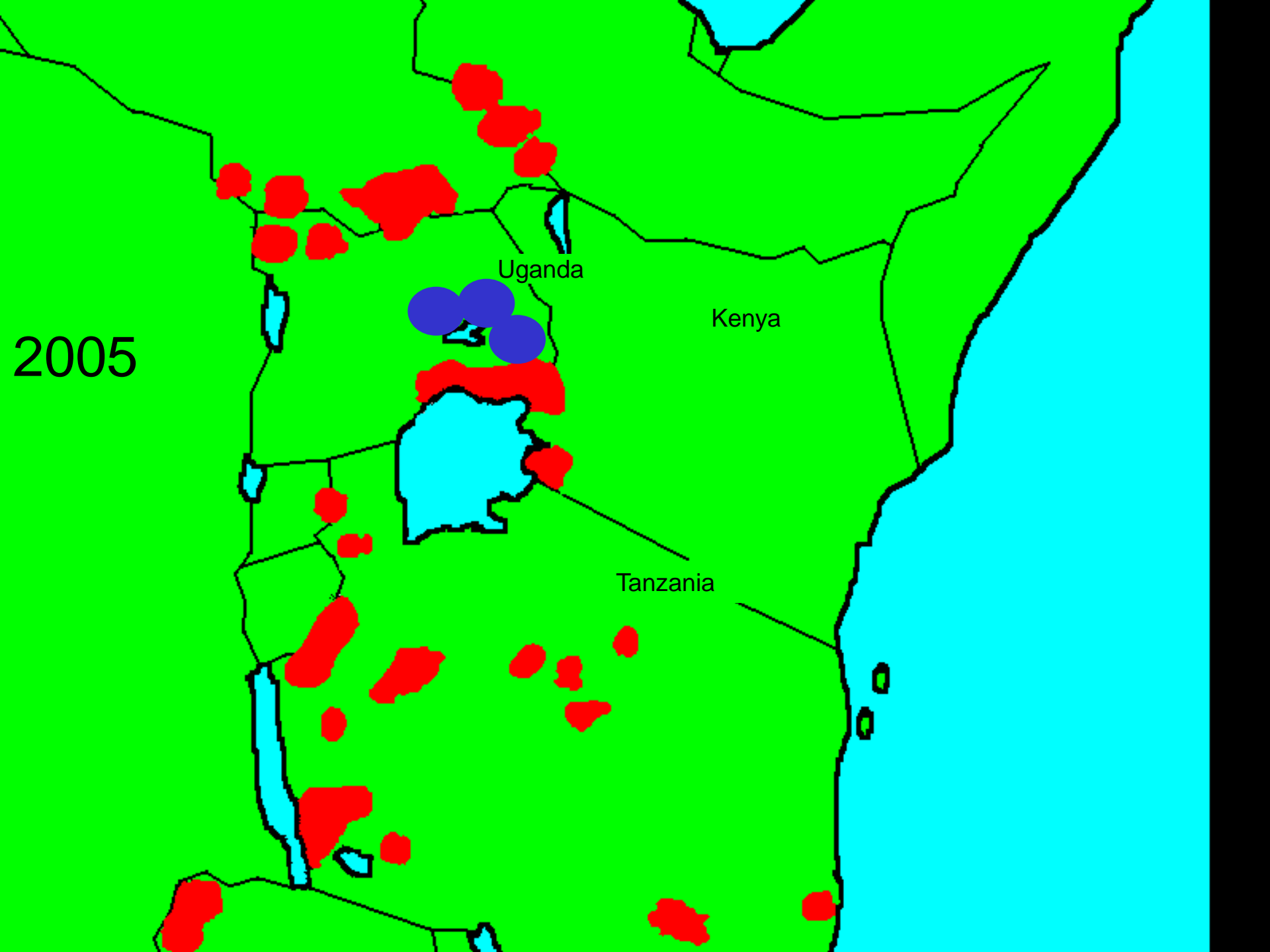


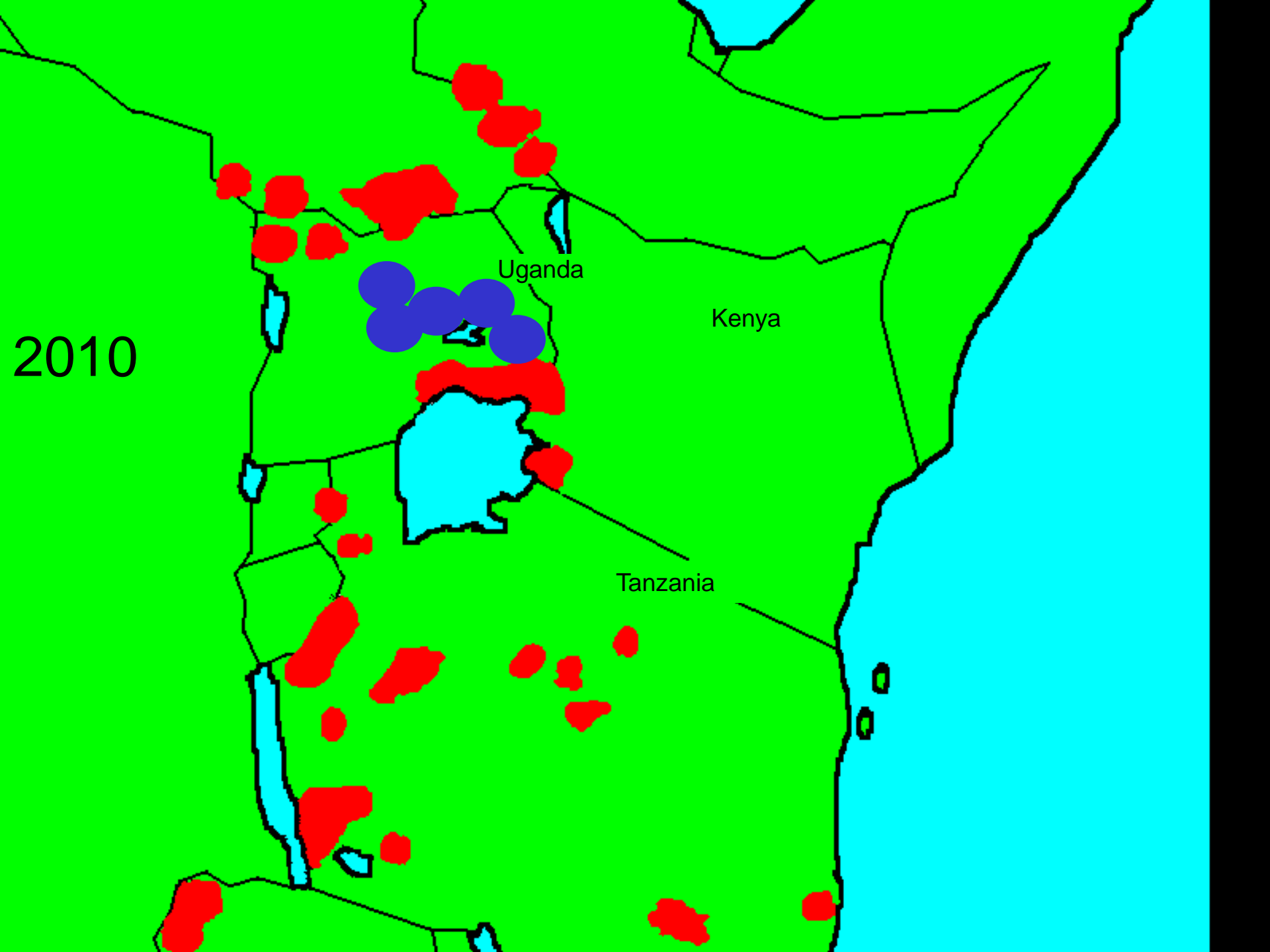
1998

Uganda

Kenya

Tanzania





2010

Uganda

Kenya

Tanzania

Recent Epidemics in Uganda

1988 - 1992 - Tororo, Uganda

200-300 cases per year

1998 to date - Soroti, Uganda

119 cases in first 18 months



Soroti

Uganda

Tororo

Kenya

Busoga

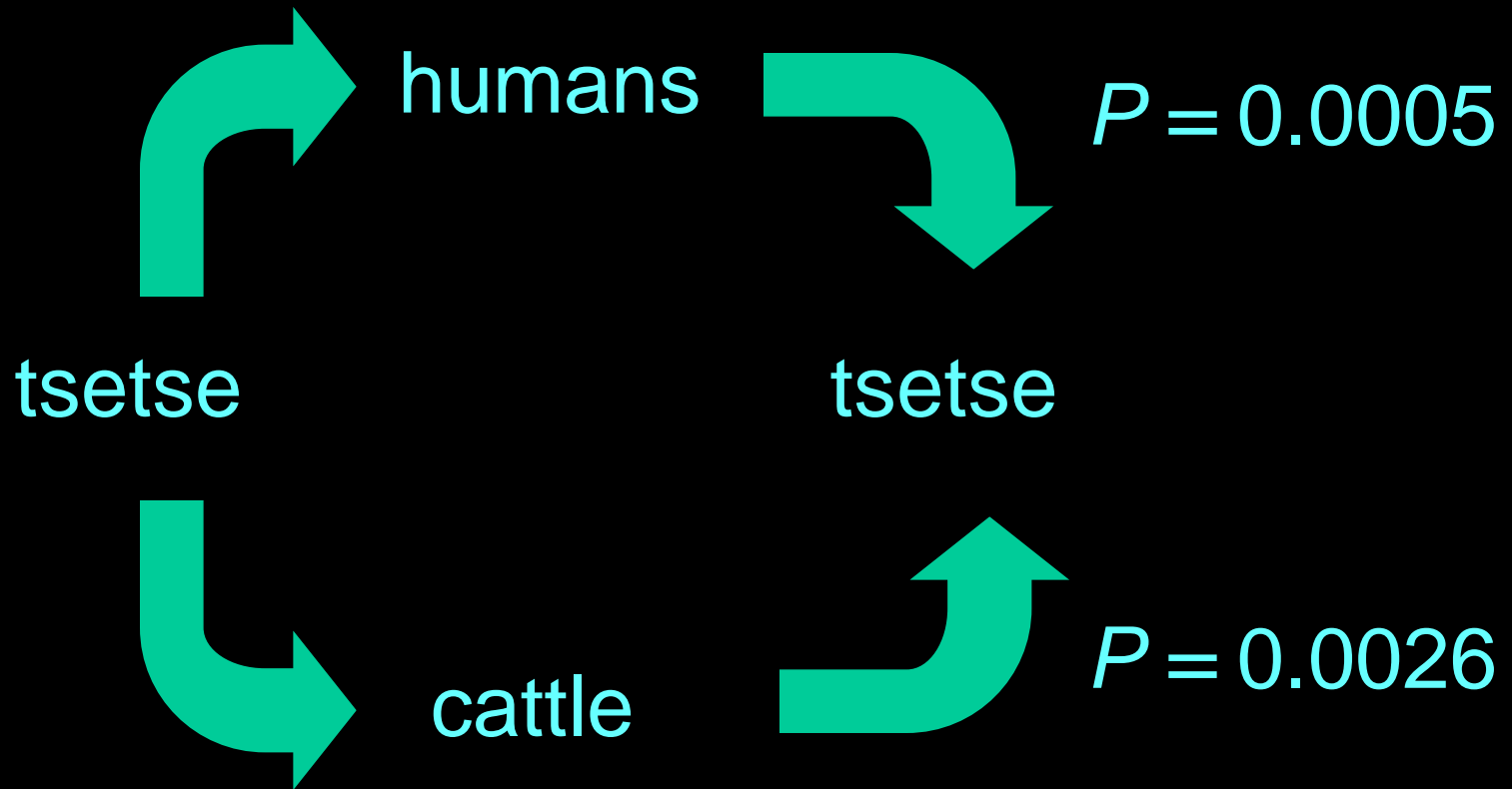
Central Nyanza

Lake Victoria

20 miles

Tororo Epidemic

- No cases prior to 1984
- Epidemic Peaked 1990
- Still a few cases



Main findings

- A Specific human strain
- Presence of human strain may determine extent of focus
- Cattle are a significant reservoir for the human strain

1998 Soroti Epidemic

- Never had sleeping sickness
- 31st Dec 1998 local nurse reported first case
- 119 cases by June 2000
- Recent movement of cattle from into the area



Soroti

Uganda

Tororo

Kenya

Busoga

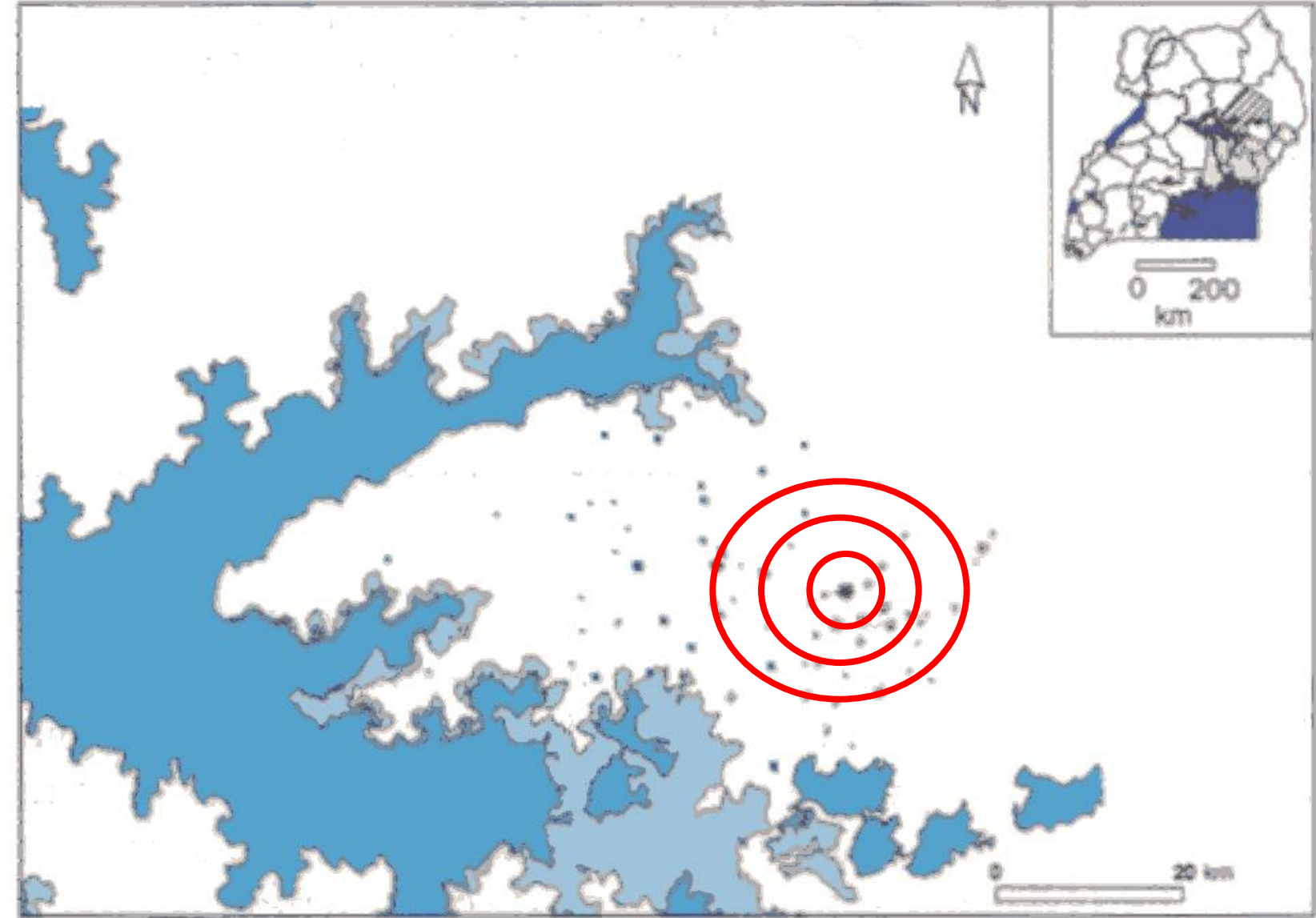
**Central
Nyanza**

Lake Victoria

20 miles

Location of disease associated with local cattle markets





Where did the Soroti epidemic come from?

- Need a method of accurately identifying strains
- MGE-PCR as a tool

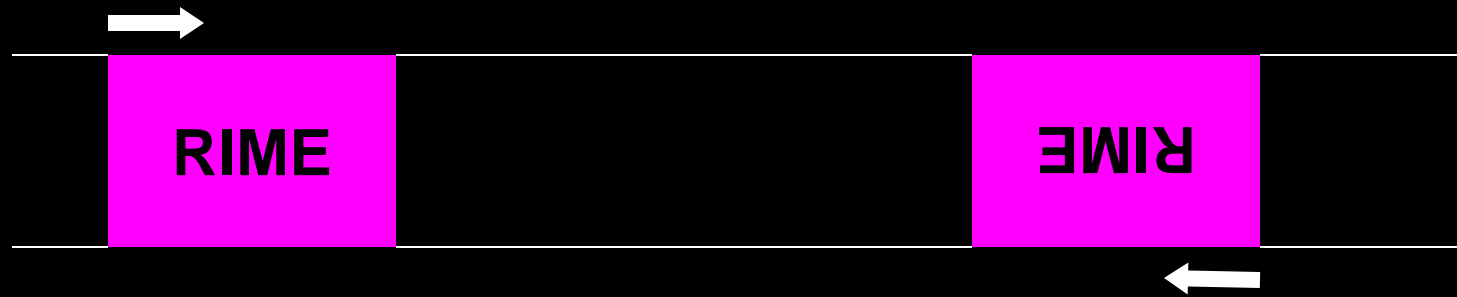
The trypanosomal mobile genetic element RIME

(b) RIME + *Ingi* MGE

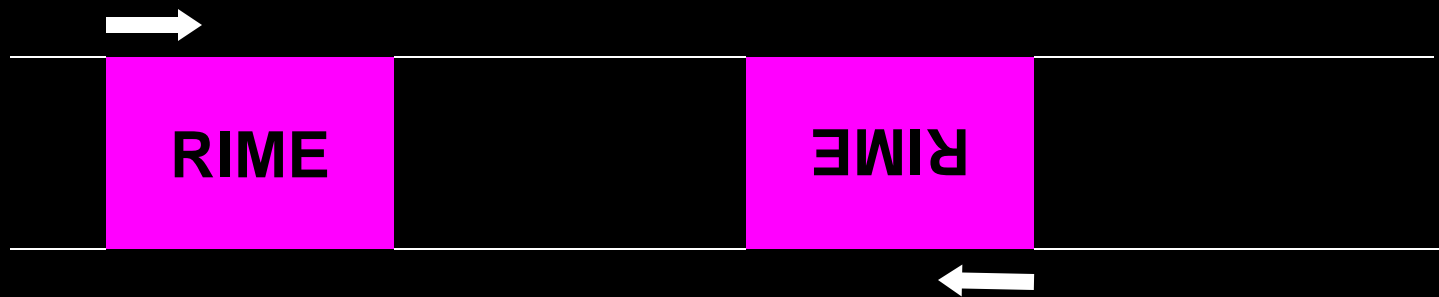


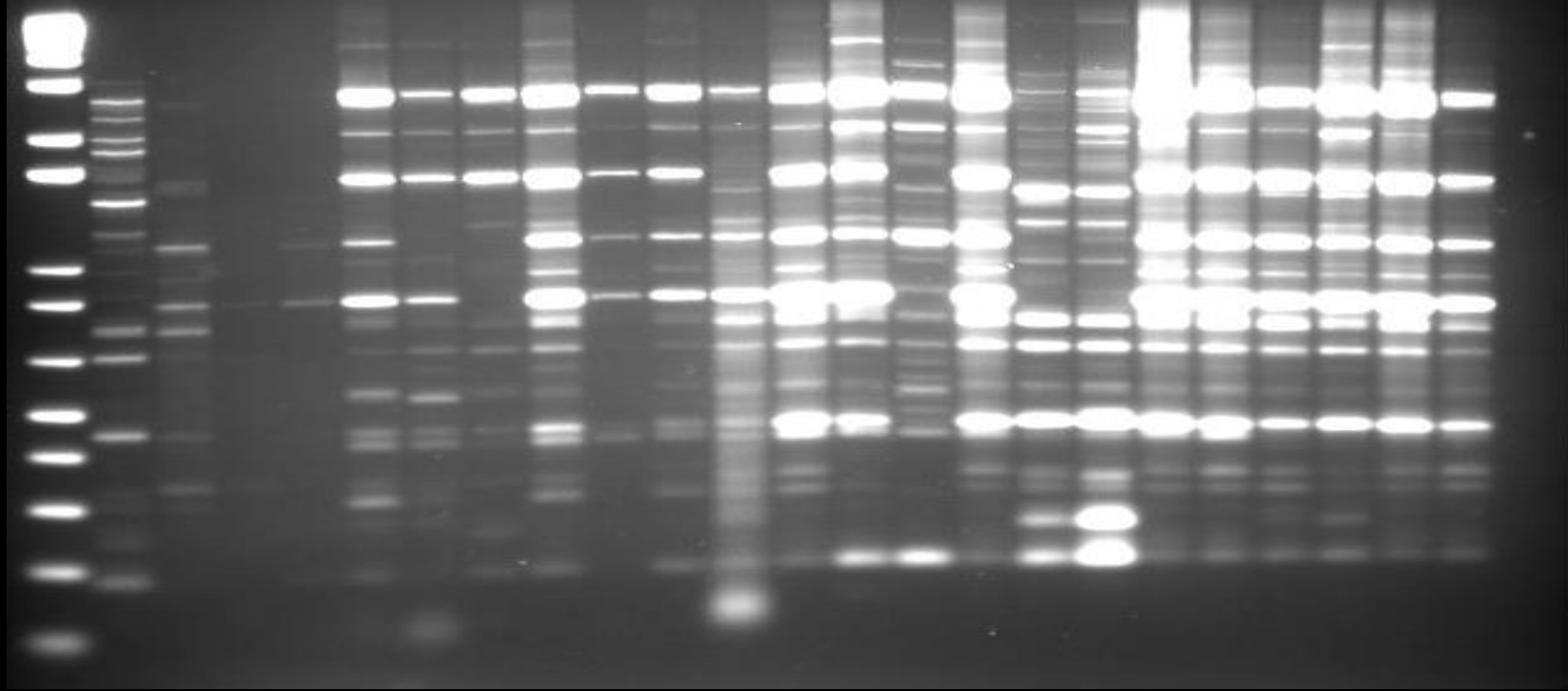
Analysis variation in RIME position between trypanosome strains - single primer

Strain a



Strain b





Marker

***a* Bumanda 146**

***a* UGL**

***c* LIRI 16**

***c* AKO C20**

***b* KINU C21**

***b* KINU P4**

***b* BUWHTK**

***b* BUG H1**

***b* BUG H2**

***b* KINU HBW**

***c* LIRI 014**

***a* UGK**

***a* Mela 27**

***a* Mela 32**

***a* Mela 71**

***a* Mela Pig1**

***a* Mela Pig 2**

***a* UGB 88**

***a* UGC 88**

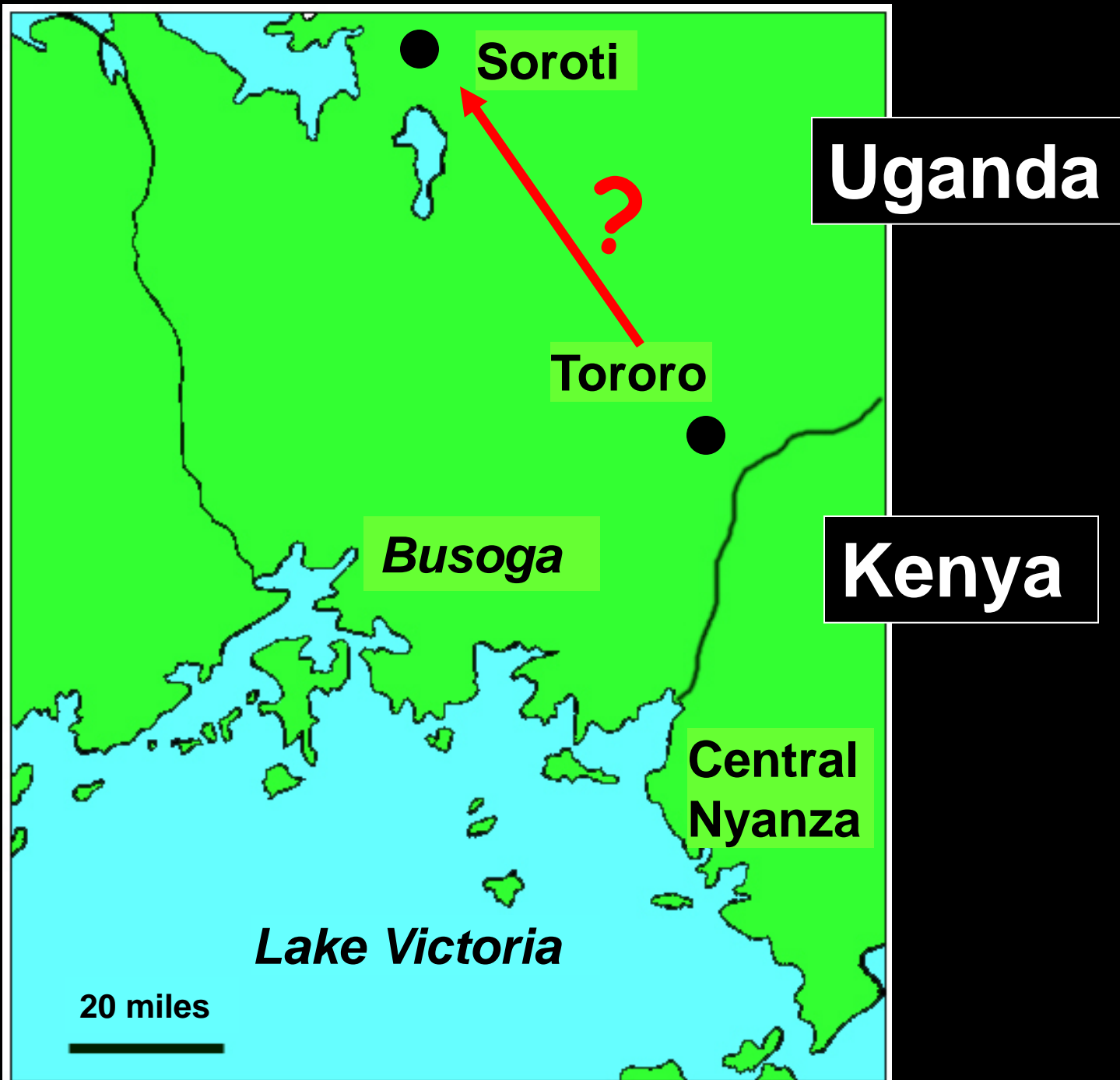
***a* UGC**

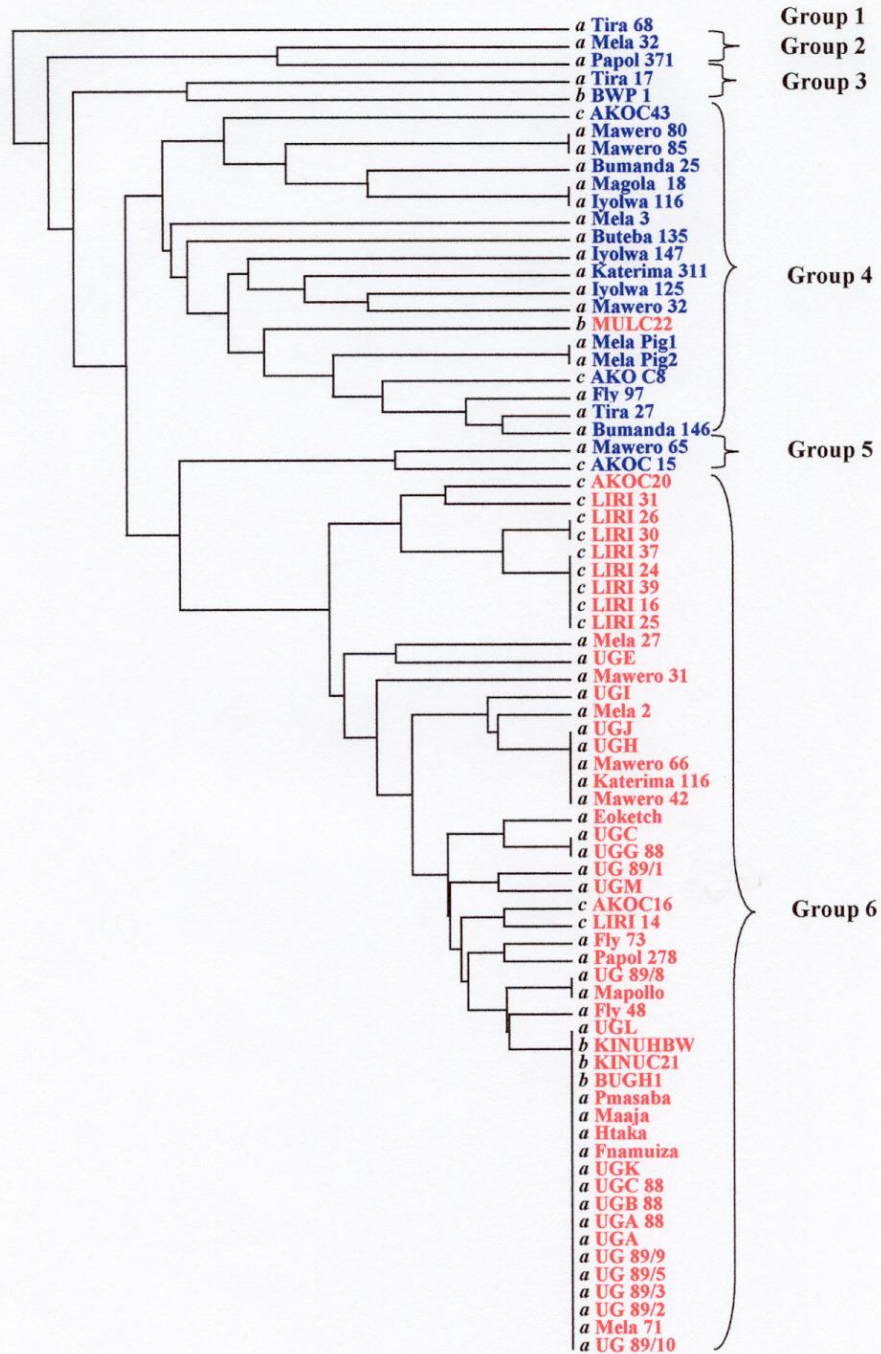
***a* UGE**

***a* UGG 88**

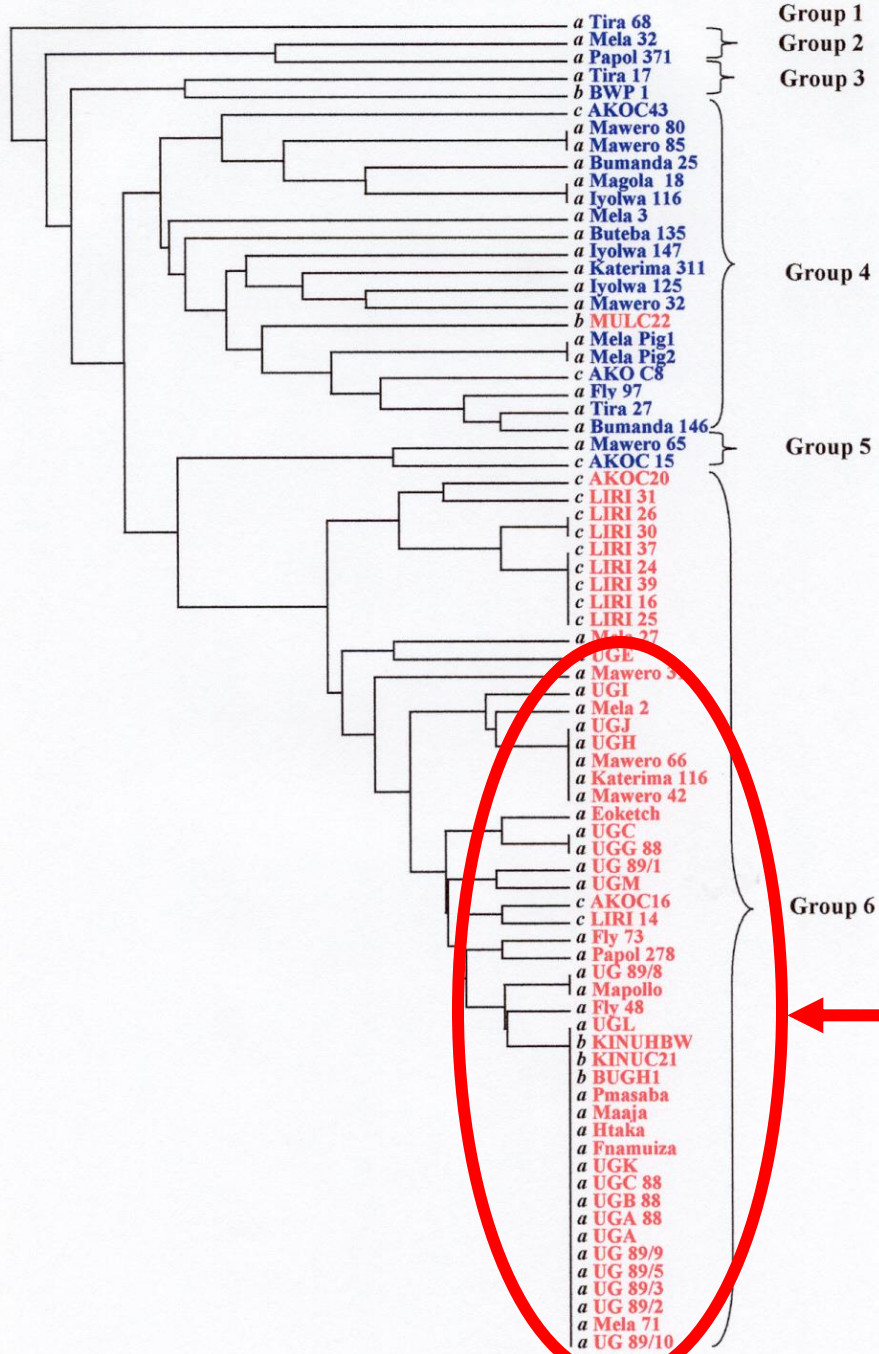
***a* UGM**

Water

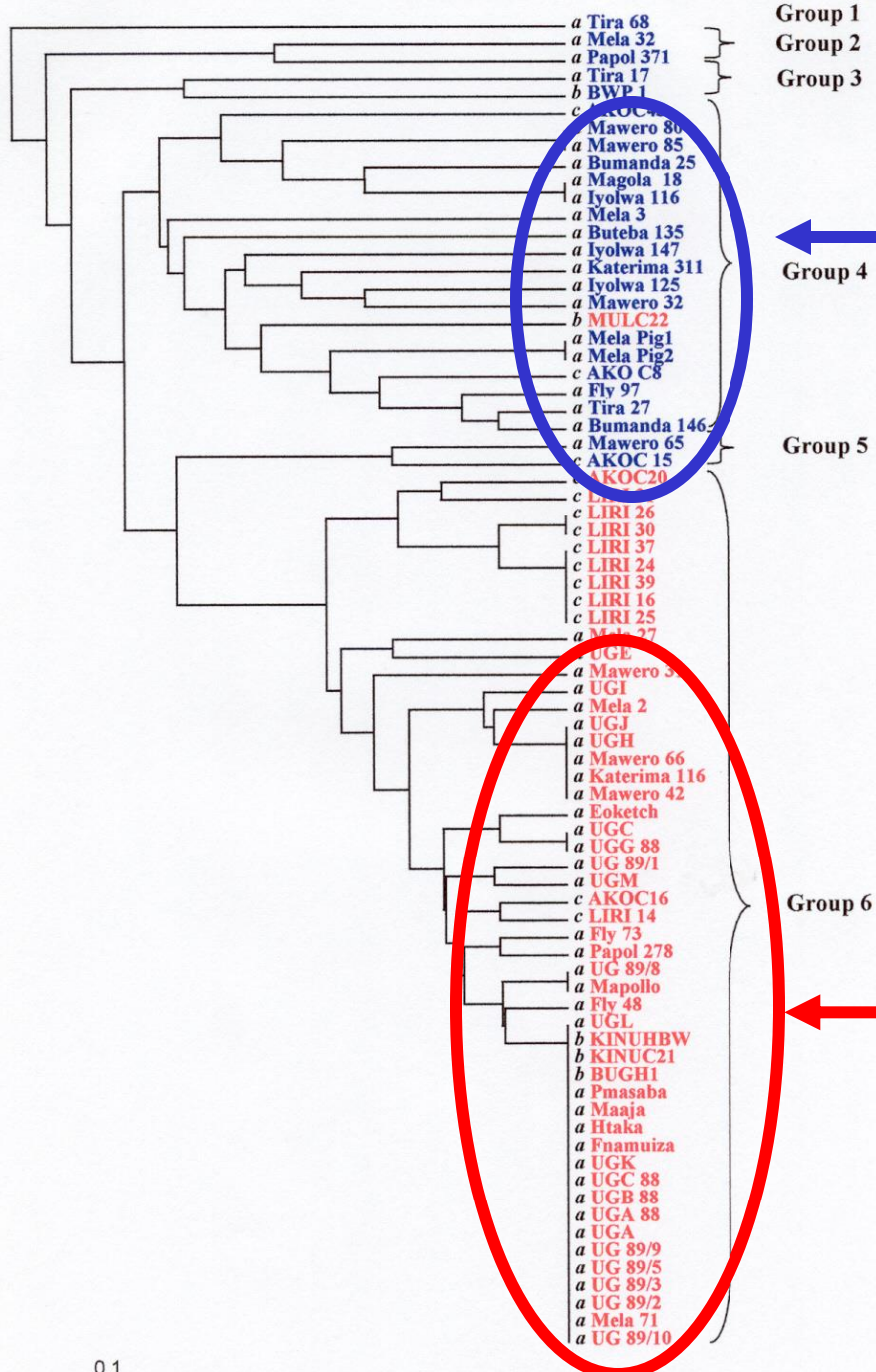




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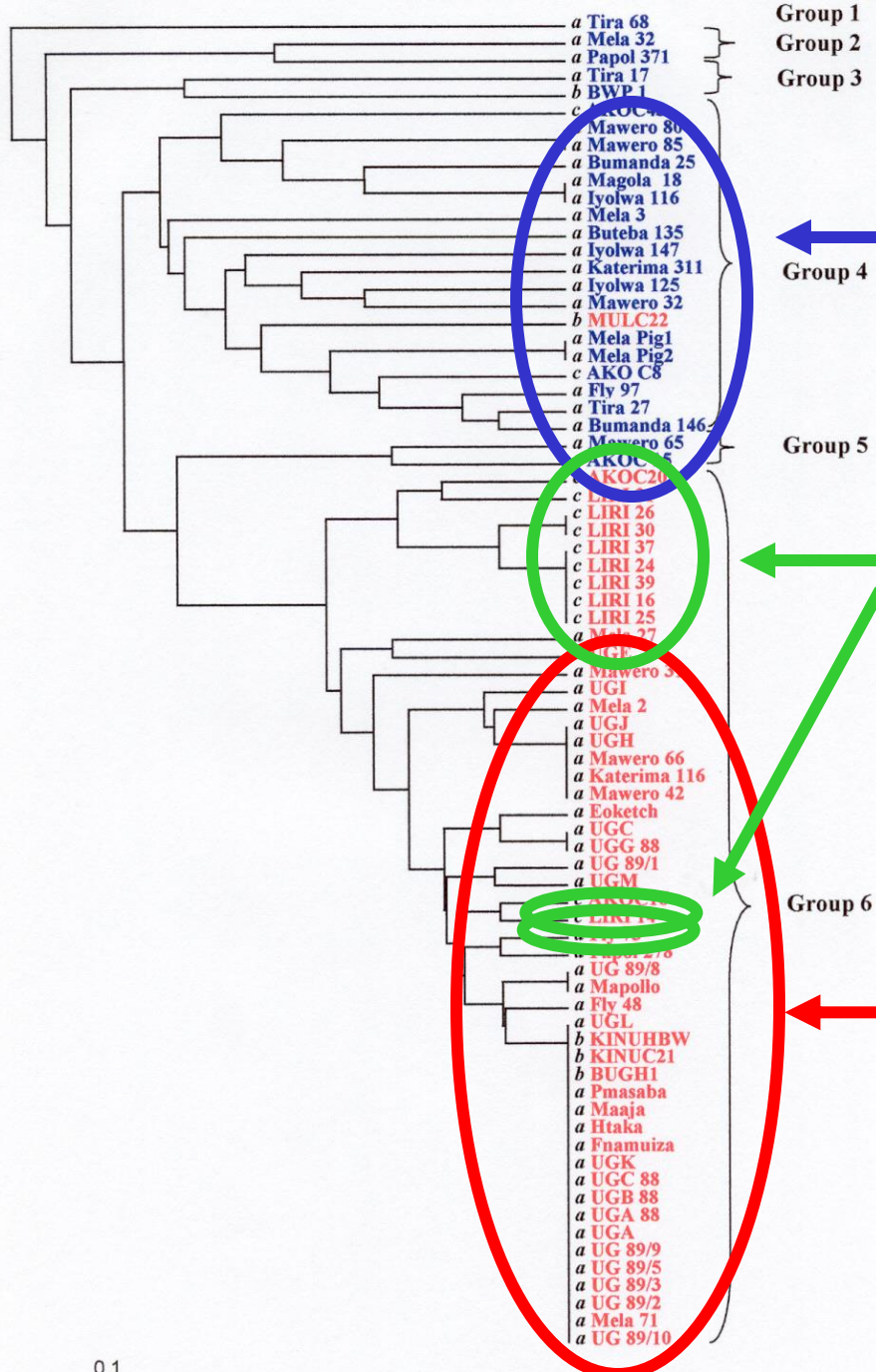


T.b.rhodesiense
from Tororo



T. b. brucei from Tororo

T. b. rhodesiense from Tororo

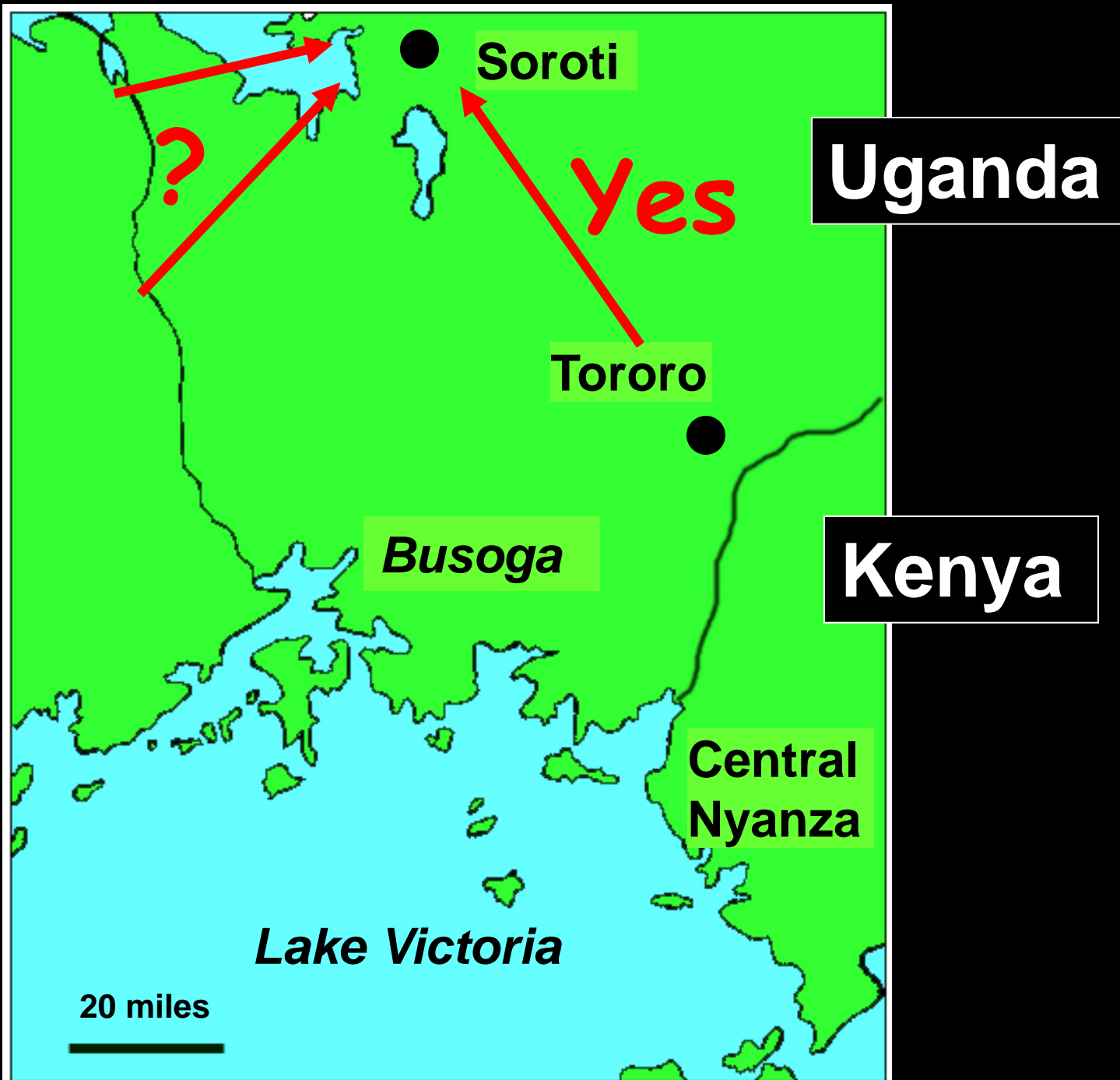


Group 1
Group 2
Group 3
Group 4
Group 5
Group 6

T. b. brucei from Tororo

T. b. rhodesiense from Soroti

T. b. rhodesiense from Tororo



Conclusions

- Two *T.b.rhodesiense* isolates in Soroti from Tororo (18%)
- Others from another source
- MGE-PCR a useful tool for tracking

Conclusions

- The origins of the Soroti epidemic may be complex
- Cattle important
- Need to identify risk areas and cattle movement

Examples

Markers for strain typing in
Toxoplasma gondii

Markers for strain typing in *Toxoplasma gondii*

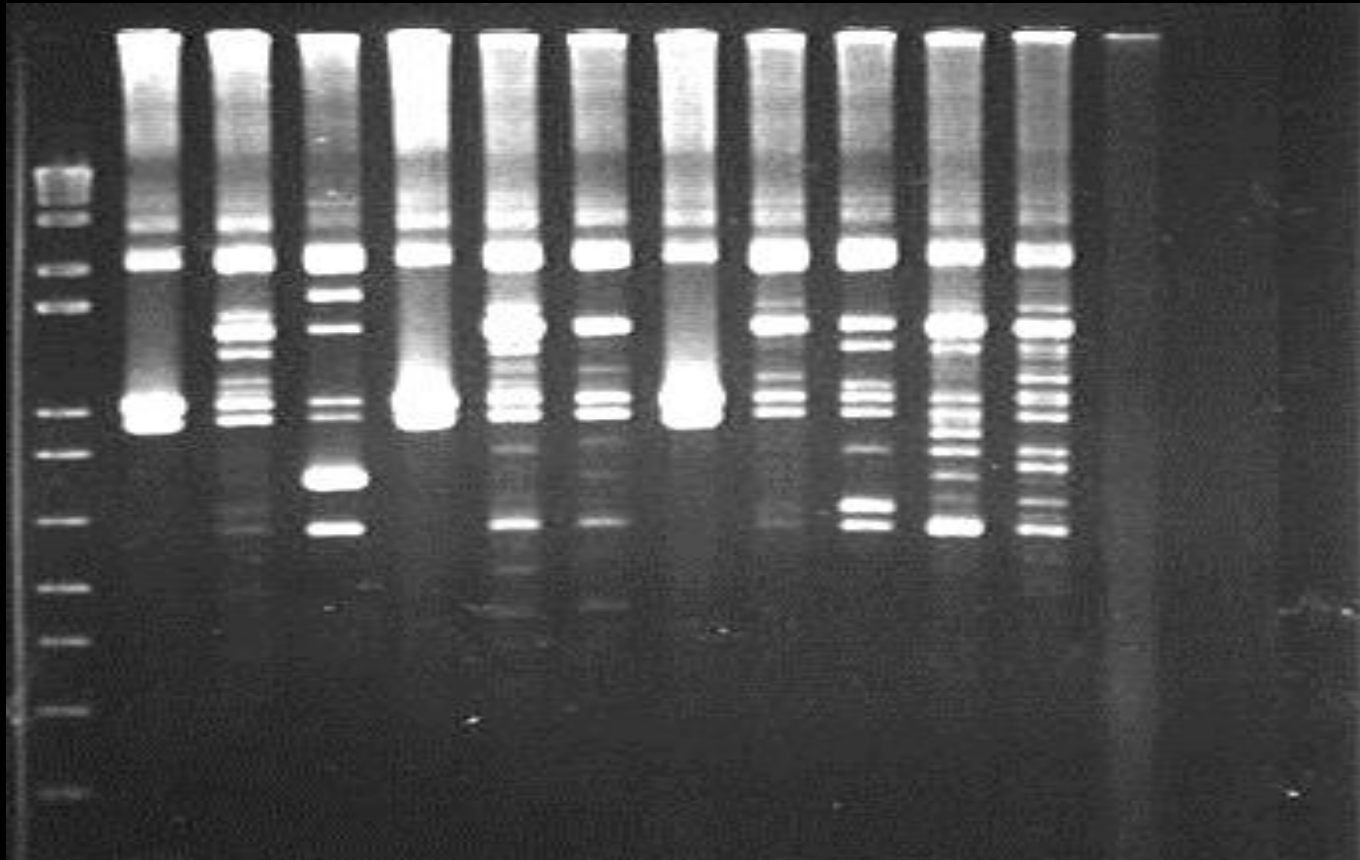
Traditional types:

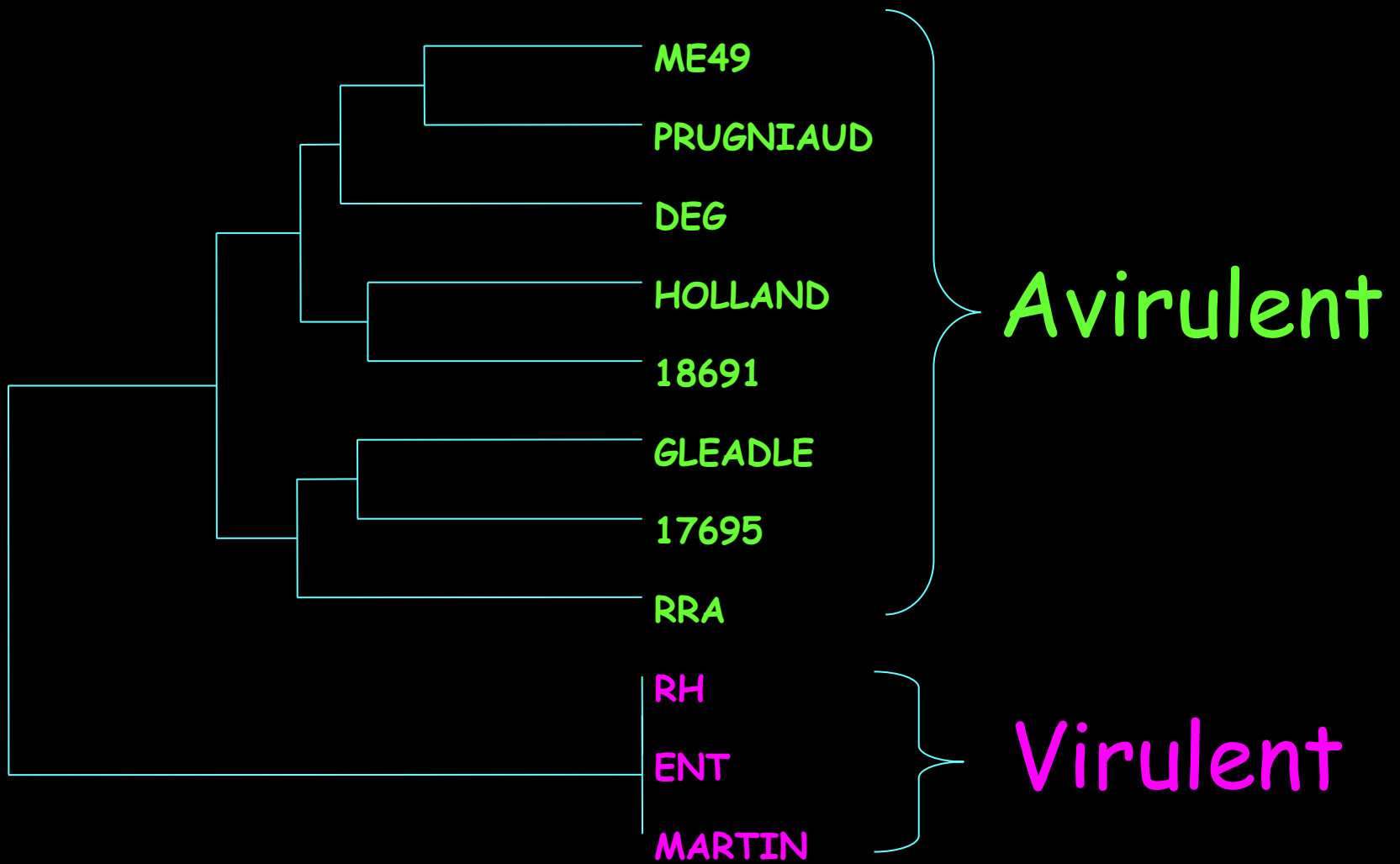
Type I virulent to mice

Type II avirulent to mice

Type III avirulent to mice

1 2 2 1 2 2 1 2 2 2 2





Conclusions

- MGE is a useful tool for tracking parasite strains and understanding epidemiology

Salford

Prof Geoff Hide

Dr Aimee Tilley

Dr Rebecca Terry

Dr Andy Cox

Dr Jackie Hughes

Edinburgh

Prof Sue Welburn

Dr Aimee Tilley

Dr Eric Fevre

Dr Kim Picozzi

Prof Ian Maudlin

Uganda

Dr Martin Odiit
and colleagues