

A RECENT OUTBREAK OF NEUROLOGICAL DISEASE IN THE EQUID POPULATION OF THE CENTRAL RIVER DISTRICT OF THE GAMBIA



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Clinical problem

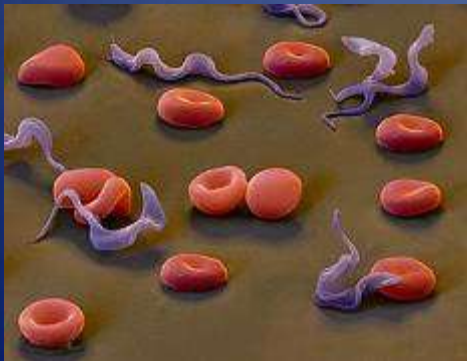
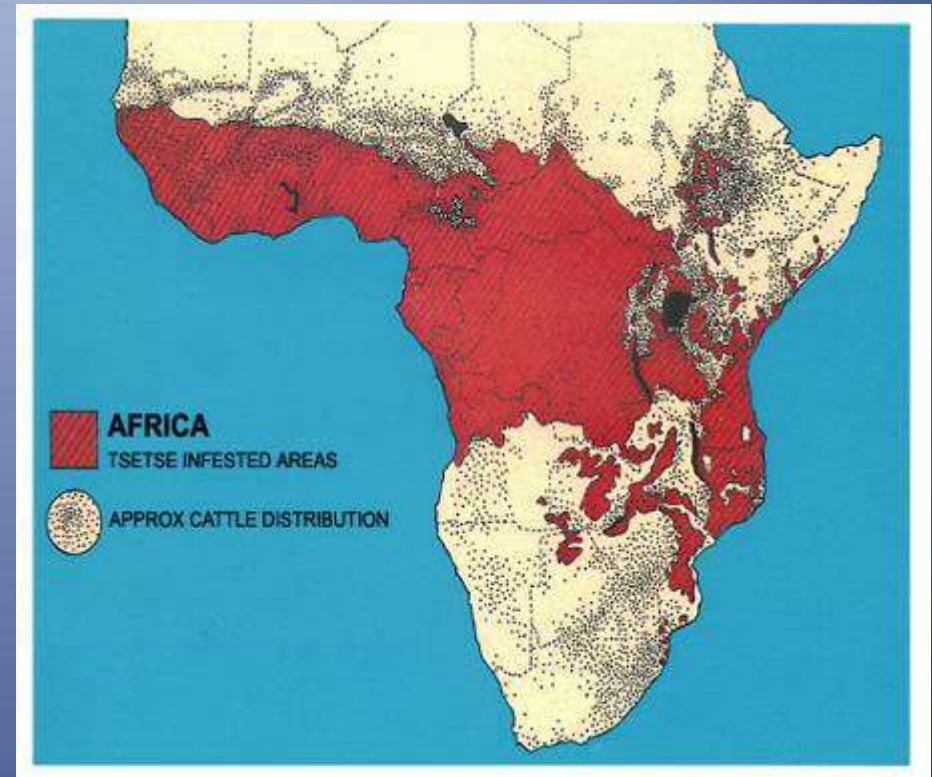
- Cases of neurological disease noticed by the Gambia Horse and Donkey Trust since 2000
- Increasing prevalence
- Unresponsive to empirical treatment
- High mortality
- Clinical presentation not well characterised before study

Possible differential diagnosis

- Equids recently introduced to the Gambia
- Very little is known about the infectious diseases that affect this population
- It is likely that they are highly susceptible to endemic livestock pathogens
- Trypanosomiasis was recently identified as a major cause of morbidity in the equid population of the Gambia

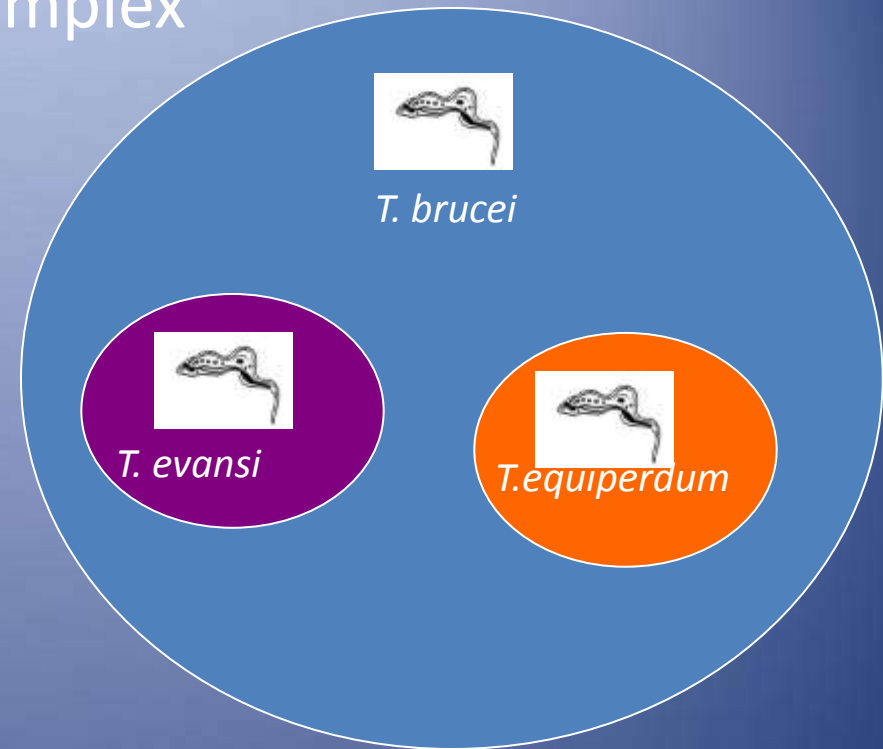
Trypanosomiasis

- Single-cell blood-borne parasite
- Three major species
- *Trypanosoma congolense*
- *Trypanosoma vivax*
- *Trypanosoma brucei*
- Transmitted by tsetse fly
- Millions livestock infected/year
- Cause huge economic losses to agriculture in Africa, estimated to be \$1.4 billion/annum



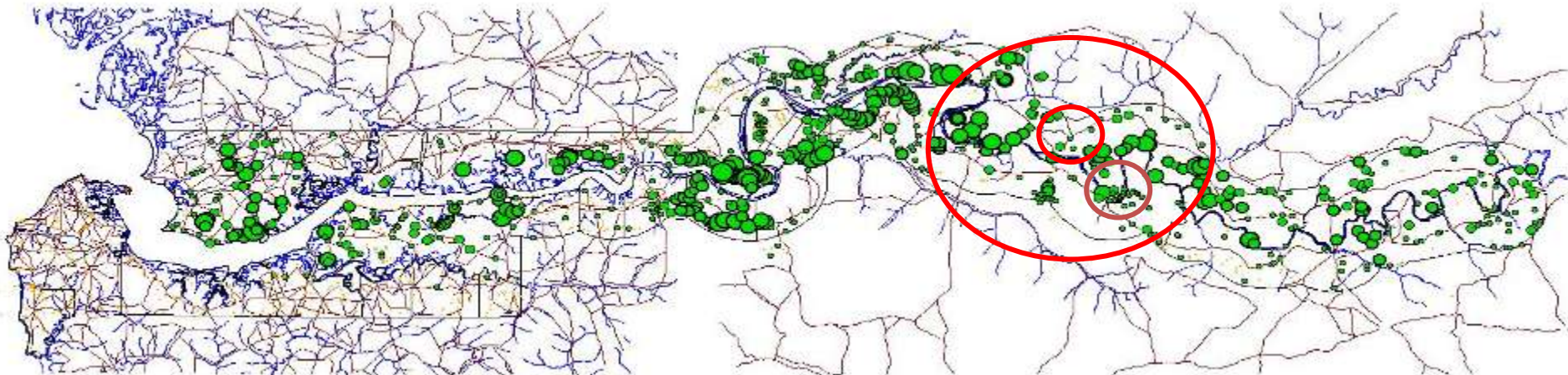
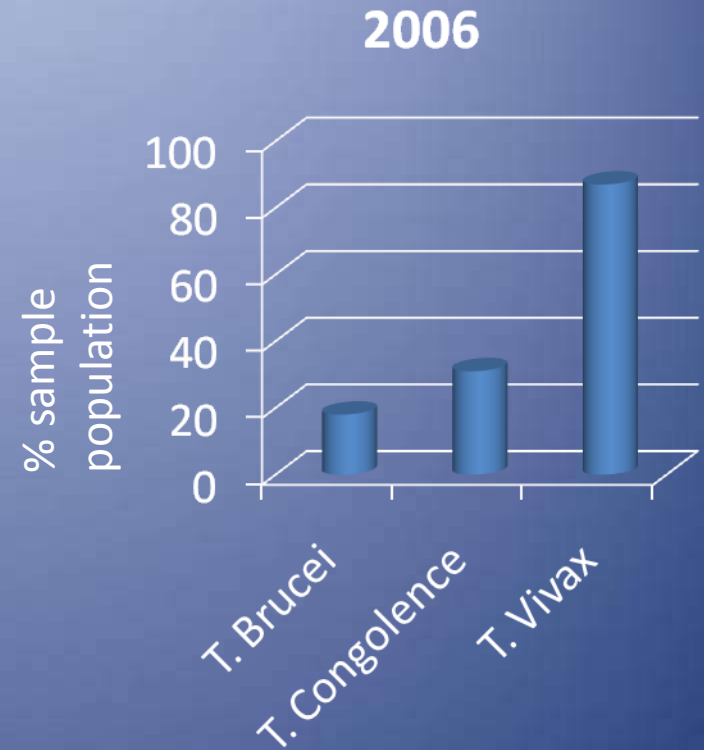
Trypanosoma brucei species complex

- *T. brucei* species are associated with neurological disease ('sleeping sickness' in humans)
- *T. evansi* and *T. equiperdum* have been shown to cause neurological disease in equids
- *T. evansi* and *T. equiperdum* are mutants of *T. brucei* and have adapted to different modes of transmission
- *T. equiperdum* is venereally transmitted ('dourine')
- *T. evansi* is transmitted mechanically by biting flies
- There is no diagnostic test that will reliably differentiate between these – all 3 may occur in The Gambia



Previous research

- Pinchbeck et al 2008
- Overall trypanosome prevalence of 91% using sensitive PCR based detection methods
- Multiple species were present in 43% of infections



Differential diagnosis

- Minimal clinical information at this point
- DDx for neurological disease in this area include:
- Trypanosomiasis
- Babesia
- West Nile virus
- Equine encephalosis virus
- Equine herpes virus
- Rabies

Study Design

- Matched case control study
 - Case definition: Any equid showing consistent neurological signs
 - MATCHED to control by species, time and village
 - 2 controls per case

Study design – data collection 1

Neurological case control study

Case ID:	Case:	Control:	Group No:	Place of sampling:

Case definition: Animal showing neurological signs. i.e. Central or peripheral (Presented at market, to clinic, or at village) Signs have developed in last 2 months.

Control selection: Animal from same area/market that is not showing neurological signs. For those at market take the next 3 cases presented (if village is nearby get village control as well). For those in village take next 3 cases presented). For those presented at GHDT get 3 x village control if possible.

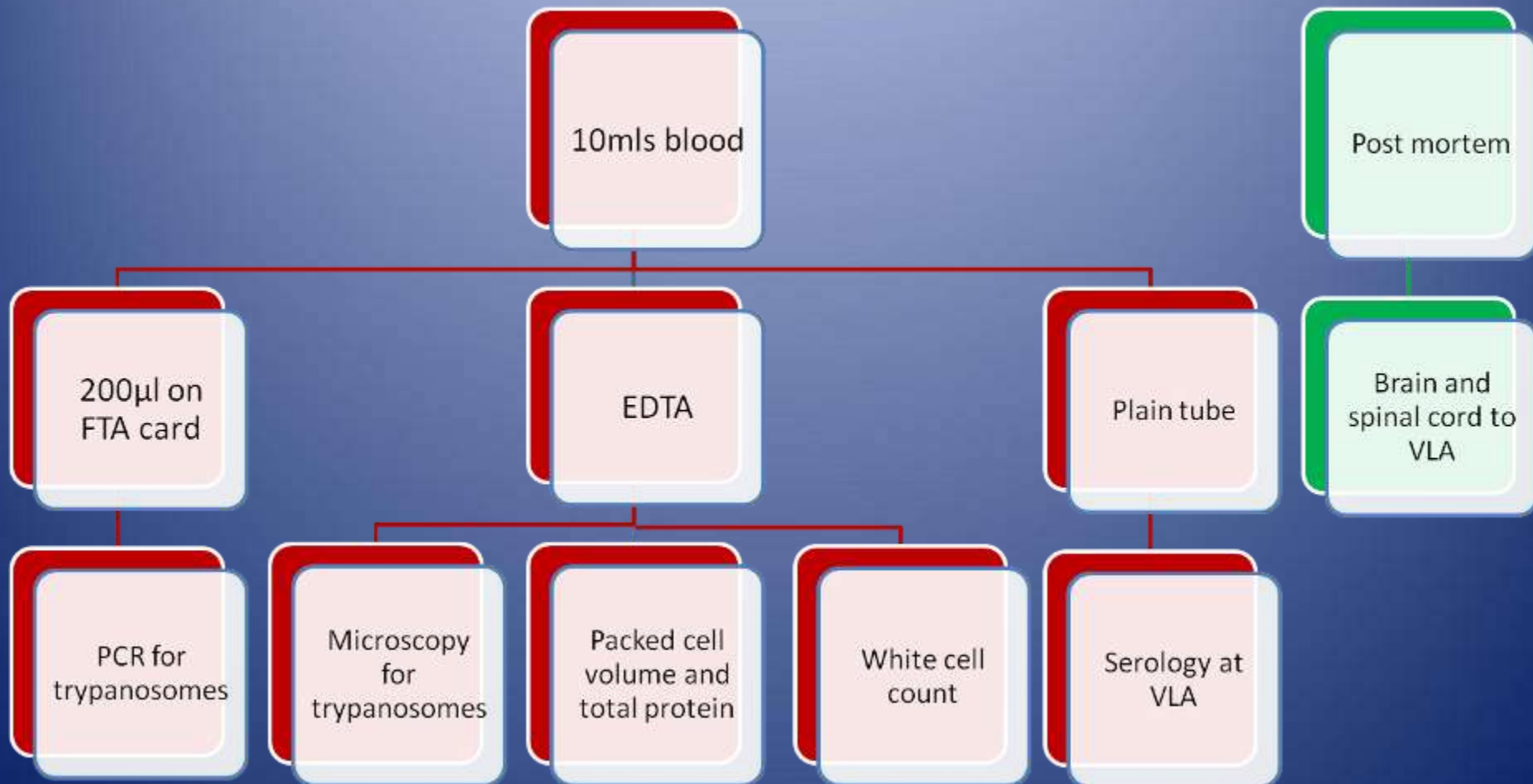
1) History:

Age:	Species:	Sex:
Owner name:	Owner village:	Hrs work/day:

- a) How long have they owned it/it recently purchased, where from:
- b) What is it fed/how much water drunk:
- c) Has it received worming treatment/if so what:
- d) Has it had any disease problems in the past (lameness/ataxia/weight loss/dull/diarrhoea/eyes/wounds...)/if so what:
- e) Has it received treatment for anything before, if so: what was it/where did they get it from/how given/who given:
- f) What are the clinical signs that animal has been showing recently in order of when they first occurred:

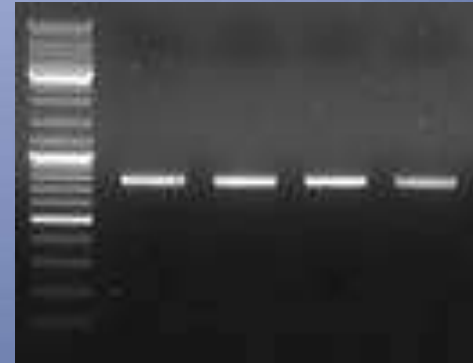
- History
 - Village
 - Length ownership
 - Age
 - Any previous disease
 - Any previous treatment
 - Duration of present disease
 - Signs of present disease
- Clinical examination
 - Full clinical examination including detailed neurological examination

Study design – data collection 2



Laboratory tests

- PCR – *T. brucei*, *T. congolense* and *T. vivax*
 - Strong positive, weak positive, negative

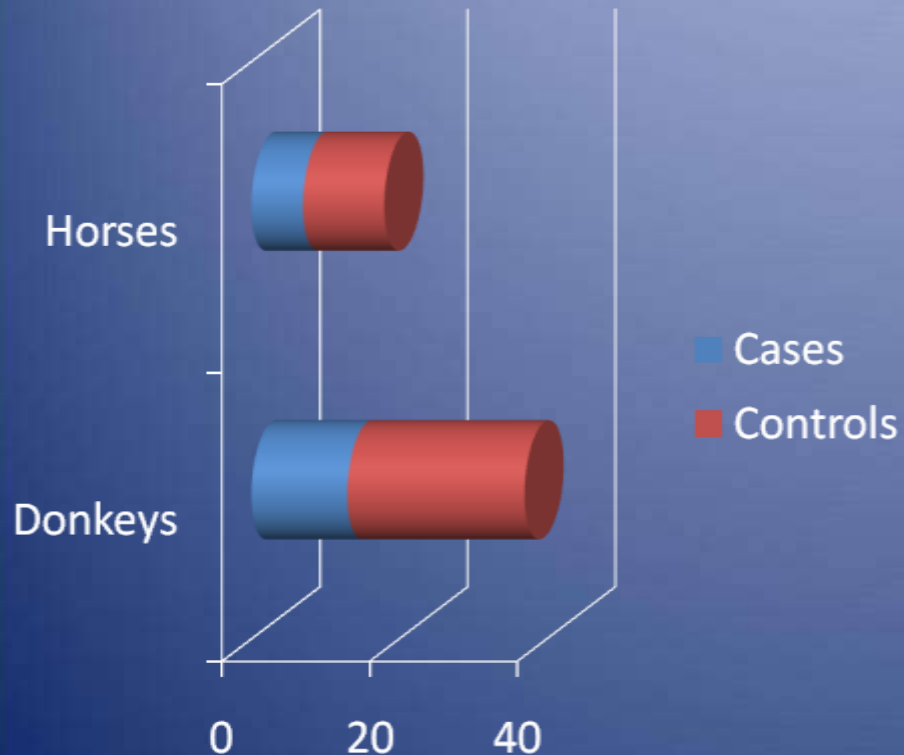


- Serology
 - *T. equiperdum*, West Nile Virus, Equine Encephalosis Virus, Equine Herpes Virus



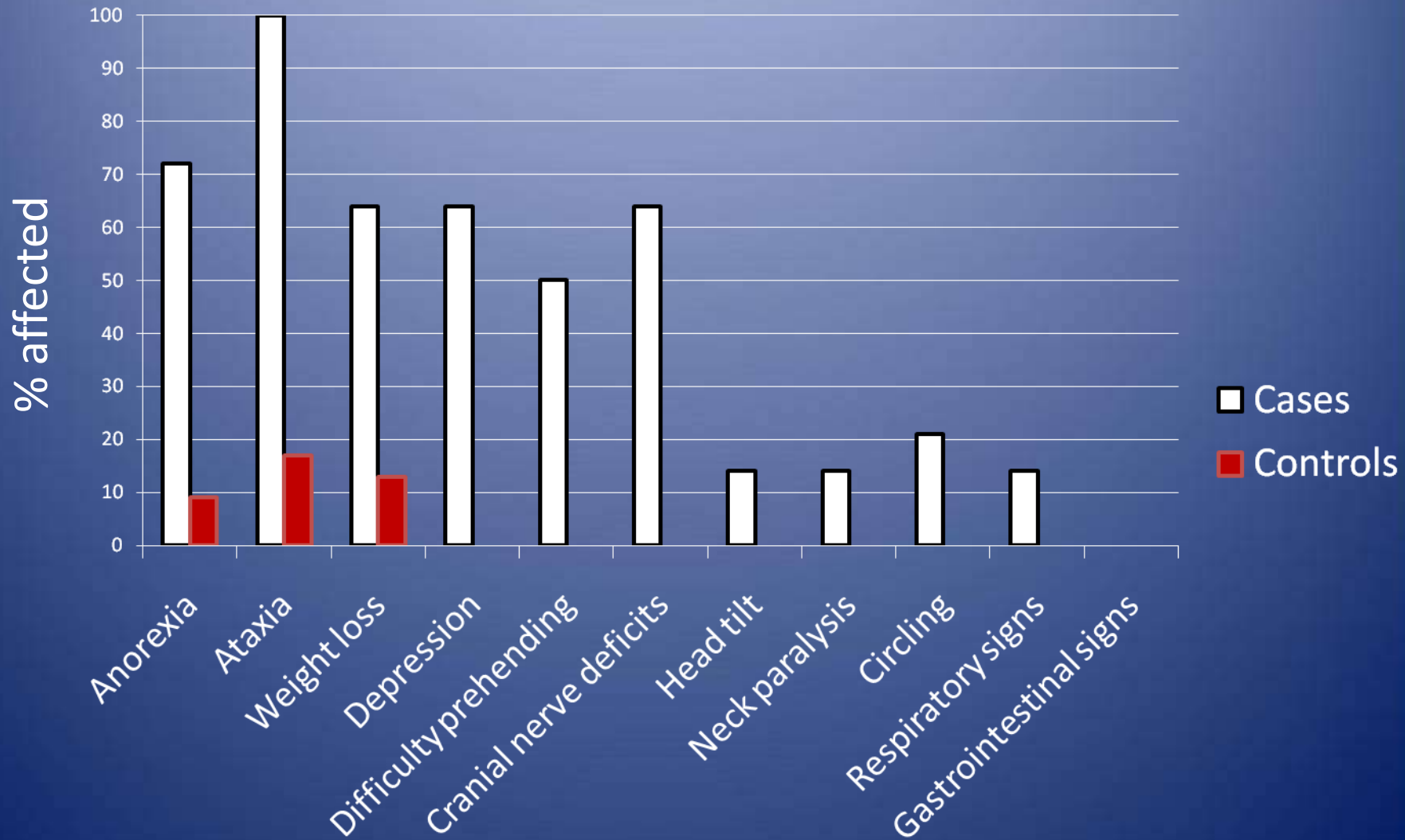


Results

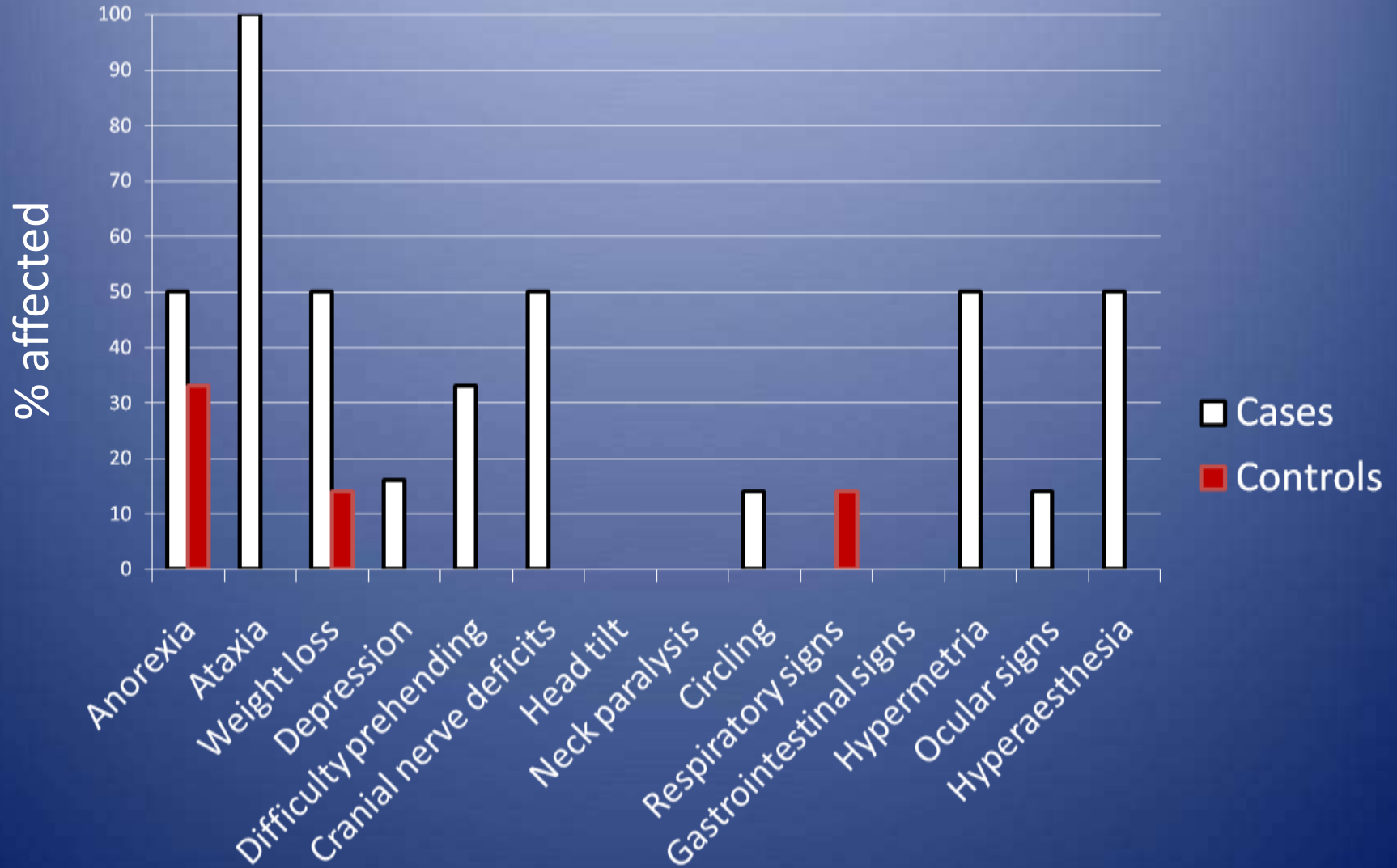


- 20 cases
 - 14 donkeys
 - outcome known in 12/14 cases
 - Mortality 86%
 - 6 horses
 - Outcome known on 4/6 cases
 - Mortality 75%
- 36 controls
 - 23 donkeys
 - 11 horses

Donkeys



Horses



Disease progression

Chronic progressive weight loss
and ataxia



Sudden deterioration

Grade 5 ataxia

Recumbency

Circling

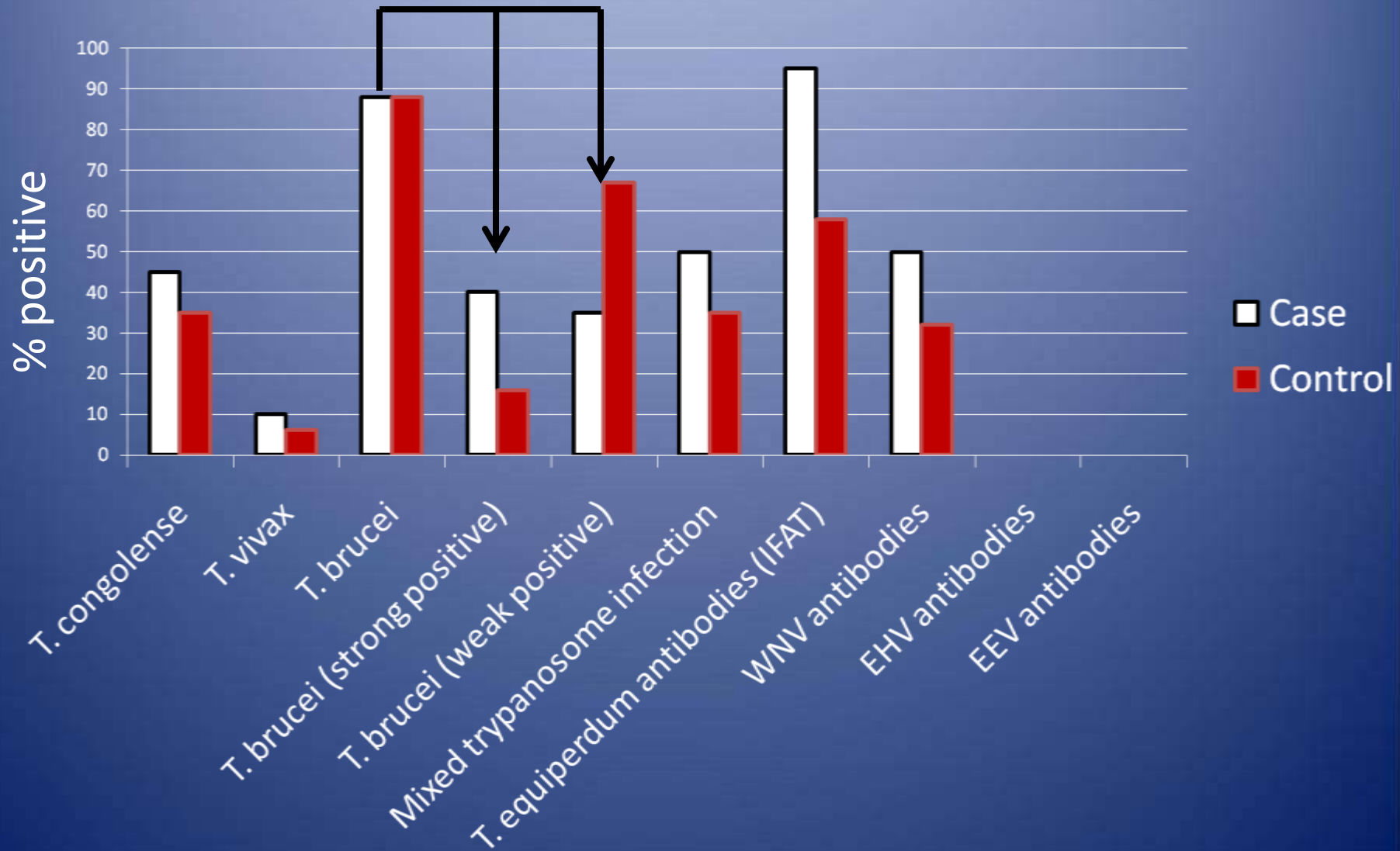
Paddling

Blindness

DEATH OR EUTHANASIA



PCR and Serology



Associations

- Uni-variable matched conditional logistic regression. $P < 0.05$

Clinical variable	Odds ratio	P value
<i>T. equiperdum</i> serology (VLA) +ve verses –ve	10.8	0.02
Recent treatment with melarsamine hydrochloride (Cymelarsan) (verses none)	18.8	0.04
Recent treatment with isometamidium chloride(Samorin) (verses none)	10.2	0.05
<i>T. brucei</i> PCR strong +ve (verses negative)	8.1	0.16
Reducing condition score (continuous)	0.08	0.008
Capillary refill time > 2	9.4	0.03
Very pale mucous membranes (verses pink mucous membranes)	10.9	0.05

Multivariable model of potential causes

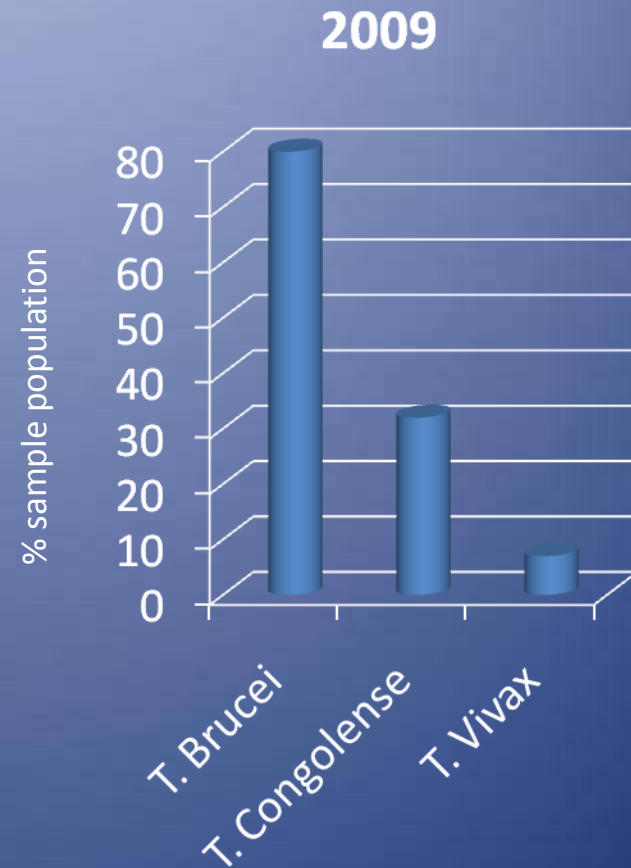
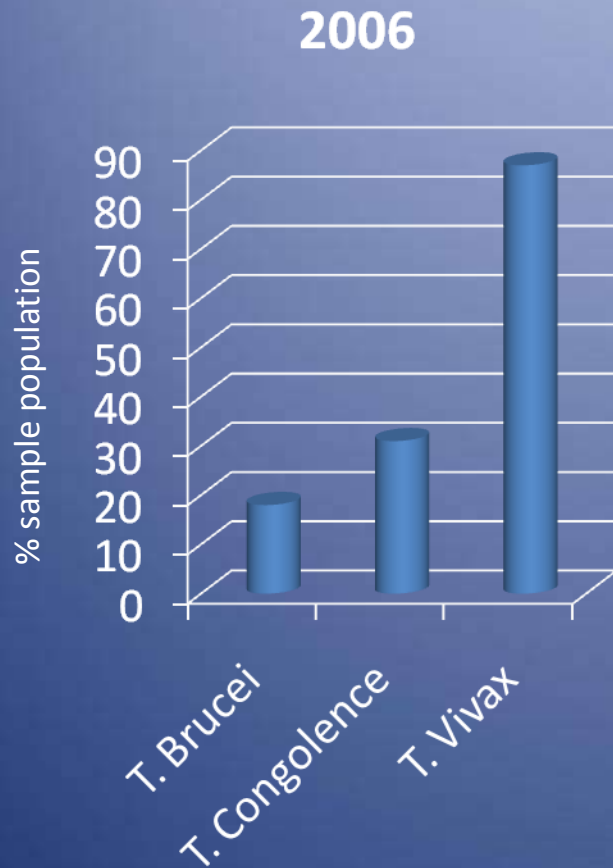
TERMS	Odds ratio	P-Value
Model 1		
<i>T. equiperdum</i> serology (VLA) +ve verses –ve	11.1	0.04
Recent treatment with melarsamine hydrochloride (Cymelarsan) (verses none)	27.5	0.05
Model 2		
<i>T. brucei</i> PCR strong +ve (verses negative)	17.7	0.1
Recent treatment with melarsamine hydrochloride (Cymelarsan) (verses none)	5.78	0.05

Summary of findings

- Aetiological agents associated with cases
 - Antibodies to *T. equiperdum*
 - Strongly positive PCR result for *T. brucei*
- Clinical presentation of the cases is consistent with cerebral trypanosomiasis previously described in equids (Berlin et al 2009; Brun et al 1998)

Clinical Sign	<i>T. equiperdum</i>	<i>T. evansi</i>	Cases
Anaemia	Green	Green	Black
Fever	Green	Green	Green
Depression	Green	Green	Green
Abortion	Green	Green	Black
Skin plaques	Green	Green	Black
Genital oedema	Green	Black	Black
Emaciation	Green	Black	Green
Spleen and lymph node enlargement	Black	Green	Black
Ataxia	Green	Green	Green
Hyperaesthesia	Green	Green	Green
Hypermetria	Green	Green	Green
Cranial nerve abnormalities	Green	Green	Green
Circling	Green	Green	Green
Head turn	Green	Green	Green
Blindness	Green	Green	Green

Prevalence of strains



Biased population OR shift in the relative prevalence of species

Treatment

- If trypanosomiasis is the cause of the symptoms in these cases they appear unresponsive to treatment
- 25% cases deteriorated rapidly after treatment with trypanocide (specifically isometamidium chloride)
- Post treatment reactive encephalopathy recognised in humans, especially with sub-curative doses (Pepin et Milord 1994)

Conclusions

- This study suggests that there is an association between trypanosome infection and the neurological disease affecting this equid population
- Not definitively diagnosed
- Other factors/pathogens may be involved

Further work

- Ongoing
- Increase case numbers
- Identify trypanosomes in central nervous system
 - Cerebral spinal fluid
 - Choroid plexus
 - Histopathology
- Identify specific species/strain involved
 - Theoretical technique using PCR
- Controlled treatment trials
- Identify any other pathogens involved

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All GHDT employees



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Dr Liam Morrison
Craig Duffy
Dr Annette MacLeod



Any questions?



Reasons for failure of treatment

- Trypanosomes are not the cause of the neurological symptoms in these cases
- Drug resistance or ineffective concentrations in CNS
- Incorrect drug choice
- Under dosing – increases risk of PTRE
- Incorrect administration
- Cryptic foci leading to re-emergence of infection

Species differentiation

- DNA (kDNA maxicircles) involved in pro-cyclic stage in tsetse fly. Absent in *T. evansi*, partially absent in *T. equiperdum* (Lai et al 2008)
- Both *T. evansi* and *T. equiperdum* have major antigen VSG, *T. brucei* has a different version
- Could potentially differentiate – but many had mixed infections and looking for a negative