Resistance to Trypanocidal drugs

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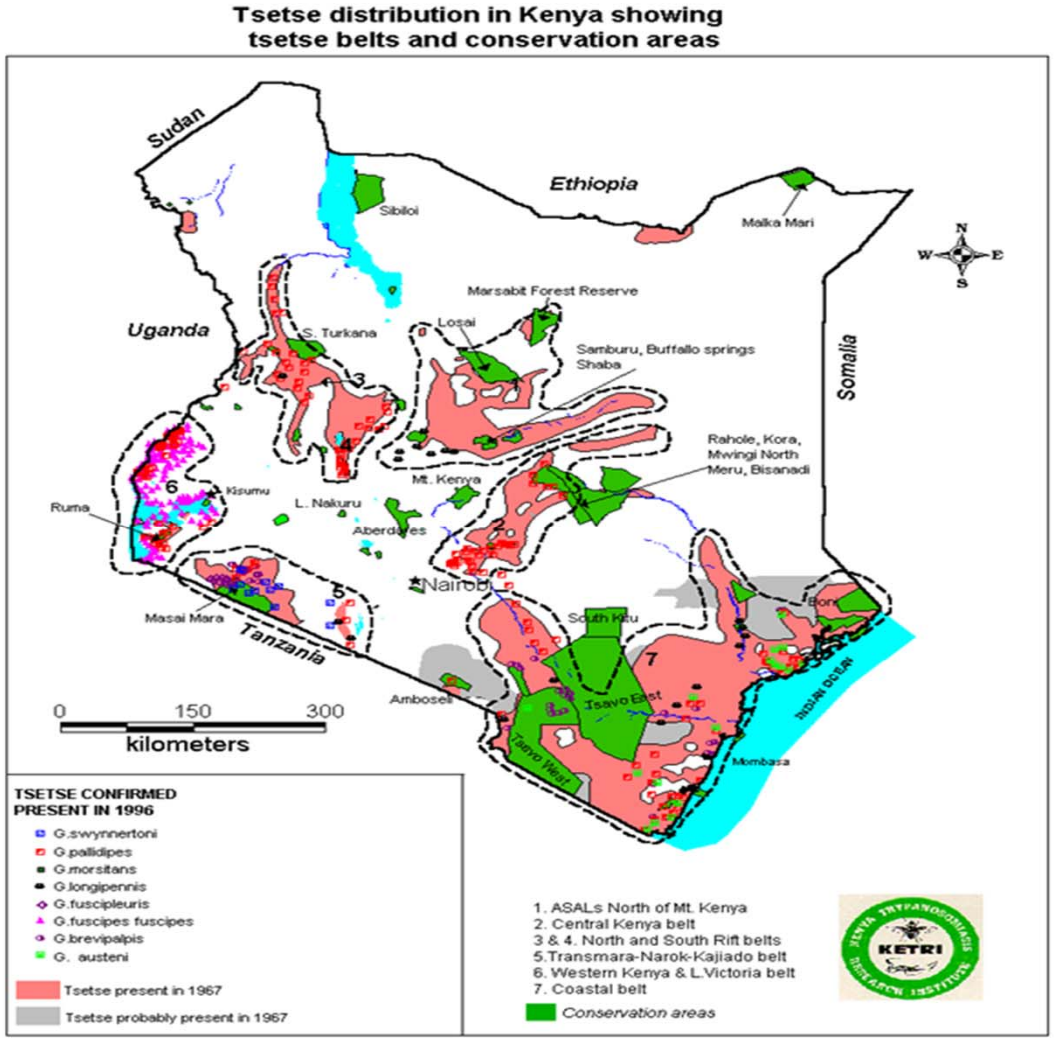
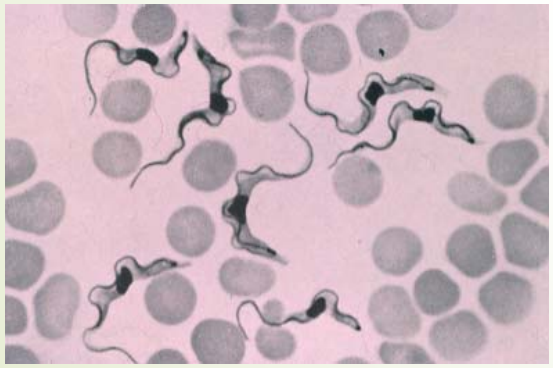

Tsetse distribution in Kenya showing
tsetse belts and conservation areas
Sudan
Ethiopia
Somalia
Uganda
Tanzania
Sibiloi
Malka Mari
Marsabit Forest Reserve
Losai
S. Turkana
Samburu, Buffallo springs
Shaba
Rahole, Kora,
Mwingi North
Meru, Bisanadi
Mt. Kenya
Kisumu
L. Nakuru
Ruma
Aberdares
Nairobi
Masai Mara
South Kitu
Amboseli
Tsavo East
Tsavo West
Mombasa
INDIAN OCEAN
0
150
300
kilometers
N
W
E
S
1
2
3
4
5
6
7
TSETSE CONFIRMED
PRESENT IN 1996
G.swynnertoni
G.pallidipes
G.morsitans
G.longipennis
G.fuscipleuris
G.fuscipes fuscipes
G.brevipalpis
G. austeni
Tsetse present in 1967
Tsetse probably present in 1967
1. ASALs North of Mt. Kenya
2. Central Kenya belt
3 & 4. North and South Rift belts
5.Transmara-Narok-Kajiado belt
6. Western Kenya & L.Victoria belt
7. Coastal belt
Conservation areas
KETRI

The disease and its Vector

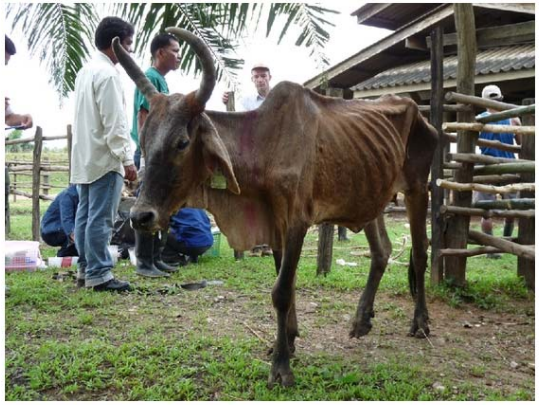
- HAT (sleeping sickness) transmitted by the tsetse fly
- Two forms of HAT exist (acute – *T.b. rhodesiense* and chronic – *T.b. gambiense*) and two stages (early and late)

In livestock the disease is caused by *T. congolense*, *T. vivax* (cattle) and *T. evansi* (camels) – tsetse and non-tsetse transmitted



trypanosomiasis in both humans and livestock is fatal but treatable

Trypanosoma brucei evansi

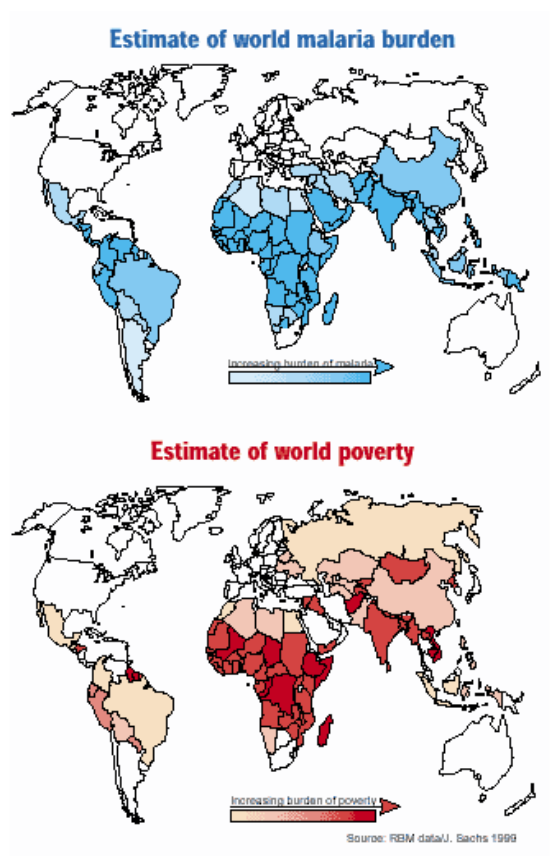


T.b. evansi, the most widely distributed parasite (Asia, Africa, S. America

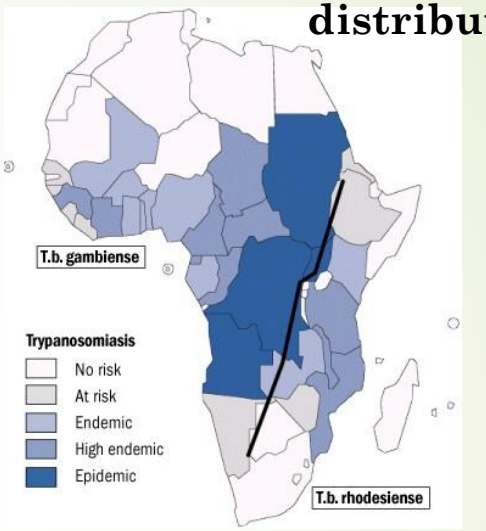
- Joshi PP el al., Am J Trop Med Hyg. 2005 Sep;73(3):491-5.
- **Human trypanosomiasis caused by *Trypanosoma evansi* in India: the first case report.**

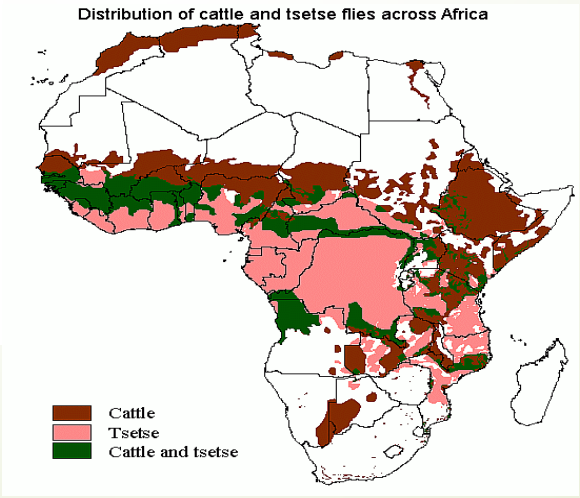
Pathogen evolution???

Diseases of poverty



Sleeping sickness distribution





Cattle & tsetse distribution

Disease Control

- Vector control methods
- Parasite control methods
  - Trypanotolerant breeds
    - Disease tolerance vs productivity
  - Vaccination
    - None due to antigenic variation
    - Transmission blocking (genomics/genetics)
  - Chemotherapy and chemoprophylaxis
    - most important strategy for the control of trypanosomiasis in African livestock

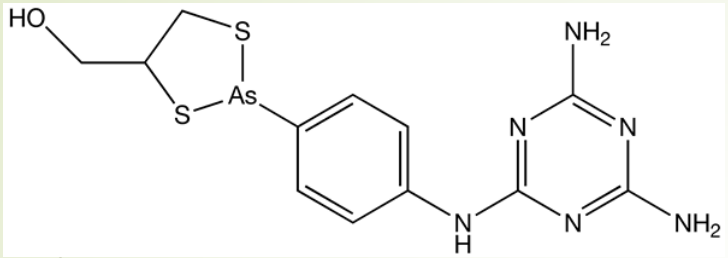
Current treatments for HAT and associated problems

Stage 1

- Pentamidine (1940)
  - 10 day injections
- Suramin (1920)
  - Used primarily for rhodesiense SS

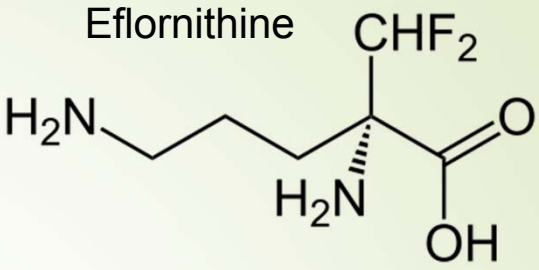
Stage 2

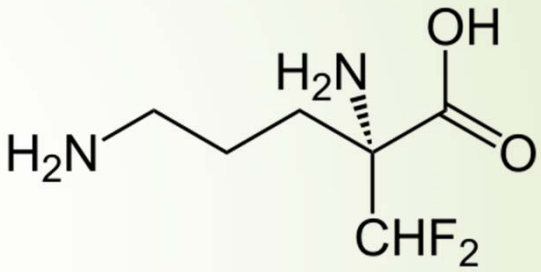
- Melarsoprol (1949)
  - highly toxic, 5% tretament related mortality; increasing treatment failure up to 30%)
- Eflornithine (1981)
  - Difficult to administer, requires 4 infusions per day for 14 days
- NECT (2009), combination therapy

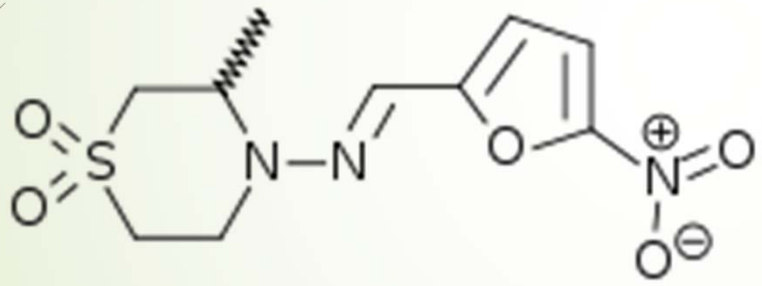


Melarsoprol

Eflornithine







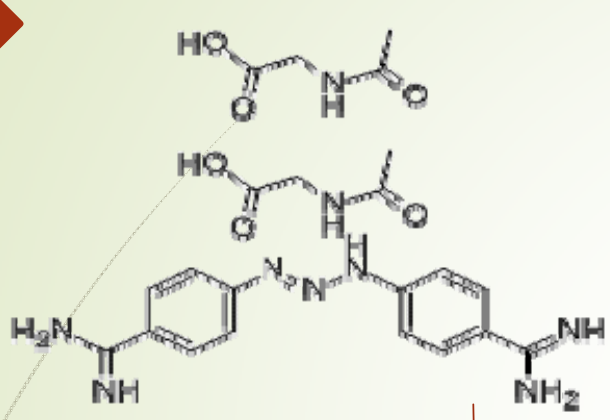
Nifurtimox

NECT = Nifurtimox Eflornithine Combination Therapy

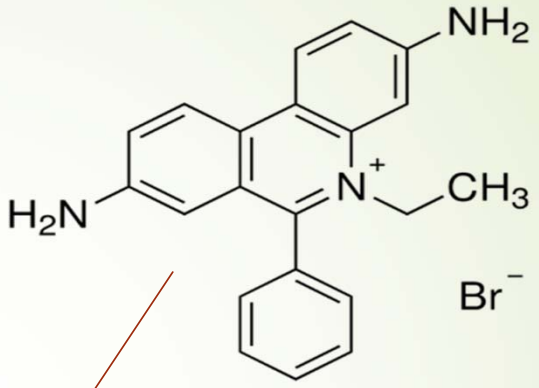
Commercial Animal trypanocides - AAT

Generic name	Trade name	Main application
Suramin	Naganol®	*T. evansi* in camels
Diminizene aceturate	Berenil, Ganaseg, Trypazen, Veriben	Cattle and small ruminants
Homidium bromide	Ethidium	Cattle and small ruminants
Homidium chloride	Ethidium C, Novidium	Cattle and small ruminants
Quinapyramine methyl sulphate	Antrycide, Trypacide, Noroquin, Quintrycide	*T. evansi* and *T. brucei* in camels and horses
Mel cy	Cymelarsan	*T. evansi* in camels
Isometamidium chloride	Samorin, Trypamidium	Cattle, as a curative at lower rates, as a prophylactic at higher rates.

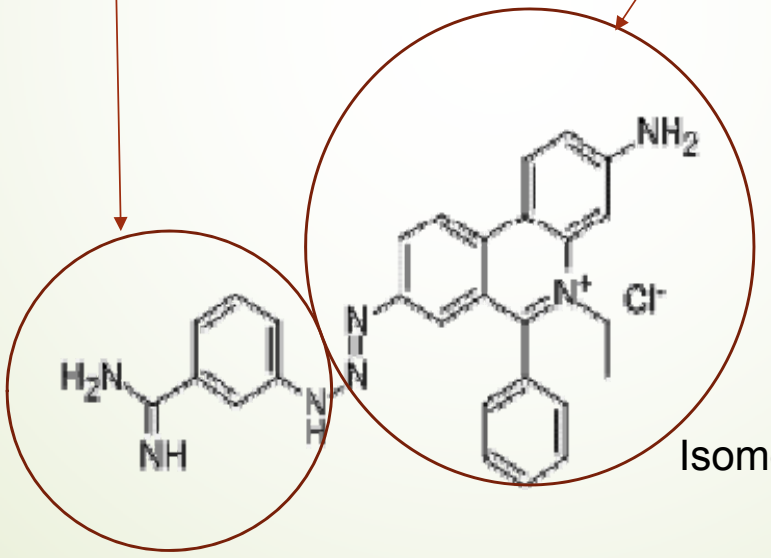
Drugs for treatment of trypanosomiasis in cattle and small ruminants



Diminazene di-aceturate
(Berenil®, Veriben®)

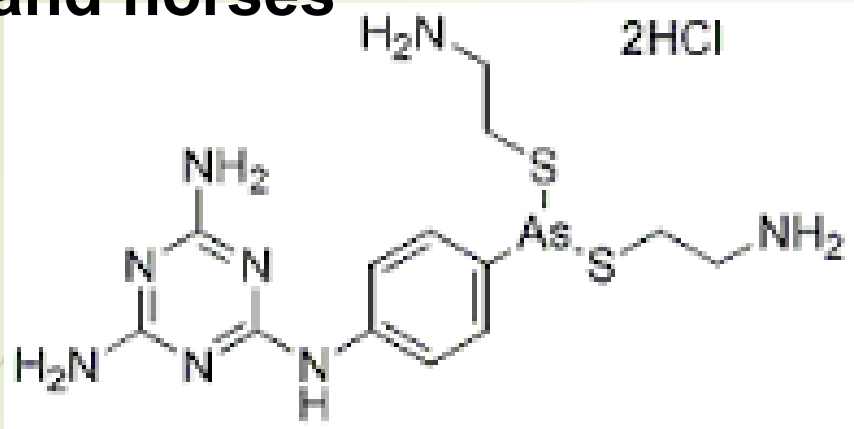


Homidium bromide / chloride
(Ethidium®, Novidium®)

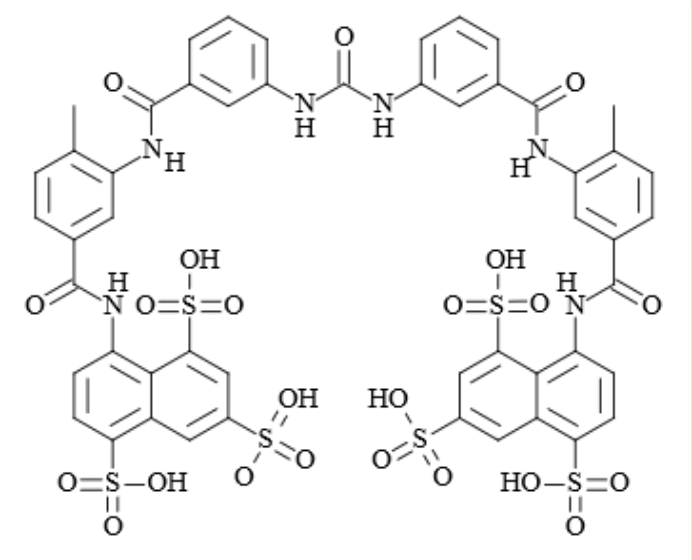


Isometamidium chloride (Samorin®)

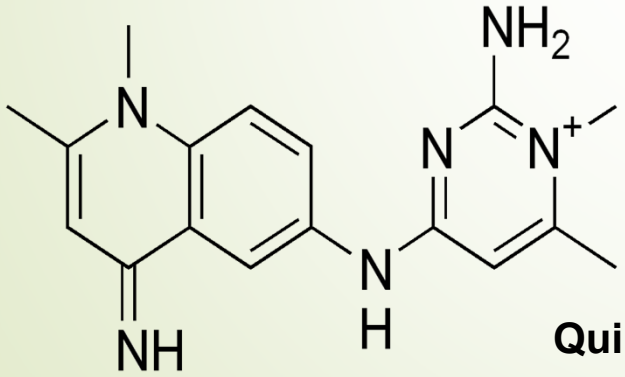
Drugs for treatment of trypanosomiasis in camels, donkeys and horses



Melarsenoxide cysteamine (Mel Cy)- 1985



Suramin (Germanin®); developed 1916, published 1924



Quinapyramine (Antrycide®)

Drug Use: Issues

- Poor diagnosis
- Poor estimation of weight
- Product Quality / counterfeits
- Access to quality products
  - Designated distribution points / private practice?
- Preparation (water quality) and administration E.g route
- Packaging – single vs multiple dosage
- Mixing of drugs (pastoral communities)
  - Antibiotics & trypanocides

- Role of immunosuppression
  - Osman AS[1], Jennings FW, Holmes PH (1992). **The rapid development of drug-resistance by *Trypanosoma evansi* in immunosuppressed mice.** Acta Trop. 50(3):249-57.
  - Using *T. evansi*, rapid development of high levels of resistance to Mel Cy, diminazene aceturate and isometamidium chloride through sub-curative treatments of infected immunosuppressed mice. Cross-resistance to pentamidine was also demonstrated. Normal immunocompetent mice infected with the same parent clones did not lead to the development of drug-resistance.

Lab: Development of drug resistance

- By repeated under-dosing and passage, drug resistance can be induced rapidly in drug-sensitive clones
- E.g.
  - Peregrine AS[1], Gray MA, Moloo SK (1997). Antimicrob Agents Chemother. 41(7):1604-6.
    - Cross-resistance associated with development of resistance to isometamidium in a clone of *Trypanosoma congolense*. Derivative was 94-fold resistant to ISMM, 3.4-fold to diminazene, 33-fold to homidium, 4.2-fold to quinapyramine
  - Ndoutamia G[1], Moloo SK, Murphy NB, Peregrine AS (1993). Antimicrob Agents Chemother. 37(5):1163-6.
    - Derivation and characterization of a quinapyramine-resistant clone of *Trypanosoma congolense*. Derivative 40-fold resistant to quinapyramine, 8-fold to ISMM, 28-fold to homidium and 5.5-fold to diminazene. The resistant clone was cyclically transmitted by *G.m. centrals*. Cross resistance was demonstrated

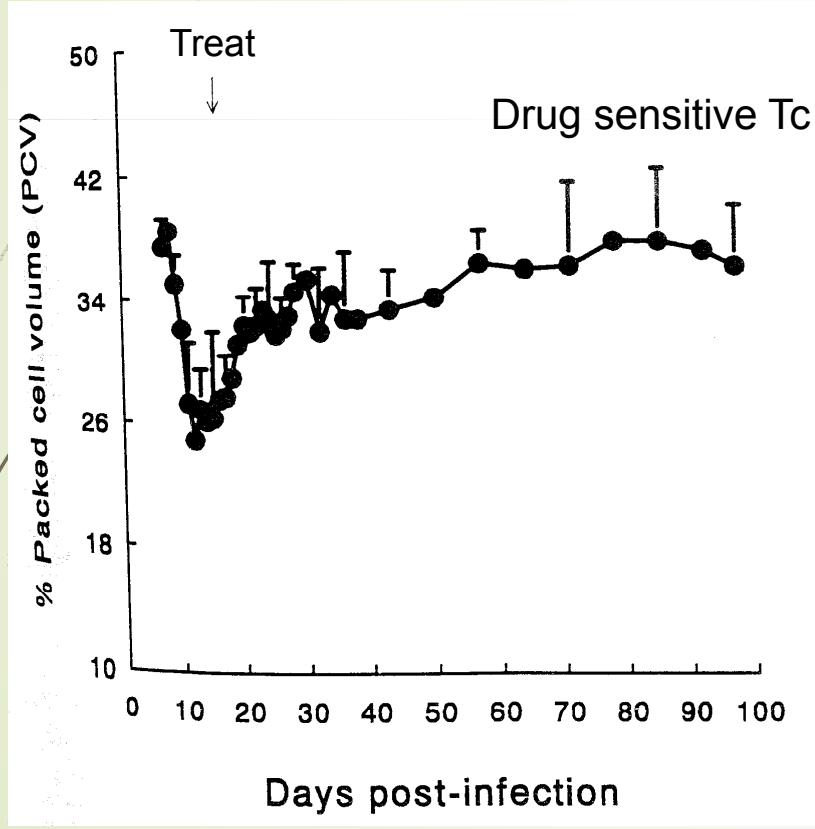
Field: Treatment failure

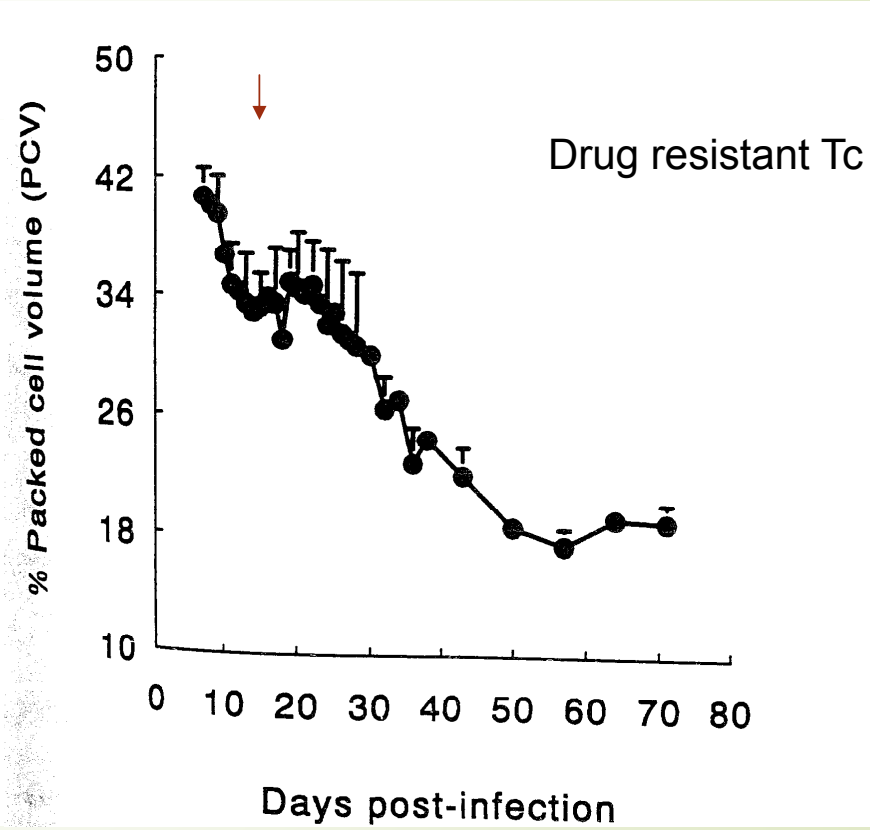
- Role of immunosuppression
  - Malnourished animals, poor body condition
- High Disease Challenge
  - Dolan RB[1], Stevenson PG, Alushula H, Okech G. (1992). Failure of chemoprophylaxis against bovine trypanosomiasis on Galana Ranch in Kenya. Acta Trop. 51(2):113-21

How does drug resistance develop in the field?

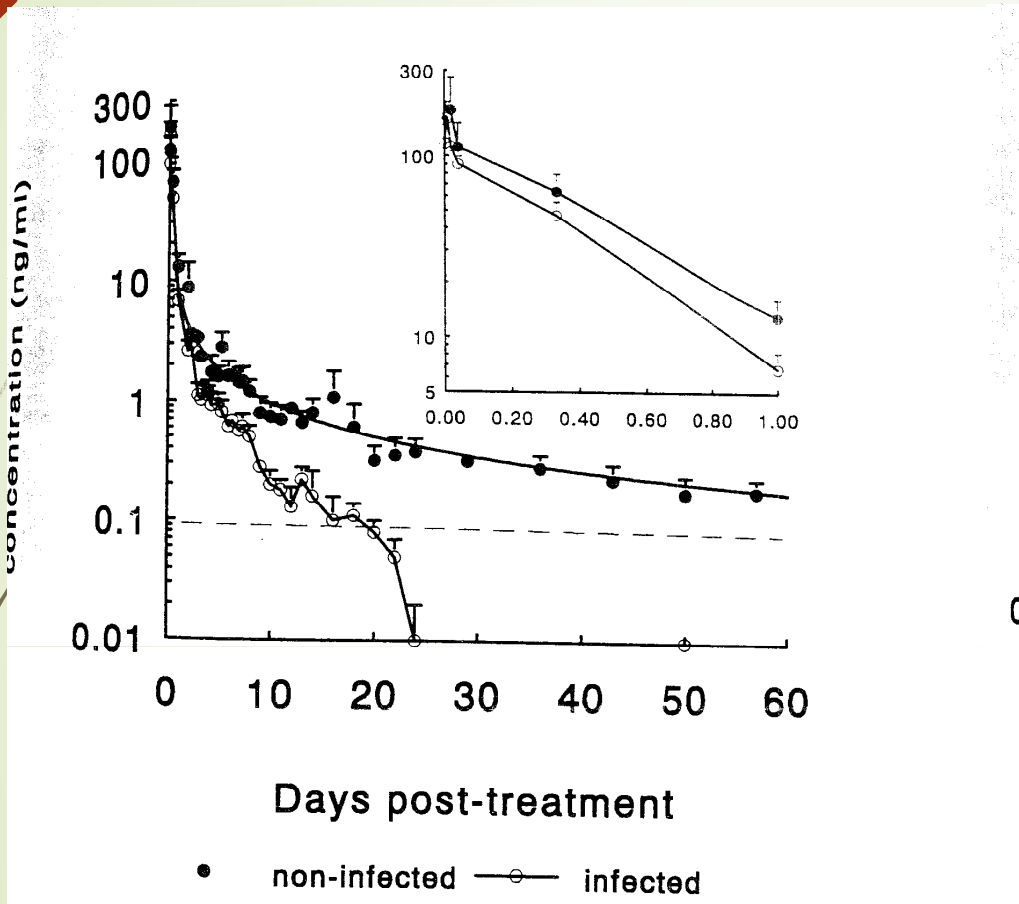
- exposure of parasites to sub-therapeutic drug levels
  - under-dosing (Whiteside, 1960; 1962; Boyt, 1986)
  - Mass treatments of cattle and frequency; high drug pressure
  - Use of substandard products / counterfeits

Packed cell volume of Boran cattle infected with *Trypanosoma congolense* (Tc) and treated with homidium chloride at 1 mg/kg bwt

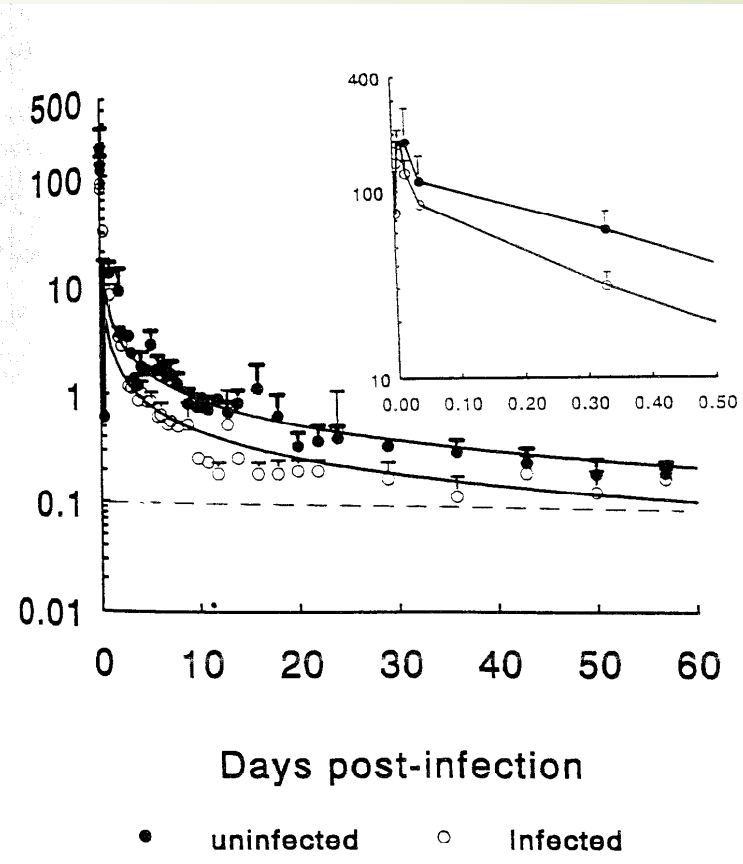




Serum drug profiles in cattle following treatment of *Trypanosoma congolense*-infected with homidium chloride at 1 mg/kg bwt

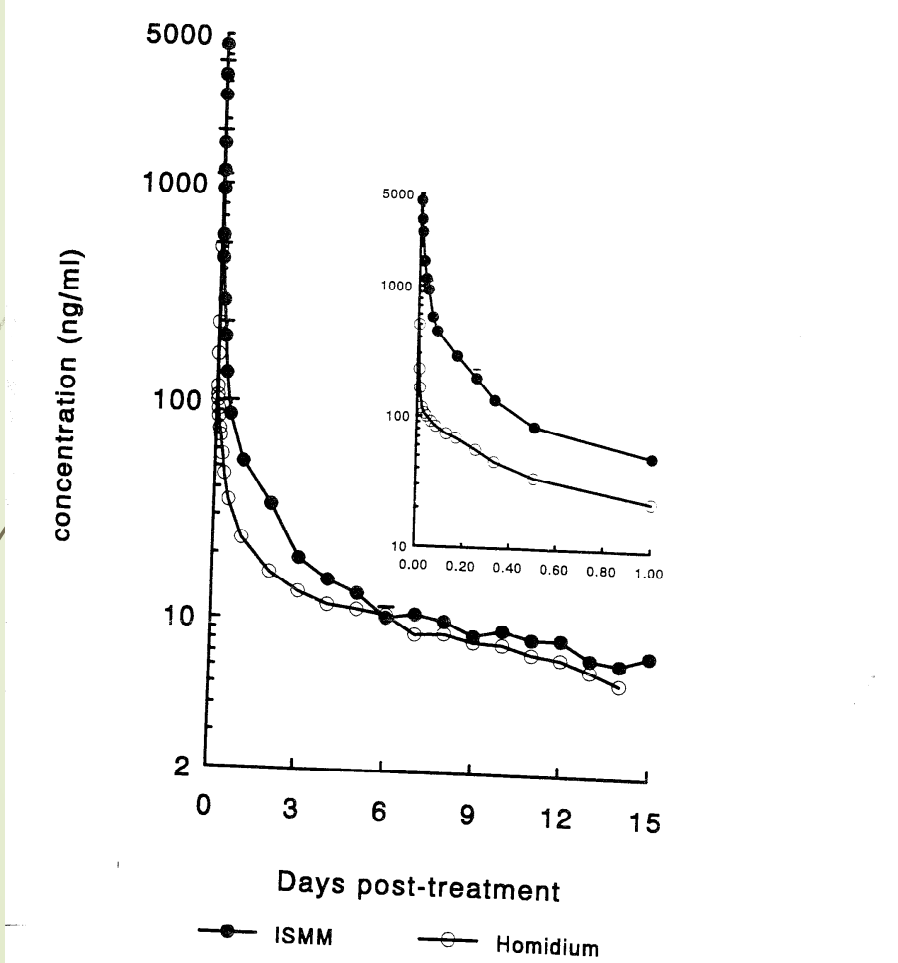


Drug resistant *T. Congolense* IL3330

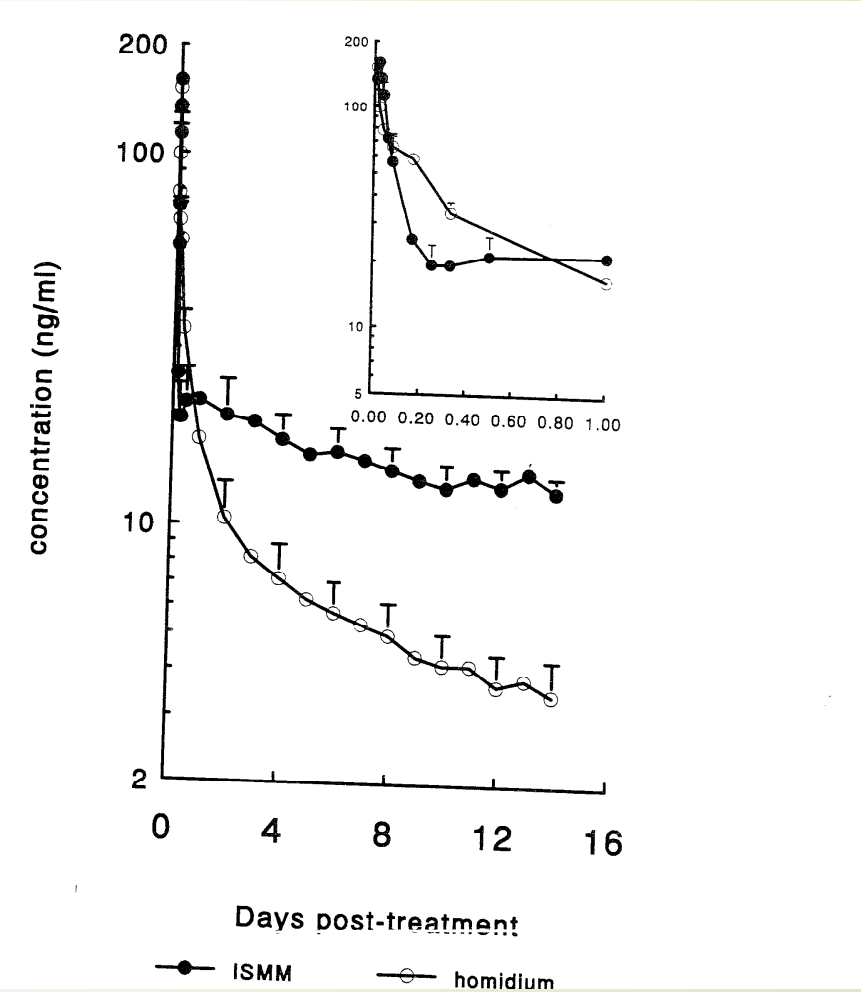


Drug sensitive *T. congolense* IL1180

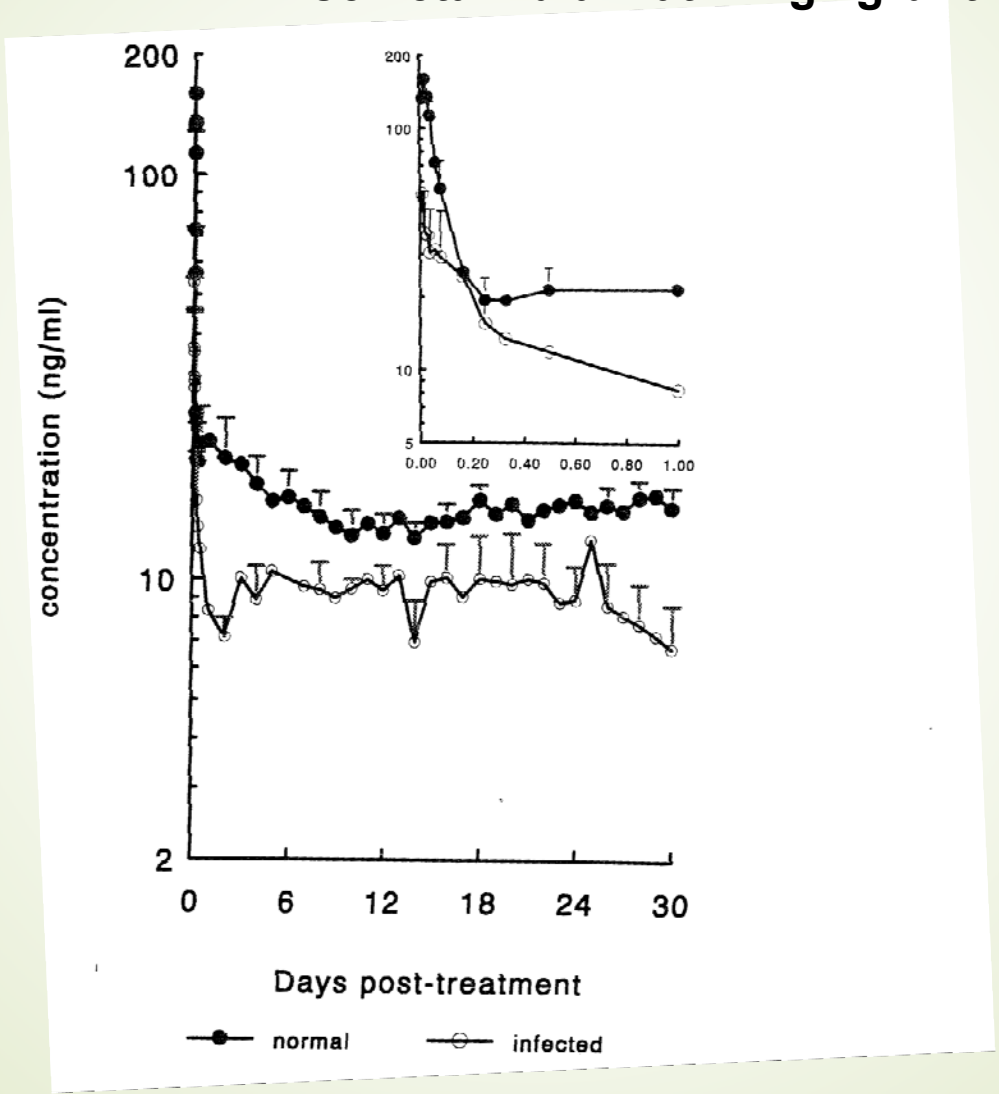
Plasma drug concentrations in Boran cattle following i.v. injection of ^{14}C radio-labelled homidium and isometamidium



Plasma drug concentrations in Boran cattle following i.m. injection of ^{14}C radio-labelled homidium and isometamidium



Plasma drug profiles in cattle following administration of ^{14}C isometamidium at 1 mg/kg bwt



Tests for drug resistance

- In vitro
- In vivo – mice
- In vivo - cattle

Reference:

- Eisler MC[1], Brandt J, Bauer B, Clausen PH, Delespaux V, Holmes PH, Ilemobade A, Machila N, Mbwambo H, McDermott J, Mehlitz D, Murilla G, Ndung'u JM, Peregrine AS, Sidibé I, Sinyangwe L, Geerts S. **Standardized tests in mice and cattle for the detection of drug resistance in tsetse-transmitted trypanosomes of African domestic cattle.** Vet Parasitol. 2001 Jun 12;97(3):171-82.

How to delay development of drug resistance

- use of the "sanative pair" of drugs
- Use correct dosage of quality drugs
- avoid exposure of trypanosomes to sub-therapeutic drug concentrations (Whiteside, 1960; Boyt, 1986).
- Improved formulations of existing drugs
- Ban use of quinapyramine in cattle
- In areas of high tsetse challenge:
  - use an integrated approach (control vector, reduce freq. of drug application (Fox *et al.,* 1993; Peregrine *et al.,* 1994).
  - use of trypanotolerant livestock and drugs (Diall *et al.,* 1992).
- Evidence-based treatments

Acknowledgements

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- OIE for the invitation
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