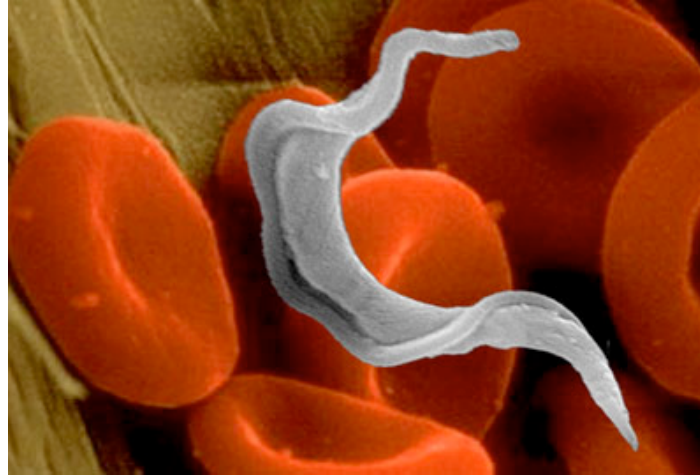


# **Trypanosomiasis**

- **African trypanosomiasis  
(Sleeping sickness)**
- **American trypanosomiasis  
(Chagas' disease)**

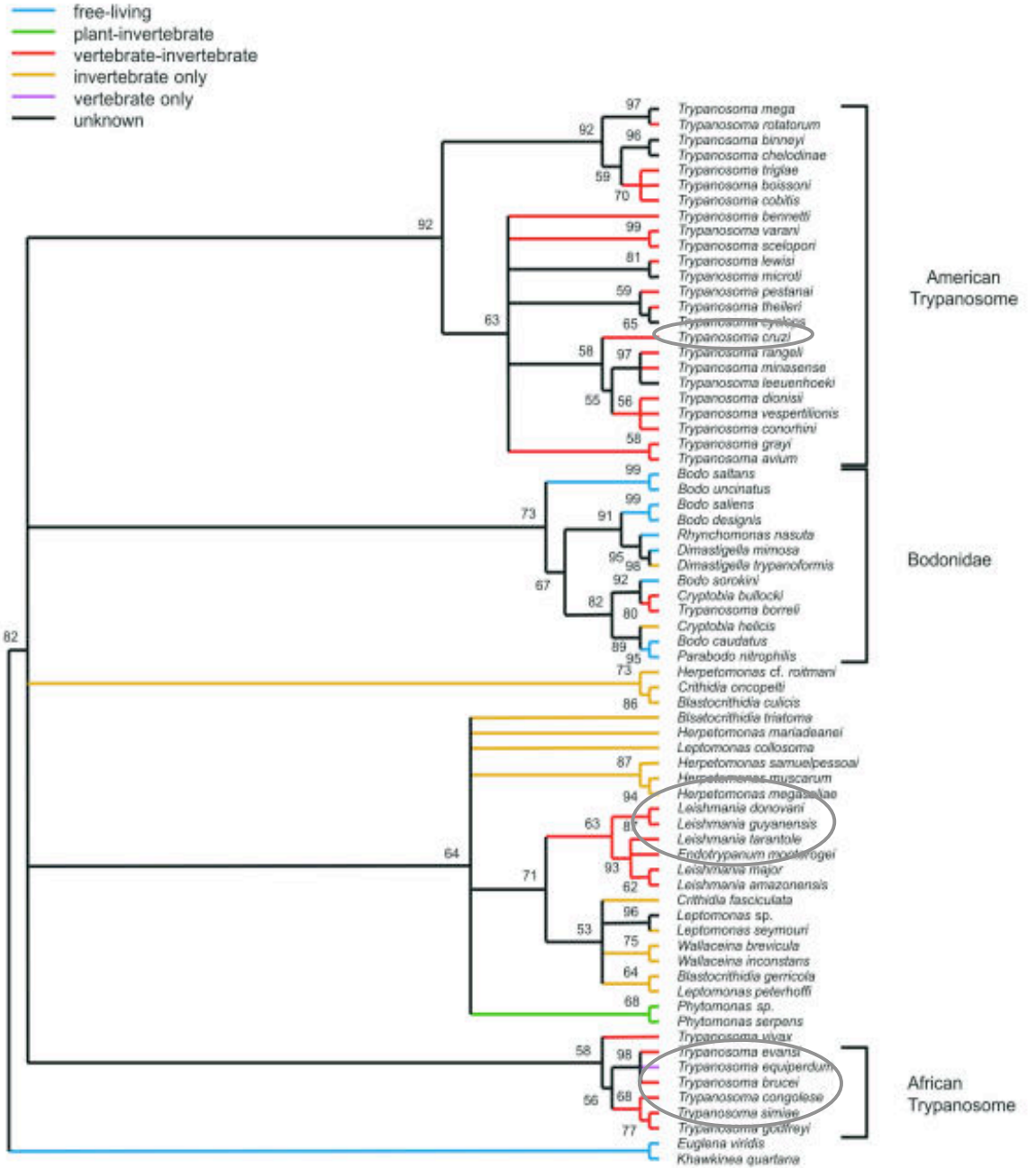


# African Trypanosomiasis

*'Blood is the originating cause of all men's diseases.'*

*The Talmud, Baba Nathra*

Kingdom  
 Protozoa  
 Phylum  
 Mastigophora  
 Class  
 Kinetoplastidae  
 Family  
 Trypanosomatidae



# Trypanosomes: Classification

- Order: Kinetoplastida
  - *kinetoplast* – disc shaped organelle containing DNA (kDNA) within a single large mitochondria
  - Genera differ in host distribution, life cycles, and clinical relevance (veterinary and human)
- Family: Trypanosomatidae
  - **Heteroxenous** (most) – require more than one living host to complete life cycle
  - **Hemoflagellates** – dependence on blood (host, invertebrate vector, culture)

# Definition

- African trypanosomiasis (AT) is the systemic disease that results from infection by either the two pathogenic subspecies of **salivarian hemoflagellates** that form part of the *Trypanosoma brucei* complex.
- *T. brucei rhodesiense*
- *T. brucei gambiense*
- *T. brucei brucei*—does not cause disease in humans.

# Definition

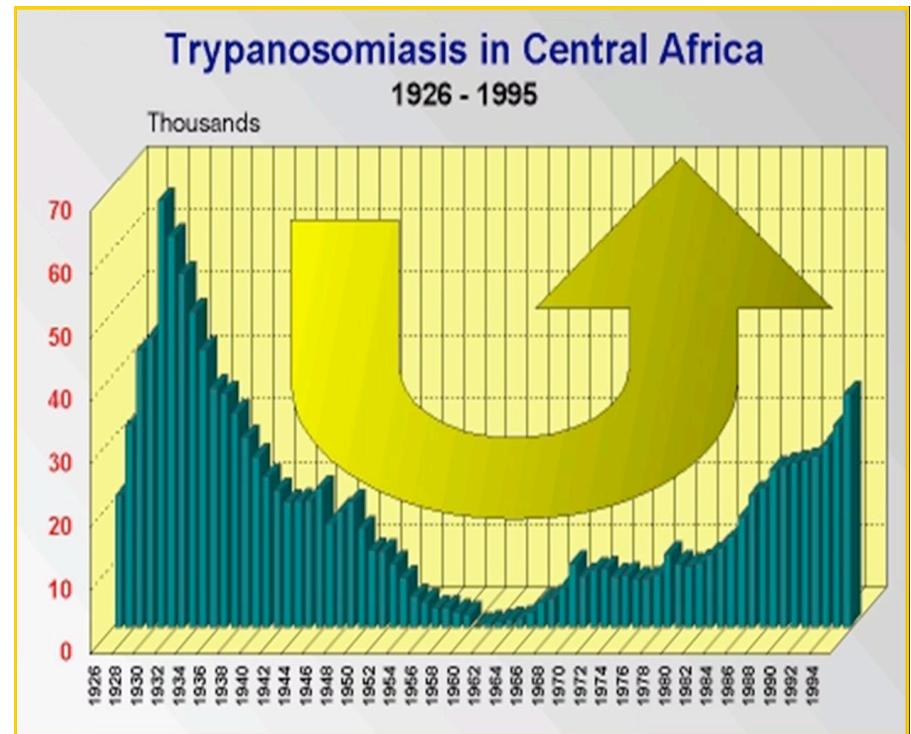
- Both *T. brucei rhodesiense* and *T. brucei gambiense* are transmitted by the tsetse fly.
- Synonyms for AT include:
  - Human African Trypanosomiasis
  - African Sleeping Sickness
  - Rhodesian Sleeping Sickness
  - Gambian Sleeping Sickness
  - West African Sleeping Sickness
  - East African Sleeping Sickness

# Historical Information

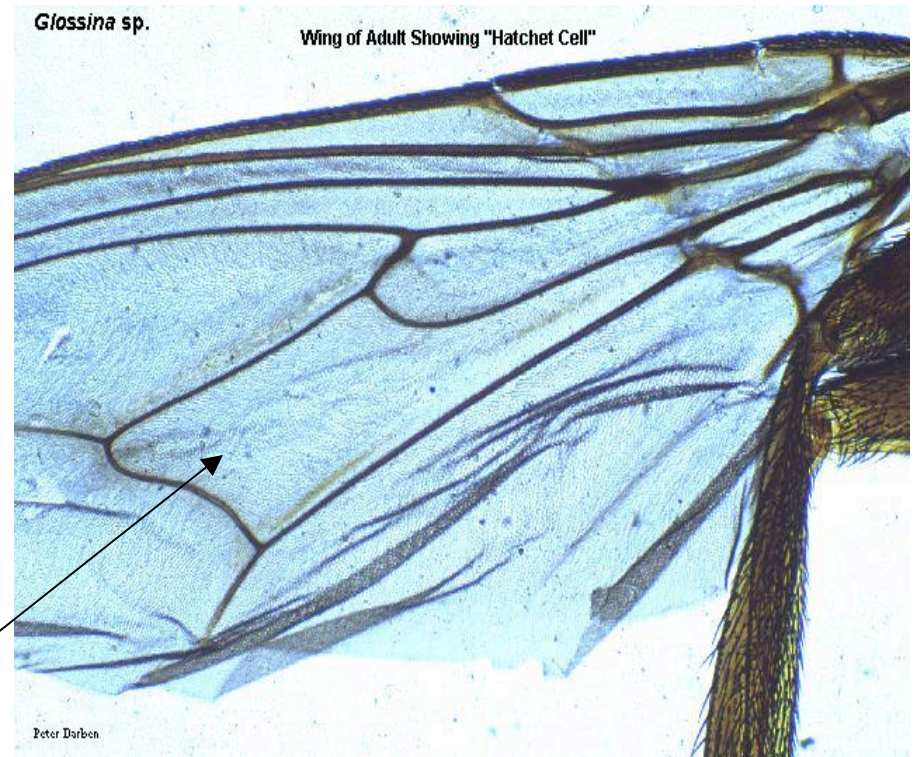
- Known in Europe since the 1700s.
- Bruce (1885) described the disease and its causative agent by showing that *nagana*, a disease of cattle, was caused by trypanosomes, and that the tsetse flies were the vectors.

# Cases

- 60 million at risk
- 450,000 cases occurred in 2005 throughout sub-Saharan Africa according to the WHO.
- This increase was caused in part to forced migrations caused by civil turmoil leading to a decrease in control measures.



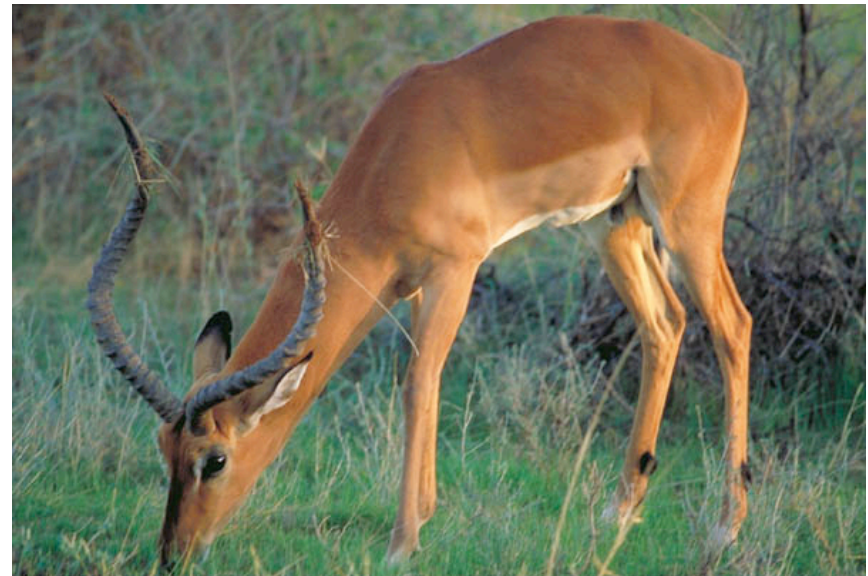
# Transmission--Tsetse Fly (*Glossina* spp.)



Characteristic 'hatchet wing'

# Transmission

- **Blood transfusion**
- **Transplacental**
- **Eating uncooked meat**

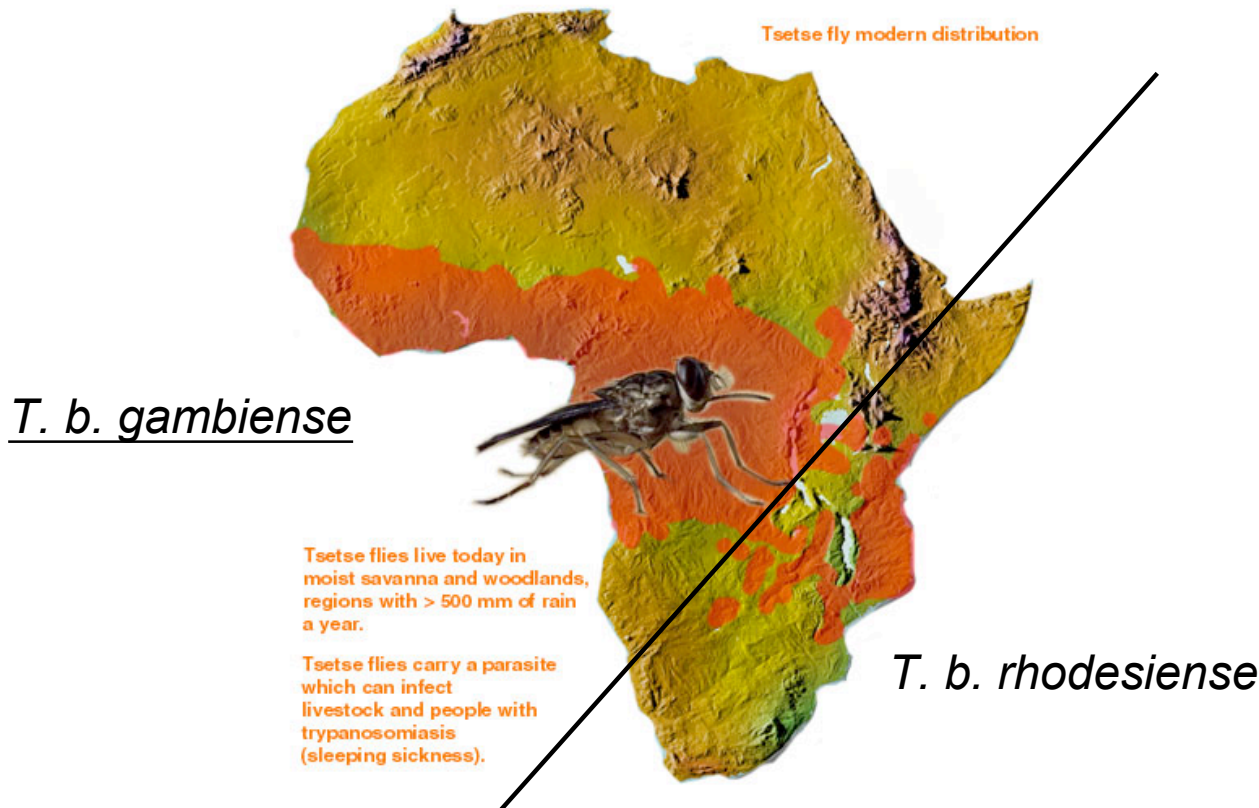


Infective dose for most hosts is 300-500 organisms.

# Comparison of *T. b. rhodesiense* and *T. b. gambiense*

	<i>T.b. rhodesiense</i>	<i>T.b. gambiense</i>
VECTORS	<b><i>G. morsitans</i></b> <i>G. fuscipes</i> <i>G. pallidipes</i> <i>G. swynertoni</i>	<b><i>G. palpalis</i></b> <b><i>G. tachinoides</i></b> <i>G. fuscipes</i>
VERTEBRATE HOST	<b>Wild game</b>	<b>Human; pig</b>
Endemic Area	Central/East Africa	West Africa
Clinical course	<b>Rapid/fulminant</b>	Indolent

# Tsetse Fly Distribution (Tsetse belt)



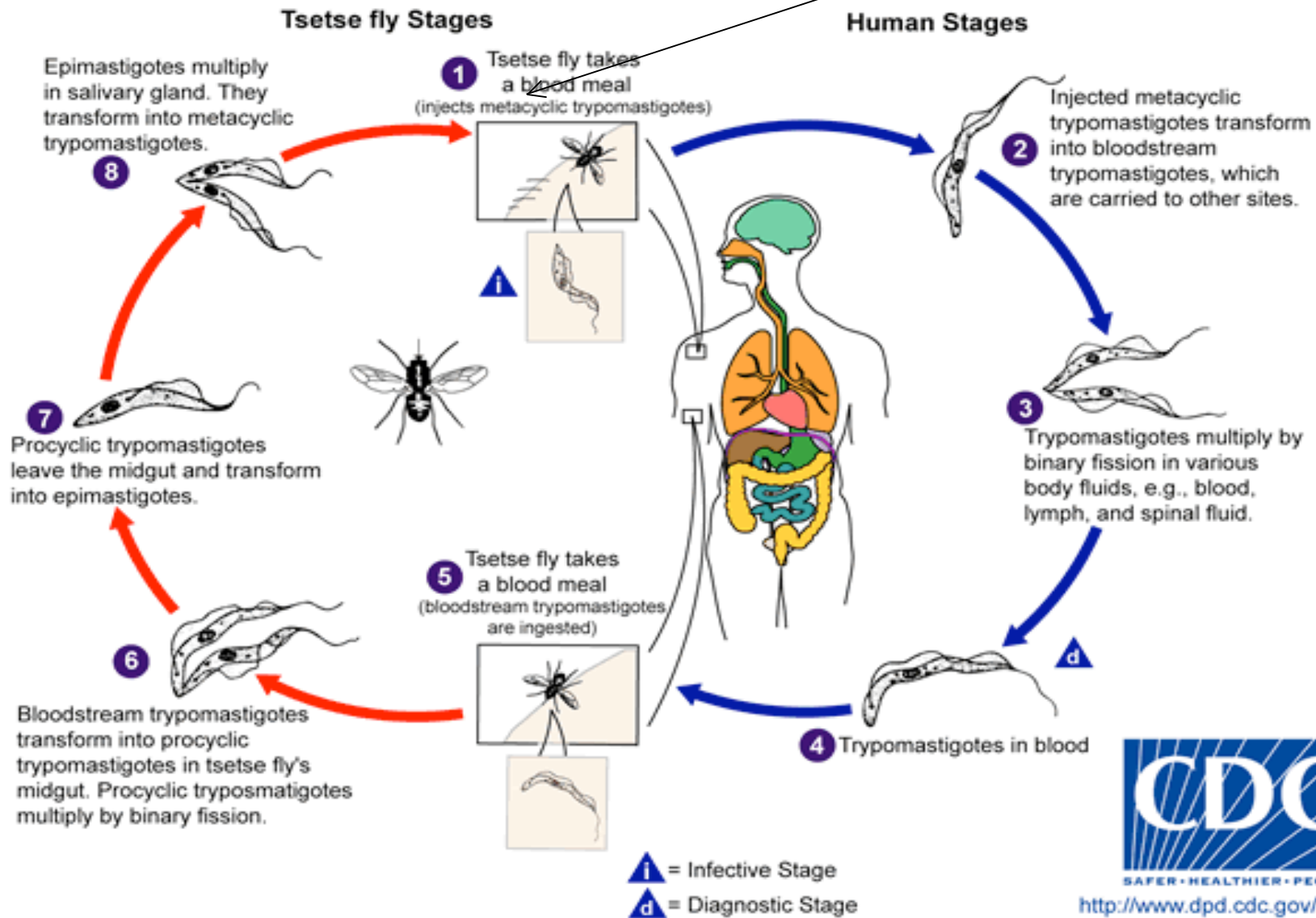
Glossina shows peaks of feeding and flight activity in the early morning and evening (avoid heat of the day).

Although not understood, seldom more than 1% of the tsetse fly population in any endemic area of trypanosomiasis carry the parasite.

Repulsed by humans, feed on them only when they have too!

# Life Cycle

40,000

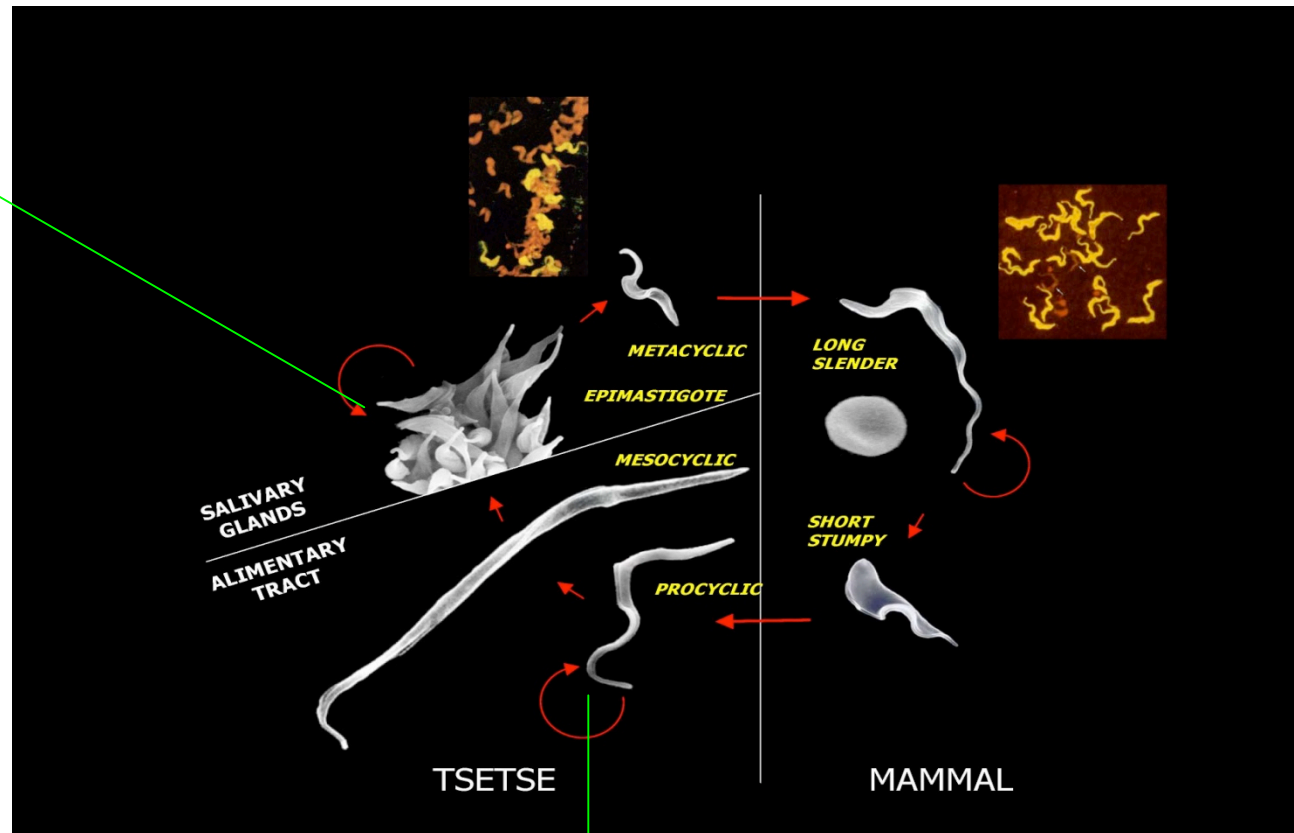


# Life Cycle

Final division in the fly.

Detach and form variable antigenic coat.

Free in lumen of salivary Gland.

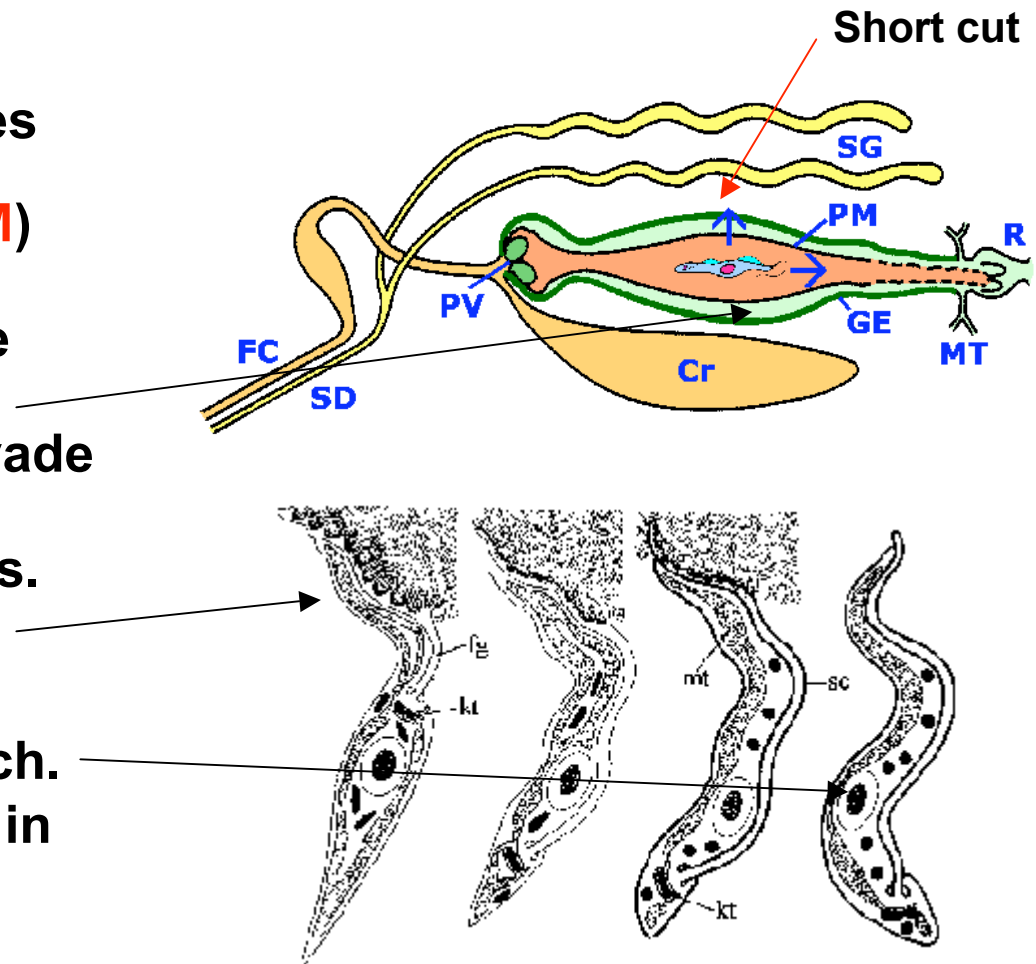


2-5 weeks in the fly

Alterations in O<sub>2</sub> utilization,  
Dietary changes, active division.  
Migration.

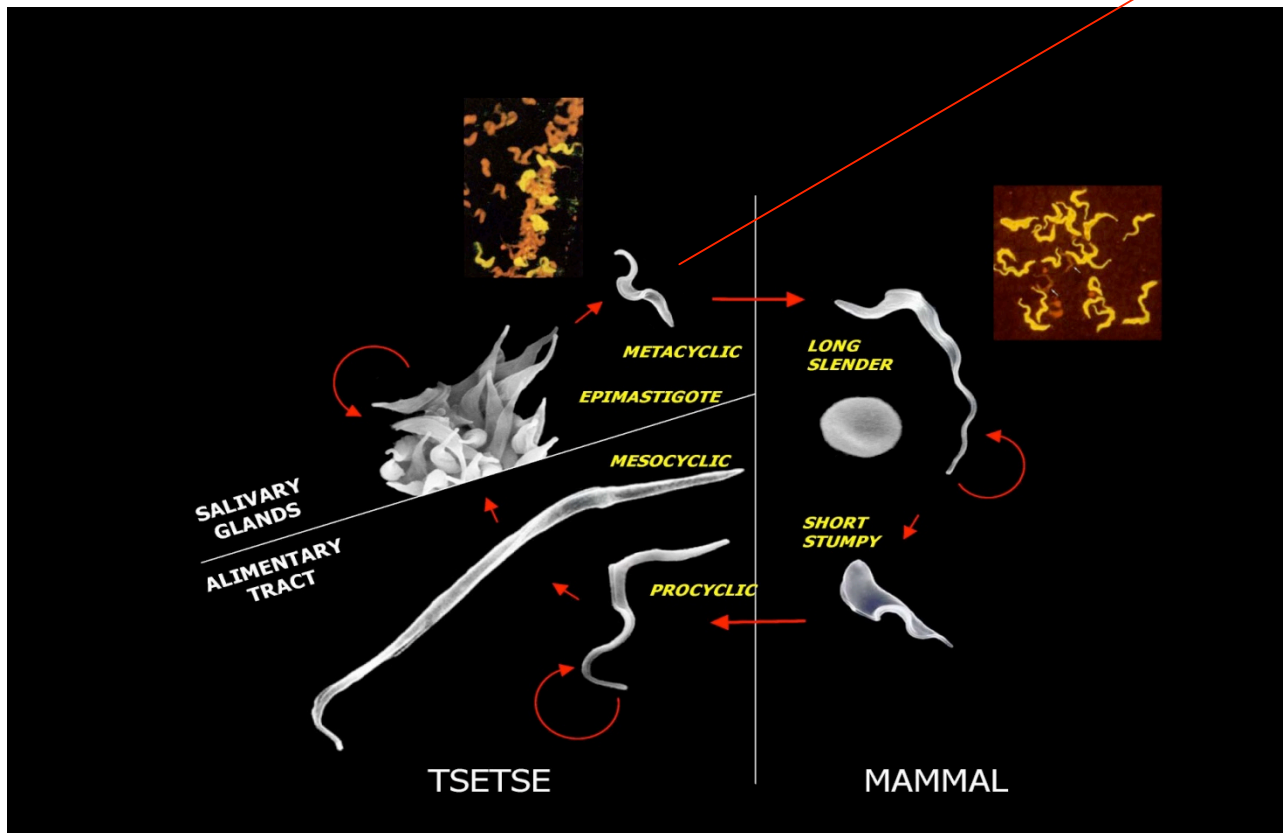
# Migrating through the fly...

- Developing trypanosomes penetrate the chitinous peritropic membrane (PM) lining the intestine.
- Enter epiperitropic space (multiply here).
- Proventricles (PV)– reinvade the endotropic space.
- Migrate to salivary glands.
- Attach via 'flagellipodia'.
- Divide once more.
- Form variable coat, detach.
- Metacyclic trypanosome in the lumen of the salivary gland (infective stage).



# Life Cycle

As few as 10 needed to establish an infection.



Remain at the site of infection for 1-2 days.

Divide and spread systemically.

### 3 stages of disease:

- Chancere
- Hemolymphatic
- Meningoencephalitic

# Chancre

- 20-50% incidence.
- More common in Caucasians and patients infected with *T. b. rhodesiense*.
- Differential with anthrax, syphilis, and certain tick bites.
- Without therapy, chancre develops for 2-3 weeks before healing with perilesional desquamation.



# Hemolymphatic Stage

- **Heralded by generalized rash, fever, and lymphadenopathy as the parasite migrates through lymphatics to enter lymph nodes and blood stream.**
- **This phase may be fulminant in patients with *T. b. rhodesiense* infection.**
- **Lymphadenopathy occurs in response to both African trypanosomes.**
- **Patients infected with *T. b. rhodesiense* may die at this stage before developing CNS involvement.**
- **In patients infected with *T. b. rhodesiense* , the hemolymphatic and meningoencephalic stages may appear clinically superimposed.**

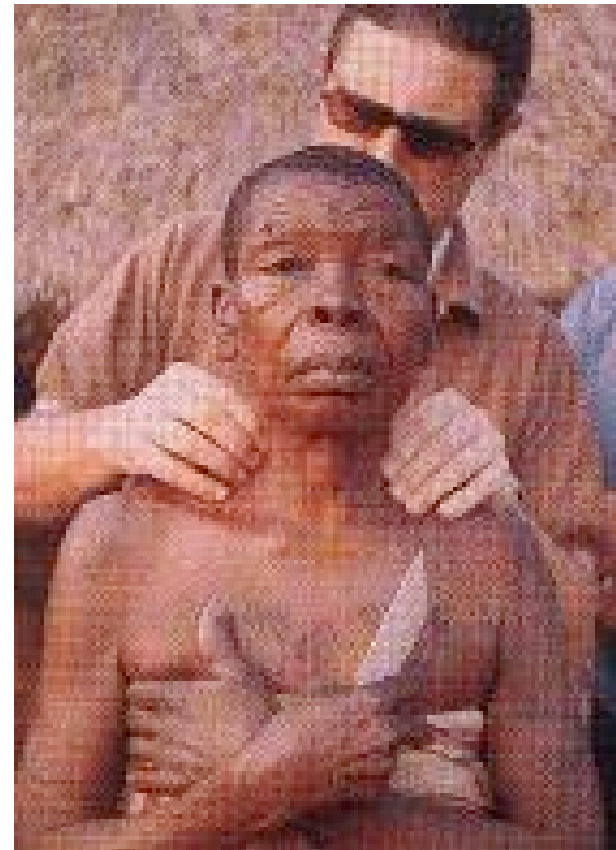
# Hemolympathatic Stage

- **Hematologic examination at this stage can reveal:**
  - **Normochromic/Normocytic anemia**
  - **High ESR**
  - **Thrombocytopenia**
  - **Winterbottom's sign**
    - **Lymphaenopathy of the posterior cervical nodes.**



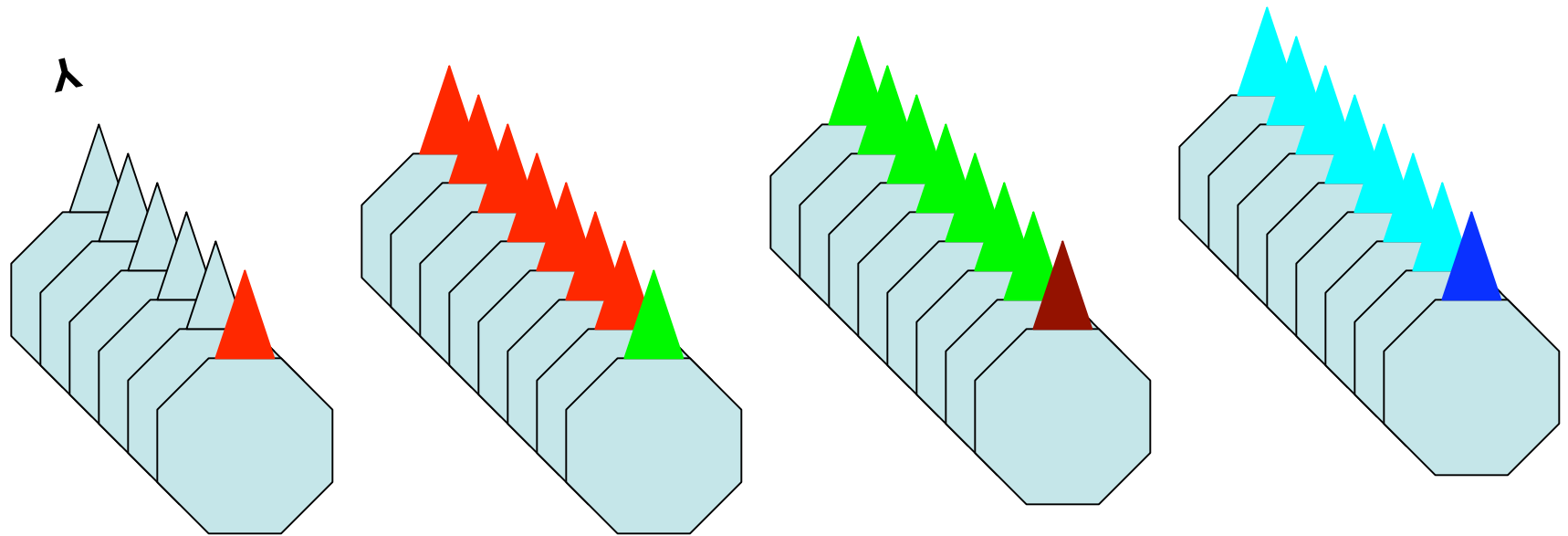
# Meningoencephalitic Stage

- Involvement of CNS.
- Psychological and behavioral changes
- Cranial nerve palsies
- Seizures
- Meningitis
- Encephalitis
- Terminally—mask-like **facies**.



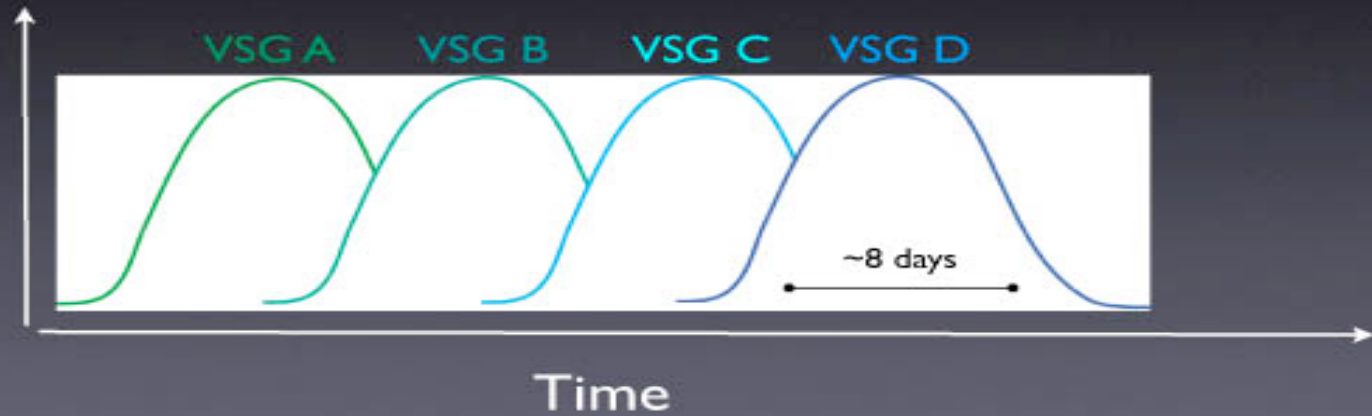
# Pathogenesis

- Trypanosome--induced disturbances of host immune control mechanism probably play the most important role in both the pathologic and clinical features of AT.
- Following infection of the vertebrate host, the parasite's **VSG** stimulate a pronounced host B cell response.
- Macrophage drives the immunosuppression.
- Within the CNS, the astrocyte has a major part in immunomodulation.
  - Produce somnogenic substances.



Antigenic variation in trypanosomes is associated with expression of distinct variant surface glycoproteins

Parasitaemia

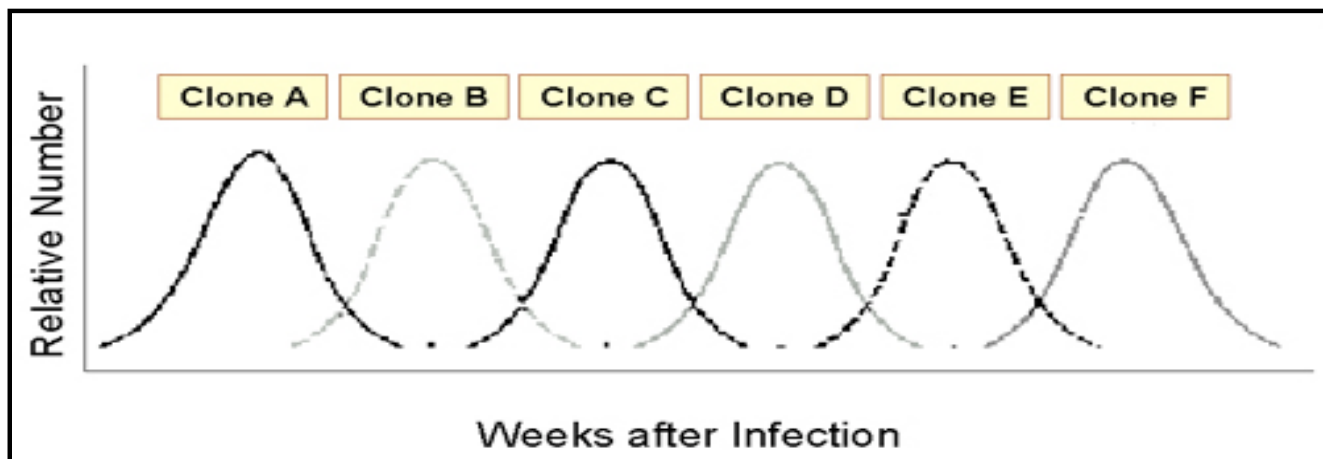


# ***Trypanosoma brucei* spp.: Immunology**

- **Protective antibody response mounted by host leads to periods of remission**
- **Ab response not sufficient to completely eliminate organisms**
- **variant antigenic types (VAT)**
  - **Mechanism for escaping host defense; conducive to survival from host defense**
  - **expression of new (unrecognized) surface glycoprotein**
  - **without treatment, only limited by lifespan of host**

# *Trypanosoma brucei* spp.: Immunology

- variant-specific surface glycoprotein (VSG)
  - released through flagella; coats entire organism
  - only one gene (of ~1,000) expressed at a time
  - in chronic infection, VSG genes are heterogeneous, although a single VAT is predominant depending on the Ab response.



# ***Trypanosoma brucei* spp.: Immunology**

- **VSG surface coat lost when ingested by fly**
- **During time promastigotes are without surface coat within fly, susceptible to antibodies taken with subsequent blood meals – possible control mechanism**

Reference on Molecular Biology of Trypanosomes  
<http://www.med.sc.edu:85/lecture/trypanosomiasis.htm>

# Pathology

- **Lymph nodes--enlarged and rubbery**
- **Splenomegaly frequent.**
- **Heart—parasite recoverable from pericardium (autopsy).**
  - **Valves fibrotic.**
- **Brain—Edematous and hyperemic**
  - **Meninges thickened and opaque.**
  - **Mononuclear infiltrates in Virchow-Robin spaces.**

# African trypanosomiasis: Economic Impact

- Cattle, other livestock, horses



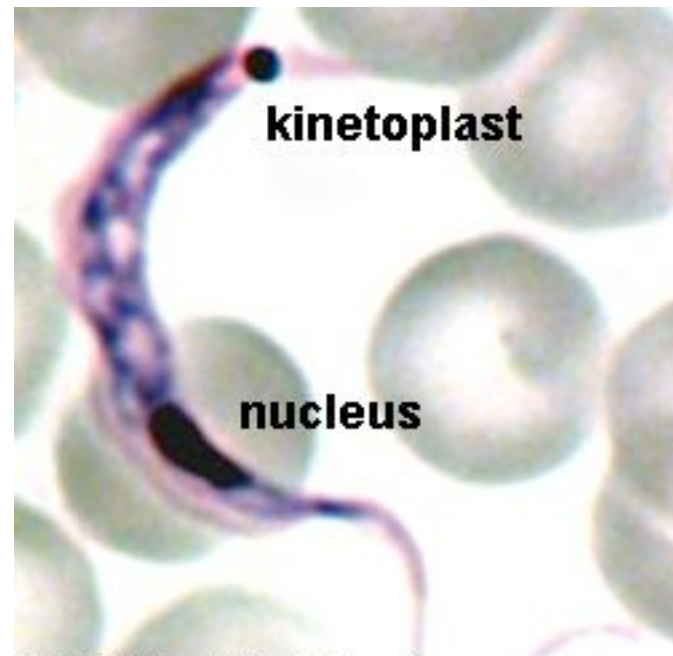
- Disease called nagana.
- Primary agents: *T. congolense*, *T. vivax*; *T.b. brucei*.

# **African trypanosomiasis: Economic Impact**

- **Economic impact on agriculture**
  - **Major reduction in food production**
    - **>3 million deaths per year**
    - **50% reduction in herd size**
    - **25% reduction in milk production**
    - **20% loss in calving**

# Diagnosis

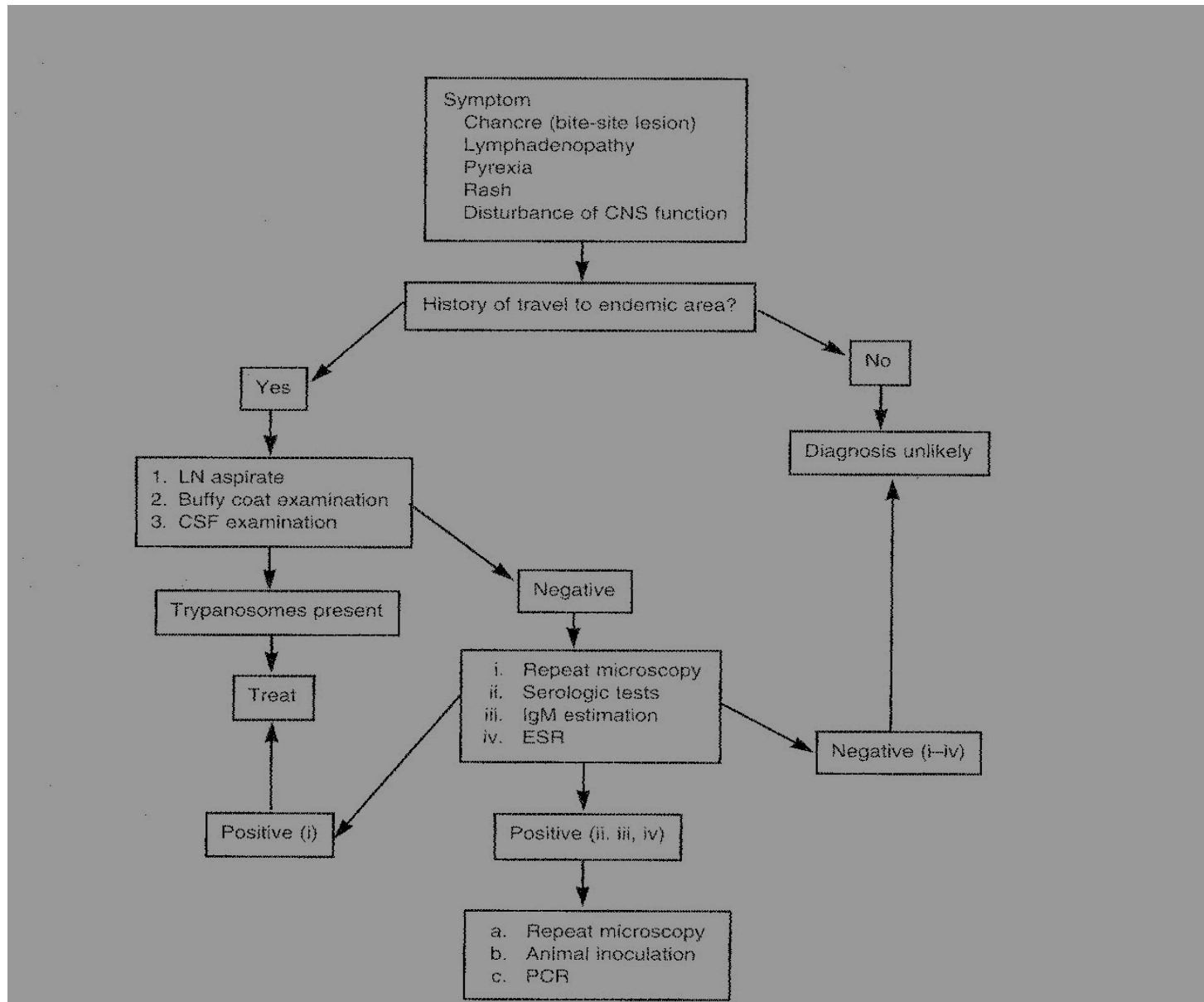
- **DIFINITIVE** diagnosis depends on demonstrating the parasite in the blood, lymph, or CNS.
- **Gimsa stain**
- **Concentration techniques** usually required before microscopic examination (centrifugation of CSF, QBC for buffy coat)
- **Tissue aspiration.**
- **CATT** (card agglutination trypanosome test) - useful for gambiense screening and surveillance
- **Serology**
  - antibodies: **IFA, ELISA;** high levels of IgM common
  - antigen: **PCR**



Mark F. Wiser, Tulane University

[www.tulane.edu/~wiser/protozoology/notes/kinet.html](http://www.tulane.edu/~wiser/protozoology/notes/kinet.html)

# AT Diagnostic Algorithm



# African trypanosomiasis: Treatment

- **Eflornithine**: difluoromethylornithine (DFMO)
  - called the resurrection drug
  - drug of choice, particularly with CNS involvement
  - Due to limited availability in many areas, only saved for later stage disease
- Hemolymphatic disease stage (no CNS involvement):
  - **suramin** (rhodesiense)
  - **pentamidine** (gambiense)

# **African trypanosomiasis: Control**

- **Education**
- **Surveillance – backbone of control**
  - **Of ~ 60 million at risk, only 5-10% under surveillance**
- **Treatment programs for human infections**
  - **Availability of effective drugs**
  - **Economic barriers**
  - **Accessibility issues**

# **African trypanosomiasis: Control**

- **Vector control, especially tsetse fly habitats reduction measures around villages.**
  - **Military action and civil unrest in Sudan, Ethiopia Sierra Leone, Congo and Liberia are responsible for forced migration of millions of people.**
  - **Control programs for tsetse fly have disappeared in these regions.**
- **Vaccine development unlikely.**
- **Breeding trypanotolerant livestock.**
- **Sterile Insect Technique/**
- **Bush clearing.**

# **American Trypanosomiasis (Chagas' Disease)**

*The real public-health problem of  
course, is poverty.*

**Wendell L. Willkie (1892-1944),  
*One World*, Chapter 2**

# Definition

- Zoonosis caused by the hemoflagellate protozoan, *Trypanosoma cruzi*.



# History

- Born 1879, Brazil
- Convinced by his uncle to abandon his engineering studies and become a doctor.
- Even though electricity had been introduced at this time, a student's dedication was measured by the number of candles burned during a night of studies.
- Carlos was a '2-candle' student.



**Carlos Chagas**

# History

- 7 years older than Chagas.
- Eradicated Yellow fever from Brazilian ports.
- After working several years at the Pasteur Institute he created in Manguinhos a model institute for vaccine production.
- Became great friends with Chagas.
- In 1908, Chagas was assigned to a new antimalarial campaign related to the construction of the Brazilian railroad where he discovered and described the clinical aspects of the disease that within years would be known as **Chagas' disease**.



Oswaldo Cruz

*‘Gentlemen, the hygienic practical applications and tropical medicine have destroyed the prejudice of a terrible climate; the scientific method has conquered the tropic’s unhealthiness.’*

Carlos Chagas, 1933



# Geography

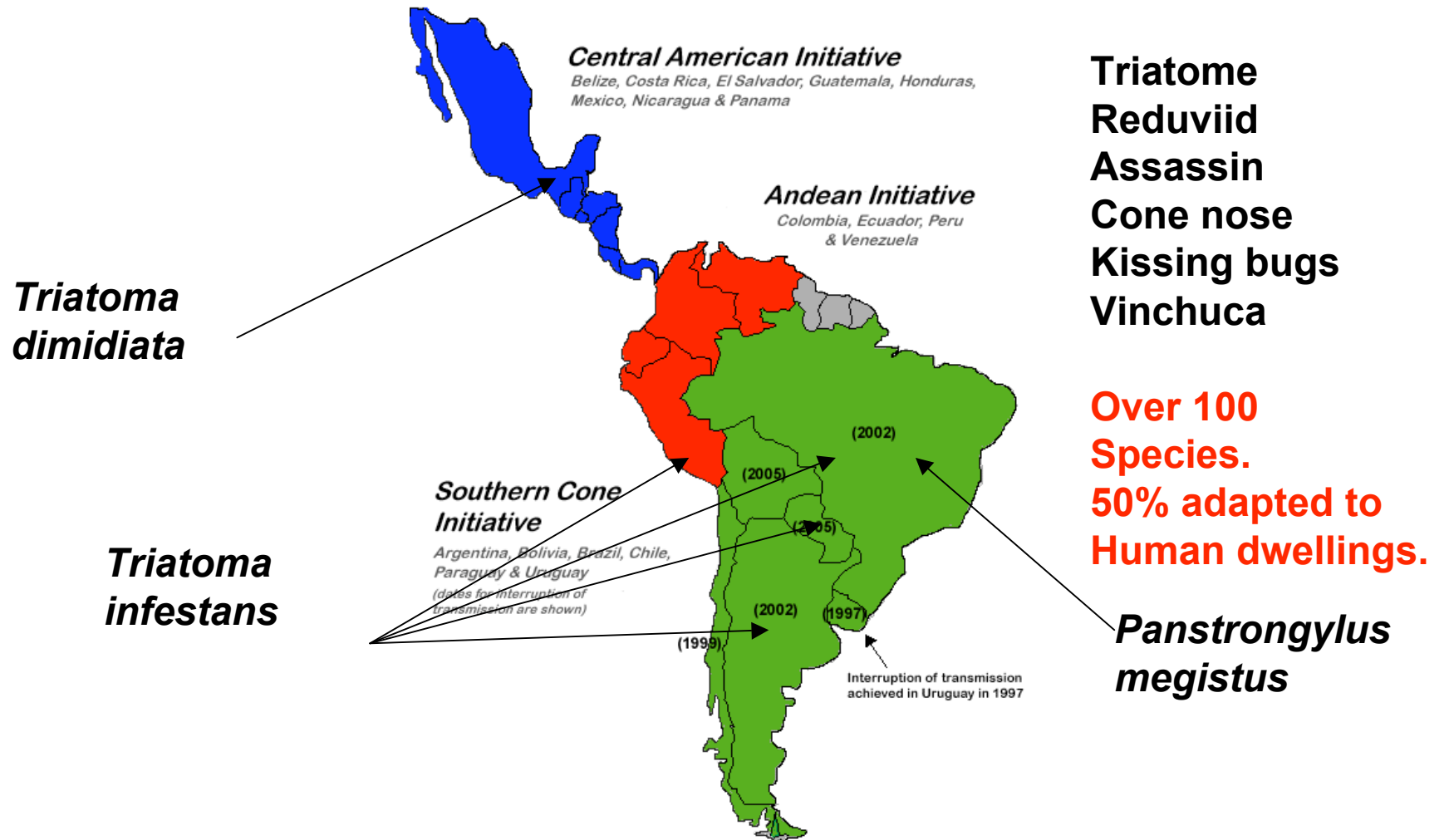
- *T. cruzi* parasitizes a variety of vertebrates including humans.
- Only in the Western Hemisphere.
- Heaviest concentration in Brazil (16-18 million people have chronic infections).
- 50,000 deaths/year.

## Regional Initiatives to eliminate Chagas disease

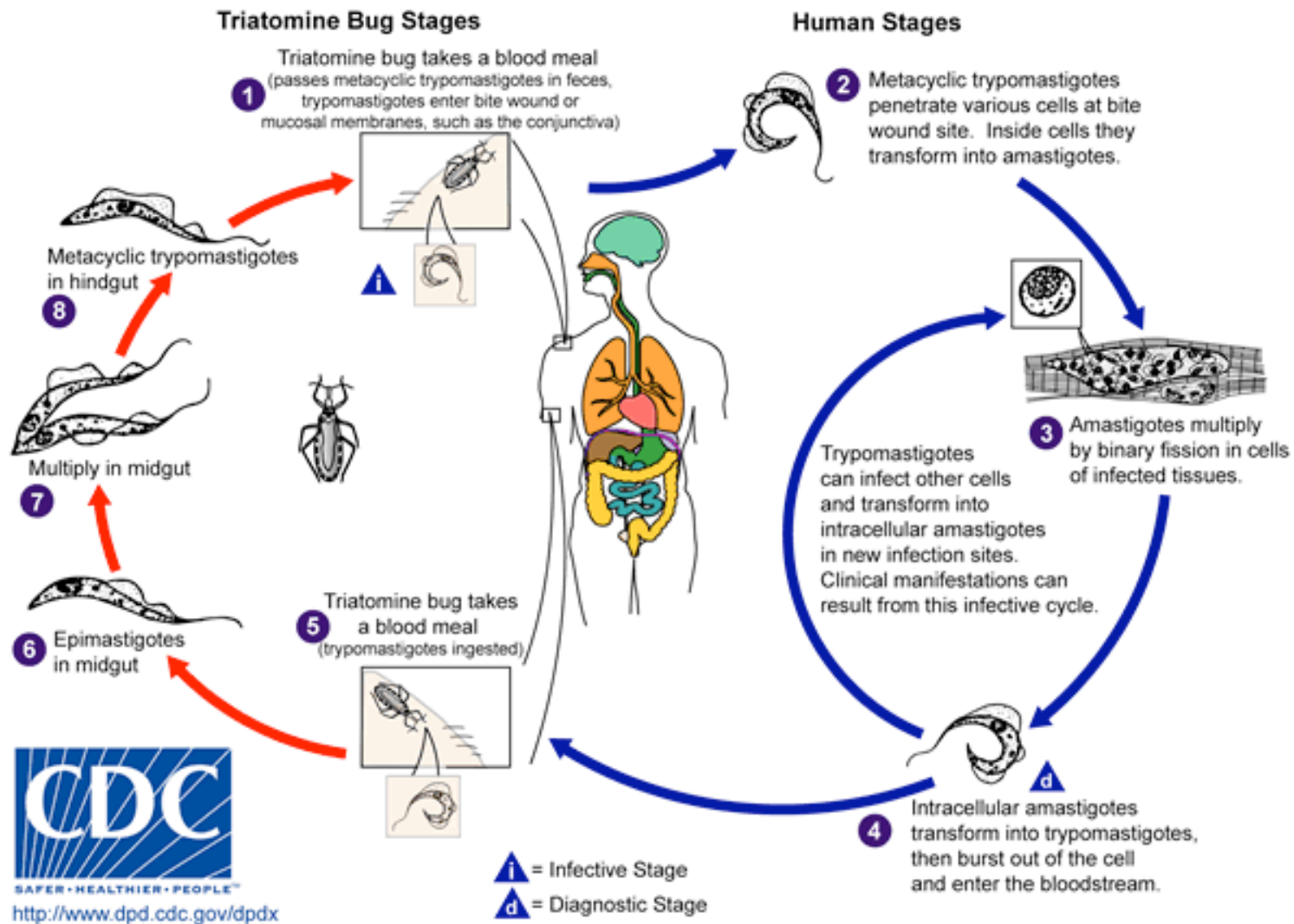


# Geography

Regional Initiatives to eliminate Chagas disease



# Life Cycle



# Epidemiology

- When a triatome bug bites a human, it defecates and the parasite, if in the feces, invades through the opening made by the bite.
- Most new infections are in children—rural areas.
- Most infections undiagnosed.

Because bugs are coprophagic and cannibalistic, bug-to-bug transmission is common.

**Some triatomines are sylvatic.**  
**Peridomestic cycle**  
**Domestic cycle**



# American Trypanosomiasis: Epidemiology

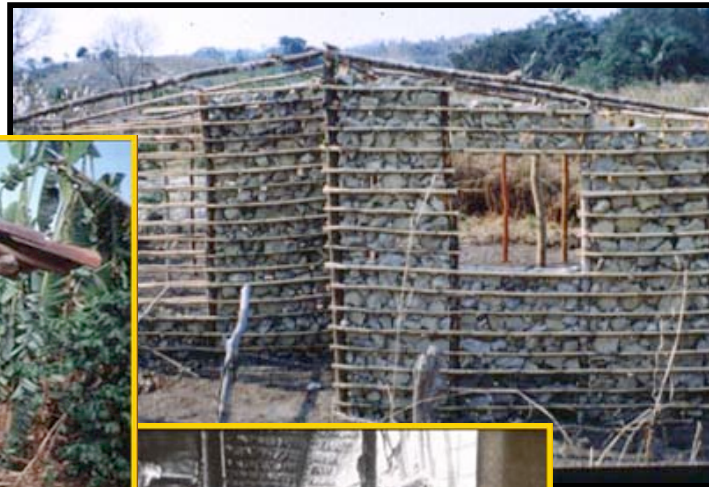
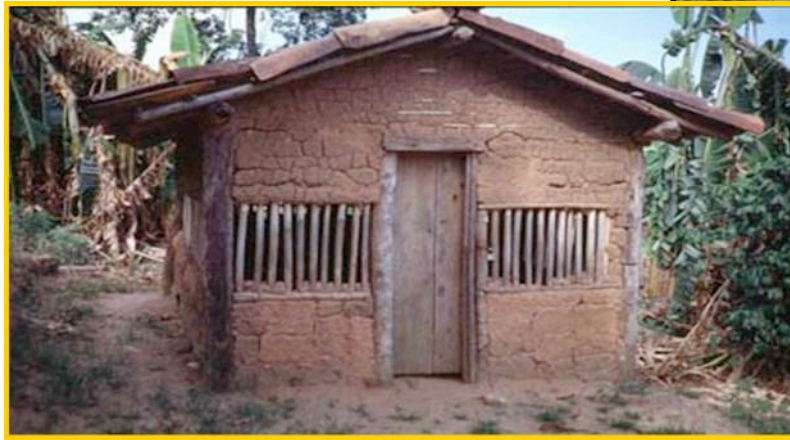
- **Mode of transmission:**
  - **contamination of wound site, conjunctiva, or mucosa by infected feces of insect**
  - **blood transfusion/organ transplantation**
  - **transplacental/congenital**
  - **ingestion of infected triatomes**

# **American Trypanosomiasis: Epidemiology**

- **Risk**
  - **Poor housing**
  - **Transplacental transmission**
  - **Blood transfusion**
  - **Culture – triatomines as aphrodisiacs (Mexico)**

# American Trypanosomiasis: Epidemiology

– Poor housing



Courtesy of WHO/TDR

# American Trypanosomiasis: Clinical

- Incubation period: 5-14 days; up to 100 days by transfusion
- Phases
  - Acute
  - Indeterminate
  - Chronic

# American Trypanosomiasis: Clinical

- **Symptoms – acute phase**
  - **Generally seen in children**
  - **variable fever, malaise, lymphadenopathy, hepatomegaly**
  - **chagoma: inflammatory response at site of infection; may last a couple of months**
  - **Romaña's sign: bipalpebral edema (unilateral)**

# American Trypanosomiasis: Clinical

- **Romaña's sign**



Courtesy of WHO/TDR

# American Trypanosomiasis: Clinical

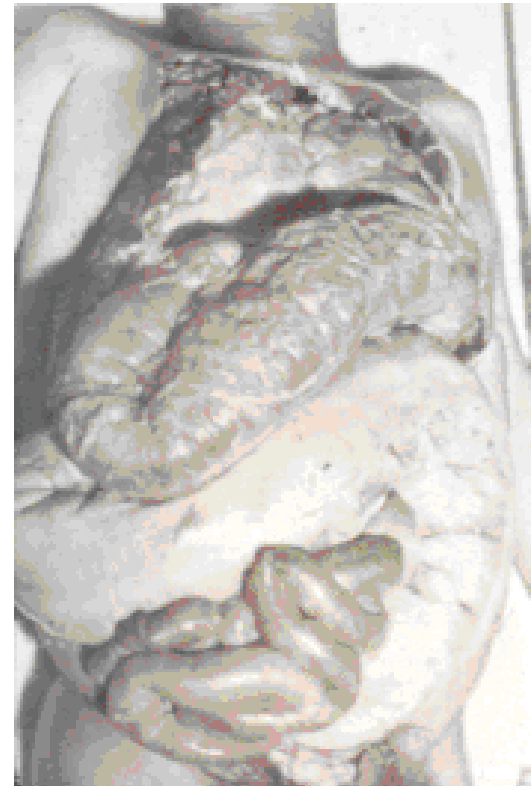
- **Symptoms – indeterminate phase**
  - **Non-treated patients and patients not responding to acute phase therapy will develop this phase**
  - **No parasite evident in blood**
  - **Amastigote “nests” in muscle tissue but no signs of progressive myocarditis**
  - **Anti-*T. cruzi* IgG present**
  - **~40% of patients remain in this phase**

# American Trypanosomiasis: Clinical

- **Symptoms – Chronic phase**
  - **Conversion rate to overt chronic disease is approximately 1% per year**
  - **Irreversible sequelae:**
    - **Cardiovascular (>80%): cardiomyopathy, arrhythmias, conduction disturbances, RBBB**
    - **Gastrointestinal (<30%): megaesophagus, megacolon**
    - **Meningoencephalitis in AIDS patients**
  - **Note: percentages represent proportion of all chronic phase patients; some have both cardiovascular and megadisease.**

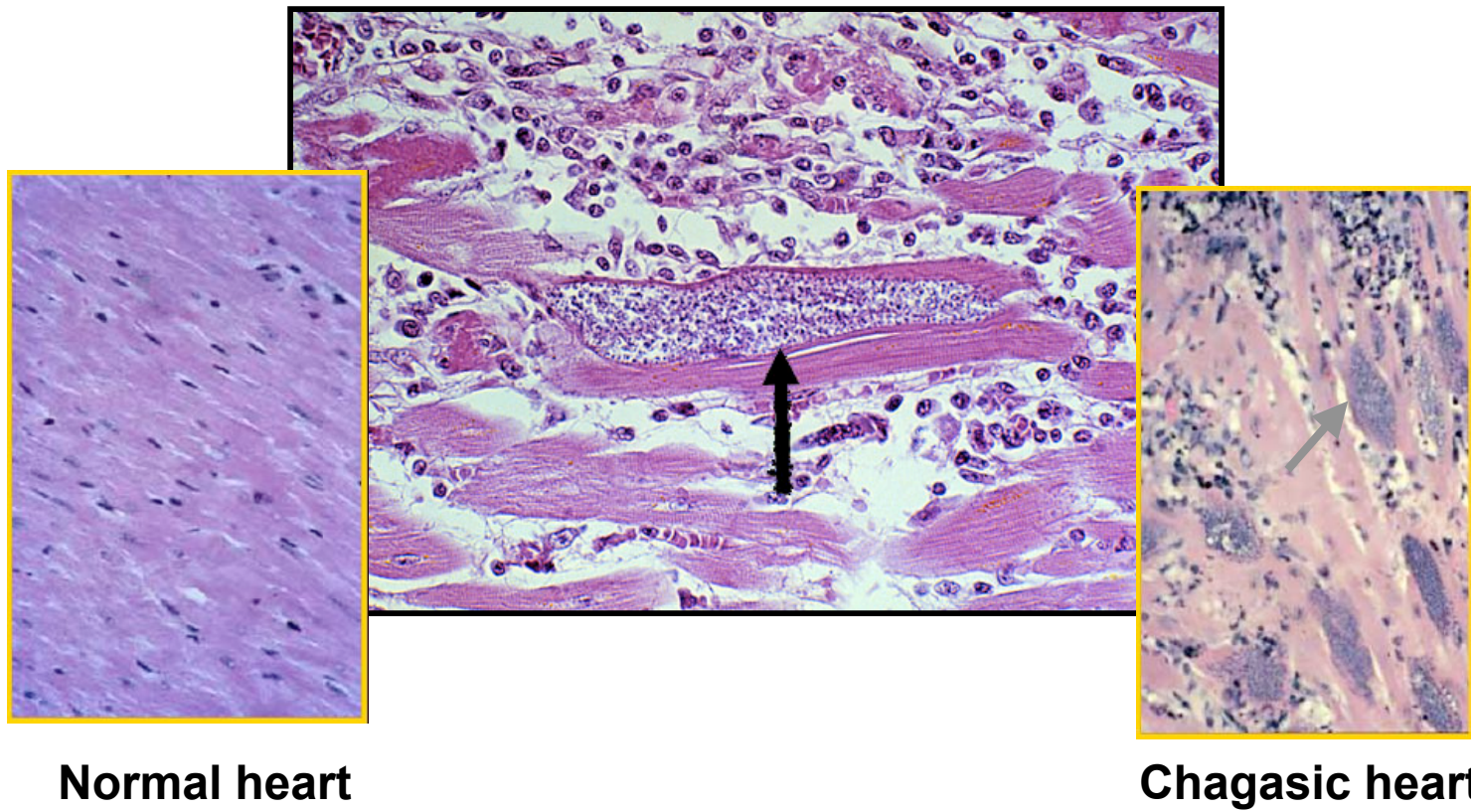
# Megacolon

- **Attributed to invasion, destruction and diminution of ganglion cells .**
  - **Megaesophagus**
  - **Megaduodenum**
  - **Megagallbladder**
  - **Megaureter**



# American Trypanosomiasis: Clinical

**Pathology: pseudocysts - amastigotes in cardiac muscle from patient with myocarditis**



**Normal heart**

**Chagasic heart**

Courtesy of WHO/TDR

# **American Trypanosomiasis: Diagnosis**

- **Demonstration of the agent is the diagnostic procedure in acute Chagas' disease; almost always yields positive results**
  - **Microscopic examination**
    - **a) fresh anticoagulated blood or buffy coat for motile parasites**
    - **b) thin and thick blood smears stained with Giemsa**

# American Trypanosomiasis: Diagnosis

- **Serology - antibody detection or antigen detection useful in screening and for chronic infections**
  - **ELISA**
  - **INNO-LIA – newly developed and tested optimized line immunoassay (recombinant and peptide antigen line); for confirmatory testing**
  - **PCR – compared to direct detection methods, provides earlier detection in acute phase; more reliable in chronic phase**

# American Trypanosomiasis: Diagnosis

- **Chagas STAT-PAK**
  - **Chembio Diagnostic Systems**
  - **Rapid immunochromatographic screening test for detection of antibodies to *T. cruzi***
  - **Three recombinant trypomastigote antigens**
  - **High degree of sensitivity and specificity compared to leading commercial tests**
    - **Recent clinical trial of 536 serum samples**
      - **ELISA/IF – Sensitivity 100%; Specificity 100%**

# Chagas STAT-PAK

Positive

Negative



Control

Patient

Patient

Control

**Sample: 5-10  $\mu$ l blood, serum, plasma**

**Add diluent**

**Read in 15 minutes**

# American Trypanosomiasis: Treatment

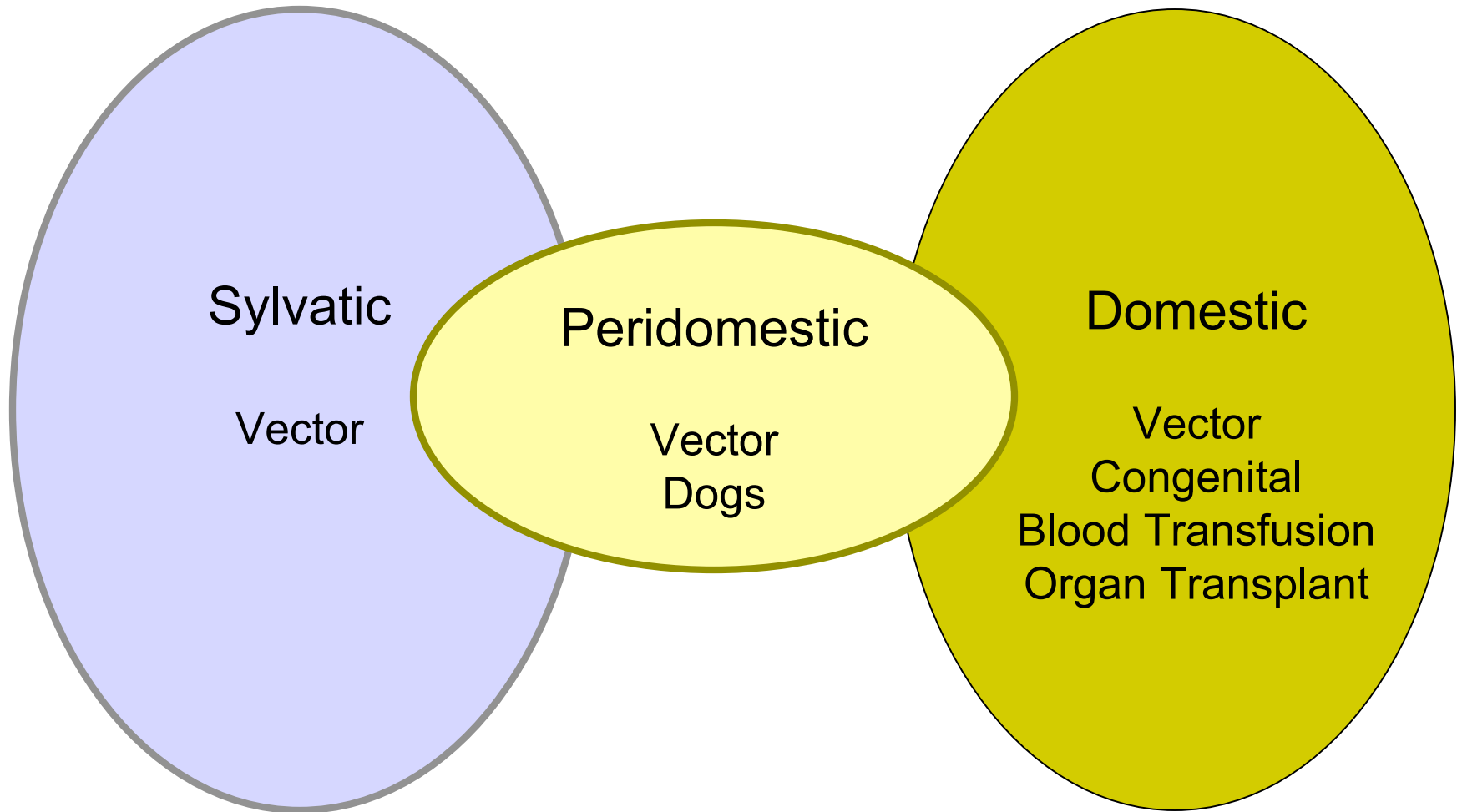
- Acute phase infections
  - Nifurtimox – many side effects.
  - Benznidazole – many side effects, but more reportedly effective.
  - Allopurinol – reportedly as effective as others, less side effects.
  - Combination therapy: benznidazole + ketoconazole.
- Treatment efficacy may vary with strain.
- Only cures ~60% of acute cases.
- Drug resistance problems
- New drug development
  - Cysteine protease inhibitors – stop replication by blocking enzyme action necessary for survival.

# **American Trypanosomiasis: Control**

- **Education**
- **Vectorial transmission:**
  - **vector control**
  - **reservoir control**
  - **improvement in housing construction**
  - **elimination of breeding habitat**
- **Transfusional transmission**
  - **Screening of blood and organ donors**

**Chagas' Disease:  
Are we missing an emerging disease in  
Texas?**

# Transmission in the US



# **Congenital Risk in Humans**

***Houston, TX, 1993-1996***

**Houston area residents**

**Seroprevalence determined by ELISA (screening)  
and HA (confirmatory Hemagen)**

**Screening assay: 22/3765 (0.6%)**

**Confirmation assay: 11/3765 (0.3%)**

**Hispanic: 9/2107 (0.4%)**

**Non-Hispanic: 2/1658 (0.1%)**

**Di Pentima et al., *Clin Inf Dis* 1999;28:1281-1285**

# **UTSPH Studies: Overview**

**Is Chagas' disease transmitted in Texas?**

**If so, what is the scope of the problem?**

**What are the major modes of transmission?**

**What steps need to be taken to:**

**Identify existing cases?**

**Prevent new cases?**

# Combined Use of Enzyme Linked Immunosorbent Assay and Flow Cytometry to Detect Antibodies to *Trypanosoma cruzi* in Domestic Canines in Texas

Shadomy, S.V., Waring, S.C., and Chappell, C.L., 2004, Clinical and Diagnostic Laboratory Immunology 11:313-319.

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## Seroprevalence of *Trypanosoma cruzi* infection in impounded canines in Corpus Christi, Texas

Charles Monney, BA, MS

*Master's Thesis, 2004*

# Previous Canine Studies in Texas

## Lower Rio Grande Valley, 1970's

Stray/wild canids: seroprevalence up to 9%

## Central Texas, 1972 – 1975

Domestic canines: 9 fatal cardiomyopathy cases

## Statewide (TVMDL), 1994 – 1998

67 of 351 (19.1%) positive clinically suspect dogs

# Sampling Methods

Companion dogs  
(peridomestic study)

**Clinics randomly selected from northwest Harris County (n=11)**  
**Healthy dogs > 6 months of age (n = 356)**  
**Convenience sample: annual checkups**

Stray dogs  
(community study)

**Blood samples obtained from Corpus Christi Animal Control.**  
**Convenience sample: canines selected for euthanasia by animal control (n=303)**  
**Study area in proximity to region where Texas isolates obtained**

# Serological Testing Methods

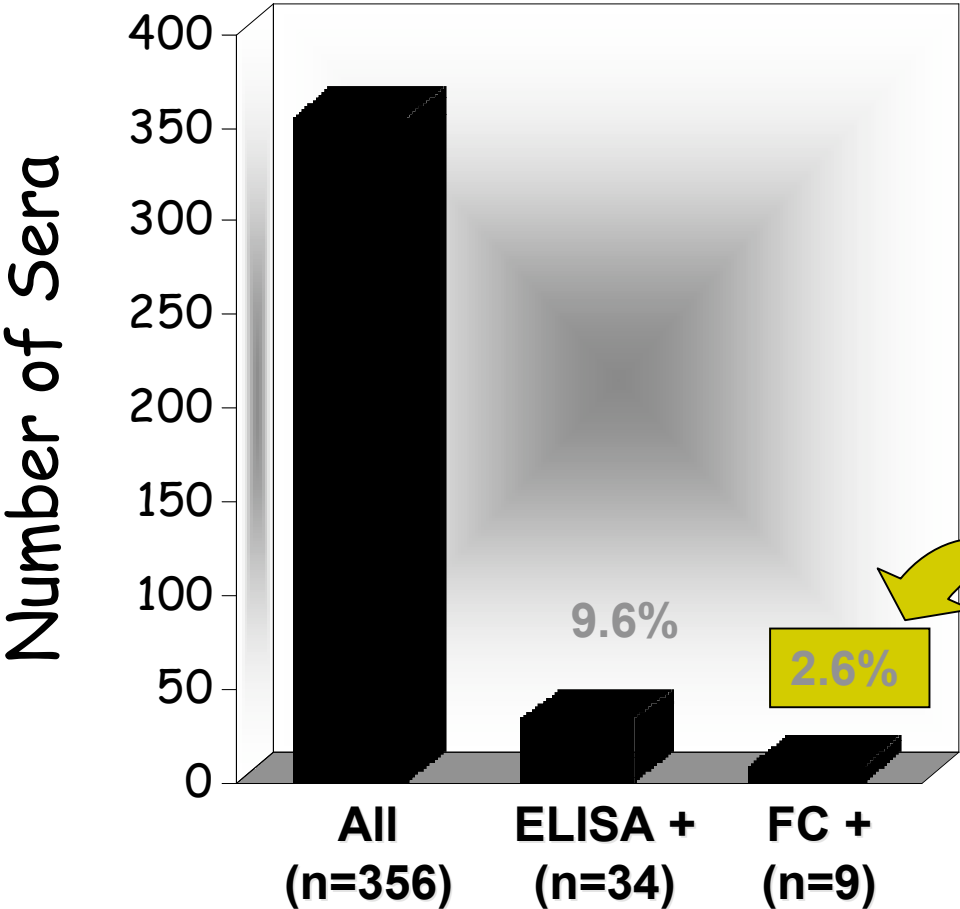
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	Companion dogs (peridomestic study)	Stray dogs (community study)
<b>Antigen</b>	<b>Brazilian strain of <i>T. cruzi</i> (YCL2)</b>	<b>TX 080801 and Bz (YCL2)</b>
<b>Screening</b>	<b>ELISA (intact epi's)</b>	<b>ELISA (intact epi's)</b>
<b>Confirmatory</b>	<b>Flow Cytometry (intact tryp's)*</b>	<b>Not yet determined</b>

---

\* established at UTSPH by O. Martins-Filho, FIOCRUZ (CPQRR), Bz

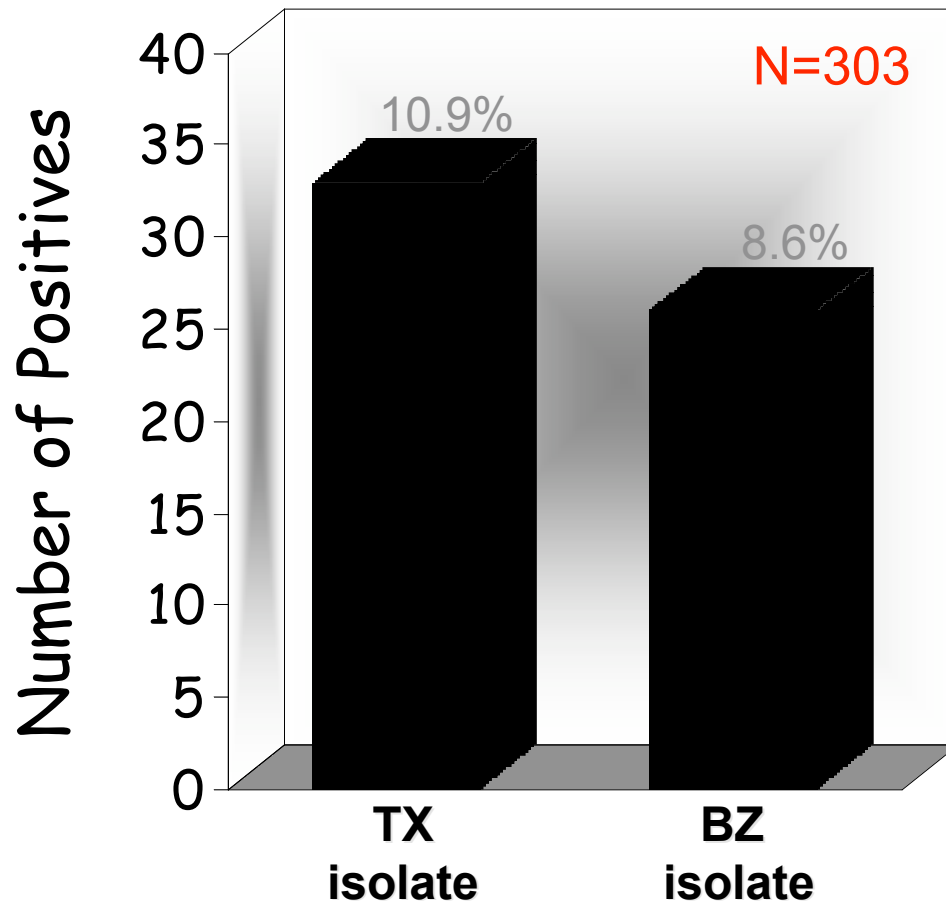
# Seroprevalence: Companion Dogs



**Seropositive dogs located throughout NW Harris County**

Evidence that *T. cruzi* is endemic in this area

# Seroprevalence: Stray Dogs



% pos (screening) similar to companion dogs  
Seroprevalence estimates higher with local isolates

**Conclusion:**  
***T. cruzi* is endemic in Nueces County**

**Comparison of two ELISA protocols for  
detection of antibodies to *Trypanosoma  
cruzi* utilizing two distinct antigen  
sources**

**Paul E. Grunenwald, Jr., BS, DVM  
Masters Thesis, 2004**

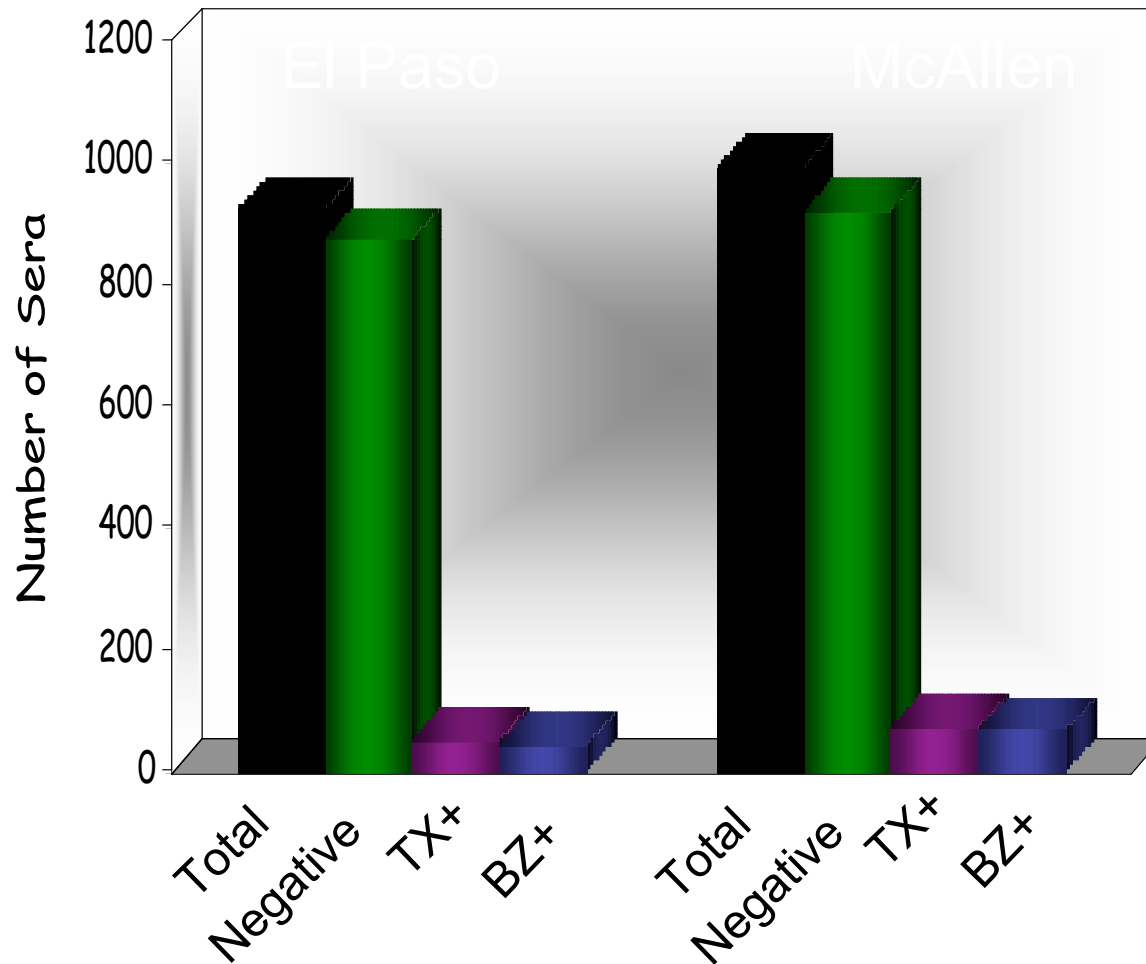
# **Study Population**

**Healthy blood donors (age > 18 years)**

**Blood-banking centers in El Paso and McAllen, Texas**

**Eligible samples were collected over a 4-month period in 2003**

# Seroprevalence: Border Areas



❖ Trend of higher seropositivity with Tx isolate compared to Bz isolate

	% Positive	
	TX+	BZ+
McAllen	7.3	6.8
El Paso	5.4	4.6

# Conclusions

**Positive sera detected in both locations  
Higher seropositivity in McAllen (LGV)  
than El Paso**

**TX isolate detected more positives than  
the BZ isolate**

**Confirmatory testing required using both  
BZ and TX isolates**

# Preliminary Confirmatory Testing

- **Chagas STAT-PAK (Chembio)**
  - **Three recombinant trypomastigote antigens**
  - **High degree of sensitivity and specificity compared to leading commercial tests**
    - **Recent clinical trial of 536 serum samples**
      - **ELISA/IF – Sensitivity 100%; Specificity 100%**

# Preliminary Confirmatory Testing

ELISA	BZ neg	BZ pos
TX neg	1,880	0
TX pos	13	109

STAT-PAK	TX+/BZ-	TX+/BZ+
Neg	11	85
Pos	2	24

15%

22%

7%

- **Positives**

- Screened:

6.5%

- Confirmed:

1.3%\*

\* True positives likely underestimated by magnitude of at least 7% using non-local strain of *T. cruzi*

# **Is Chagas' disease a potentially emerging problem in Texas?**

***T. cruzi* is endemic in Texas.**

**Transmission associated with community and peridomestic settings.**

**Screening assays in human sera suggest existence of undiagnosed, asymptomatic cases.**

# Where do we go from here?

## Variation

### Surface antigens:

- Sequence polymorphism
- Epitopes
- Immune response



## Vectors

### Influence of vector species on:

- Transmission
- Infectivity
- Virulence



## Virulence

Strain variation and pathogenesis in vitro and in vivo