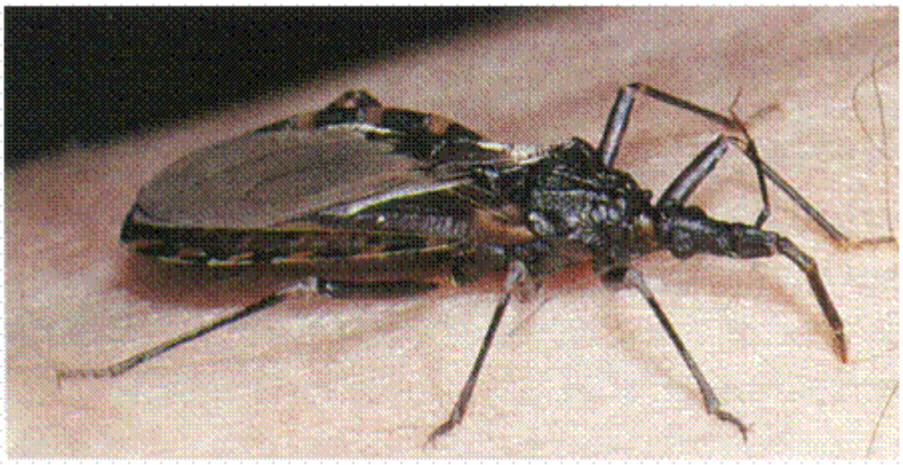
Discovery of “kissing bugs” infected with trypanosomes By Carlos Chagas

Chagas was sent to Northern Brazil to try to stop a malaria epidemic. After he had been there for one year, a railroad engineer told him about blood sucking bugs in the local huts, which were called "barbeiros" or "kissing bugs" due to their behavior of biting sleeping people on the face. Chagas then became interested in seeing if this bug could be transmitting some disease to humans or animals. He examined the hindgut contents of a bloodsucking triatomine bug (*Panstrongylus megistus)*, and found numerous flagellates which resembled stages of a trypanosome he has described previously from a marmoset. Chagas sent infected triatomine bugs to the Institute in Rio where they were allowed to bite monkeys and after 30 days large numbers of trypanosomes were found in the peripheral blood. Subsequently, it was found that other animals i.e. rabbits, pigs, dogs and other monkeys, could also be infected.

Kissing bugs

During the colonial and missionary period in Latin America there are descriptions in the writings from Portuguese and Spanish missionaries and historians of the conquistadors that describe attacks by bugs called vinchucas, with biting and blood-sucking habits: *"Instead of ordinary bedbugs. . these are bugs bigger and more pernicious to the inhabitants.. they are as big as the tip of a little finger, long brownish and in the shape of beetles. They live in the ceiling of the houses and get out at night guided by the smell of people asleep, and getting down on the beds, bite cruelly, making a big wheal and sucking up to a half a thimble full of blood. While they suck blood they do it with such care and sweetness that it cannot be felt; but when they withdraw full they leave an unbearable pain and itching."*



Triatoma infestans

Discovery of trypanosomes in blood of sick people

Though Chagas was convinced he had found the vector of a human disease he did not know what that disease was. In 1909, two or three weeks after finding triatomines and a cat infected with *T. cruzi* he was called to treat a seriously ill 2 year old child named Berenice. She was feverish, had an enlarged spleen, liver and swollen lymph nodes and her blood teemed with trypanosomes similar in morphology to those found in the marmoset. He wrote: *"Examination between cover glass and slide revealed the existence of flagellates in good number and fixing and staining of blood films made it possible to characterize the parasite's morphology and to identify it with Schizotrypanum cruzi"*.

Fig. 6. A historic photograph—a hut in Lassance. Carlos Chagas is examining a sick child, one of the first cases of the disease bearing his name. In the background is the railroad car in which the Brazilian master lived for two years. Reproduced from Bacellar, R.C.: *Brazil's Contributions to Tropical Medicine and Malaria. Personalities and Institutions*. Rio de Janeiro Brazil, 1963, p. 163.

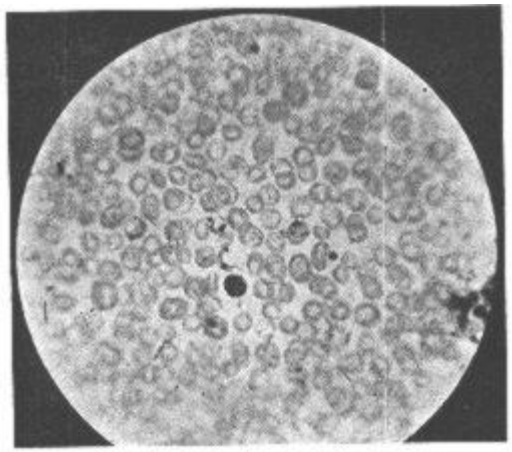
Knowing the domiciliary habits of the insect, its hematophageous nature, and its abundance in all the human habitations of the region, we immediately stayed on, interested in finding out the exact biology of the *barbeiro*, and principally to check the hypothesis, which came up immediately, that it was perhaps the transmissor of some parasite to man or to another vertebrate.

The role of the various hematophageous insects in the transmission of human diseases and of some of the mammalian trypanosomiases now guided my reasoning and urged me to get new species of the insect in order to do research on their digestive tubes or salivary glands, for any parasite for which the *barbeiro* should be an intermediate host.

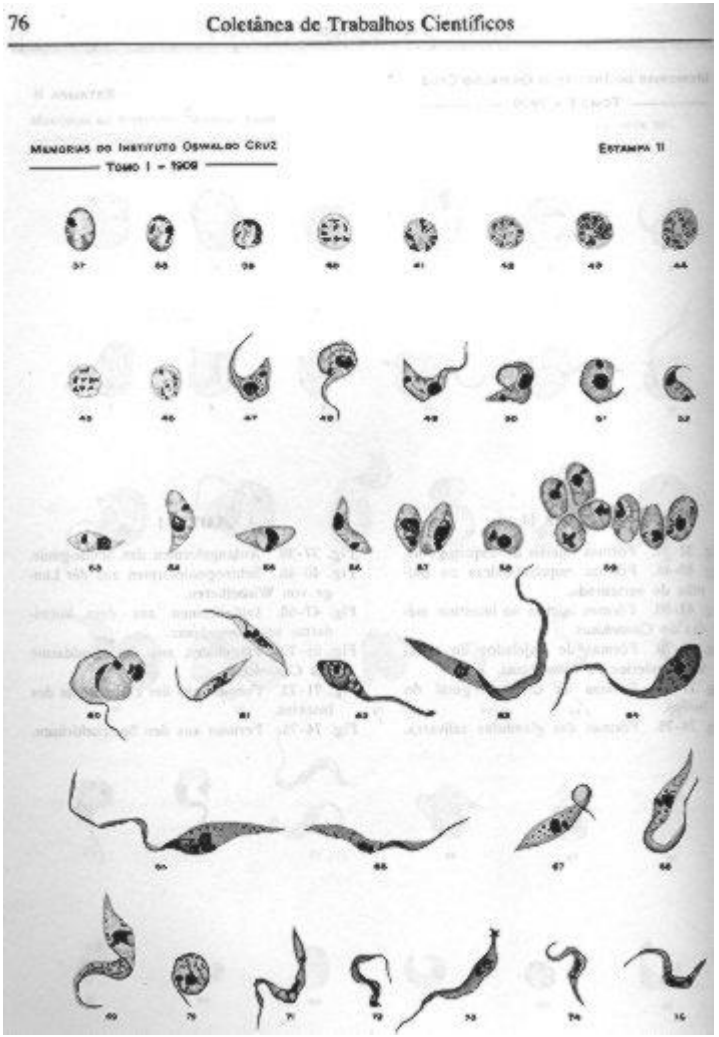
Drying the insects, I found in the posterior intestine of each one numerous flagellates, which showed the morphological characteristics of critidias. This confirmation led me to two hypotheses: either the flagellate observed was a natural parasite of the insect, without any pathogenic action, or it represented a phase of a vertebrate hemoflagellate, perhaps of man himself.[18]

Discovery of trypanosomes in heart muscle of sick people

In 1911 Chagas described the dividing forms in heart muscle. The connection between human disease and the blood-sucking bug had been made. In 1912 Chagas found that the armadillo was a reservoir host.



An original micrograph of T. cruzi in human blood



Drawings of the types of cells he found

At 29 years of age, Carlos Chagas had described the agent, the vectors, clinical symptoms in humans and animals, and the existence of a new disease. The species name was given to honor Oswaldo Cruz, who was the Director of the Oswaldo Cruz Institute where Chagas worked.

Oswaldo Cruz stamp

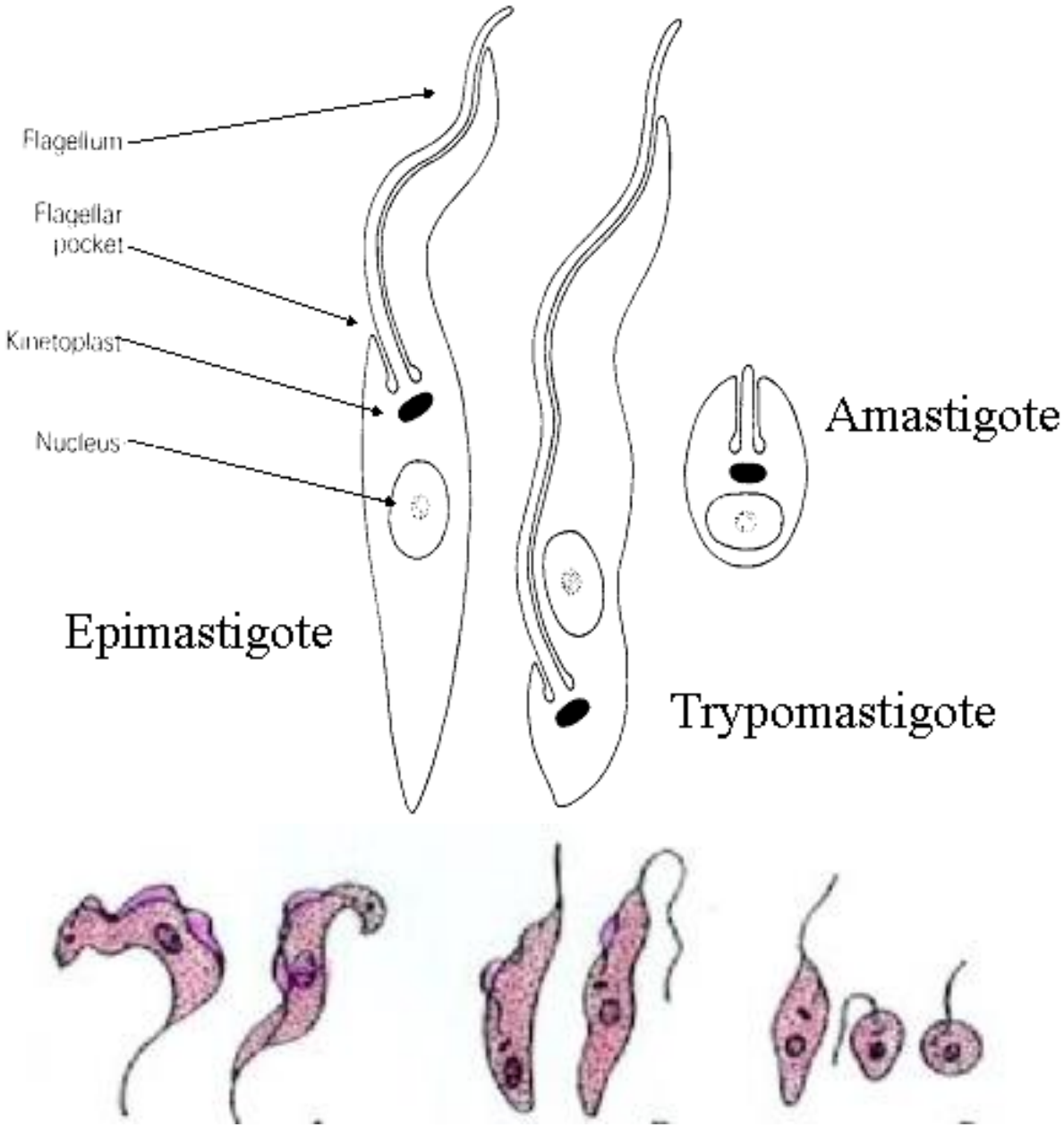
Carlos Chagas

However Chagas had made some enemies. For example, in the same year, Charles Donovan, who was trying to find the vector for transmission of *Leishmania*, wrote in the journal Lancet:

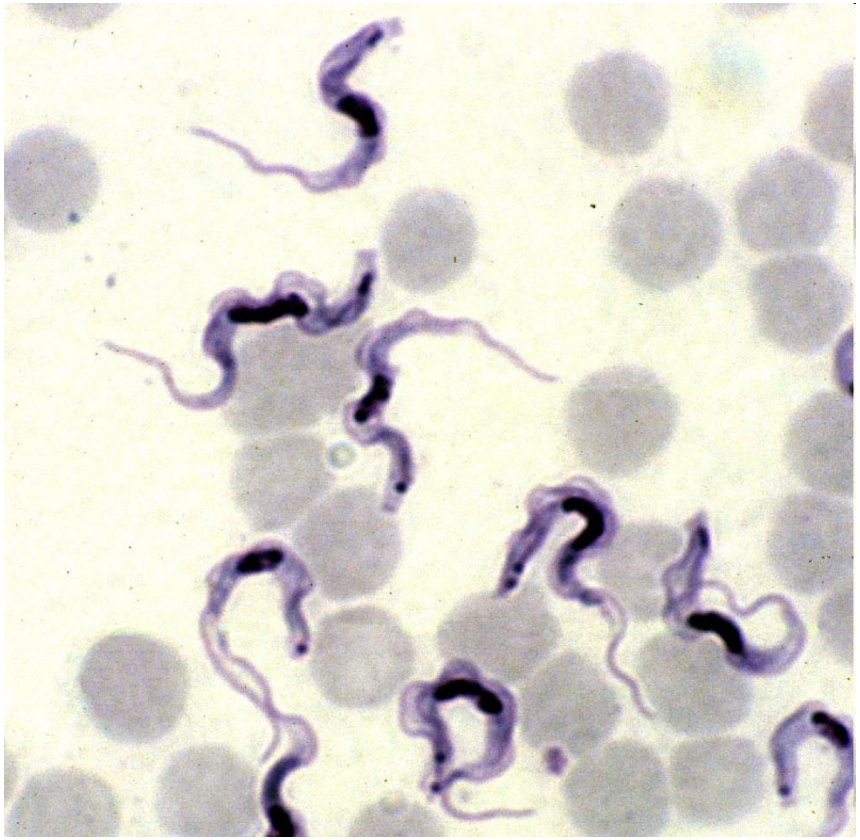
"The interest centering round this reduviid bug is the coincidence of the find in Brazil of an insect of the same genus transmitting trypanosoma to human beings. It is true Chagas's statements are astounding; time will however clear any doubts that at present exist on his discovery. As mentioned in my report of 1908, Conorrhinus, to the extent of 90 per cent, harbours the flagellate, Crithidia. Could Chagas have mistaken these parasites for trypanosomes?"

And in 1916 at a major International Meeting in Argentina, a prominent German microbiologist named Krause, denounced Chagas' findings, and the work of Chagas was forgotten for almost 20 years. Finally, in the early 1930's, Johnson described more than 1000 cases of Chagas Disease in Panama and Argentina.

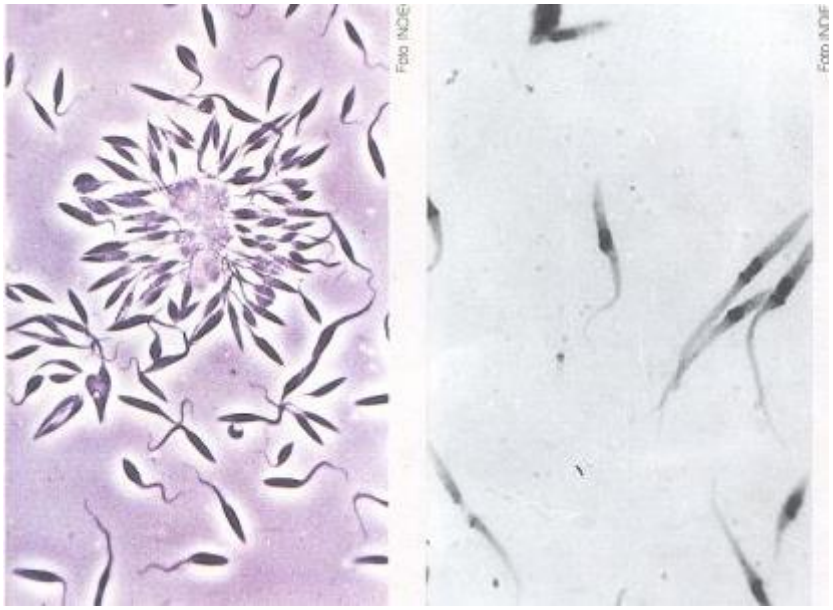
Morphology of T. cruzi

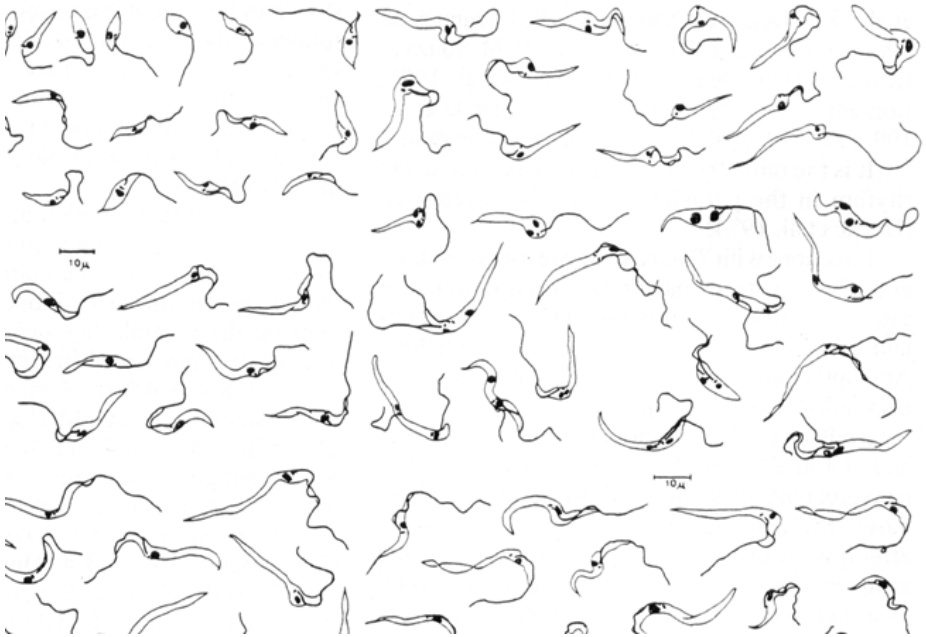


trypomastigote



epimastigote





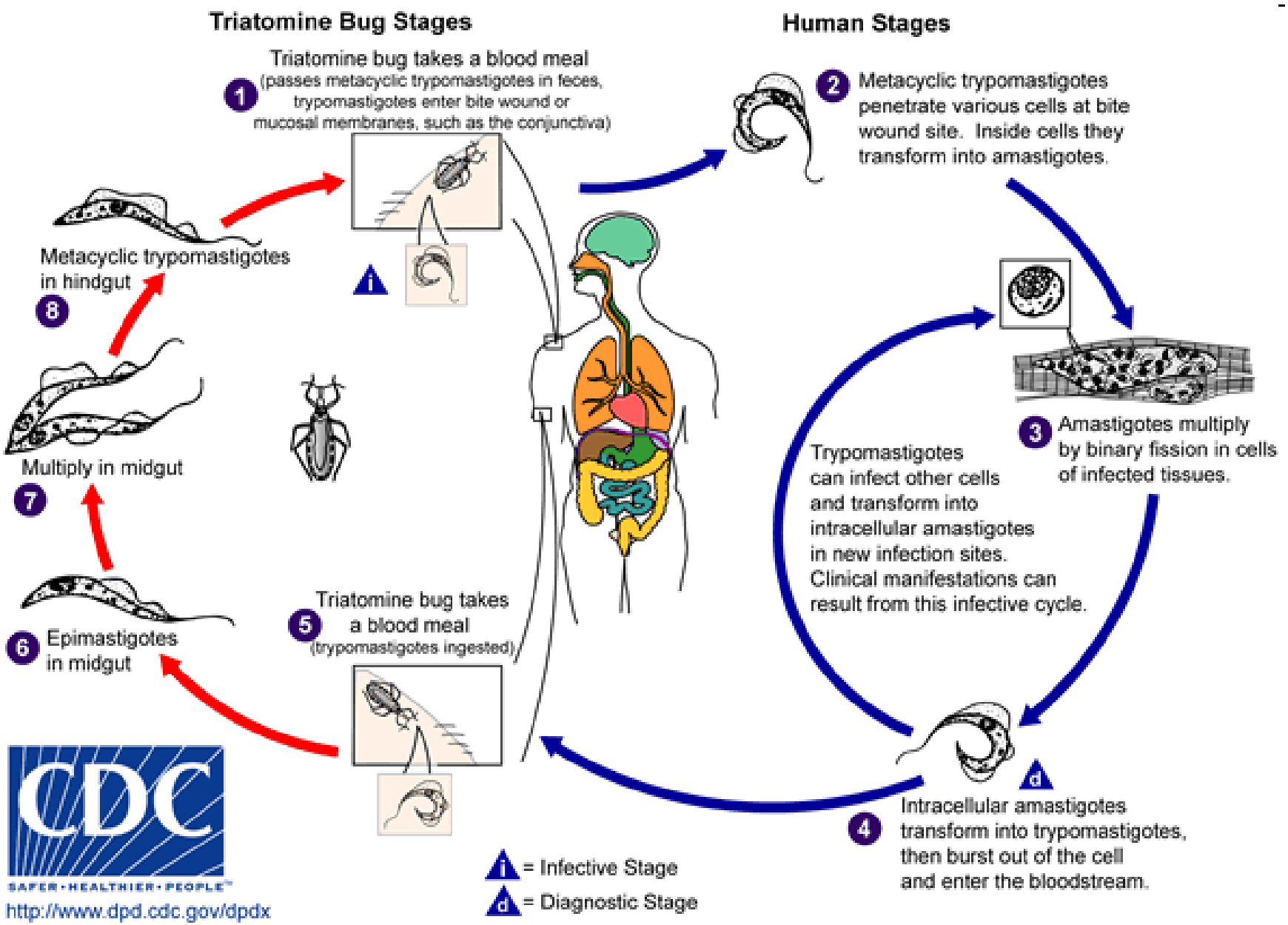
Chagas Disease

Chagas Disease is prevalent throughout South and Central America. An estimated 15-20 million people are infected and over 100 million people are at risk. In some endemic areas up to 60% of the population is serologically positive for *T. cruzi*. It was once thought to be an exotic rare disease, but with improved diagnostic methods it is now known to be one of the most widespread infectious diseases in Latin America. In one hospital in Goiania, Brasil, more than 20% of the patients had Chagas Disease. Most cases of sudden death in young adults in parts of Latin America can be attributed to chronic Chagas Disease.

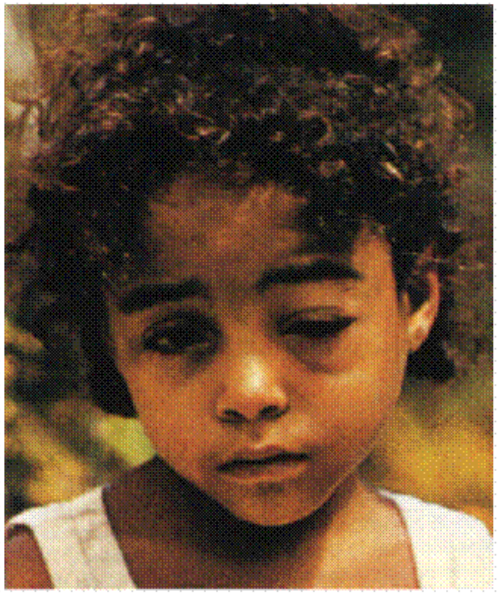
The disease was known in colonial times

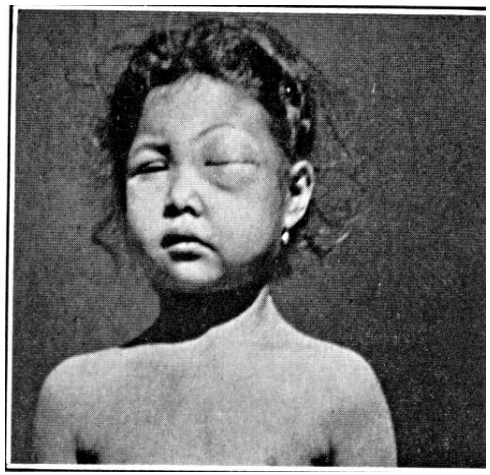
In 1735 the physician Gomes Ferreira wrote: *"the corruption of bicho is nothing else but an enlargement and distention of the rectum."* These descriptions suggest that patients in Brazil suffered from a disease that resulted in megaesophagus and megacolon—now recognized as a signal character of American trypanosomiasis.

Life Cycle

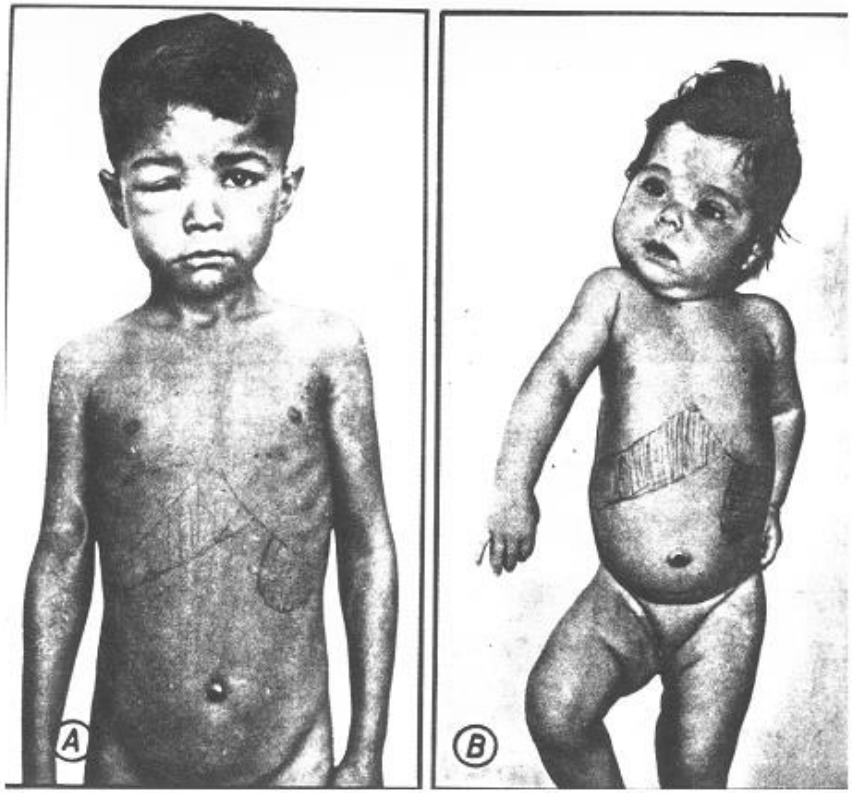

Triatomine Bug Stages
Human Stages
Triatomine bug takes a blood meal
(passes metacyclic trypomastigotes in feces, trypomastigotes enter bite wound or mucosal membranes, such as the conjunctiva)
1
2
Metacyclic trypomastigotes penetrate various cells at bite wound site. Inside cells they transform into amastigotes.
Metacyclic trypomastigotes in hindgut
8
Multiply in midgut
7
3
Amastigotes multiply by binary fission in cells of infected tissues.
Trypomastigotes can infect other cells and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle.
Epimastigotes in midgut
6
Triatomine bug takes a blood meal
(trypomastigotes ingested)
5
4
Intracellular amastigotes transform into trypomastigotes, then burst out of the cell and enter the bloodstream.
i = Infective Stage
d = Diagnostic Stage
CDC
SAFER·HEALTHIER·PEOPLE™
http://www.dpd.cdc.gov/dpdx

Romaña's Sign





Hepatosplenomegaly



The ACUTE phase of *Trypanosoma cruzi* infection

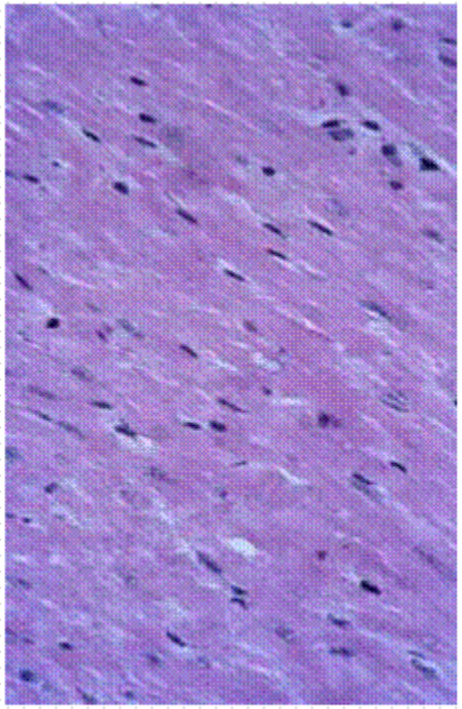
1. **Romaña's Sign**
2. **Fever**
3. **Hepatosplenomegaly**
4. **Trypomastigotes in Blood**
5. **Lasts 2-8 weeks**
6. **10% Mortality**

The INDETERMINATE phase

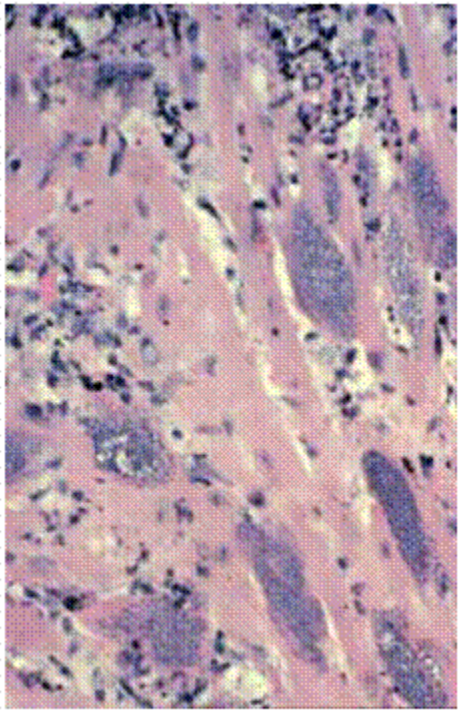
1. **No parasite evident in blood**

2. **Amastigote nests in muscle tissue**

3. **Anti-*T. cruzi* IgG present**

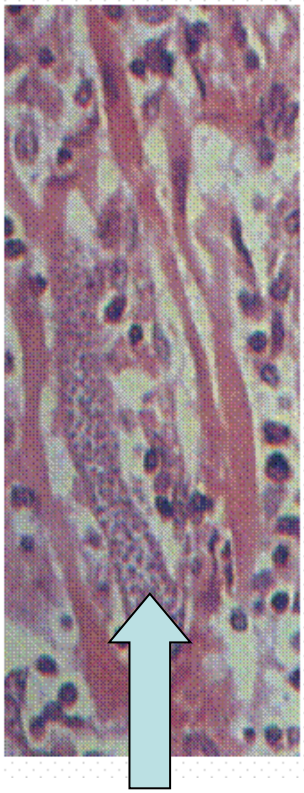


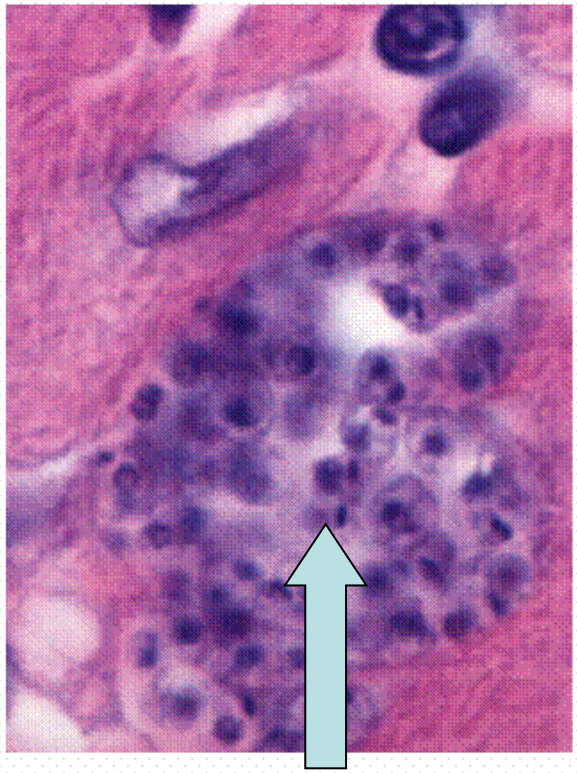
Normal Heart

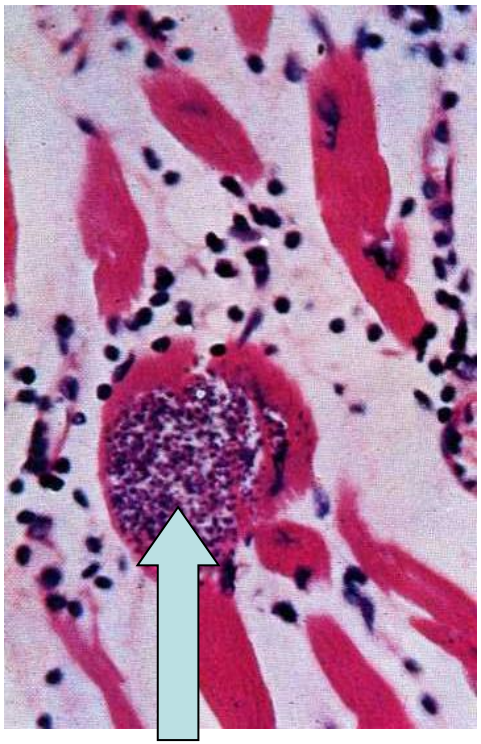


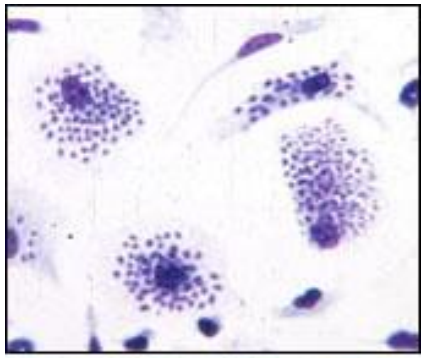
Chagasic Heart

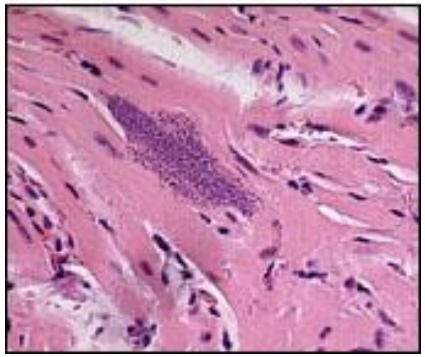
Trypanosoma cruzi amastigotes in heart muscle



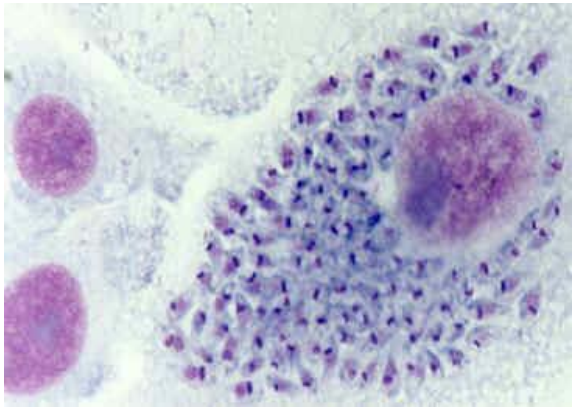




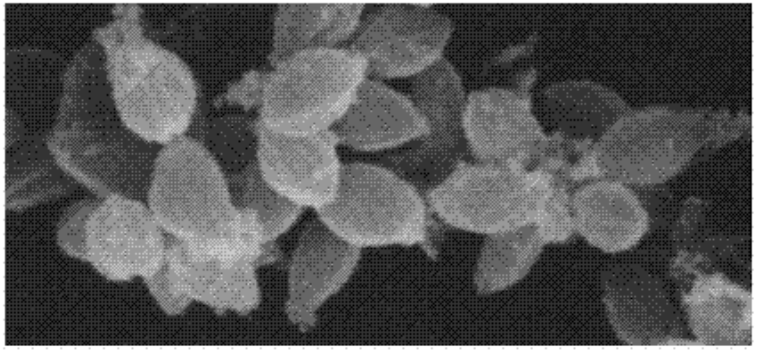




Amastigotes in a tissue culture cell

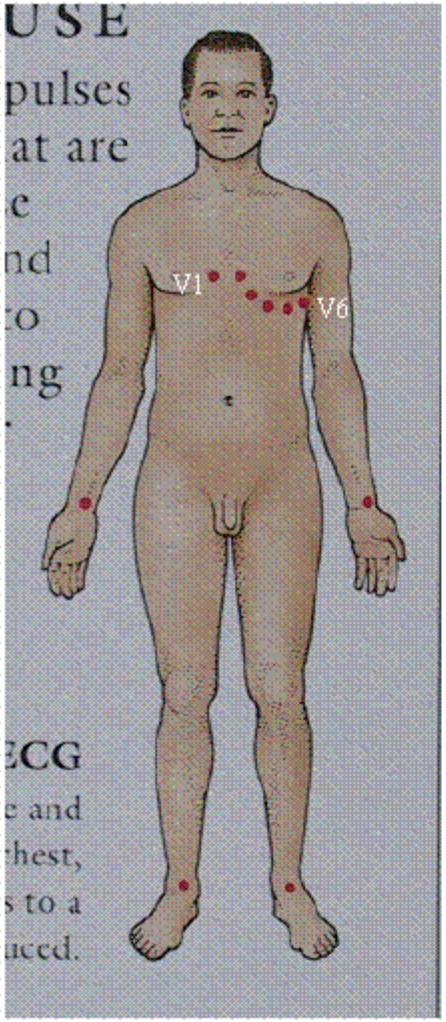


Scanning EM of isolated amastigotes



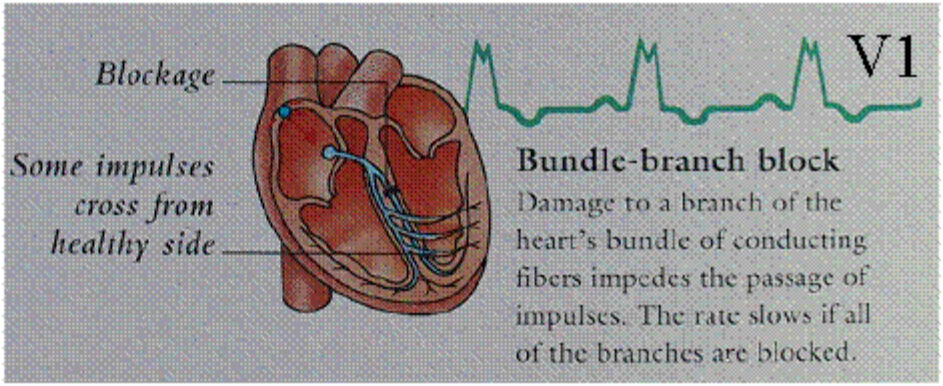
CHRONIC phase of Chagas Disease

1. **Nerve Degeneration**
2. **Cardiomyopathy (80%)**
   - **Heart arrhythmia and blocks**
   - **Heart enlargement (cardiomegaly)**
   - **Apical aneurism**
3. **Megaesophagus (25%)**
4. **Megacolon (30%)**

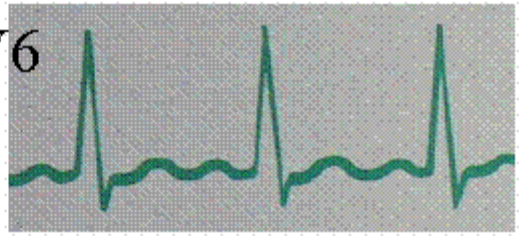


Chagas Disease

Right Branch-bundle Block



Normal and V6



Cardiomegaly

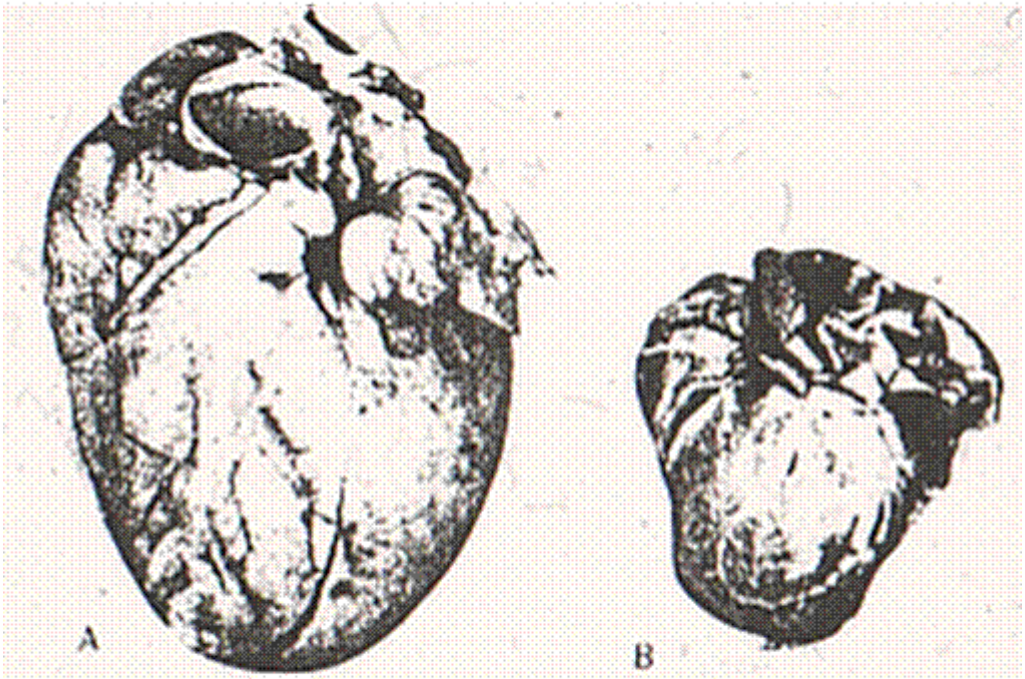
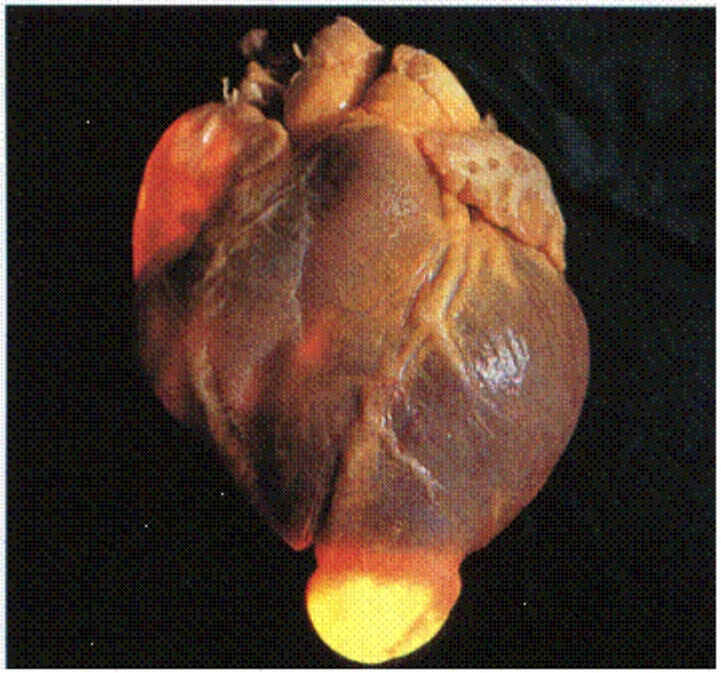
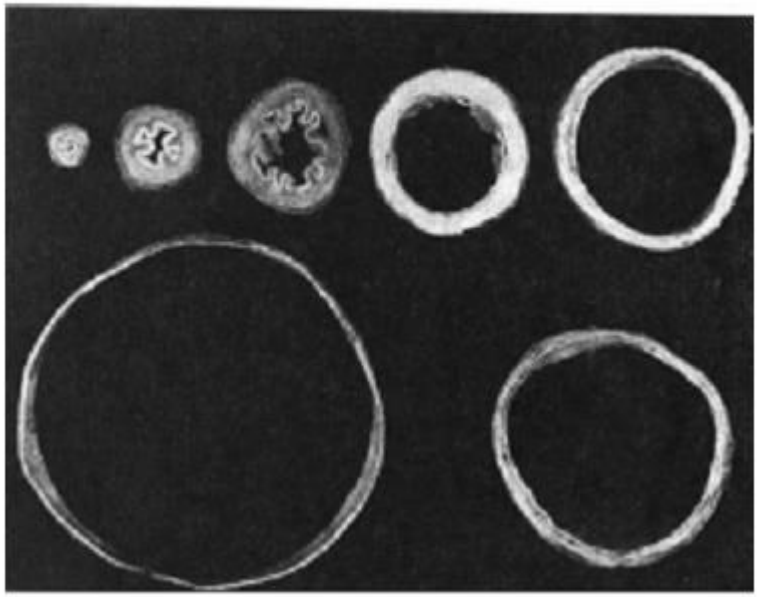


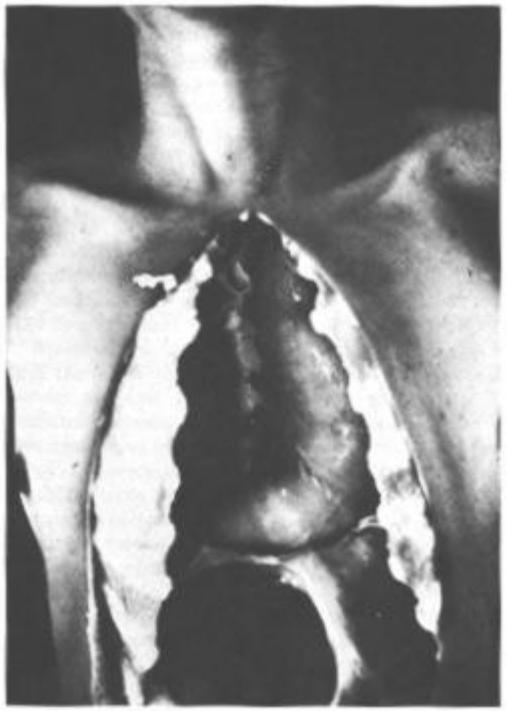
Fig. 13.11 — A — Coração de indivíduo falecido com miocardite chagásica crônica; notar a cardiomegalia. B — Coração de indivíduo normal. (Segundo Jairo Ramos e cols.)

Apical Aneurysm



Megaesophagus



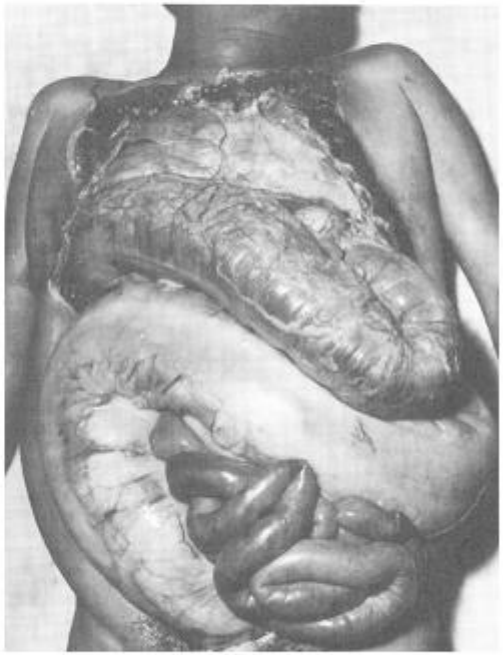


5. Chagasic megaesophagus at necropsy. [Courtesy of Dr. F. Köberle.]

Megacolon

Constipation

Negative control



Autopsy

Definitions

ANTHROPONOSIS - disease with humans as only vertebrate hosts

ZOONOSIS - disease transmitted among wild animals (reservoir hosts) and humans

RESERVOIR HOST - wild animal that maintains infection in nature

Fecal transmission of *T. cruzi*

There are several genera of Reduviidae that can transmit the protozoan. *Triatoma infestans*, as its name suggests, frequently invades homes (mud and stick huts) and is responsible for what is termed the domestic transmission cycle. Other reduviids, such as *Rhodnius prolixus,* reside in rural settings or forests, they are associated with the silvatic cycle of transmission, which includes many wild animals as vertebrate hosts. As mentioned above, the common reduviid vectors *R. prolixus* and *Panstrongylus megistus* defecate during the blood meal, allowing efficient infection by the parasite.

Distribution of Triatomine vectors

Chagas Disease as a Socio-Economic Disease

Chagas disease is mainly a disease of third world countries with substandard housing with dirt floors, mud walls and thatched roofs, that allows infestation with the reduviid vectors. Prevention involves the destruction of the vectors by spraying insecticides in houses and also by replacement of adobe houses (see below) with modern houses. The best insecticide seems to be BHC (hexochlorocyclohexane), in terms of low cost, low toxicity to humans and animals, and activity in the mud walls of houses. Most other insecticides are rapidly inactivated when sprayed on such walls. The number of applications required vary from twice per year to every month.

Habitat of *T. infestans*

Infection of Kissing Bugs with *T. cruzi* in Tucson, Arizona

Collected triatomines inside and aound human houses in Tucson.
Analyzed for parasites using PCR

Adult female kissing bug of the species *Triatoma rubida*, the most abundant triatomine species in southern Arizona. Scale bar = 1 cm.

Collection sites and collected insects per area, triatomine insects survey, metropolitan Tucson, Arizona, USA, 2006*

Area	No. collection sites (% with insects infected with *Trypanosoma cruzi*)	No. insects collected (% infected with *T. cruzi*)
Central	2 (100)	2 (100)
North	1 (0)	2 (0)
Northeast	1 (100)	2 (50)
Northwest	6 (66)	14 (43)
South	0	0
Southeast	3 (66)	11 (45)
Southwest	2 (100)	19 (42)
East	0	0
West	4 (100)	88 (40)

Alternative Methods of Transmission

Rural migrations to urban areas in South and Central America during the 1970s and 1980s changed the traditional epidemiological pattern of Chagas disease: it became an urban disease, as unscreened blood transfusion created a second way of transmission. Between 1960 and 1989, the prevalence of infected blood in blood banks in selected cities of South America ranged from 1.7% in Sao Paulo, Brazil to 53.0% in Santa Cruz, Bolivia, a percentage far higher than that of hepatitis or HIV infection. Transmission by blood transfusion has also become a potential problem in the Los Angeles area due to immigration from Central America where the disease is endemic. In Los Angeles, 2% of the blood donors in a 1993 study were seropositive. Five cases of Chagas in the US in 1990-1993 came from blood transfusion or organ transplants.

Congenital transmission also occurs. 0.5-6.3% of infants born to Chagasic mothers are positive for T. cruzi.

“Sugar Cane Juice Causes Deadly Outbreak of Chagas in Brazil” !

Contaminated sugar cane juice is thought to be the source of a Brazilian outbreak of Chagas disease, a potentially fatal parasitic disease normally transmitted to people by insect bites. In the past few days, health officials in the state of Santa Catarina have recorded 45 cases of patients developing symptoms of Chagas disease after drinking the juice. At least five of the patients died. The patients initially reported having fever, migraine, and muscle pain, with some going on to develop jaundice, abdominal pain, internal bleeding, fluid in the lungs and heart failure. Blood tests confirmed the presence of Trypanosoma cruzi in 31 of the 45 suspected cases.
Ninety per cent of cases had consumed sugar cane juice from the Kiosk # 2 along the northern beaches of Santa Catarina.

Oral Transmission of Chagas Disease by Consumption of Açaí Palm Fruit, Brazil

Aglaêr A. Nóbrega, Marcio H. Garcia, Erica Tatto, Marcos T. Obara, Elenild Costa, Jeremy Sobel, and Wildo N. Araujo

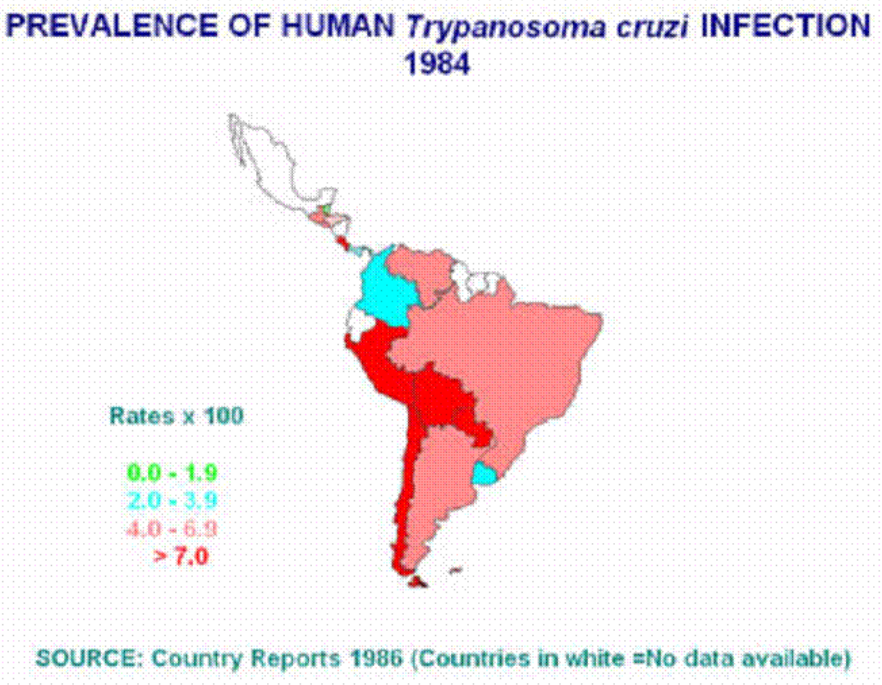
Emerg Infect Dis. 2009 April; 15(4): 653–655.

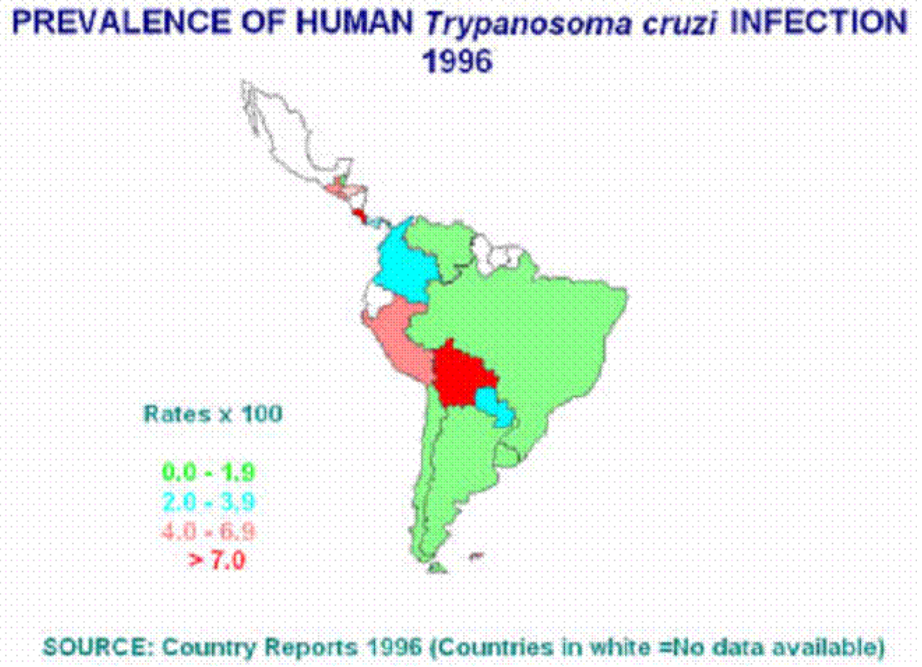
Chagas' disease as a foodborne illness.

Pereira KS, Schmidt FL, Guaraldo AM, Franco RM, Dias VL, Passos LA.

J Food Prot. 2009 Feb;72(2):441-6.

The Southern CONE initiative





Diagnosis of Chagas Disease and detection of the parasite

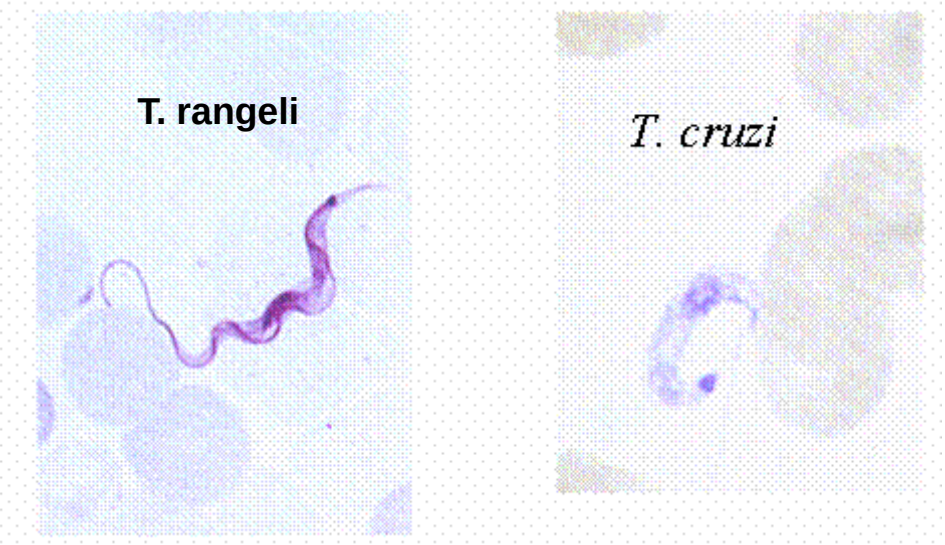
Assay must be:
Specific (No False Positives or crossreactivity, e.g. *T. rangeli*)
Sensitive (Single Cell)

Indirect Assays Detect Antibody
i.e. evidence of past (present?) infection
Direct Assays Detect Parasite

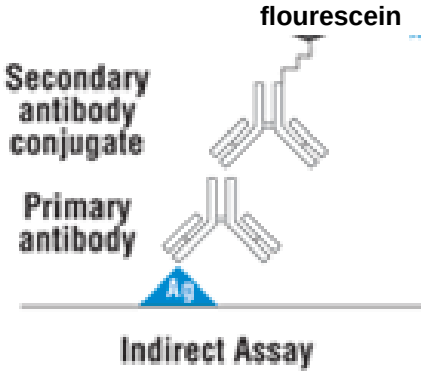
Microscopy – Direct Detection Method

This is generally made directly on blood smears, or following culture in synthetic medium. When the parasite is not abundant, one can concentrate the parasites first by centrifugation; they are found in the "buffy coat" or white cell layer. The real problem is that the parasite is not abundant in the blood during the indeterminate and chronic stages. This method probably can only detect less than 1% of chronic infections.

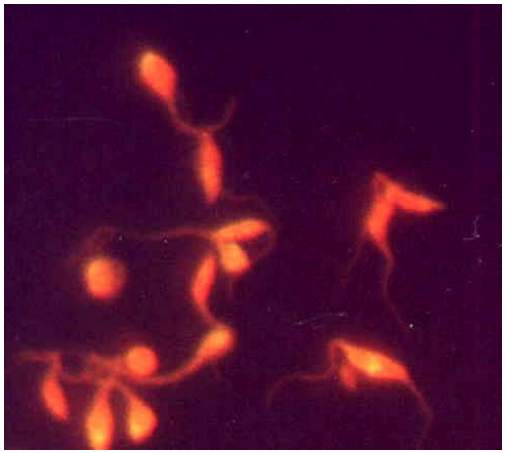
Another problem is that *Trypanosoma rangeli* looks very much like *T. cruzi* and can lead to misdiagnosis. *T. rangeli* is a trypanosome that is infective for humans and other animals but is nonpathogeneic to humans (but detrimental to the insect!). The chief insect host is *R. prolixus*. The trypomastigotes are discharged in the saliva rather than the feces. It looks morphologically like *T. cruzi* and has an overlapping geographical distribution.



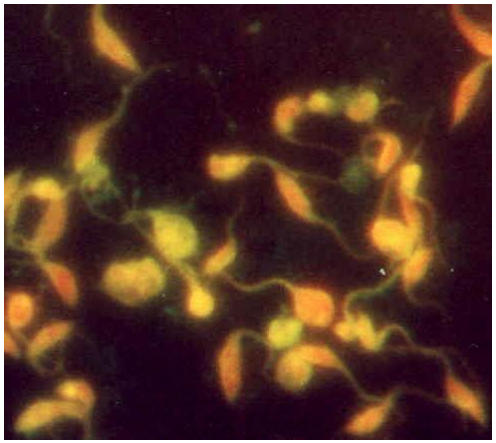
Indirect Immunofluorescence



57% of 87 raccoons in South Georgia were seropositive



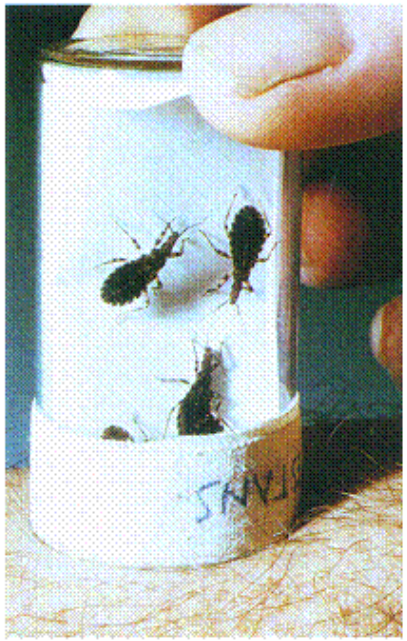
Negative control. Stained with Evans blue

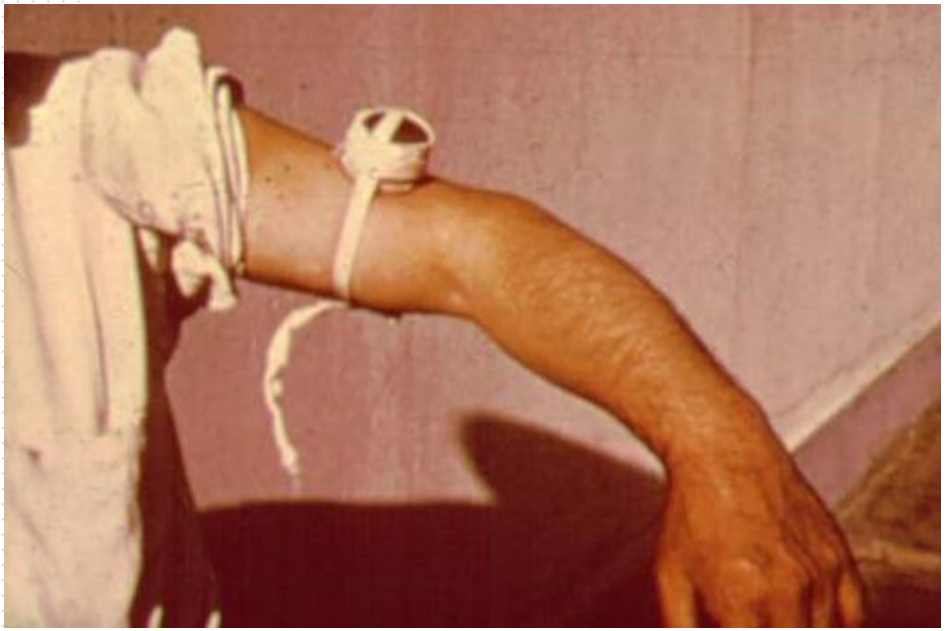


Flourescein conjugated anti-racoon Igg Ab's binding to anti-T. cruzi Ab's in the serum

From Yabsley et al (1999)

Xenodiagnosis (Direct)





Xenodiagnosis is a method of diagnosis first described in 1877. Laboratory-reared non-infected triatomines are allowed to feed on patients suspected to have Chagas disease. The bugs are then examined 3-4 weeks later for the presence of *T. cruzi* in the hind gut/excreta; *T. rangeli* will be found in the salivary glands. Although this method is quite efficient in diagnosing the acute disease, it may be only 50% efficient in the chronic stage. Whereas 1 µl of blood can be viewed on a microscope slide, 10 bugs can sample 1 ml of blood. The efficiency of this technique is complicated by variable growth of different *T. cruzi* isolates in different genera and species of reduviids.

DNA-based assays (Direct)

There are two major criteria for successful PCR detection of a parasite (or any microorganism):

<u>Specificity</u> -- must detect only the *T. cruzi* parasite

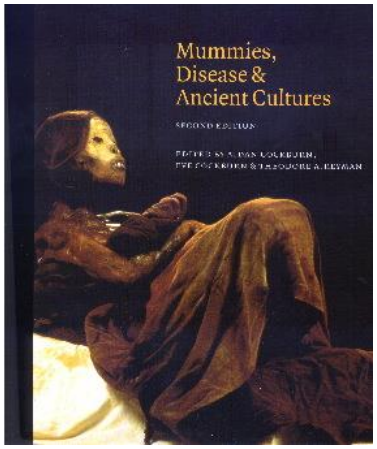
and

<u>Sensitivity</u> -- how few parasites can the assay detect?

A recent study showed a 9000 year record of Chagas Disease

Studied 283 mummies from the Atacama desert region of Northern Chile and Southern Peru.

Culture	Time range	No. tested	Percent positive
Early Chinchorro	7050–3000 BC	18	39
Late Chinchorro	3000–1500 BC	53	43
Early Alto Ramirez	1000 BC–0	16	25
Late Alto Ramirez	0–400 AD	20	35
Cabuza	400–1050 AD	27	41
Maitas	1000–1250 AD	25	40
Chiribaya	1050–1250 AD	70	47
M8 (upper Chiribaya)	1050–1250 AD	16	19
San Miguel	1250–1350 AD	9	33
Inca	1450–1550 AD	26	50
Colonial	1550–1850 AD	3	67
All cultures	7050 BC–1850 AD	283	40.6



DNA was extracted from mummy tissue and PCR performed using primers specific for the unusual mitochondrial DNA from *T. cruzi*.

Results: 115 of the 283 mummies were positive = 40.6%.
No sex differences or time period differences.

In the 1970's the *T. cruzi* infection rates in Bolivia, Peru and Venezuela were 15-60%

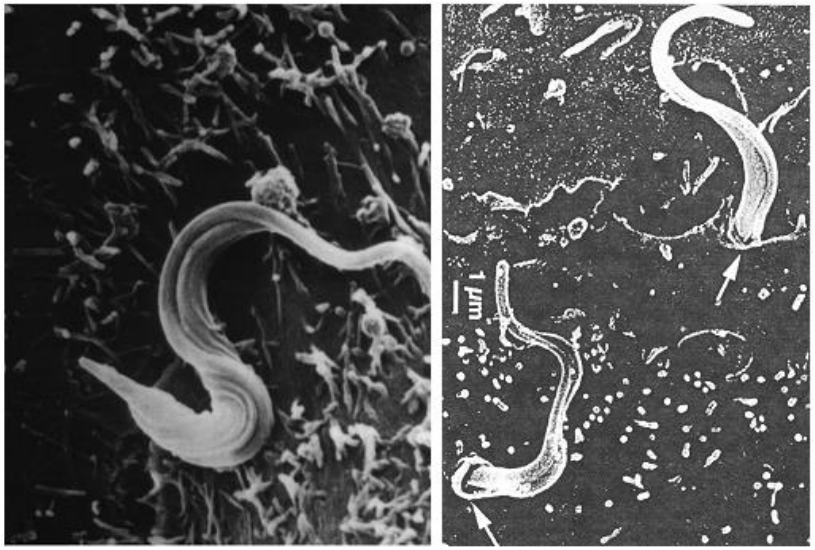
Possible reasons for the ancient transmission of Chagas Disease:

1. **People built wattle and thatch houses until present time.**
2. **People kept domestic animals in houses.**

How do *T. cruzi* parasites enter the cell?

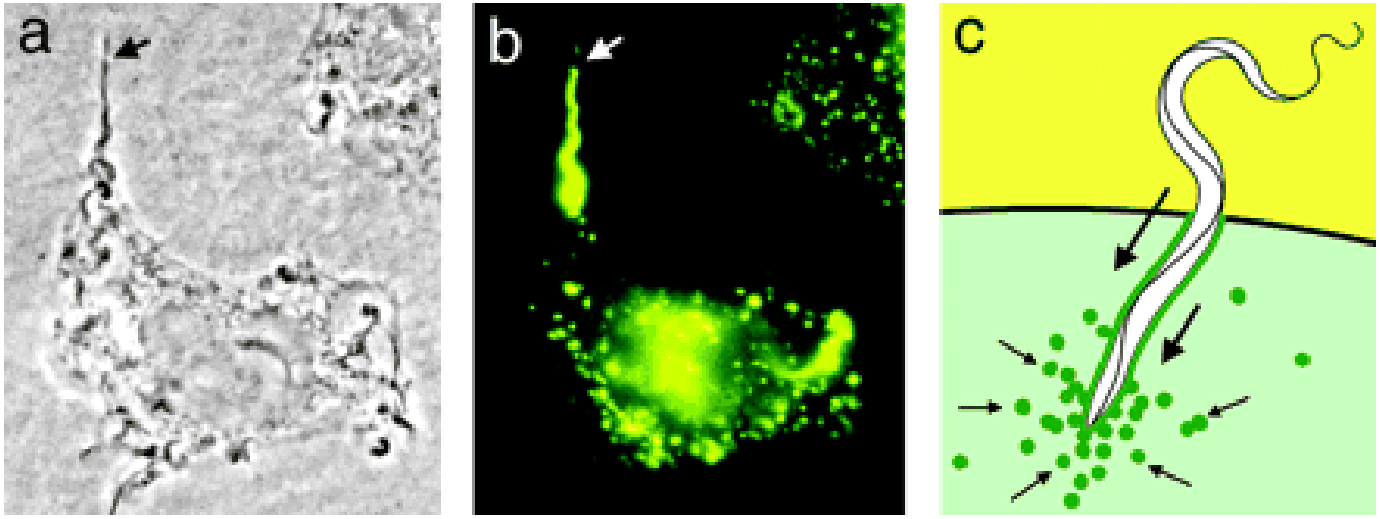
Metacyclic trypomastigote parasites from the feces of the infected triatomid bug enter the vertebrate host through the bite wound or the mucosal membrane. These invade cells through the formation of a membrane vacuole. This vacuole is disrupted and the trypomastigotes are released and differentiate into amastigotes. The amastigotes go through nine cycles of intracellular replication in 4-5 days, and then differentiate into trypomastigotes. The host cell ruptures and the parasites are released into the bloodstream, where they are disseminated throughout the body.

T cruzi entry into cell

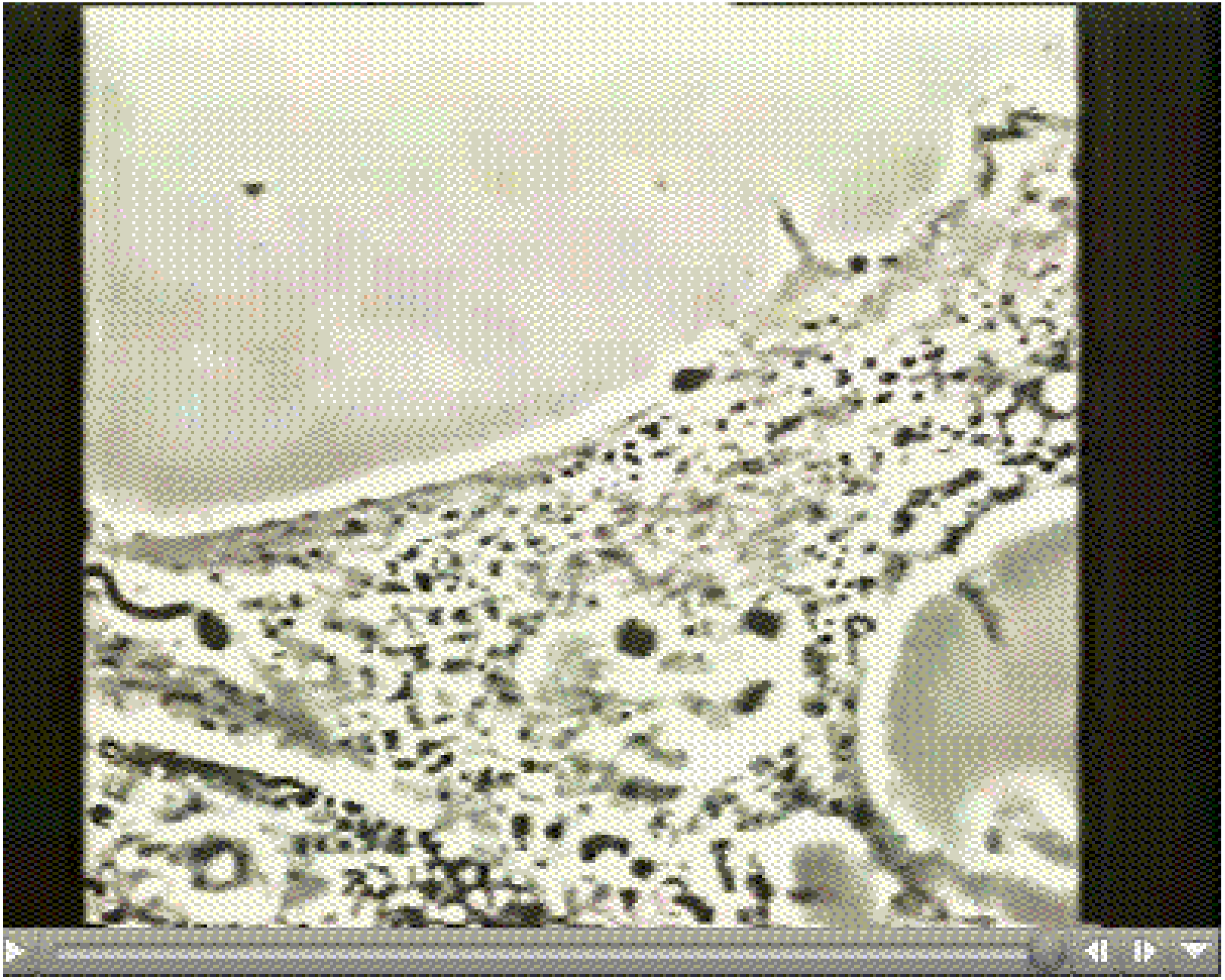


No engulfing pseudopodia
Resistant to Cytochalasin D

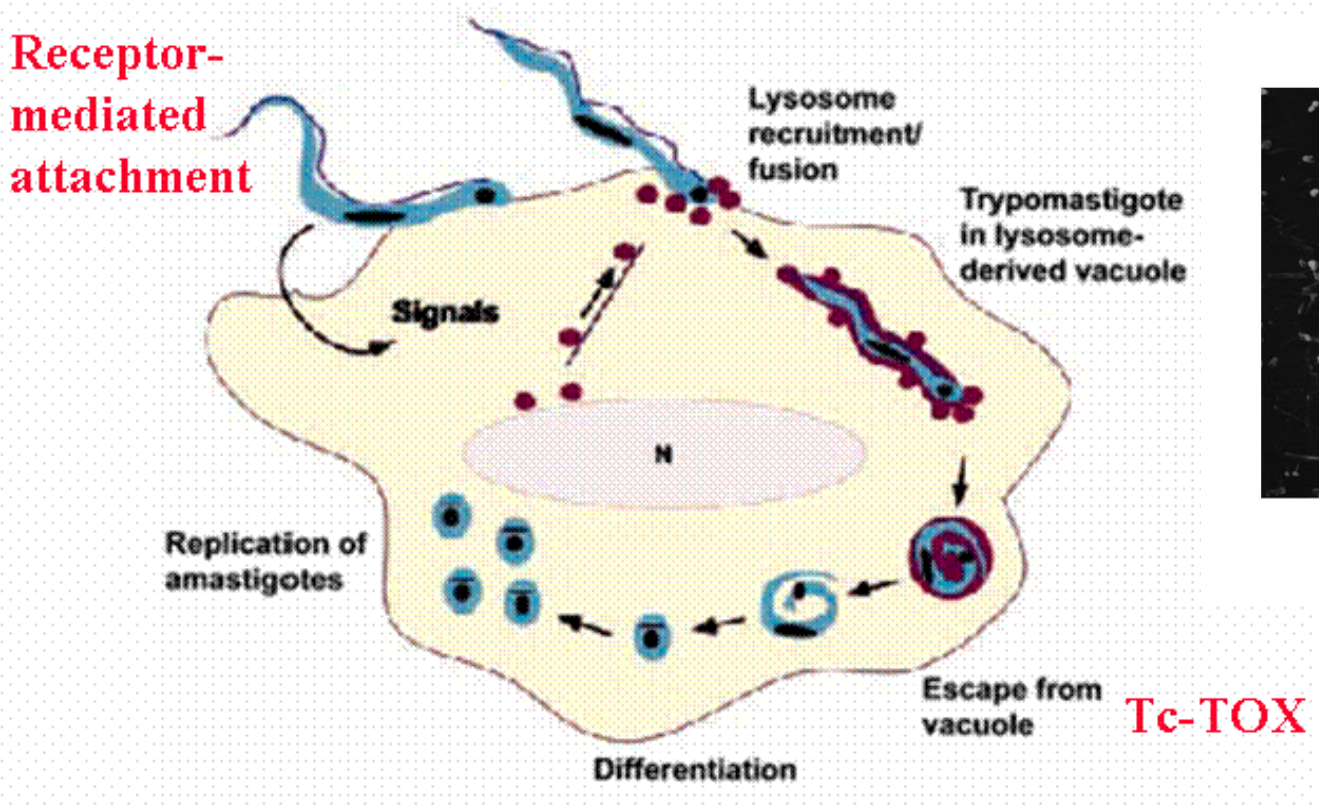
Recruitment of lysosomes to the invading parasite



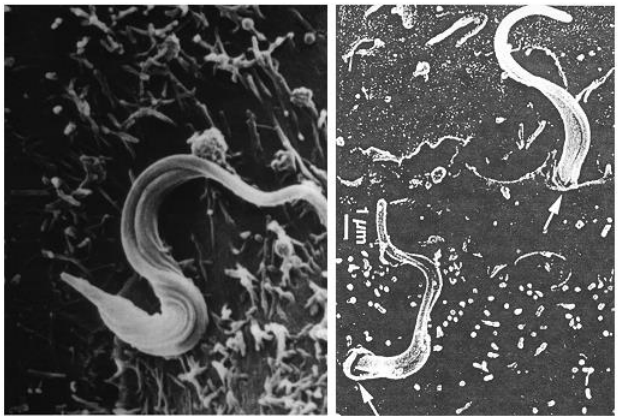
(a) Phase-contrast image of a trypomastigote (arrow) in the process of entering a HeLa cell. (b) Immunofluorescence image of the same cell shown stained with antibodies against the lysosome-specific protein,Lamp-1. (c) The green line represents lysosomal membranes that are gradually incorporated into the vacuole, the small arrows indicate the direction of lysosome movement, and the large arrows indicate the direction of parasite movement. (Tardieux et al, 1992)



Release of the parasites from the vacuole



T cruzi entry into cell



No engulfing pseudopodia
Resistant to Cytochalasin D

A secreted protein, TcTOX, which has antigenic relatedness with the lytic complex C9 of the complement system, lyses the vacuole, and the metacyclic trypomastigote enters the cytoplasm where it differentiates into an amastigote and divides.

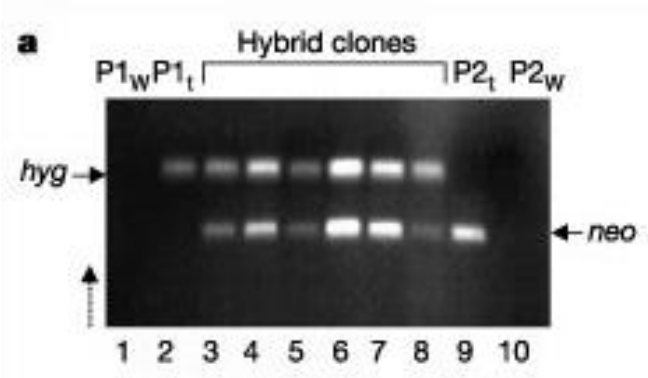
Evidence for Genetic Exchange in *T. cruzi*

Two clones of *T. cruzi* from the Amazon forest were transfected with plasmids containing either the Neomycin marker or the Hygromycin marker.

These were passaged either singly or together through the life cycle stages. The parasites were recovered and cultured with both drugs and cloned.

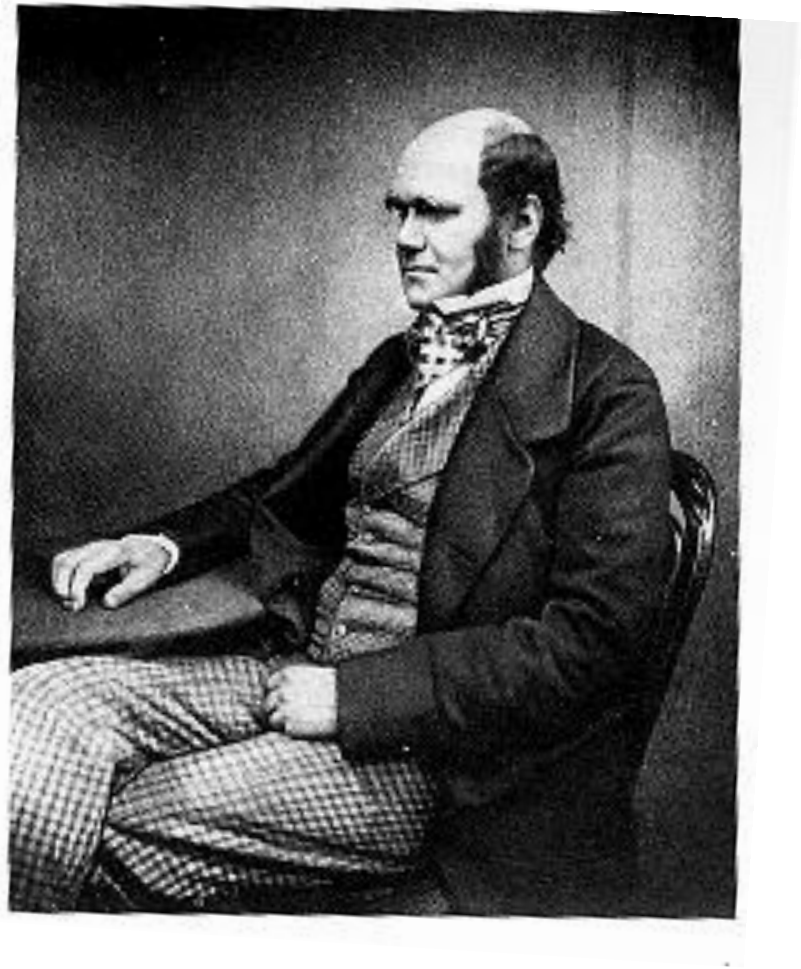
Results:

1. 50 tissue cultures infected with the mixture of strains gave populations resistant to both drugs. Six *T. cruzi* clones contained both drug resistance genes. The clones were not binucleate.



2. No double resistant populations were obtained from mixed axenic epimastigote cultures, from mixed passage through triatomids, or from mixed passage in mice.

The Case of Charles Darwin

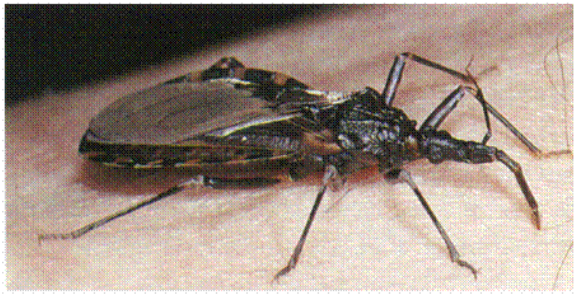


Mendoza, Argentina

In his own words:

"We slept in the village, which is a small place surrounded by gardens, and forms the most southern part, that is cultivated, of the province of Mendoza; it is five leagues south of the capital. At night I experienced an attack (for it deserves no less a name) of the Benchuca (a species of Reduvius) the great black bug of the Pampas. It is most disgusting to feel soft wingless insects, about an inch long, crawling over one's body. Before sucking they are quite thin, but afterwards they become round and bloated with blood, and in this state are easily crushed. They are also found in the northern parts of Chile and in Peru. One which I caught at Iquique, was very empty. When placed on the table, and though surrounded by people, if a finger was presented, the bold insect would immediately draw its sucker, make a charge, and if allowed, draw blood. No pain was caused by the wound. It was curious to watch its body during the act of sucking, as it changed in less than ten minutes, from being as flat as a wafer to a globular form. This one feast, for which the benchuca was indebted to one of the officers, kept it fat during four whole months; but, after the first fortnight, the insect was quite ready to have another suck."

This insect was the triatomid, *Triamtoma infestans*, of which today more than 70% of the insects in that region are infected with *T. cruzi*. Also 12% of the population in Mendoza today has antibodies against *T. cruzi*.



Darwin was at that time one of the most active members of the Beagle's crew. He often took long overland expeditions and was a mountain climber. He retuned to England in 1836 and in 1838 his health suddenly became poor. His health became progressively worse and he suffered from periodic vomiting, fatigue and flatulence. After social dinners he had violent shivering and vomiting attacks, and mainly for these reasons he gave up all social interactions. His diaries are full of descriptions of his mysterious illness.

He wrote to the Botanist, Joseph Hooker, in 1845: *"I believe that I have not had a whole day, or rather night, without my stomach being greatly disordered, during these last three years, and most days great prostration of strength.“* In 1849 he was too ill to attend his father's funeral.
He wrote:
" I was quite broken down, head swimmy, hands trembling and never a week without violent vomiting."

Emma's Diary

APRIL, 1865

23 SUNDAY. [Low Sunday.]
Les. M.-Numbers 16, Acts 20. E.-Numbers 22, 2 Peter 2.

C. sick

24 MONDAY.

Lizzy & boys to sch

C sick

25 TUESDAY.

Flen. G. & Clem to London

C. sl

26 WEDNESDAY. [Ox. Eas. Term begins.]

C 3 fits of sickness

last 6.30

Hope came

APRIL, 1865.

27 THURSDAY.

C. better

Therm. 70.

sick twice at 8. p. m.

28 FRIDAY.

sick 10. 30. a. m.

1 in night

29 SATURDAY.

Better all day

Hope went

9 - sick

Took blue pill

Table 1 Proposed causes of Darwin's disease

Causal type	Specific cause	Reference
Organic	Heart disease	Darwin himself (1831–1882)[1 2] and *Diary of Health*
	Nervous indigestion	Obituary (1882)[2]
	Chronic from sea sickness	Obituary (1882)[4]
	Chronic neurasthenia	Johnston, 1901[5]
	Chronic eye strain	Gould, 1903[6]
	Aftermath of Chilean fever	Leonard Huxley, 1927, see Colp[7]
	Pyorrhoea	Leonard Darwin, 1927, see Colp[7]
	Brucellosis	Simpson, 1958[8]
	Chagas' disease	Adler, 1959[9]
	Metabolic disease	Stetten, 1959[10]
	Acute intermittent porphyria	With, 1960, see King-Hele[11]
	Diaphragmatic hernia	Kohn, 1963[12]
	Narcolepsy (diabetes)	Roberts, 1966[13]
	Arsenic poisoning	Winslow, 1971[14]
	Pigeon allergy	Gruber and Barrett, 1974, see Colp[7]
	Peptic ulcer	See Colp p130[7]
	Duodenal ulcer	See Colp p130[7]
	Appendicitis	see Colp p130[7]
	Smouldering hepatitis	See Colp p130[7]
	Cholecystitis	See Colp p130[7]
	Amoeba infection	See Colp[7]
	Allergy	Smith, 1990, 1992[15 16]
	Systemic lactose intolerance	Campbell and Matthews,[17 18]

Psycosomatic		
	1st psychoanalytical theory	Kemf, 1918[19]
	Hypochondria	Hubble, 1943[20]
	Psychoneurosis	Hubble, 1943[20]
	Chronic depression	Alvarez, 1959[21]
	Psychosomatic	Woodruff 1965[22]
	Bereavement syndrome	Bolby, 1965, 1990[23,24]
	Neurosis	Colp, 1977[7]
	Mixed psychosomatic	Colp, 1977[7]
	Anxiety state	Bernstein, 1982[25]
	Panic syndrome	Barloon and Noyes, 1997[26]

A recent suggestion is *lactose intolerance*

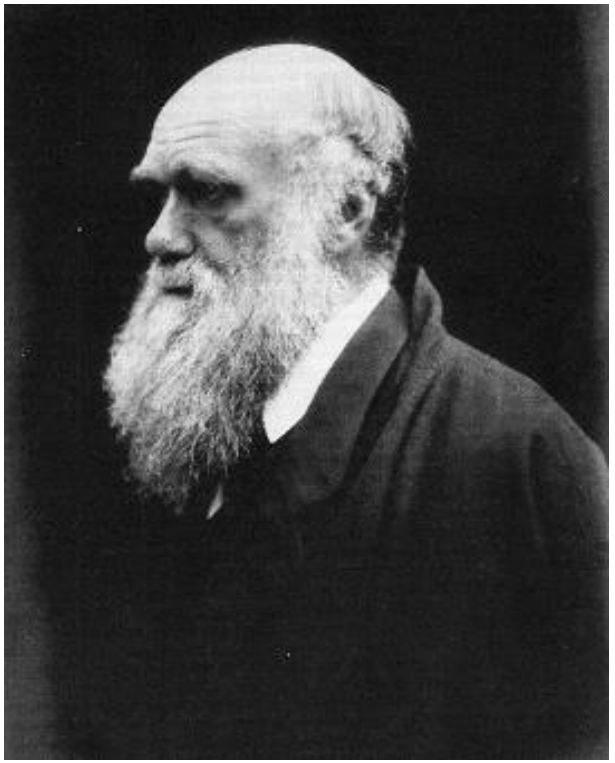
Darwin wrote that : "The sickness starts usually about two hours after a meal."
His wife, Emma, had a cookbook that confirmed his love of sugar and rich foods.

Table 2 Systemic lactose intolerance compared with Darwin's disease

Symptoms of systemic lactose intolerance	% People with lactose intolerance who have this symptom*	Darwin's description of his symptoms	Occurrence of Darwin's symptoms
Gut symptoms (pain, bloating, diarrhoea)	100	Stomach ache	Common
Flatulence	100	Flatulence (belching)	Common
Headache	86	Headache	Common
Light headedness and loss of concentration	82	Swimming head and difficulty to concentrate	Common
Nausea and vomiting	78	Vomiting	Very common
Muscle and joint pain	71	Rheumatic pain	Often
Tiredness and chronic fatigue	63	Chronic fatigue and exhaustion	Very common
Allergy (eczema, hay fever, rhinitis, sinusitis)	40	Skin rash and boils	Often
Mouth ulcers	30	Mouth sores	Common
Heart palpitations	24	Palpitations in the chest	Common
Depression	Common, but not quantified	Depression	Frequent

*Represents proportion of people diagnosed as lactose intolerant who have this particular symptom within 48 hours of taking lactose. Darwin's occurrence is based on his notes and letters during periods of the episodes. The systemic symptoms of lactose intolerance are described previously.[17 18]

Or - Did Darwin have Chagas Disease stemming from his stay in Mendoza during the Beagle voyage?



CERTIFIED COPY OF AN ENTRY OF DEATH

GIVEN AT THE GENERAL REGISTER OFFICE

Application Number PAS 303697

REGISTRATION DISTRICT Bromley

1882 DEATH in the Sub-district of Bromley in the County of Kent

Columns: 1	2	3	4	5	6	7	8	9
No.	When and where died	Name and surname	Sex	Age	Occupation	Cause of death	Signature, description and residence of informant	When registered	Signature of registrar
20	Nineteenth April 1882 Down R.S.O.	Charles Robert Darwin	Male	73 years	J.P. M.A. L.L.D. F.R.S.	Angina Pectoris Syncope Certified by C. H. Alleyne M.D.	Frances Darwin Son present at the Death Down	Twenty fifth April 1882	Henry J. Rose Registrar

CERTIFIED to be a true copy of an entry in the certified copy of a Register of Deaths in the District above mentioned.

Given at the GENERAL REGISTER OFFICE, under the Seal of the said Office, the 31st day of August 1994

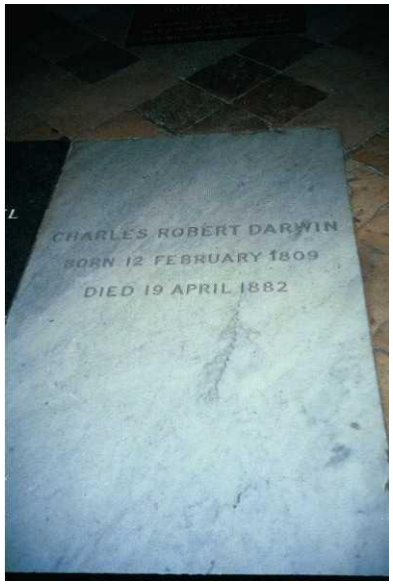
DXZ 215122

See note overleaf

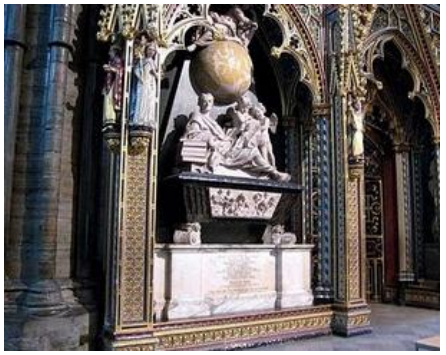
CAUTION: It is an offence to falsify a certificate or to make or knowingly use a false certificate or a copy of a false certificate intending it to be accepted as genuine to the prejudice of any person or to possess a certificate knowing it to be false without lawful authority.

He died in 1882 from an apparent heart attack.

Darwin is buried in Westminster Abbey - just next to Isaac Newton



Darwin's gravestone



Newton's tomb

PCR could perhaps be used to distinguish between Chagas Disease and a C to T mutation in the lactase gene!