

Review of Chagas Disease in Paraguay, South America with A survey on the public concern for Chagas' disease in Paraguay

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1. Biology of *Trypanosoma cruzi* and epidemiology of Chagas' disease

I Historical notes on Chagas' disease in Paraguay

A. Identification of *Trypanosoma cruzi* as an etiological agent

In 1939, 30 years after the first identification of *T. cruzi* by Chagas in Brazil in 1909, the first acute case of Chagas' disease in a soldier from Chaco, Paraguay was reported by G. Gonzalez and J.B. Rivarola, as "Enfermedad de Chagas aguda. Primer caso autóctono identificado en el Paraguay" in. An. Fac. Cien. Med. Univ. Nac. Asuncion., 7 (12) : 39-56. In the same year (1939), Dr. Denjamin Vorgas Pena, a physician of Puerto Guarani, on the west bank of the Paraguay river, sent pictures and fresh blood samples of 3 children and an adult who developed eyelid edema to Gonzalez and Rivarola laboratory, and they were confirmed as having Chagas' disease due to the infection by *T. cruzi*.

In 1942, Dr. Ricardo Odriolola discovered Chagas' disease in an infant. He reported the case in Comnica a la sociedad de Pediatría y Puericulture del Paraguay, as the first congenital case of Chagas' disease.

After that, Dr. Gonzalez made epidemiological surveys of the disease based on clinical symptoms and efforts were made by Prof. Dr. Canese and Prof. Dr. Rosner of the School of Medicine, Asuncion University to develop immunological and epidemiological test and/or immunological assay methods for Chagas' disease.

Furthermore, immunological survey of Chagas' disease was also conducted by El Servicio Nacional de Erradicacion del Paludismo (SENEPA) in the Ministry of Health of Paraguay, along combatting with malaria. Similar efforts were made by the section of the Tropical Diseases in Instituto de Investigaciones en Ciencias de la Salud (IICS) and Laboratorio Central de Instituto de Medicina Tropical (LACIMET).

B. Studies on *Triatoma* bugs

Studies on the insect vector, *Triatoma* spp. was said to

be initiated in San Bernardino in the following year after the identification of Chagas' disease in 1909. At present, however, none of the results of those studies remained in this country. In 1941, Gonzalez et al. investigated the presence of Triatomine bugs in 63 houses in Marisca Estigarribia district, Puerto Casado, Chaco Paraguay, and found that 59 (94%) were positive. Moreover, of the 265 Triatomine bugs caught at those houses that were examined for the presence of *Trypanosoma*, 60 (22.2%) were found to be infected with *T. cruzi*. Studies on the inhabitants of these houses revealed 4 acute cases of Chagas' disease.

In 1942, Gonzalez et al. conducted an epidemiological studies of Chagas' disease in San Bernardino, and found 24 (38.7) out of 62 Triatomine bugs examined were infected with *T. cruzi*, they also found a patient with an acute case of Chagas' disease. In the 1960s, Canese et al. made a series of studies on the distribution of Triatomine bugs in Paraguay and reported them in Revista Paraguaya de Microbiologia, followed by a review in 1978. Recently, surveys are also being made by SENEPA (1984-) and IICS (1970-).

II Biology of *Trypanosoma cruzi* Chagas, 1909

A. Classification

Trypanosome in mammalian hosts have been classified into STERCORARIA and SALIVARIA, and divided into 7 subgenus (by Dr. A. Miyata, 1979).

1. Section STERCORARIA

This group of the parasites always have a free flagellum and the kinetoplast is usually large and extends outside of the body. Posterior tip of the organism is sharp. The parasite propagates in the posterior part of the Triatomine gut and metacyclic trypomastigotes are shed along with the feces of the bug. The parasite is then transmitted to the mammalian host through the wound of the skin.

T. rangeli belongs to this section, but the organisms

propagate at the anterior part of the gut of the bug. Moreover, it can also be transmitted to another host when the bug bites with its contaminated mouthparts.

1) Subgenus *Megatrypanus* Hoare, 1964

Type species: *Trypanosoma (Megatrypanus) theileri* Lavern, 1902

Large, the nucleus is close to the kinetoplast and located rather far from the posterior end of the organism.

2) Subgenus *Herpetosoma* Doflein, 1901

Type species: *Trypanosoma (Herpetosoma) lewisi* (Kert, 1880)

Medium size, location of the kinetoplast is middle of the body and slightly far from the posterior end of the organism.

3) Subgenus *Schizotrypanum* Chagas, 1909

Type species: *Trypanosoma (Schizotrypanum) cruzi* Chagas, 1909

Comparatively small, in stained specimen the organism forms C shape, the kinetoplast is large and the nucleus is located in the middle of the body.

2. Section SALIVARIA

Organisms with free flagellum and also these without flagellum are found in this section. They have kinetoplast in the middle part of the body and the posterior tip of the organism is not sharp. In the vertebrate host, the organism form trypomastigote stage, and in the insect vector it propagates at the anterior part of the body and transmit to another host when the vector has a blood meal.

1) Subgenus *Duttonella* Chalmers, 1918

Type species: *Trypanosoma (Duttonella) vivax* Ziemann, 1905

Only trypomastigote stage of the organism is seen, and always has a free flagellum. Posterior end of the organism is round and the kinetoplast is large and present in the middle of the body.

2) Subgenus *Nannomonas* Hoare, 1964

Type species: *Trypanosoma (Nannomonas) congolense* Broden, 1904

Body of the organism is small and has no free flagellum. The middle size kinetoplast is present in

the midmost of the body.

3) Subgenus *Trypanozoon* Luhe, 1906

Type species: *Trypanosoma (Trypanosoon) brucei* Plimmer et Bradford, 1809

There are various forms with free flagellum and/or without free flagellum. The kinetoplast is small and present in the midmost of the body.

4) Subgenus *Pycnomonas* Hoare, 1964

Type species: *Trypanosoma (Pycnomonas) suis* Ochmann, 1905

Having short free flagellum, and the kinetoplast is present in the middle of the body. Transformation of the organism is rare.

B. Description of the forms of *Trypanosoma*

Description of the forms of *Trypanosoma* is made by measuring each part of the organism, e.g., from posterior end to the center of the kinetoplast (P-K), or to the center of the nucleus (P-N), from the center of the nucleus to the anterior end (A-N), and length of free flagellum (F) and length of the nucleus (N), from kinetoplast to the center of the nucleus (K-N), and the width (W) of the body. From the abovementioned parameters, the nuclear index (NI) and kinetoplast index (KI) can be calculated.

$$NI = P-N/N = N \quad KI = P-K/K-N$$

C. Life cycle and morphology of *T. cruzi*

In 1907 and 1908, Dr. Carlos Chagas traveled to the Brazilian state of Mines Cerais for the investigation of malaria. He found flagellates of epimastigote stage in the gut of Triatomine bugs *Panstrongylus megistus*. He inoculated the flagellates to *Callithrix penicillata*. One

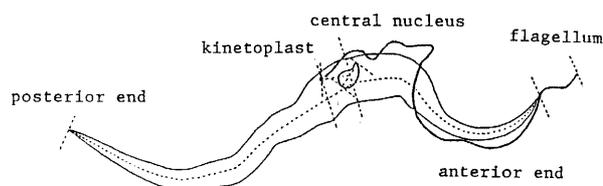


Fig. 1 The form of *Trypanosoma* sp. (Miyata, A., [The Parasitic Protozoa] Inst. Trop. Med., Nagasaki University Press, 1906pp, 1979)

month after the inoculation, he found a new species of *Trypanosoma*. He named the protozoan as *Trypanosoma cruzi* and reported it in 1909. The area where *T. cruzi* was found coincided with the area where an unknown disease in children, which is represented by the manifestation of anemia, fever, bipalpebral edema and cardiac disease, is observed. Thus, Chagas thought *T. cruzi* is the etiologic agent, and Miguel Couto proposed to call the disease, Chagas' disease.

Although in 1909, Chagas reported the organism as *T. cruzi*, in the same year, he proposed to classify it as a new genus *Schizotrypanum*. In 1911, however, Chagas himself withdrew the genus *Schizotrypanum* and reassigned the protozoan to the genus *Trypanosoma*.

Morphology of *T. cruzi* in mammalian blood are trypomastigote stage and epimastigote stage, and the intracellular one is amastigote stage. *T. cruzi* in its insect vector Triatomine bugs are trypomastigote stage, amastigote stage, promastigote stage, spheromastigote stage, and metacyclic trypomastigote stage.

1. Phase of *T. cruzi* in mammalian host

- a) Trypomastigote stage: The trypomastigote stage may be divided into 2 forms, one is the slender form with slim nucleus and a short flagellum. The kinetoplast, which is at the end of the body, measures 1.2 μm in diameter. The trypomastigote usually moves very rapidly. The other is the broad form with subspherical nucleus and the kinetoplast is at the posterior end of the body. This form of trypomastigote usually moves slowly. The former is considered to be a young organism and the latter, adult one. In fixed specimen, the organism shows C shape, measuring 11.7–30.4 μm in length 0.7–5.9 μm in width, and their NI are 0.9–1.7. However, these morphometric measurement varied slightly according to the various reports. The nucleus lies in the center of the maximum width of the body.
- b) Amastigote stage: In the host cell, the organism measures 2.4–6.5 μm in diameter. Without an undulating membrane, its nucleus is spherical or subspherical and its kinetoplast is stick or bean

shape.

2. Phase of *T. cruzi* in vector insect (Triatomine bugs)
 - a) Trypomastigote stage: Two forms could be observed; the one seen in mammalian blood and a slightly shorter one.
 - b) Amastigote stage: The form with short flagellum, which lost its undulating membrane, appeared 14–20 hours after the blood meal of the vector.
 - c) Spheromastigote stage: The form seen several hours after the amastigote stage, spherical shape with nucleus in the center and having kinetoplast and short flagellum. This form of the organism moves from anterior gut to middlemost gut, then it proceeds to multiply by binary fission and changes into promastigote stage.
 - d) Epimastigote stage: After the binary fission of promastigote stage, the organism changes into epimastigote stage that measures 10–20 μm in length. The elongated one has a length of 24–40 μm . This organism has large kinetoplast.
 - e) Metacyclic trypomastigote stage: Seven or eight days after the sucking of infected blood, the organism with a length of 17–20 μm appears in the feces of Triatomine bugs. The organism moves rapidly, but do not propagate at this stage.

D. *Trypanosoma* reported from vertebrates in South America

1. Mammalian *Trypanosoma*

T. rangeli Tejera, 1920

Host: Mammals

Vector: *Triatoma* spp.

T. diasi Dean et Martin, 1952

Host: Monkey, *Cebus apella*

Vector: Triatomine bugs, *P. megistus*, *T. infestans*,
R. prolixus

T. saimari Rodhain, 1941

Host: *Saimiri sciureus*

Vector: Triatomine bugs, *P. megistus*, *R. prolixus*

T. mycetiae Brumpt, 1913

Host: *Alouatta* spp.

- Vector: ? (Unknown)
T. mymecophagae Floch et Abonnenc, 1948
 (= *T. rangeli*?)
 Host: *Mymecophaga tridactyla*
 Vector: Triatomine bug, *R. prolixus*
- T. freitasi* Rego, Magalthes et Siqueira, 1957
 Host: Opossum, *Didelphis azarae*, *D. marsupialis*
 Vector: ?
- T. pessoai* Deane et Sugay, 1963
 Host: Bats, *Desmodus rotundus*, *Artibeus jamaicensis*, *A. cinereus*
- T. minasense* Chagas, 1909
 Host: Marmoset, *Callithrix penicillata* and Monkey, *Cebuella pygmaea*, *Tamarinus nigricollis*, *Saimiri boliviensis*, *Lagothrix infumata*, *Oedipomidas oedipus*, *Cebus apella*, *C. albifrons*
 Vector: ?
- T. lambrechtii* Marinkelle, 1968
 Host: Monkeys, *Cebus griseus*, *C. albifrons*
 Vector: ?
- T. devei* Leger et Porry, 1918
 Host: Monkeys, *Leontocebus midas*, *Midas rufimanus*, *Leontocebus tamarin*
 Vector: ?
- T. lewisi* (Kent, 1880)
 Host: Rats, *Rattus* spp.
 Vector: Fleas, *Nosopsyllus fasciatus*, *Xenopsylla cheopsis*
- T. prowazeki* Bernberg-Gossler, 1908
 Host: Monkey, *Cacajao clavivus*
 Vector: ?
- T. lesowrdi* Lger et Porry, 1918
 Host: Monkey, *Ateles paniscus*
 Vector: ?
- T. sanmartini* Garham et Gonzales-Mugabulu, 1962
 Host: Monkey, *Saimiri sciureus*
 Vector: *Triatoma* bug, *R. prolixus*
- T. evansi* (Steel, 1885)
 Host: Horse
 Vector: Horsefly
- T. pifanoi* Marinkelle et Duarte, 1968
 Host: Bats, *Artibeus lituratus*, *Phyllotomus hastatus*
 Vector: ?
- T. akodoni* Carini et Maciel, 1915
 Host: Rodent, *Akodon nigrita*
 Vector: ?
- T. coutinhoi* Deane, 1961
 Host: Rodent, *Cuniculus paca*
 Vector: ?
- T. forattinii* Coutinho et Pattolli, 1964
 Host: Rodent, *Oryzomys eliurus*
 Vector: ?
- T. lineatum* Iturbe et Gonzalez, 1916
 Host: Bat, *Vampyrops lineatus*
 Vector: ?
- T. mesnibrimonti* Deane, 1961
 Host: Sloth, *Choloepus didactylus*
 Vector: ?
- T. pecarii* Pessoa et Basi, 1972
 Host: Wild boar, *Tayassu tajacu tajacu*
 Vector: ?
- T. renjifoii* Deane, 1961
 Host: Rodent, *Procehimys cayennensis*
 Vector: ?
2. Avian *Trypanosoma*
T. pedrozi Carini et Botelho, 1914
 Host: *Crax sclateri*
 Vector: ?
- T. schistochlamydis* Splendore, 1910
 Host: *Shistochlamys capistrata*
 Vector: ?
- T. zonotrichae* Splendore, 1916
 Host: *Zonotricha pileata*
 Vector: ?
3. Reptiles *Trypanosoma*
T. superciliosae Walliker, 1965
 Host: Iguana, *Uruoscodon superciliosa*
 Vector: ?
- T. plicae* Lainson, Shaw and Landau, 1975
 Host: Lizard, *Plica umbra*
 Vector: ?
- T. brazili* Brumpt, 1914
 Host: Water snake, *Helicops modestus*

Vector: Leeches, *Placobdella brasiliensis*, *P. catenigera*

T. platemysi Folch et Abonnenc, 1942

Host: *Platemys platicephala*

Vector: ?

T. ocumarensis Scorza et Dagert, 1955

Host: Gecko, *Thecadactylus rapicaudus*

Vector: ?

T. plicae Lainson, Shaw and Landau, 1975

Host: *Plica umbra*

Vector: ?

T. rudolphi Carini et Rudolphi, 1912

Host: *Mabuia agilis*

Vector: ?

T. superciliosae Walliker, 1965

Host: *Uranoscodon superciliosa*

Vector: ?

T. butantanense Arantes et Da Fonseca, 1931

Host: *Ophis merremii*

Vector: ?

T. cascavelli Pessoa et Da Biasi, 1971

Host: *Crodalus durissus terrificus*

Vector: ?

T. constrictor Pessoa et Fleury, 1969

Host: *Boa constrictor amarali*

Vector: ?

T. erythrolampri Wenyon, 1908

Host: *Erythrolamprus aesculapii*

Vector: ?

T. hoguei Pessoa, 1968

Host: *Rachidelus brazili*

Vector: ?

T. mattogrossense Da Fonseca, 1935

Host: *Cyclagras gigas*

Vector: ?

T. merremii Arantes et Da Fonseca, 1931

Host: *Ophis merremii*

Vector: ?

T. philodraiasi Pessoa, 1928

Host: *Philodrayas nattereri*

Vector: ?

T. vitali Brumpt in Lavier, 1943

Host: *Helicops modestus*

Vector: ?

4. Amphibians *Trypanosoma*

T. rotatorium (Mayer, 1843)

Host: Frogs,

Vector: ?

T. arcei Mazza, Conzaies, Franke and Alvarado, 1927

Host: Frogs, *Leptodactylus ocellatus*

Vector: ?

T. borrelli Marchoux et Salimbeni, 1907

Host: Tree frogs, *Hyla* sp.

Vector: ?

T. leptodactyli Carini, 1907

Host: *Leptodactylus ocellatus*

Vector: ?

As listed above, many trypanosomes had been reported from South American countries (Miyata, 1979), but little is known of their insect vectors. However, some trypanosome species are known to share the same vector with that of *T. cruzi*, as shown in an epidemiological survey of Chagas' disease. Thus, care must be taken to differentiate the trypanosome found in experimental animals. In Paraguay, there was an attempt to use *Cebus apella* for the study of Chagas' disease. However, since isolation of *T. minasense* and *T. diasi* have been reported from this monkey in Brazil, it is very important to reconfirm the species of trypanosome found in the monkey during the experiment.

E. Ecology of *T. cruzi*

Hoare (1972) reported that *T. cruzi* can parasitize in the blood and tissue cells of more than 100 mammalian species. Triatomine bugs belonging to the genus *Triatoma*, *Panstrongylus*, *Rhodnicus*, *Tratyrus*, *Netriatoma*, *Dipetalogaster*, *Garabelminus* and *Psannolestes* are the vectors and they are distributed between the latitudes of North 42 and South 4. *T. cruzi* is generally considered to be found only in North and South American continents, but some reports indicate its occurrence in monkeys, *Macaca* spp., in Malaysia and Indonesia. However, these *T. cruzi* in Asia are reported to be the strains which hardly infect humans, similar to those found in North America, which has been known to be non-infective to humans.

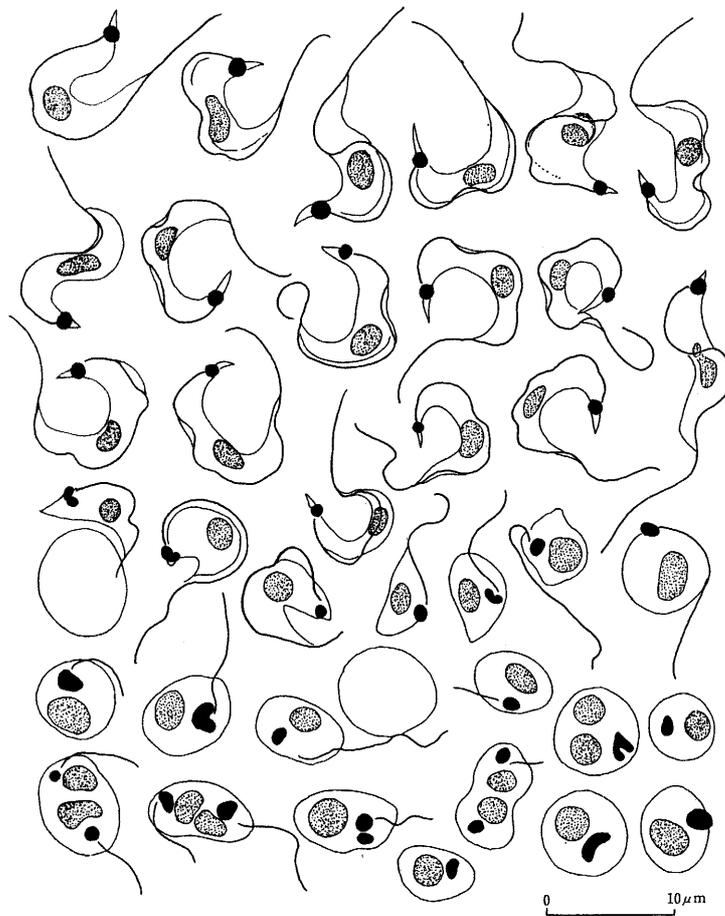


Fig.2 The variation forms of *T. cruzi* in blood (Miyata, A.,1979)

Metacyclic-trypomastigote stage of *T. cruzi* in mammalian blood penetrate into tissue cell where it transforms into amastigote stage and proceed to multiply. The amastigote stage changes into epimastigote stage and then again into trypomastigote stage. They are released into the blood as the host cell rupture. Major site of predilection are the cardiac muscle, liver, neuromuscular tissue, secretory organs, seminal glands and ovary glands. Trypomastigote stage of *T. cruzi* penetrates into tissue cells, and multiply to several hundreds by binary fission within 3-4 days. At this time, host cells were enlarged and their cytoplasm were liquefied. It is interesting to note that a number of amastigote that transformed from trypomastigote moved into blood stream and likely to die without changing to trypomastigote stage after the propagation.

T. cruzi in triatomine bugs requires 6–15 days to

propagate. It is transmitted to man by triatomine bugs. The bugs hide during the daytime in the crack of walls, or back of ceiling, and after dark they come out to feed. Not only both sexes of adult triatomine bugs but also the nymph will suck blood. And after sucking blood, they will not feed for several days. Nymph stage of the insects lasted 50-150 days and their life span are 90-695 days. According to the author's experiments, 50 *T. infestans* were fed blood and then kept for 7 months without feeding. During the period of the experiment, only 2 of them died, and some lived on to lay eggs. The nymphs that hatched from the eggs could live for more than 3 months without feeding. Animals were said to be infected by eating infected bugs. Furthermore, an experiment proved that carnivores could be infected by eating infected animal meat. Infection to man occurred through the feces of an infected bug, while he is sleeping. The bug frequently defecate while feeding on blood around

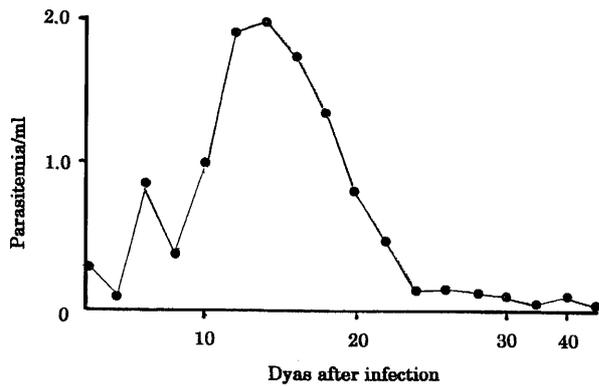


Fig. 3 Number of Tripomastigote forms in mice infected with *T. cruzi* (JAG strain)(Fleitas, 1991)

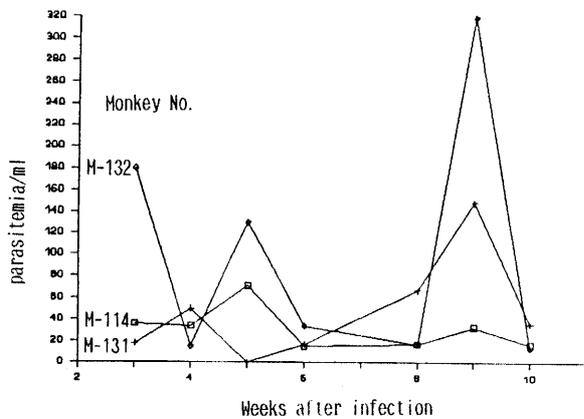


Fig. 4 Parasitemia of infected *Cebus paella* monkeys (Inchausti et Schinini, 1991)

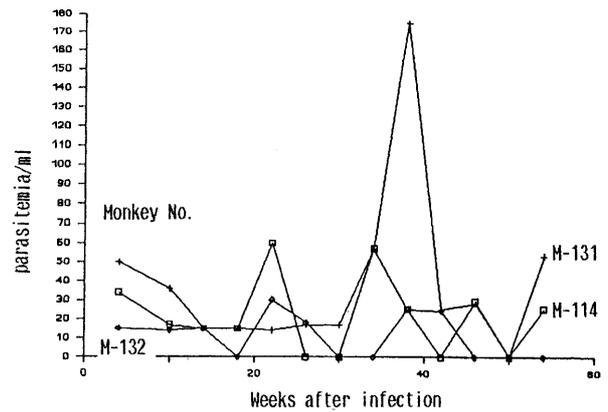


Fig. 5 Parasitemia of infected *Cebus paella* monkeys (Inchausti et Schinini, 1991)

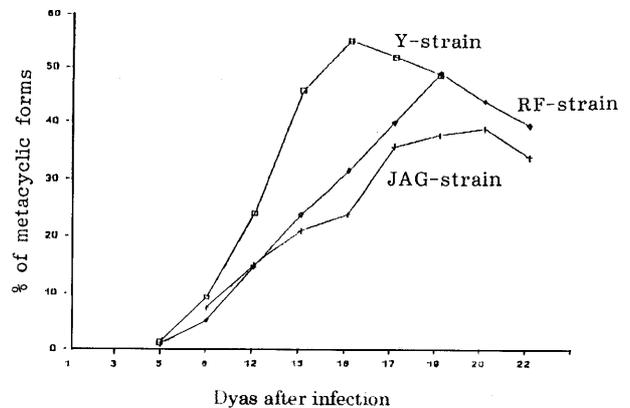


Fig. 6 Yield of metacyclic forms of the Y, RF and JAG strain (Yaluff et al., 1991)

the face or lip. Metacyclic-trypomastigote stage present in the feces of bug penetrate into the new host through the scratch of the skin.

Romaña (1963) investigated the infection sites on 560 patients of Chagas' disease, and found that most of them were infected through the conjunctiva, and rest through the skin of the face. The triatomine bugs in South America defecate while feeding. In contrast, the triatomine bugs in North America never defecate while feeding. In the latter, after feeding, they moved from the feeding place and then defecate, thus rendering the manner of the infection still remaining unknown. Furthermore, in South American countries, considerable number of patients had been reported to be infected through blood transfusion. In Paraguay, most of the studies on *T. cruzi* or ecology of triatomine bugs commence several years ago in IICS. They are the fruit of the JICA-PARAGUAY joint research

programs.

Fleitas (1991) reported the results of experimental infection of 1×10^6 p/ml of *T. cruzi* JAG in BALB/c mice. He observed that at day 14 post infection, the parasitemia reached its peak (Fig. 3).

On the other hand, Inchausti & Schinini (1991) conducted similar experiment with *Cebus apella*, by infecting, 3 monkeys were infected with 3.5×10^6 p/ml of the organisms and their parasitemia were monitored. Peak parasitemia were seen 5–20 weeks after the infection (Fig. 4 & 5).

In 1990, Yaluff et al. reported on the growth characteristics of the metacyclic-trypomastigote stage in vitro for JAG, RF and Y-strain of *T. cruzi*. Consequently, it was found that the parasitemia peak varied accordingly to the strain used (Fig. 6).

Despite the few reports on the morphology of triatomine

bugs, Arias et al. (1990) and Hirai et al. (1991) examined the chromosome of *Triatoma infestans* and *Rhodnius neglectus*, and found that both of them were $2n=22$. However, they reported that the morphology of the karyotype of *T. infestans* and *R. neglectus* were apparently different when they were stained with C-Band staining method. At present, mice or rats have been used as experimental animals for the study of Chagas' disease. In Paraguay, Rosner et al. (1990–91), and Ferro et al. (1990) demonstrated that *Cebus apella* could be infected with *T. cruzi* and the infected animal showed symptoms quite similar to that of man. They then continued to study the symptoms and movement of the organisms in monkeys.

F. *Trypanosoma rangeli* Tejera, 1920

In the American continent, *T. cruzi* is well known as a *Trypanosoma* species infective to man. However, *T. rangeli* also has a wide range of mammalian host comparable to that *T. cruzi*. Furthermore, *T. rangeli* shares the same vector insect with that for *T. cruzi*. Thus, differentiation of these 2 species is important from the viewpoint of public health. At present, 919 cases of *T. rangeli* infection in man have been reported from Venezuela, 130 from Colombia, 70 from Guatemala, 70 from Panama, and several cases from El Salvador, Brazil, Costa Rica and Paraguay.

Morphology of *T. rangeli* is quite similar with that of *T. cruzi*. In blood, it resembles *T. lewisi*, which is parasitic in rat but having more developed undulating membrane. The nucleus is located anterior-centrally, and the kinetoplast is near the posterior end. It is 27.0–32.0 μm in length, 1.8–7.0 μm from kinetoplast to posterior end, and 7.9–9.5 μm in its free flagellum. Triatomine bugs known to have been naturally infected with *T. cruzi* are *Rhodnius prolixus*, *R. pallescens*, *Triatoma infestans*, *T. dimidiata*, *T. phyllosomae*, *T. nitida*, *Panstrongylus geniculatus* and *Cavernicola pilosa*.

After infecting man or animals metacyclic trypomastigote stage had been reported to transform to the epimastigote stage in blood, and remained there without propagation or penetrating into tissue cells. On the other hand, *T. cruzi* is thought to propagate somewhere else in

the body. When *T. cruzi* entered into the midgut of the triatomine bugs, they began to propagate. Although some remained in the gut and the rest moved into the blood-body cavity and propagated in hemolymph. They then entered into the salivary gland and transformed to the metacyclic trypomastigote stage, ready to infect the mammalian host. The transmission route to mammalian host includes:

- 1) infection with metacyclic stage in the feces of triatomine bugs, and
- 2) infection with metacyclic stage from the salivary gland during the feeding of blood have been known.

Mammalian hosts that have been reported to be naturally infected with *T. cruzi* are man, dog, squirrel-monkey, opossum, anteater, raccoon and cat. Experimentally infected hosts includes *Cebus capucinus*, Macaque monkey, dog, mouse, rat, guinea pig, hamster, opossum, anteater and horse. Among them, dog is very important.

Detection of *T. cruzi* from man is usually made by hemoculture and xenodiagnosis, because the parasitemia is too low to be detected in the thick blood films. Xenodiagnosis is a method which employs clean, laboratory-reared triatomine bugs, and they are allowed to suck the patient's blood. Several weeks later, the feces and body fluid of the bugs are examined for the presence of *T. cruzi*. *T. rangeli* is not pathogenic to man, but its distribution area is similar with that of *T. cruzi*. Moreover, the morphology of the organism also resembles that of *T. cruzi*. Thus, differentiation of these two species is very important from the viewpoint of public health. Morphological difference between these two species includes the kinetoplast of *T. cruzi* being always larger than that of *T. rangeli*, and the epimastigote stage of *T. rangeli* is bigger and more elongated than that of *T. cruzi*. *T. cruzi* could not be detected from the body fluid of triatomine bugs by xenodiagnosis. However, *T. cruzi* can multiply well when cultured using Warren medium but not for *T. rangeli*. On the contrary, when Nystatin (Mycostatin) was added to the medium, *T. rangeli* can grow but not *T. cruzi*. In South America, *Trypanosoma* species resembling *T. rangeli* in various hosts are known. They are:

T. diasi Dean et Martin, 1952

Host: *Cebus apella*

- Vector: *P. megistus*, *T. infestans*, *R. prolixus*
T. saimari Rodhain, 1941
 Host: *Saimiri sciureus*
 Vector: *P. megistus*, *R. prolixus*
T. mycelae Brumpt, 1913
 Host: *Alouatta* spp.
 Vector: ?
T. mymecophagae Floch et Abonnenc, 1948
 Host: *Mymecophaga tridactyla*
 Vector: *R. Prolixus*

III Symptoms

Metacyclic trypomastigote stage of *T. cruzi* usually penetrate into the cells of reticuloendothelial system, where they transformed into amastigote stage and then proceed to multiply. After several days, rupture of the host cells releases the trypomastigote stage. Edema (Chagoma) is usually observed near the portal of entry, accompanied by the swelling of the adjacent lymph nodes. A unilateral bipalpebral edema is called Romana sign. Chagas' disease could be separated into two groups; one is acute infection and the other, chronic infection. In an acute infection, parasitemia and rupture of the cells due to multiplication of the organisms in various organs cause many symptoms in patients, such as, fever, swelling of lymph nodes, hypertrophy of liver and spleen, serious myocarditis and nervous disorder. Among the patients with acute symptoms, particularly in infants, about 10% of them were fatal due to serious myocarditis. On the other hand, in chronic infection, no parasitemia was observed and the infection of *T. cruzi* could be proved only by immunological examination. Major symptoms are heart disorder, such as, hypertrophy of heart, tachycardia, and slow pulse. Known electrocardiogram (ECG) changes includes blocking of right hand and of left hand-leg, and abnormalities of ST and T waves.

Velazquez and Gonzalez (1959) described the symptoms of Chagas' disease in 59 patients in Paraguay. They found that 48 (81.4%) were acute cases and the remaining 11 (18.6%) were chronic cases. Symptoms observed on the 48 patients were, 41 having parotitis, 35 with fever, 34 with edema of spleen, 21 with hypertrophy

Table 1 Symptoms of Chagas' disease in acute case of patients

Symptoms	No. of cases (%)
Parotitis	41 (85.4)
Fever	35 (72.9)
Edema of spleen	34 (70.8)
Llypertrophy of local lymphnodes	21 (43.8)
Llypertrophy of liver	18 (37.5)
Inflammation of lachrymal gland	18 (37.5)
Lymphadentitis of whole body	18 (37.5)
Dermal eruption	4 (8.3)
Chagoma	4 (8.3)
Congenital chagoma	1 (2.1)

(Velazquez et Gonzalez, 1959)

of local lymph nodes, 18 with hypertrophy of liver, 18 with inflammation of lacrymal gland, 18 with lymphadenitis of whole body, 4 with dermal eruption, 4 with chagoma, 1 with congenital Chagoma, and 48 with bipalpebral edema (Table 1). ECG test on 9 chronic patients revealed both abnormal ST and T waves.

Velazquez (1990) reported the symptoms of acute case patients as fever, headache, muscle pains, arthritis, vomiting, diarrhea and tachycardia. Lipalpebral edema (Chagoma) occurs 4–12 days after the sting by triatomine bugs, and the ratio of the patient developing acute symptoms is 5%, while 30% of the acute patients proceeded to chronic forms.

Arrura (1986) reported that 17 of 50 adults inhabitants of Guaza-Gua of Paraguari Province showed some ECG abnormalities, and 26% of them were stage I or stage II chronic heart disease patients. However, when he conducted ECG test on 33 inhabitants of Macharety of Boqueron Province, who were positive for antibody to *T. cruzi* by ELISA, only 5 of them showed some abnormalities on ECG.

Megacolon is another known symptom of Chagas' disease. Many patients with this symptom have been reported in Bolivia and Brazil. In Paraguay, Recald (1981) also reported that megacolon was observed on 0.7% of 114 autopsy cases at the Department of Pathology, School of Medicine, Asuncion University, during the period from 1961 to 1979. The cases from which megacolon were detected were observed among the inhabitants of a specific

Table 2 Parasitemia, serology, ECG and cardiothoracic index of *C. apella* infected with the Y strain of *T. cruzi*

	Date of sacrifice (months)							
	4			12				
Monkey No.	85	42	90	35	83	25	32	
Sex	F	F	M	M	M	F	F	
Date of 1 st positive direct parasitemia (days)	6	6	7	5	5	5	7	
Date of peak of direct Parasitemia (days)	21	14	14	12	12	19	12	
No. of <i>T. cruzi</i> /ml	920	4060	160	1280	4800	5600	9600	
Date of last positive Direct parasitemia (days)	84	77	110	166	152	103	96	
Date of last positive Xenodiagnosis (weeks)	17	17	17	52	52	52	39	
Date of 1 st positive serology (days)	14	14	14	28	28	14	28	
Date of highest serology titer (days)	84	49	56	40	33	47	33	
highest serology titer	1:256	1:128	1:128	1:512	1:512	1:256	1:1024	
Date of 1 st abnormal ECG (days)	7	7	7	7	47	7	7	
Cardiothoracic index (%)	0.48	0.54	0.49	0.50	0.55	0.51	0.54	

(Rosner et al. 1990)

Table 3 Parasitemia serology, ECG and cardiothoracic index of *C. apella* infected with the RA strain of *T. cruzi*

	Months after				
	48				
Monkey No.	54	60	5	48	53
Sex	M	M	M	M	M
Date of 1 st positive direct parasitemia (days)	5	7	3	7	4
Date of peak of direct parasitemia (days)	12	21	17	21	14
No. of <i>T. cruzi</i> /ml	100	950	176	1830	432
Date of last positive direct parasitemia (days)	106	50	68	90	177
Date of last positive xenodiagnosis (weeks)	65	81	65	91	91
Date of 1 st positive serology (days)	28	28	14	28	7
Date of highest serology titer (days)	70	31	92	125	31
highest serology titer	1:512	1:512	1:1024	1:128	1:1024
Date of 1 st abnormal ECG (days)	750	15	7	15	90
Cardiothoracic index (%)	0.56	0.54	0.65	0.58	

(Rosner et al. 1990)

area where many people showed positive results by immunological tests. Transmission of *T. cruzi* through mother's placenta to fetus is known. In most of the cases, miscarriage or stillbirth of the infected fetuses occurred. However, some of the fetuses born with congenital cardiac disease hardly live long.

On the other hand, Rosner et al. (1990) reported on the clinical symptoms and parasitemia of *Cebus apella* infected with *T. cruzi* in Paraguay (Table 2 & 3).

IV Diagnosis

Accurate diagnosis of Chagas' disease is considerably difficult. Rosner (1990) proposed that diagnostic methods using various techniques should to be used for acute and

chronic cases (Fig. 7, 8).

Arrura (1986 & 1990) proposed a set of diagnostic criteria for Chagas' disease by dividing them into 4 categories, with 42% of the patients in Paraguay being classified into categories I and II. Following are the diagnostic methods used in Paraguay, and the advantages and disadvantages are discussed.

	Serology (+)	Serology (+)
	E.C.G. (-)	E.C.G. (+)
Stage 0	Radiology (-)	Stage II Radiology (+)
	Clinic (-)	Clinic (-)
	Serology (+)	Serology (+)
	E.C.G. (+)	E.C.G. (+)
Stage I	Radiology (-)	Stage III Radiology (+)
	Clinic (-)	Clinic (+)

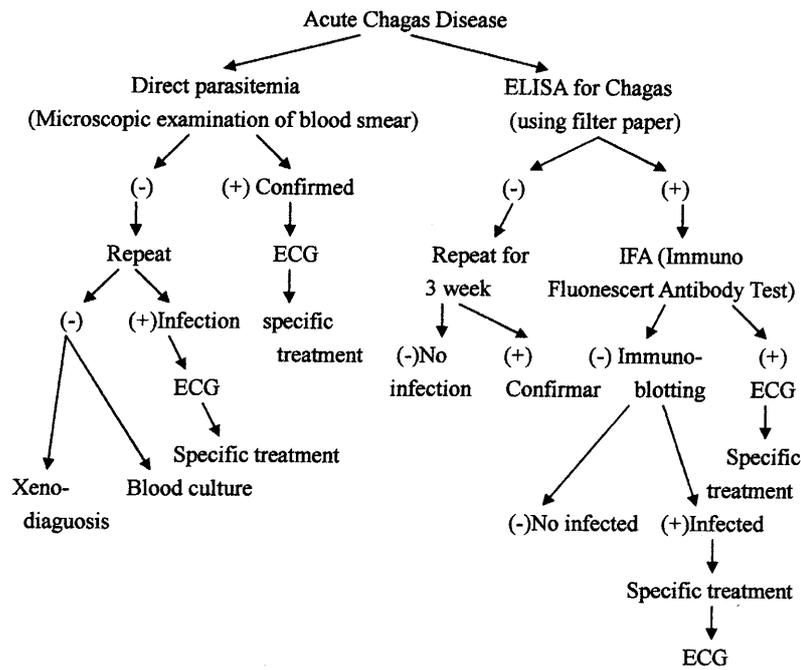


Fig.7 Diagnostic/Treatment protocol for Acute Chagas' Disease (Rosner, 1990)

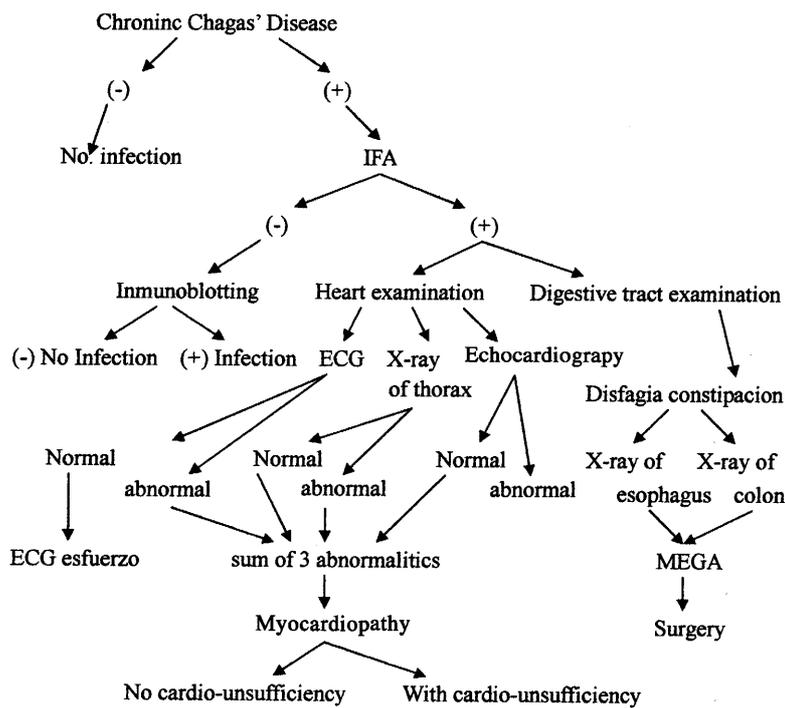


Fig.8 Diagnostic/Treatment protocol for Chronic Chagas' Disease (Rosner, 1990)

1. Xenodiagnosis

Xenodiagnosis is a method developed by Brumpt (1913) that employs non-infected, clean, laboratory-reared

triatomine bugs. Several weeks after the feeding of bugs on patients, feces of the bugs are checked for the presence of metacyclic trypomastigote stage. Velazquez (1961) reported that 32 (84.2%) of 38 acute Chagas' disease

Table 4 Results obtained through Xenodiagnosis

Subject	No. tested	No. positive for <i>T. cruzi</i> infection (%)	
General public	162	11	6.8
Babies & children	78	0	-
Acute patients	72	70	97.2
Suspicious patients	218	5	2.3
Total	530	86	16.2

(A. Canese, 1969)

patients showed positive results by xenodiagnosis. On the other hand, Canese (1969) reported on the results of xenodiagnosis on a total of 530 persons, that consisted of 162 persons from the general public, 78 babies and children, 72 acute *T. cruzi* patients and 218 suspected *T. cruzi* patients. Results obtained from this diagnostic method are shown in Table 4.

Furthermore, detection rate of *T. cruzi* by xenodiagnosis was compared with those of blood smears. It was found that the positive ratio of former method was 97% while the latter was 73%. This indicates the superiority of xenodiagnosis in the diagnosis for Chagas' disease.

Canese (1966) described the detection method for *T. cruzi* from feces of triatomine bugs.

The method is as follows:

- 1) Place several drops of 1% formalin on a slide glass. Place the abdomen of triatomine bugs on the slide glass and squeeze out the feces. After mixing the feces well with the formalin, the slide glass was air dried.
- 2) Fix with ethanol: ether (3:1) for 10 minutes at 40–50°C.
- 3) Stain for 15 minutes with a solution, which consisted of 0.5g of basic fuschin, 5 ml of ethanol and 100 ml of carbonic acid.
- 4) Wash with water.
- 5) Stain for 15 minutes with a solution, which consisted of 0.5g of basic fuschin, 5 ml of ethanol and 100 ml of carbonic acid.
- 6) Wash with water.
- 7) Giemsa stain (1ml of water + drops of basic Giemsa solution).
- 8) Wash with water and dry.

- 9) Observe under light microscope.

In xenodiagnosis, detection ratio of *T. cruzi* is higher than the other methods. This is because the minute number of *T. cruzi* in the blood of patients is allowed to multiply until they reached a sufficiently high number to detect. However, the disadvantageous points are that:

- a) the triatomine bugs must be kept clean and free of infection,
- b) the patients must endure the pain during the feeding of the bugs,
- c) the method cannot be used in epidemiological survey, because bugs need 30 minutes to feed and
- d) it takes a long time to get the final results.

2. Detection from thin blood films

Advantage: confirmation of *T. cruzi* is easy if parasitemia is sufficiently high.

Disadvantage: Frequently, the parasites are too few in the blood.

In Central and South America, *T. rangeli* is also present. Thus, differentiation of *T. cruzi* from *T. rangeli* is necessary.

3. Detection of parasite from thick blood films

Advantage: Probability to detect *T. cruzi* is higher than that of the thin blood films.

Disadvantage: As the film is thick, technical identification of *T. cruzi* is difficult.

4. Detection of parasite from fresh blood preparation

Advantage: Simple and easy to detect, the parasite under microscope because the parasite moves vigorously in fresh blood.

Disadvantage: Species identification is difficult, since internal structure of the parasite could not be seen.

5. Concentration method

Add 1% lithium oxalate ($\text{Li}_2\text{C}_2\text{O}_4$) to blood sample to agglutinate the erythrocytes and let the parasites concentrate in serum.

Advantage: Relatively large amount of blood can be examined, and without the interference of the erythrocytes,

it is easy to find the parasites.

Disadvantage: Nothing particular at present. Several concentration techniques have been described. Ceriola et al. (1974) made a comparison of these methods and reported the following efficiency:

Examination of fresh blood	52.4%
Gross drop method	47.6%
Triple centrifugation method	71.4%
Strout method	95.2%
Silicon gradient method	71.4%
Differential lysis method	19.0%
Xenodiagnosis	100.0%
Hematocrit method	100.0%

Consequently, both xenodiagnosis and hematocrit method showed good results. In the latter method, blood sample is put into a hematocrit tube and centrifuged at 3000g for 40 seconds followed by observing the organisms in the layer above the leukocytes.

6. Culture technique

It is easy to cultivate *T. cruzi* using many different types of culture media. In many of the culture media, epimastigote stage of the organism appeared 4 days after incubation at 25–28°C, and 9 days later, metacyclic trypomastigote stage will come out. In tissue culture at 37°C, amastigote stage can be also obtained too.

Advantage: Relatively large amount of blood specimens can be tested for the detection of the organisms. The method is appropriate for the preparation of antigen, since enough amount of organisms can be obtained easily.

Disadvantage: Only specific institutions can afford to conduct the test because special facilities and equipments are required for the cultivation of the organisms.

Russomando and Chiller (1990) reported the use of 2 different culture media, namely, 1) DMEM (Dubelco Minimum Essential Medium) with 10% FCS (Fetal Calf Serum) and antibiotics for LLC-MK2 cell line, and 2) MEM with 5% FCS and antibiotics for BHK cell line. After inoculation of the specimen, both media were cultured at 37°C in an incubator with 5% CO₂.

In chronic Chagas' disease, detection of the parasites is said to be difficult. However, Ferreira et al. (1990) cultured 50 blood specimens from chronic cases (ELISA and IF positive) for 15–120 days, and detected the parasites from 5 cases.

7. Polymerase Chain Reaction (PCR) method

The method uses a specific primer set derived from sequences of *T. cruzi* DNA. After electrophoresis of the PCR product, identification of the specific band was made.

Primers known at present are;

TC4-1	GCCGTTCTCTCTAAGCCACTCCG
TC4-2	ATTGTCCGCATCATCTCTTTACGGT
TC22-1	GCAAATAAAAGAAAGTGTTTCATGAG
TC22-2	AACTTCCTTGACGTCCATGACTGT
TCZ-1	CGAGCTCTTGCCCACACGGGTGCT
TCZ-2	CCTCCAAGCAGCGGATAGTTCAGG

Advantage: Theoretically, diagnosis could be made from the presence of a single organism in the specimen because the method detects and amplifies DNA of the parasite.

Disadvantage: Only specific institution can afford to conduct the test because special facilities and equipments are required.

Russomando et al. (1991) reported that the use of TC-4 and TC-22 (primer of *T. cruzi*) were quite sensitive and effective for the detection of *T. cruzi*. Eight days after the administration of Denzomidazole (5mg/kg body weight) to a patient with Chagas' disease, the effect of the medication was tested by means of ELISA, IIF (Indirect Immunofluorescence Test), PCR, hemoculture, and direct parasitemia tests. Consequently, it was found that PCR test was the most appropriate for evaluating the effect of the medication, because PCR showed negative result one week after the administration of drug, while ELISA and IIF showed positive results even at 401 day post-treatment (Table 5).

^{a)} IIF, indirect immunofluorescence ^{b)} Optical density IgM OD=0.285 and IgG OD=0.291, for negative controls ^{c)} Days required for a positive diagnosis ^{d)} Represented as parasites per ml NT: Not tested ¹⁾ Days after administration of Denzomidazole (Russomando et al. 1991)

Table 5 Diagnostic test results of sequential serum samples of the treated acute patient

Day ¹	IIF titer ^a		ELISA titer ^b		Hemo- ^c culture	Dire ^d Parasitemia	PCR Reaction
	IgM	IgG	IgM	IgG			
0	1:160	1:160	1.100	1.180	45	140	+
8	1:640	1:320	1.781	1.195	NT	NT	-
29	1:320	1:40	1.215	1.080	-	-	-
45	1:80	1:40	0.950	1.000	NT	NT	-
60	1:80	1:40	0.777	0.915	-	-	-
86	1:80	-	0.777	0.915	NT	NT	-
120	1:40	-	0.737	0.747	NT	NT	-
149	1:80	-	0.654	0.749	-	-	-
179	1:80	-	0.566	0.801	NT	NT	-
256	1:40	-	0.576	0.633	-	-	-

^a) IIF, indirect immunofluorescence; ^b) Optical density IgM OD=0.285 and IgG OD=0.291, for negative controls; ^c) Days required for a positive diagnosis; ^d) Represented as parasites per ml
NT: Not tested; ¹) Days after administration of Denzonidazole (Russomando et al. 1991)

Table 6 Specificity complement fixation test (CF) in Chagas' disease patients

Disease	No. of tested	No of negative negative	CF titer						
			1/20	/40	/80	/160	/320	/640	/1280
Chagas' patient	21	-			5	4	9	2	1
Chagas' heal patient	95	63	17	15					
Leper	7	6		1					
Syphilitic patient	3	3				4			
Toxoplasmosis	5	5							
Chagas' endemia Persons	416	308			54	11	10	16	17
Total	554	308	17	70	16	18	25	19	1

(Arias et al. 1984)

8. Simple fluorescent method with barrier filter

Blood smear stained with acryl-orange is observed under a microscope with 515 nm barrier filter in the eyepieces. This method is being employed to detect malaria parasites at present. It is possible to use this method for the detection of trypanosome but no information is available on its advantageous point or disadvantageous point.

9. Immunological diagnosis

Antibody detection by immunofluorescence antibody test (IF-test), haemagglutination test, Ouchterlony method, Enzyme linked immunosorbent assay (ELISA) are used.

Advantage: Only a small amount of the patient's or animal's serum is needed.

Disadvantage: There is cross-reaction with sera of patients who are infected with parasite other than with *T. cruzi*.

In Paraguay, diagnosis of sera by indirect immunofluorescence test and indirect haemagglutination test have been used in clinical and epidemiological studies.

Monzon (1990) evaluated the specificity of complement fixation test (CF) using epimastigote-derived antigen in Chagas' disease patients. All of the acute phase patients were positive. The positive ratio of the recovered patients by drug administration were 66.3%. On the other hand, when the CF-test was carried out on 7 leishmaniasis patients, four were found to be positive.

Arias et al. (1984) had reported on the variation as *T. cruzi* antibody titers in patients sera and also the cross-reactivity of sera of patients with between Chagas' disease and those with other diseases. They observed the presence of cross-reactivity among the ser especially with those of the leishmaniasis patients (Table 6). Presently, in Paraguay, the IHA and ELISA-tests are used in immunodiagnostic studies.

Table 7 Recognition of antigen bands in lysates of *T. cruzi* by sera of patients infected with either *T. cruzi* or *Leishmania*

Patient group	Percentage of patients recognizing <i>T. cruzi</i> epimastigote antigen bands					
	25K	38K	66-72K	85K	90K	97-110K
1. <i>T. cruzi</i> infection						
Parasitemia (n=10)	100	100	100	60	70	100
Cardiomyopathy (n=11)	100	100	100	100	66	100
Megacolon (n=30)	100	100	100	100	66	100
Non0specific	90	100	100	85	50	92
2. <i>Leishmania</i> infection						
Leishmaniasis (skin test +) (<i>T. cruzi</i> ELISA -) (n=30)	0	0	70	25	50	0
Suspected double Infection (skin test +) (<i>T. cruzi</i> ELISA +) (n=8)	100	100	100	87	75	100

(Chiller et al. 1990)

There were also some reports on co-operative studies. The joint research project on Chagas' disease and other parasitic diseases between JICA and University of Asuncion in Paraguay started in 1989. It has been reported that crude antigen of *T. cruzi* reacted against the sera of patients with other protozoal disease. In order to produce the specific antigen of *T. cruzi*, the production of monoclonal antibody (MoAb) to *T. cruzi* was carried out.

Zarate et al. (1990) established six hybridoma clones to produce MoAb to epimastigotes-derived antigens of *T. cruzi*, two of which were IgG2b and others were IgM. IgM class antibodies reacted with a number of epimastigote protein molecules of different molecular weights. Proteins of 60Kda from trypomastigotes were also recognized as well. Antibodies of IgG2b subclass reacted with a low molecular weight protein (4Kda) from epimastigotes. No trypomastigote stage protein was recognized by IgG2b antibodies.

Maldonado et al. (1991) compared two sources of *T. cruzi* amastigote stage antigen; one derived from fibroblast cultures and the other from cell-free liquid medium, for their usefulness as solid phase antigens of ELISA for

Chagas' disease. They demonstrated that the amastigote stage antigen derived from the cell-free culture were more useful than those from the cell culture for the ELISA-test. In that study, however, one acute case was detected as positive by the aforementioned method, despite that it was negative by the usual ELISA using epimastigote stage antigen.

Chiller et al. (1990) reported that the specific antigen bands of 25K, 38K and 97-110K in *T. cruzi* lysates were detected by immunoblotting method with sera of Chagas' patient. Although the bands of 66-72K, 85K and 72K were detected in almost all sera from Chagas' patients, they were also detected in the study by up to half of the sera from Leishmaniasis patients (Table 7).

Yamashita et al. (1992) reported that the MoAb (TCY-3) that recognized the 53K band of *T. cruzi* trypomastigote stage lysates did not react against the antigens of *L. (m.) amazonensis*, *L. (b.) panamensis* and ten other parasites. Some of chronic phase Chagas' patients developed myocardopathies and/or megacolon. These clinical signs were found to be associated with specific autoantibodies that reacted against those organs and tissues. However, it is

Table 8 Rheumatoid factor of IgG, IgA, and IgE types in Chagas' disease patient.

Clinical sign	No. of patients	Rheumatoid factor (%)			
		IgG (+)	IgA (+)	IgM (+)	IgE (+)
Non-specific	19	32	16	53	5
Myocardopathies	16	12	0	56	6
Gastrointestinal Lesions	6	33	17	17	0
Healthy controls	18	0	0	28	0

Note: one of the acute patient showed all types of RF.
(Cabello et al. 1990)

still not clear which kind of autoantibodies are present in the sera of Chagas' patients.

It is known that Rheumatoid factors (RF) were observed in an elevated level in many autoimmune diseases.

Cabello et al. (1990) reported the presence of IgG, IgA, IgM and IgE types of RF in Chagas' patients, both during the acute and chronic phase, as detected by ELISA. An elevated frequency of IgG and IgM type of RF was found in the sera of chronic phase patients. They suggested that the presence of RF during infection with *T. cruzi* would further support the theory of the autoimmune characteristic of Chagas' disease, which could result in auto-antibodies production by polyclonal B-cell activation (Table 8).

Picagua et al. (1990) studied the productivity of tumor necrosis factor (TNF) in mice infected with *T. cruzi*. They reported an active production of TNF by peritoneal cells from infected mice, but a reduced TNF production by spleen cells. They concluded that TNF production was not due to spleen T-cells.

V Epidemiology

1. Disease in human

Since Gustavo Gonzalez and J.B. Rivarola reported the first human case of Chagas' disease in Paraguay in 1939, many epidemiological surveys, during the period from 1939 to 1961, had been carried out. Velazquez (1861) reported the occurrence of 77 cases of acute patients in various provinces in Paraguay (Table 9). Among them the 57 acute cases consisted of 52 male and 5 female, while the 19 chronic cases were made up of 14 male and 5 female,

indicating that male is likely to be more susceptible to the disease. The remaining one case was unknown due to lack of data. Most of the acute patients were 10-20 years old, while chronic cases were mostly middle age or older people (Table 10).

On the other hand, Canese (1971) reported that during the period from 1939 to 1971, a total of 170 acute patients of Chagas' disease were detected in Paraguay (Table 11). Furthermore, Canese (1978) reported the results of surveillance on the incidence of antibody by means of indirect fluorescent antibody technique. He found that inhabitants of Chaco area showed higher incidence than the inhabitants of other areas. The Chaco area was where the first patient of Chagas' disease was reported. It was found to be an epidemic area (Table 12). Incidence of antibody to Chagas' disease tested by CF and IHA were also reported (Table 13).

SENEPA (1984), which is an affiliated organ of the Ministry of Health, Paraguay, conducted immunological tests on a total of 2859 inhabitants by indirect fluorescent antibody technique (IFA) and indirect haemagglutination (IHA) technique. It was observed that 654 (22.9%) of them showed positive results by IFA and 655 (22.9%) by IHA (Table 14).

Rosner et al. (1990) reported that during the period from 1984-1989, a total of 562 persons, consisting of 283 male and 279 female, were tested by ELISA. Consequently, it was found that 325 (48%) showed positive results. Among them, 12 were acute cases, 15 congenital cases, 9 megaesophagus and 30 megacolon cases (Fig. 9). Moreover, 40 cases of heart disease associated with Chagas' disease were seen at University hospital (HC) and 75 in IICS, (Instituto de Investigaciones en Ciencias de la salud, Health Science Research Institute.) respectively (Fig.10). Patients analysed by age in IICS are shown in Fig. 11.

Furthermore, Rosner et al. (1990) reported that 69 (21.6%) of 319 inhabitants in Nandua, 63 (17.1%) of 368 in Yapbu and 26 (15.8%) of 165 in Canada had FA antibody, respectively. In addition, 42 from Nandua, 47 from Yaphu and 33 from Canada showed abnormal electrocardiogram.

Arias et al. (1990) conducted surveys on the incidence

Table 9 Incidence of Chagas' disease patients by area (1939-1961)

(Province) Area	No. of patients	(Province) Area	No. of patients
(Capital y Parroquias)			
Tacumbu	1	(Alto Parana)	0
Beterete-cue	1	(Central)	
Barrio Hospital Clinicas	1	Luque	1
(Concepcion)	0	Mariano Roque	1
(San Pedro)		(Neembucu)	0
Itacurubi del Rosairo	1	(Amambay)	0
(Cordillera)		(President Ilayes)	
Emboscada	1	Fortin General Briguez	1
Tobati	1	(Chaco)	1
Itacurubi de la cordillera	1	(Boqueron)	
Atyra	1	Mariscal Estigarribia	27
Caraguatay	1	Fortin General Diaz	4
(Guaira)		Fortin Linares	1
Colonia Independencia	1	Fortin Tte. Montania	4
Villarrica	1	Fortin Ballivian	1
(Caaguazu)		Oruro	1
San Jose de los Arroyos	2	Fortin Rojas Silva	1
Cornel Oviedo	1	Puesto Casado	1
(Caazapa)		Puesto Sota	1
San Juan Nepomuceno	1	Centeno	1
(Itapua)		Fortin Guachalla	1
Encarnacion	1	Puesto Guarani	4
(Misiones)		Territorio Militar del Chaco	2
San Ignacio	1		
(Paraguari)			
Mubuyapey	1		
Colonia la colmena	2		
Pirayu	1		
Quyquyho	1		
Quiindy	1		
Ybtimi	1		
Total			77

(Velazquez, 1961)

Table 10 Incidence of Chagas' disease patients by age

Age	Acute cases	Chronic cases
1-10	6 (10.5%)	0
11-20	42 (73.7)	2 (10.5)
21-30	2 (3.5)	0
31-40	2 (3.5)	1 (5.3)
41-50	2 (3.5)	3 (15.8)
51-60	1 (1.8)	7 (36.8)
61-70	1 (1.8)	4 (21.1)
71-80	1 (1.8)	2 (10.5)
Total	57	19

(Velazquez, 1961)

antibody to *T. cruzi* by IFA in Loma Plata of Boqueron (Chaco district). They found that 71 (18.8%), consisting of 27 male and 44 female, out of 377 persons in Paratodo area, and 82 (20.8%), consisting of 34 male and 46 female, out of 385 persons in Aceval area, were positive for the antibody (Table 15 & 16).

IIF: Indirect Immunofluorescence Test

Roveria et al. (1991) carried out an ELISA test on 300 military personals, aged 15-20 years, from the Infantry (from Paraguari and Concepcion provinces), alvay (from Central, Caaguazu, Guaira, Cordillera and Paraguari provinces), and Navy (Paraguari, Cordillera, Central, Neebucu and San Pedro provinces). They found 2 (2%)

Table 11 Incidence of Chagas' disease patients by area (1939-1971)

(Province) Area	No. of patients	(Province) Area	No. of patients
(Distrito de la Capital)		(Villa Hayes)	
Tacumbu	1	Bruguez	1
Baterette-cue	3	Chaco-I	1
H. Clinicas	1	Pilcomayo	2
Asuncion	2	Lopez de Filippis	5
(Cordillera)		Carlos Rivarola	1
Cmboscada	1	Pozo Colorado	2
Lobati	2	Nanawa	2
Itacurlubi data C.	2	Salto Palmar	1
Atyra	2	Fortin Delgado	4
Caraguatay	2	Estancia el Carmen	1
Lusrbio Ayala	6	Colonia Falcon	1
San Bernardino	1	Benjamin Aceval	1
Valenzuela	1	Nueva Flor	1
Caacupe	1	Est. San Remigio	1
Piribebuy	2	(Boqueron)	
(Guaira)		Mariscal Estigarribia	28
Villarrica	2	General Diaz	5
Independencia	1	Tte. Montania	4
(Caaguazu)		Puerto Gurani	4
S. Jose de los Arroyos	3	Rojas Silva	2
Coronel Oviedo	2	F. Guachalla	2
Caaguazu	2	Filadelfia	2
(Caazapa)		Saavedra	2
Z. Juan Nepomuceno	1	Linares	1
(Itapua)		Ballivian	1
Encarnacion	2	Oruro	1
(Misiones)		Sosa	1
San Ignacio	1	Zenteno	1
(Paraguari)		Juan de Salazar	1
Mubuyapey	1	Pozo Azul	1
La Colmena	2	Rendague	1
Pirayu	1	Puerto Pinasco	1
Quyquyo	1	Pedro Pena	1
Quiindy	1	F. Garay	1
Ybytimi	1	(Bahia Negra)	
Ybycui	3	Behia Negra	1
Yaguaron	5		
Sapucaí	1		
Achay	1		
Caballero	1		
(Central)			
Luque	2		
Marino R. Alonso	1		
Ypane	3		
Itagua	1		
Nebmy	1		
Villeta	1		
San Lorenzo	1		
No specification of living in Chaco Paraguayo	7		
Total	180		

(Canese, 1971)

from Infantry, 9 (9%) from Cavalry, 10 (10%) from Navy were positive for *T. cruzi* antibody. Of these, 2 were 15 years old, 4 were 16 years old, 12 were 17 years old and 3 were 18 years old respectively. None of the 19 and 20 years old military personnels were positive for the antibody.

Nunez et al. (1990) examined 158 inhabitants of Escobar district of Paraguari province, consisting of 4 months old babies to 13 years old children, by ELISA test (1:50). they

Table 12 Incidence of antibody to Chagas' disease by IFA

Subjects	No. of tested	Positive rate (%)
Inhabitants of San Lorenzo	267	22.9
Inhabitants of Yaguaron	63	41.2
Out patients of hospital	248	13.6
Inhabitants where bugs present	63	33.6
Inhabitants of Asuncion	156	2.0
Psychiatric hospital patients	256	10.2
Blood from blood bank	562	11.3
Inhabitants of Chaco area (A)	197	62.2
Children in kindergarden	408	11.5
Inhabitants of San Lorenzo	50	14.0
Inhabitants of Chaco area (B)	140	73.6

(Canese, 1978)

Table 13 Incidence of antibody against Chagas' disease in man (1978)

Province	No. of tested	Positive rate (%)	
		CF	IHA
Concepcion	54	7.4	7.4
San Pedro	350	14.3	14.3
De las Cordilleras	931	16.2	16.2
Guaira	223	9.4	9.4
Caazapa	453	12.4	12.4
Caasapa	218	8.3	8.3
Itapua	280	8.2	8.2
Misiones	151	1.3	1.3
Paraguari	781	12.8	12.8
Alto Parana	76	6.6	6.6
Central	2333	6.0	6.0
Neembucu	49	4.1	4.1
Amambay	16	12.5	12.5
Pte. Hayes	27	7.4	7.4
Boqueron	38	18.4	18.4
Olimpo	11	9.1	9.1
Argentina	1	-	-
Not specified	8	-	-
Total	6000	9.7	9.7

(Canese, 1978)

found that, 12 (7.6%) showed, positive results, with 3 (1.3%) less than 4 years of age, and 9 (5.7%) between 5-13 years of age. A total of 400 mothers and their newborn babies at Asuncion National Maternity Hospital were investigated by Canese, J. (1979) for incidence of antibodies against Chagas' disease. It was found that 16.5% of the mothers and 2 of their newborns (0.5%) were positive, indicating that the babies might have been infected through the placenta.

Arias et al. (1990) checked for the incidence of IgM antibody in 141 infants of less than 2 months old in a maternity hospital. They found 7 were positive for antibody to *T. cruzi* but without parasitemia.

As for the 10 IgM positive children, who were diagnosed at IICS (Health Science Research Institute), 7 showed parasitemia with 4 having abnormal electrocardiogram, 1

Table 14 Incidence of antibody against Chagas' disease in man (1984)

Province	Area	No. of tested	Positive rate (%)		
			IIF	IHA	
Concepcion	Belen	202	68 (33.7)	69 (34.2)	
	Loreto	72	10 (13.9)	10 (13.9)	
	Concepcion	37	6 (16.2)	6 (16.2)	
	San Pedro	Union	96	40 (41.7)	40 (41.7)
		Gual. Aquino	188	86 (45.7)	86 (45.7)
Corillera	Villa del Rosario	91	13 (14.3)	13 (14.3)	
	Itac. Del Rosario	98	31 (31.6)	31 (31.6)	
	San Pedro	225	55 (21.6)	55 (21.6)	
	N. Germania	50	6 (12.0)	6 (12.0)	
	Caaguazu	Juan de Mena	32	11 (34.4)	11 (34.4)
Central	Carayao	90	21 (23.3)	21 (23.3)	
	San jose	150	50 (33.3)	50 (33.3)	
	Cecilio Baez	79	18 (22.8)	18 (22.8)	
	Lambare	115	13 (11.3)	13 (11.3)	
	Villa Elisa	24	8 (33.3)	8 (33.3)	
	Nemby	25	1 (4.0)	1 (4.0)	
	San Antonio	38	9 (23.7)	9 (23.7)	
	Nueva Italia	30	25 (83.3)	25 (83.3)	
	Villeata	80	17 (21.3)	17 (21.3)	
	Guarambare	23	6 (26.1)	6 (26.1)	
Amambay	Ita	126	16 (12.7)	16 (12.7)	
	Capiata	127	36 (28.3)	36 (28.3)	
	Itaugua	124	13 (10.5)	13 (10.3)	
	Ypacarai	77	13 (16.9)	13 (16.9)	
	P. J. Caballero	466	42 (9.0)	42 (9.0)	
	Yby Yau	53	5 (9.4)	5 (9.4)	
	Total	2859	654 (22.9)	655 (22.9)	

(SENEPA, 1984)

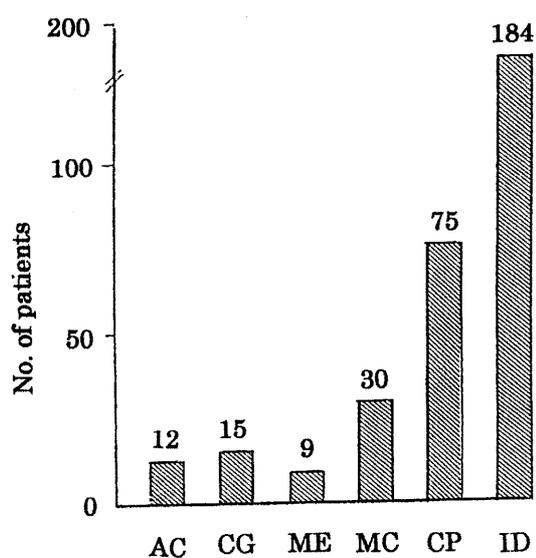


Fig.9 Frequency of the different pathological manifestations of Chagas' disease diagnosed at the Tropical Medicine Unit of IICS.

Ac:Acute, CG:Congenital, Me:Megaseophagus, Mc:Megacolon, CP:Cardiopathy, ID: Inderminate (Rosner et al., 1990)

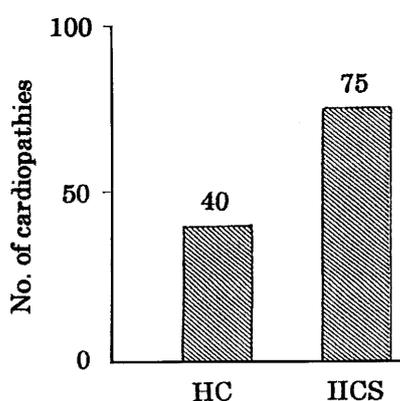


Fig.10 Chagasic myocardiopathy cases diagnosed at the Hospital of Clinics (HC) over 30 years, and at the IICS over 5 years (Rosner et al., 1990)

megacolon, 1 retro-ocular bleeding and 1 pneumonitis. Kasamatsu et al. (1991) detected amastigotes of *T. cruzi* from the placenta of 15 pregnant women who were positive for antibody against Chagas' disease.

Arias et al. (1991) investigated the incidence of *T. cruzi* in the inhabitants and in triatomine bugs in Guazu Cua and Pozo-Hondo districts. They found that 81 (29.0%) out of 282 inhabitants of Guazu-Cua, and 13 (20.0%) out of 65 in Pozo-Hondo were positive for FA antibodies (Table 17 & 18).

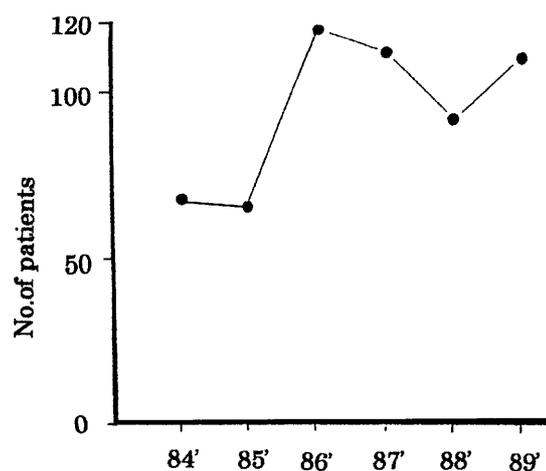


Fig.11 Number of patients seen at the tropical Medicine Unit from 1984 to 1989 (Rosner et al., 1990)

Table 15 Sero-epidemiological survey of antibody to *T. cruzi* in Prartodo

Age	No. examined	IIF positive		total positive	(%)
		Male	Female		
0-4	50	2	5	7	14.0
5-14	96	4	6	10	10.4
15-24	96	7	9	16	16.7
25-34	35	6	6	12	34.4
35-44	53	3	7	10	18.9
45-54	26	4	6	10	38.5
55-64	10	1	3	4	40.0
65-73	11	0	2	2	18.2
Total	337	27	44	71	18.8

(Arias et al. 1990)

Table 16 Sero-epidemiological survey of antibody to *T. cruzi* in Campo Aceval

Age	No. examined	IIF positive		total positive	(%)
		Male	Female		
0-4	80	4	7	11	13.8
5-14	157	10	22	32	21.1
15-24	52	4	5	9	17.3
25-34	37	6	3	9	24.3
35-44	33	3	5	8	24.2
45-54	23	2	5	7	23.8
55-64	10	3	1	4	40.0
65-73	3	2	0	2	66.7
Total	395	34	46	82	20.8

(Arias et al. 1990)

2. Triatomine bugs

The major insect vectors of *T. cruzi* are *Triatoma infestans*, *T. sordida*, *T. guasayana*, *T. delpontei*, *T.*

Table 17 Sero-epidemiological survey for *T. cruzi* antibody in Guazu-Cua, 1982

Age	No. examed	IIF positive		total positive	(%)
		Male	Female		
0-4	38	3	2	5	13.0
5-14	90	7	11	18	20.0
15-24	52	8	9	17	32.6
25-34	20	2	2	4	20.0
35-44	45	9	9	18	40.0
45-54	21	4	3	7	33.3
55-88	16	7	5	12	12.0
Total	282	40	42	82	29.0

(Arias et al. 1991)

Table 18 Sero-epidemiological survey for *T. cruzi* antibody in Pozo Hondo, 1982

Age	No. examed	IIF positive		total positive	(%)
		Male	Female		
0-4	15	-	-	-	-
5-14	7	1	2	3	42.9
15-24	11	1	1	2	18.1
25-34	11	2	3	5	45.4
35-54	8	-	1	1	12.5
55y+	3	1	1	2	66.6
Total	65	5	8	13	20.0

(Arias et al. 1991)

platensis, *T. guazu*, *Panstrongylus geniculatus*, *P. megistus*, *P. oswaldoi*, *P. lignarius*, and *Almoestes coreodes*. In Paraguay, Velazquez et Gonzalez (1959), Canese (1961-81), Da Silva et al. (1979), Gonzalez (1981), IICS (1986), SENEPA (1987), IDRC (1988), and WHO (1987) reported the presence of the following species of Triatomine bugs and their distributions as follow:

T. infestans: Concepcion, San Pedro, Cordillera, Guaiia, Caaguazu, Caazaoa, Itapua, Misiones, Paraguari Alto Pranan, Central, Neembucu, Amambay, Canindeyu, Pte. Hayes, Alto Paraguay, Chaco, Nueva Asuncion and Boquiren provinces.

T. sordida: Concepcion, Alto Paraguay, Cordillera, Paraguari, Central and Nueva Asuncion provinces.

T. guasayana: Paraguari, Central, Alto Paraguay, Boqueron, Pte. Hayes.

T. guazu: Guaira

P. geniculatus: Central, Guaira, Pte. Hayes.

P. negustys: Cordillera, Paraguari

P. oswaldoi: Itapua

A. coreodes: Central

However, *T. delpontei*, *T. platensis* and *P. lignarius* are not yet known to be the vector for *T. cruzi* in Paraguay.

Canese (1978) reviewed the distribution of triatomine bugs in the houses in Paraguay, and also studied the incidence of *T. cruzi* in the bugs. According to his survey, triatomine bugs tend to be distributed in the north-west direction. However, the incidence of the bugs in houses varied according to the villages, for example, 100% in Arroyas y Esteros while only 3.3% in Villa Hayes. Moreover, 58% of the triatomine bugs caught at Emboscada were infected with *T. cruzi* (Table 19).

On the other hand, SENEPA (1984) surveyed the incidence of *T. cruzi* in triatomine bugs, and reported a positive rate of 29.4% in the village of Santa Elena (Table 20), which is different from the report of Canese (1978).

Arias et al. (1991) examined *T. cruzi* infection in *T. infestans* at Guazu-Cua and Pozo Hondo districts, and found that 68 out of 333 in the former and 4 out of 19 in the latter district harbored *T. cruzi*, respectively (Table 21).

3. Reservoir host

Mammals that are prone to being bitten by *T. infestans* infected with *T. cruzi*, such as dogs, cats, armadillos and opossum etc. are known, but no survey on the prevalence of the protozoan in these animals in Paraguay had been reported.

VI Prevention and control

1. Treatment in man and animal

At present, Nifurtimox (Lampit) is reported to be effective for the treatment of *T. cruzi* infections in human. However, administration of 8-11mg/kg/day of the drug for 120 days consecutively is impracticable in Paraguay.

Rosner et al. (1990 & 1991), Muniagurria et al. (1990), and Schinini et al. (1991) reported the effectiveness Spirogermanium for the treatment of *Cebus apella* monkeys experimentally infected with *T. cruzi*.

On the other hand, Arias et al. (1991) reported the efficacy of various drugs extracted from various plants

Table 19 Surveys on Triatomine bugs (1940-1976)

Year	Area	No. of Examine House	Positive house (%)	Tc infected positive house (%)	No. of bugs	bugs with Tc (%)
Department: San Pedro						
	Volonia Hutteriana					
	General Aquino	80	-	-	250	30.1
1946	San Estanislao					
	Villa Rosario					
1963	Itacurubi del Ros.	48	87.0	18.0	238	7.0
Department: Cordillera						
1940	San Bernardino	-	-	-	62	38
1976	San Bernardino	40	62.0	25.0	56	19.0
1959	Caacupe	80	35.0	-	161	15.0
1977	Caacupe	70	91.0	27.0	149	16.0
1977	Caacupe	55	96.0	73.0	495	30.0
1970	Eusebio Ayala	163	17.5	0.6	36	2.8
1974	Altos	123	39.0	13.0	193	27.0
1977	Altos	65	46.0	23.0	89	39.0
1975	Piribebuy	20	15.0	-	3	-
1975	Arroyos y Esteros	24	100.0	8.3	120	2.8
1976	Arroyos Y Esteros	11	36.0	-	-	-
1977	Emboscada	60	90.0	25.0	115	58.0
1976	Emboscada	94	55.0	21.0	113	32.0
1977	Tobati	70	61.0	35.0	124	36.0
Department: Caaguazu						
1965	Caaguazu	249	29.7	4.4	145	16.6
1976	San Jose	62	21.0	3.0	26	19.0
1977	Col. Blas Garay	60	55.0	11.0	46	19.0
Department: Caazapa						
1976	S. Juan Nepomuceno	12	-	-	-	-
Department: Misiones						
1975	San Ignacio	17	11.7	-	7	-
Department: Central						
1958	Mariano R. Alonso	53	32.0	4.0	-	-
1960	Lambare	86	26.0	2.0	-	-
1962	San Lorenzo	268	32.8	11.5	857	14.2
1963	San Lorenzo	1000	58.4	8.9	3700	9.5
1964	Villeta	274	60.9	14.6	680	8.0
1975	Villeta	127	7.8	3.9	70	10.0
1976	Villeta	62	44.0	2.0	62	6.0
1964	Ita	222	62.2	13.9	712	10.1
1975	Ita	10	-	-	-	-
1969	Aregua	242	17.6	5.7	135	47.0
1975	Aregua	10	60.0	-	8	-
1975	Aregua	25	20.0	-	7	-
1973	Capiata	199	4.0	0.5	24	13.0
1974	Capiata	100	5.0	1.0	47	4.0
1974	Capiata	161	16.0	4.9	36	27.0
1974	Luque	135	14.0	2.0	35	8.5
1975	Ypacarai	25	28.0	12.0	37	24.0
1976	Ypane	107	-	-	-	-
1972	Limpio	-	-	-	90	40.0
Department: Pte. Hayes						
1974	Villa Hayes	50	26.0	-	40	-
1976	Villa Hayes	30	3.3	-	5	-
1976	Villa Hayes	11	64.0	35.0	15	33.0
1974	Benjamin Aceval	51	-	-	-	-
1974	Gral. Bruguez	100	69.0	22.0	128	26.0
Department: Boqueron						
1940	Mcal. Estigarribia	63	93.0	-	265	22.8
1974	Fortin Linares	42	70.0	42.5	54	33.0
1975	Filadelfia	20	50.0	15.0	36	7.5

Tc: *Trypanosoma cruzi*

(Canese, 1978)

Table 20 Surveys triatomine bugs (1984)

Area	Town and Village	No. of bugs	No. of examined	Bugs with <i>T. cruzi</i> (%)
Department: Concepcion				
Belen	Santa Elena	160	160	47 (29.4)
Concepcion	Tatare	15	15	0
Department: San Pedro				
Union	Potrero Jardin	33	33	2 (6.1)
Gral. Aquino	Lo de Marso	183	0	
Itac. Del Rosario	Mbocayaty	21	18	0
San Pedro	Nanducua. Curupayty	15	0	
N. Germania	Ypayere	11	11	0
Department: Caaguazu				
San Jose	Sta. Cruz. Col. Pte. Franco	39	33	2 (6.1)
Can Jose	Poterero Irala	147	120	6 (5.0)
H. Stroessner	Guavira	52	36	2 (5.6)
Department: Amambay				
P. J. Caballero	Fortuna	23	23	1 (4.3)
Yby Yau	Paso Jhu	1	1	0

(SENEPA, 1984)

Table 21 Housing infested with *T. infestans* and natural infection of *T. cruzi* in these bugs in Goazu-cus and Pozo Hondo, Paraguay, Feb.-May, 1982

Localities	Dwellings		T. infestans		
	No. of Examined	No. of infested	No. captured	No. examined	No. positive for <i>T. cruzi</i>
Guazu-cua	60	47 (8.3%)	435	333 (76.5%)	68 (20.4%)
Pozo Hondo	14	7 (50.0)	19	19 (100.0)	4 (21.0)

(Arias et al, 1991)

against trypanostigote stages in blood, after observing considerable effects of the plant extracts *in vitro*.

2. Insecticides for triatomine bugs

Arias (1991) studied the insecticidal effects of Scoparone- C₁₁H₁₀O₄, 1H-NMR (400 MHz, CDCl₃), 3.92 (3H, S), 3.94 (3H, S), 6.23 (1H, d, J9.5, HZ), 6.84 (1H, S), 6.89 (1H, d, J9.5, HZ) and Isofraxidin- C₁₁H₁₉O₅, 1H-NMR (400MHz, CDCl₃), 3.94 (3H, S), 4.05 (3H, S), 6.25 (1H, d J9.5, HZ), 6.65 (1H, d, J9.5, HZ). Furthermore, she also studied the effects of the extracts obtained from related plants such as Cabratea and Salvia.

VII Preventive measures

In 1967, SENEPA developed the eradication program for Chagas' disease along with that of the malaria

eradication program. However, in 1993, the division was busy with the control of malaria and no effort has been channeled into the eradication of Chagas' disease. Furthermore, IICS also did not conduct any particular activity, besides preparing a booklet with the cooperation of CIDA, Canada, and distributed them to the relevant agencies to provide the information on the disease and its prevention.

VIII A Chagas' Disease research project Japan to Paraguay

(Published by Isao Tada, 2003, Progress of Medical Parasitology in Japan, Vol. 8, edited by Otsuru, M., Kamegai, S. and Hayashi, S., Chapter V. 8, 634-637, Meguro Parasitological Museum, Tokyo, 2003)

This project began in 1983 and ended in 1987 at the above-mentioned institute, which was donated to the

government of Paraguay (Central Laboratory and Institute of Medicine, Ministry of Health, LACIMET) by a grant-in-aid from the Japanese government. In a comprehensive collaboration in medicine, the parasitology division was programmed to carry out laboratory and field research mainly on Chagas' disease and leishmaniasis.

In a survey of Chagas' disease in La Colmena, Paraguari Province, Yamasaki et al (1988a,b) observed that 9.3% of the 884 inhabitants tested were serologically positive. However, that positive rate ranged from 16 to 21% among those who lived in adobe-walled or wooden houses but was only 2% among the ethnic Japanese residents of the colony, ECG analysis of 64 seropositive patients revealed that 20 showed abnormalities in their cardiac function. Characteristic abnormalities were complete or incomplete right bundle block and ventricular extrasystole that were probably caused by carditis. These were considered as the characteristic findings during the chronic stage of Chagas' disease. Thus, they estimated that the prevalence of chronic Chagas' disease among the seropositive inhabitants in La Colmena was 5%. Of the 1,001 residents of Ayoras, Misiones Province, who were serologically tested for Chagas' disease, 4.8% were found to be positive (Yamasaki et al., 1988a,b). Furthermore, Yamasaki et al. (1990) analyzed the schizodeme of 3 isolates of Paraguayan *T. cruzi* and concluded that they resembled the FI strain isolated from *Triatoma infestans* in South Brazil. Moreover, they also reported that *T. cruzi* could be easily differentiated from *T. rangeli* based on the schizodeme patterns produced by using the restriction enzymes Hae III, EcoRI and Msp I. During the process of antigen isolation from *T. cruzi*, Kita et al. (1987) found that the endogenous protease activity of the protozoan was very high. Armed with this knowledge, Aoki et al. (1988) were able to obtain intact antigen from the epimastigote homogenate by adding serum bovine serum albumin and PMSF (phenylmethyl sulfonylfluoride), a serine protease inhibitor. Furthermore, using the protease inhibitors, Furushima et al. (1991) were able to obtain the mitochondria-containing membrane fraction of *T. cruzi* (Y strain) for use as antigen in western blotting to analyze the sera Chagas' disease patients. From the sera of 38 Brazilian patients who had been confirmed to

be positive by both IFA and ELISA, they observed that all the patients serum samples showed the relevant bands with molecular weights between 50 to 10 kd. Such bands were not seen in the sera of suspected or negative cases. Sera of persons with helminthic infection did not produce any band on the western blot for Chagas' disease.

A research project for Chagas' disease and parasitic infections supported by JICA was carried out at Instituto de Investigaciones en Ciencias de la Salud (IICS), Universidad de Asuncion, Asuncion in Paraguay between 1988 and 1993. For the purpose of establishing serodiagnostic technique, Maldonado et al. (1990) compared two strains of *T. cruzi* amastigotes for use as antigens in ELISA. They found that the antigen derived from a high virulent strain produced a stronger reaction and the amastigote cultured in cell-free liquid medium, when used as antigen, showed higher specificity in ELISA. Watanabe et al. (1991) found that TNF (Tumor necrosis factor) production activity was enhanced in *T. cruzi* infected mice. This phenomenon was thought to be associated with protective immunity. Later, they established a monoclonal antibody, TCY-3, which is specific to *T. cruzi*, for use in serodiagnosis (Watanabe et al., 1992). Furthermore, Watanabe et al. (1994a,b, 1995) found that IL-6 and INF- γ acted as accelerators for production of *T. cruzi* epimastigotes. They suggested that this reflects the wisdom of the parasite in using the host response for their proliferation. Mimori et al. (1994) compared the schizodeme patterns of kDNA samples from 11 strains of *T. cruzi* derived from man and triatomine bugs using restriction enzymes (Hae III, Msp I, Eco RI, Hinf I, Taq I and Rsa I). They were able to identify four different schizodeme profile groups. Group 1 showed a much simpler profile than the others and did not match with any standard strains of *T. cruzi* hitherto reported in Brazil, Chile and Colombia. Group 2 was similar to the Bolivian Z2 and Groups 3 and 4, to the Brazilian Z2 zymodemes. Russomondo et al. (1992) attempted to detect *T. cruzi* DNA from the whole blood and serum of 13 acute and chronic Chagasic patients by using Polymerase Chain Reaction (PCR). Since they noted that they could successfully detect the *T. cruzi* DNA even in serum samples,

they concluded that this technique would be convenient under tropical field condition. Acosta et al. (1995) characterized five Paraguayan strains of *T. cruzi* isolated from acute cases and found that all these strains belonged to WGA type in the lection agglutination assay, despite differences in their morphometric measurements. Yamashita et al. (1994) examined the usefulness of GPAT (Gelatin particle agglutination test) by comparing it with ELISA using epimastigote antigen from the Paraguayan RF strain of *T. cruzi*. They observed that of 10 parasitemic patients tested, all showed seropositive reaction in GPAT but two showed negative reaction in ELISA. When the sera of ten patients at chronic stage without parasitemia were tested with both methods, all were found to be positive but the titer of the individuals in GPAT were higher than that ELISA. They concluded that GPAT is a more sensitive method and convenient for field use. However, later, Sendo (1996) reported that when 22 Leishmaniasis cases from Ecuador were tested by GPAT for *T. cruzi*, 5 showed positive reaction albeit their titers were rather low. In a field survey at Macharety, Boqueron Province involving 121 inhabitants, Uchida et al. (1994) detected 33 seropositive cases of *T. cruzi* by ELISA and 8 cases with abnormal ECG in 121 inhabitants. Based on this survey, they considered that the endemicity of Chagas' disease had been reduced as compared with the situation before 1980. However, they failed to isolate any *T. cruzi* from

triatomine bugs. Furthermore, Abe et al. (1995) found that 4.9% out of 224 inhabitants in Guaria Province were seropositive for *T. cruzi*. By developing a PCR technique to detect *T. cruzi* in the feces of triatomine bugs, Russomando et al. (1996) showed that the detection rate of the protozoan by this method was 84%, while that by microscopic examination was 26%. Furthermore, the former method was not only more effective but was able to detect the protozoan faster than xenodiagnosis.

Reservoir host animals are considered to play a very important role in the transmission of Chagas' disease. Along this line of thought, Fujita et al. (1994) examined the blood samples of 108 domestic animals (33 cattle, 2 horses, 1 donkey, 20 pigs, 44 dogs and 8 cats) and 3 wild animals (1 opossum, 1 yellow armadillo and 1 long nose armadillo) from 5 endemic areas in San Pedro Province by direct blood smears and culture with LIT medium for *T. cruzi* infection. They found that all the direct blood smears were negative but one blood sample from a yellow armadillo, *Euphractus esxintus*, was shown to be positive for *T. cruzi* after culture. When specific IgG in the blood samples of the aforementioned animals were examined by Chagatest AD Color kit, those of cattle, pig, dog and cat were found to be positive. However, all the wild animals were negative. This suggests that domestic animals be involved in the transmission of *T. cruzi*

2. A survey on public concern for Chagas' disease in Paraguay

Introduction

Chagas' disease in Paraguay was first reported by Gonzalez et Rivarola in 1939, which occurred at the north-western area of Chaco. When Chagas' disease was first recognized in Paraguay, in the disease had also been reported neighbouring countries, such as Argentina (1926), Bolivia (1937), Brazil (1909), Chile (1931), Colombia (1937), Ecuador (1930), Peru (1917), Uruguay (1923) and Venezuela (1934)(Us.Dept.Agr., 1972). Studies on the clinics and Immunological diagnosis of Chagas' disease have been reported by Canese (1962–1987), Velazquez (1959–1990), SENEPA (1984), Rosner (1986–1989), and Arias (1989–1991). Infection rate of *Trypanosoma cruzi* in

the endemic areas of Chagas' disease in Paraguay varied from 5.0% to a peak of 78%.

The Ministry of Public Health, Paraguay realized the importance of the disease and had intended to promote programs for its control. However, no effective control measures has yet been taken due to the lack of information on its incidence in the rural community. In this paper we tried to analyse if there is any relationship between the basic knowledge on Chagas' disease of representative Paraguayan and their socioeconomic background. The results of field survey and of laboratory diagnosis were compared, and some aspects of Chagas' disease in this country were discussed.



Fig.12 Places where the survey was conducted

Method of field survey

Questionnaire were delivered to the member of Japanese Overseas Cooperation Volunteers (JOCV) who were stationed in various parts of Paraguay, during the period from January through March 1992. Members of JOCV interviewed people living in the endemic areas that shared high prevalence and/or non endemic control areas. Prior to the field survey, careful selection of the survey areas to make the survey more representative was considered.

The questionnaire delivered to the interviewers are as follows:

- 1) Name: Sex: Age:
- 2) Education (graduated from)
 - a: Primary school
 - b: Middle school
 - c: High school, college and/or University
 - d: No education
- 3) Do you know anything about Chagas' disease?
- 4) Ever been told by your doctor that you were suffering from Chagas' disease?
- 5) Did you know about Romana sign?
- 6) Did you see any person with Romana sign?
- 7) Were you taught anything about Chagas' disease in your school?
- 8) If you knew anything about Chagas' disease, please tell us.
 - a) Do you know the disease is transmitted by the insect vector, triatomine bugs?
 - b) Do you know *T. cruzi*, the pathogen of the disease, is in the feces of triatomine bugs?
 - c) Do you know that dog, cat, and armadillo are also susceptible?
- 9) Did ever you see triatomine bugs in your house?
- 10) Whoever had seen triatomine bugs, please tell us.
 - a) Ever been sting by triatomine bugs?
 - b) At what time of the day had you been stung by the bugs?
 - c) At what part of the house did you see the bugs?
A: Floor b: Bed c: Wall d: Ceiling

Results of the questionnaire survey

1) Representative places at where the survey was conducted

The following 14 places, namely Boqueron Province (Macharety), Concepcion Province (Concecion, Santa Elena), Caasapa Province (Caasapa), Central province (Asuncion, San Lorenzo, Nemby, Villeta), Paraguari province (Mubuyapey), Itapua Province (Hoenau), San Pedro Province (Chore), Caaguaza Province (Oviedo, Col. Blas. Garay), and Amambay Province (Amambay) were selected for the survey). In these areas, a total of 948 people, with age of 6 to 78 were interviewed and requested to answer the questionnaire.

2) Level of final education received

Of the 948, persons interviewed 293 (30.9%) were graduates of primary school, 352 (37.1%) middle school, and 248 (26.2%) high school, college, and/or university, respectively. The remaining 55 persons (5.8%) did not receive any education at all. Level of the final education received varied according to the areas surveyed that is more residents in urban area received higher education but only a few in the rural area. Particularly, in some of the indigeneous Indio villages, more than half of the residents were found to be without education.

Level of the final education received also varied accordingly with age, that is, the younger generation tend to receive higher education, while the older generation received less education. Particularly, many women with age greater than 50 were found to be without any education.

3) Knowledge of Chagas' disease

Among the 948 people interviewed, only 515 (54.3%) knew about Chagas' disease. However, knowledge of the disease varied from area to area. It is noteworthy that fewer inhabitants of rural (endemic) areas, such as Macharety and Concepcion knew about Chagas' disease, while, 70-80% of the people living in urban area knew about the disease. This is likely to be attributed to the lack of education in the rural areas.

As for the age difference of the inhabitants with regard to the knowledge on Chagas' disease, it increased with age until 40 years old, and then declined.

4) Education on Chagas' disease in school;

Out of the 948 people surveyed, 251 (26.4%) had received lecture concerning Chagas' disease were. The ratio of those who received the lecture varied according to the areas, for example, 43% of primary and middle school graduates in Macharety replied that they received some lecture concerning the disease, while that number were 30% in Concepcion and 22% in Oviedo, respectively. However, 28% of the people from urban areas and 38% of the inhabitants from a non-prevalent area, Hoenau, also replied that they received some lecture, indicating the education in school varied from one to another.

Social awareness of Chagas' disease seems to have a close relationship with the level of education received. This is reflected in 22% of primary school, 32% of middle school, and 35% of high school, college and/or university graduates replying that they had received education on the disease.

As for the difference on the knowledge by sex, 73 (24.7%) out of 296 men replied that they had received the education, while 178 (29.9%) out of 597 women replied so.

5) Persons who were previously diagnosed as having Chagas' disease

a) Distribution

Chagas' disease is primarily a disease of rural areas where the insect vectors, animal reservoirs, and humans are in close contact. However, in average, the person who had been diagnosed with Chagas' disease were only 22 (7.2%) out of 307 men and 36 (5.6%) out of 641 women, with none being reported from Hoenau, Nemby, Chore, San Lorenzo, Santa Elena and Caasapa. In some area, sex difference was observed, and the ratio of the infected person increased with age.

b) Romana sign, (unilateral bipalpebral edema)

Only 8 (2.6%) man and 18 (2.8%) women replied that they developed Romana sign. However, there is

a possibility of confusing with other disease such as conjunctivitis. In rural endemic area of Macharety, 3 (7.0%) out of 43 men and 8 (17.0%) out of 47 women replied that they Romana sign.

The incidence of the Romana sign was also seen in Col. Blas. Garay, where 2 (4.2%) out of 48 men and 6 (12.5%) of 48 women; and in Oviedo, 3 (4.5%) out of 45 men and 2 (4.4%) out of 45 women were reported to experience Romana sign, respectively. Majority of such cases replied that they acquired the disease during childhood.

Romana sign occurs during the acute stage of the infection, and between the period from 1933 to 1971, a total of 180 acute infection was reported in Paraguay (Canese, 1971). In average, it means 5.6 person per year. However, the result of this survey revealed the figure is much smaller than expected.

c) Have you seen anybody with Romana sign?

In reply to this question, 147(15.5%) out of 948 questioned said yes. The ratio is higher than that of the former question at 2.6% in men and 2.8% in women. This might be attributed to the probability of one patient with Romana being seen by many people. However, more men(20.8%) saw the patients while only 12.9% of women did so, indicating that men have more chance to see the patient outside of their residence. Furthermore, most of them replied that they saw the patient when visiting the rural endemic areas.

6) Knowledge on the vector insect of disease

The following questions were asked to 515 people who replied that they knew about Chagas' disease.

a) Do you know that the disease is transmitted by the insect vector triatomine bugs?

Of the 515 persons asked, a total of 263 (51.1%), consisting of 58 men and 205 women, answered "yes." However, almost all of the inhabitants of rural endemic area did not know this fact.

b) Do you know that *T. cruzi*, the pathogen of the disease, is in the feces of triatomine bugs?

Among the 515 persons questioned, 176 (34.2%)

consisting of 33 men and 143 women replied that they knew the fact.

- c) Do you know that dog, cat, and armadillo are also susceptible?

Among the 515 persons questioned, 112 (21.7%) consisting of 30 men and 82 women replied that they knew that the majority of the mammals, including dogs and cats, can be naturally infected with *T. cruzi*. The remaining did not know that the disease is a parasitic zoonosis. It is beyond the scope of this paper to describe the level of the epidemiological knowledge of ordinary people.

However, when the answers to the preceding 3 questions on Chagas' disease were assessed, only 11 (7.5%) men and 19 (5.2%) women answered all 3 questions correctly. This indicates that only 3.2% of the population interviewed understood that the parasite the life-cycle involves reservoir animals and the insect vectors in nature).

7) Sighting of the insect vector, the triatomine bugs

Of the 948 persons who replied, 134 (43.6%) out of 307 men and 249 (40.4%) out of 641 women said that they had seen the bugs. However, people living in urban areas said that they have not seen the bugs in their own houses, but had seen them in houses located in rural areas, such as Macharety, Concepcion, or Col. Blas. Garay.

- a) Ever been bitten by triatomine bugs?

Among the 948 questioned, 47 (15.3%) out of 307 men and 58 (9.0%) out of 641 women said "yes." In Macharety, more than 80% of the inhabitants replied that they had been bitten by the bugs before. At what time part of the day had you seen the bugs?

All of them replied that they saw the bugs at the night, but a few said they also saw the bugs in the afternoon.

- b) At what part of the house did you see the bugs?

About half (47.8%) of them replied that they saw the bugs on the wall, followed by bed, floor and ceiling.

8) Effect of education in relation to the prevention of Chagas' disease

Two groups, one which had received education on

Chagas' disease in school and the other, which received no education, were compared for their history of infection with *T. cruzi*.

Among the 251 educated, 41 (56.2%) out of 73 men and 36 (20.2%) out of 178 women reported that they had asquired the disease before, while among the 697 non-educated, 62 (26.5%) out of 234 men and 142 (30.7%) out of 463 women reported that they had had the disease before, indicating that there is no relationship between education and prevention of the infection.

9) Comparison of the results of field survey and of laboratory diagnosis

A total of 121 (2-78 years old) inhabitants of Macharety, that includes 47 men and 54 women, were examined immunologically and parasitologically, in conjunction with the field survey being conducted. The following are the results:

- a) Immunological test

Results of immunological (ELISA) test revealed that sera from 15 men and 18 women were positive at dilution of 1:40, while 10 of them replied that they were diagnosed as having Chagas' disease before.

- b) Romana sign

Although 11 replied that they had Romana sign, only 4 of them showed positive results by ELISA. A unilateral bipalpebral edema in the remaining 7 person were likely due to be caused by other diseases.

- c) Electrocardiographic dysfunctions (ECG)

Serological studies were performed on 121 persons, from which 55 were subjected to ECG studies. Eight of them were ELISA positive, while the remaining three were ELISA negative.

The ECG abnormalities found (just 9.0%) were rather low when compared with other studies. The abnormalities found were as follows:

1. Sinusal Bradicardy with left-deviation (less than 55 beats per minute),
2. Sinusal Bradicardy,
3. Left-diviation,
4. Complete blocking of right branch with forward-left hemiblocking. The remaining individuals who were negative for *T. cruzi* antibodies showed:

1. Incomplete blocking of right branch with left-deviation.
2. Incomplete blocking of right branch with right-deviation.
3. Sinusal tachycardy (more than 170 beats per minute).

Even though, all serological positive cum ECG-abnormal patients showed left-deviation abnormalities, an increase in the voltage, enlarging of the QRS complex, segment ST or negative T wave depression were not observed that could make suspectable cardiac dilatation.

d) Presence of triatomine bugs in house

As to the question of the presence of triatomine bugs in their houses, 84(93.3%) out of 90 replied yes, and 72(88.0%) said that they been bitten by the bugs when they were in bed during the night. In every houses surveyed, between 2 to 26 (average of 11.6) bugs were found and they were indentified as *Traitoma infestans*, the major vector of *T. cruzi*.

Discussion and recommendation

As Chagas' disease is primarily a zoonotic disease where the insect vectors, animal reservoirs, and man are in close contact, it occurs mostly only in the rural area. Incidence of human Chagas' disease in Paraguay varied according to areas, for example, 7.4% in Concepcion, 16.2% in De Las Cordilleras, 12.4% in Caazapa, 8.2% in Itapua, 12.8% in Paraguari, 8.3% in Caasapa, 1.3% in Misiones, 6.6% in Alto-Parana, 4.1% in Neembucu, 7.4% in Hayes and 9.1% in Olimpo (Canese, 1978).

During the period from 1939 to 1971, Canese (1971) reported a total of 170 acute cases of the human infection. However, our present survey revealed that the people previously diagnosed as having Chagas' disease were only 58(6.1%) out of 948 peoples interviewed. Furthermore, among the 58 patients, only 26 reported that they had Romana sign. Thus, the level of endemicity of the disease is likely to be less in Paraguay than was originally estimated by the govermental insutitutions or widely believed.

Furthermore, the results of our present survey revealed most of the inhabitants of rural areas were not knowledgeable on or familiar with Chagas' disease, with only 3.2% of them knowing the correct information of the diseas. Therefore, more intensive health education on the disease by the health authority is thought to be necessary to provide expertise to the inhabitants of rural areas in combatting the disease.

Diagnosis depending only on the result of immunological test is not enough to confirm *T. cruzi* infection in human, particularly in the areas of very low endemicity. Simultaneous determination of Romana sign, edema on hand and foot, enlargement of lymph nodes, liver and spleen, abnormal electrocardiogram, and megacolon, as well as verification by serological test and detection of *T. cruzi* itself are needed to establish the actual diagnosis.

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Remarks:

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