CENTENNIAL REVIEW

An update on Chagas disease (human American trypanosomiasis)

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Human American trypanosomiasis or Chagas disease — named after Carlos Chagas who first described it in 1909 — exists only on the American continent. It is caused by a parasite, *Trypanosoma cruzi*, that is transmitted to humans by blood-sucking triatomine bugs, by blood transfusion, and transplacentally.

Chagas disease has two, successive phases: acute and chronic. The acute phase lasts 6–8 weeks. After several years of starting the chronic phase, 20%–35% of infected individuals (the percentage varying with geographical area) develop irreversible lesions of the autonomous nervous system in the heart, the oesophagus, the colon and/or the peripheral nervous system. Data on the prevalence and distribution of Chagas disease markedly improved in quality during the 1980s, as a result of demographically representative, cross-sectional studies carried out in countries where no accurate information on these parameters was available. Experts had previously met in Brasilia, in 1979, and devised standard protocols for carrying out country-wide studies not only on the prevalence of human infection with *T. cruzi* but also on house infestation with the triatomine vectors.

Thanks to a co-ordinated programme in the southernmost countries of South America (i.e. the 'Southern Cone'), transmission of *T. cruzi* by the vectors or blood transfusion has been successfully interrupted in Uruguay (from 1997), Chile (from 1999) and Brazil (from 2005), and the global incidence of new human infection with *T. cruzi* has decreased by 67%. Similar multi-country control initiatives have been launched in the Andean countries and in Central America, with the goal of interrupting all transmission of *T. cruzi* to humans by 2010 — a goal set, in 1998, as a resolution of the World Health Assembly (WHO, 1998*a*).

Recent advances in basic research on *T. cruzi* include the genetic characterization of populations of the parasite and the sequencing of its genome.

Chagas disease, named after Carlos Chagas, the Brazilian clinician who first described the disease (Chagas, 1909), exists only on the American continent. It is caused by the parasite *Trypanosoma cruzi*, which is transmitted to humans by blood-sucking triatomine bugs, by blood transfusion, and through the placenta.

There are two stages of the human disease: the acute stage, which appears shortly after the infection, and the chronic stage, which may last several years. After a silent asymptomatic period lasting several years, about 25% of those infected develop cardiac symptoms that may lead to chronic heart failure and sudden death, 6% develop digestive lesions, mainly mega-colon and mega-oesophagus, and 3% suffer peripheral nervous damage (Coura *et al.*, 1985; Pereira *et al.*, 1985).

Acute Phase

Acute cases of Chagas disease are usually produced by the bites of infected triatomine bugs when they puncture the skin to suck blood and simultaneously deposit faeces containing trypomastigotes of *Try. cruzi*. Most humans infected with the parasite never appear to become symptomatic but some develop an acute form of the disease, shortly after they are infected. Most cases of

acute Chagas disease are seen in individuals aged <15 years, with the highest frequency in young children aged 1–5 years. The acute phase of the disease starts when the parasite enters the body. A local reaction known as a chagoma often develops at the point of entry, and a general malaise, with fever, lymphadenopathies, hepatomegaly and splenomegaly, follows. There is sometimes a painless conjunctival reaction, with oedema of both eyelids on one side of the face ('Romaña's sign'), and lymphadenitis of the pre-auricular ganglia. A few cases develop much more severe problems, of acute myocarditis or meningo–encephalitis, which are occasionally fatal. In most affected individuals, however, all the clinical manifestations of the acute phase disappear 4–8 weeks after they have begun.

Congenital or oral transmission of *Try. cruzi* and accidental transmission of the parasite via blood transfusions or laboratory accidents may also lead to acute cases of Chagas disease. In a patient who has been given a transfusion of blood from an infected donor, the clinical manifestations of acute Chagas disease may appear at any time from a few days to several weeks after the blood transfer. Most patients infected by transfusion stay asymptomatic, however, and none develops a chagoma or other local reaction to the parasites at the transfusion site. The risk of developing clinical disease appears to increase with the number of transfusions with infected blood. The risk of being infected by a single transfusion from an infected donor varies with the donor involved but does not exceed 25%.

Congenital Chagas disease is produced by the transmission of *Try. cruzi* from an infected mother to her child. Infection of the foetus may occur at any time during the pregnancy and in different pregnancies of the same woman. In endemic areas, 1%–10% of neonates may be infected with *Try. cruzi*. As control interventions become more successful and transmission by vectors and blood transmission is greatly reduced, such transplacental transmission is gaining in importance. Fortunately, in areas with successful vector control, the incidence of congenital Chagas disease is falling as the prevalence of infection in women of child-bearing age is reduced (Muñoz *et al.*, 1992).

Chronic Phase

The chronic phase of Chagas disease begins at the point when the clinical manifestations of the acute phase (if any) disappear. At this time, the number of trypomastigotes in the patients' bloodstream falls away, usually to undetectable levels. In untreated individuals, it is thought that the host's immune responses to the parasite cause this loss of parasitaemia and subsequently keep the bloodstream relatively free of parasites, sometimes for the rest of the subject's life. Antibodies (IgG) against *Try. cruzi* can often be detected for many years post-infection. At least 50% of infected individuals have some viable parasites circulating in their bloodstream for several years after the original infection, although these may only be detectable using parasitological tests, such as xenodiagnosis or haemoculture, or PCR-based assays.

INDETERMINATE FORM

In all infected individuals, the chronic phase of Chagas disease initially takes on an asymptomatic 'indeterminate form'. At this stage of the infection there are no objective manifestations of any organ damage (at least, none detectable using routine clinical procedures) and the results of electrocardiography and radiological studies of the heart, oesophagus and colon generally appear normal.

About 50%–70% of infected individuals — the majority of those with chronic infection — stay at this indeterminate stage for the rest of their lives. Many never realise that they are, or ever have been, infected and most only discover the truth (typically when aged 20–50 years) as the result of epidemiological surveys or circumstantial medical or serological examinations, such

as tests used, in endemic areas, to check potential blood donors for *Try. cruzi* infection.

Although they may appear to have normal hearts when checked, over about 1 min, by routine electrocardiography, some patients with the indeterminate form of *Try. cruzi* infection may be found to have abnormalities (in beat frequency control, rhythm and/or cardiac conduction) when investigated by ambulatory electrocardiographic monitoring over a 24-h period, while they carry out their normal daily activities.

A minority of individuals at the indeterminate stage of infection eventually develop symptomatic disease, which may affect the heart, digestive system and/or peripheral nervous system, sometimes fatally.

CARDIAC FORM
Chagasic cardiomyopathy is the most important clinical consequence of *Try. cruzi* infection. The results of epidemiological studies indicate that 10%–30% of individuals who are seropositive for *Try. cruzi* infection have some characteristic electrocardiographic abnormalities that point to cardiac damage caused by the parasite. These alterations, which usually occur 10–20 years after the acute phase of the disease, represent a broad spectrum of heart injury. The clinical manifestations of this damage vary from mild symptoms to heart failure and, frequently, sudden death. There may be inflammation with accompanying fibrosis scattered throughout the myocardium.

The main clinical manifestations of chronic chagasic cardiomyopathy are heart failure, cardiac arrhythmias and thromboembolism. Heart failure usually gives rise to dyspnoea and oedemas. The myocardial lesions affect the right ventricle, as well as the left, and advanced cases present with predominantly right-ventricle insufficiency, producing oedemas and congestive hepatomegaly. Electrocardiography nearly always reveals right-bundle-branch blocks, left anterior hemiblocks, prolonged A–V conduction times, primary T-wave changes and abnormal Q waves. Ventricular extrasystoles are the most common of the rhythm alterations.

DIGESTIVE FORM
Destruction by *Try. cruzi* of the autonomic enteric nerves may lead to dysfunction of the digestive tract. Although anatomical and functional alterations can be observed at various points in the tract, abnormalities are most frequently seen in the oesophagus and colon, which are often much enlarged.

The digestive form of chronic Chagas disease has been described in all endemic populations below the Equator but its prevalence varies markedly from one country to the next. In hospital-based observations, mega-oesophagus is more frequently diagnosed than mega-colon but this may just represent sampling bias, since patients with dysphagia are more likely to seek medical care than patients with constipation (Rezende and Luquetti, 1994).

ALTERATIONS IN THE PERIPHERAL NERVOUS SYSTEM
In about 10% of patients with chronic Chagas disease, neurological examination reveals the clinical manifestations of damage to the peripheral nervous system. Most of the affected subjects show a combination of sensory impairment and diminished tendon reflexes that mainly involve the lower limbs (Sica, 1986).

PATHOLOGY

Indeterminate and Cardiac Forms
There have been few pathological studies focusing on individuals in the indeterminate stage of the chronic disease. Given the prevalence of the disease, many cases at the indeterminate stage must die (for causes other than Chagas disease) and be autopsied. It is rare, however, for the published results of such autopsies to provide enough

information to permit cases at the indeterminate stage to be distinguished from other cases.

Endomyocardial biopsies can be taken from living patients and these can provide information on cardiological abnormalities in individuals at the indeterminate stage. The alterations observed can then be correlated with clinical observations. The biopsies can only be collected from limited zones of the endocardium, however, and cannot be considered representative of the whole cardiac mass. Material obtained through endomyocardial biopsies can be investigated by electron microscopy, histochemistry and immunohistochemistry. Light and electron microscopic studies of such material have revealed myocyte hypertrophy, degenerative changes, inflammation and fibrosis in some individuals at the indeterminate stage of the chronic disease. Intact parasites are rarely seen but *Try. cruzi* DNA can be demonstrated in the samples of myocardium, by PCR, even in the absence of local inflammation.

Mild myocarditis can often be observed, as scattered small foci of interstitial infiltration by lymphocytes, macrophages and plasma cells, in patients at the indeterminate stage. This does not have the intimate association with degenerating myocytes observed during the acute phase of the disease, and sometimes develops a granulomatous structure (Andrade, 2000). The inflammatory cell reaction has been found to consist mainly of cytotoxic $CD8^+$ T-lymphocytes and such cells appear to be the main T-cell type responsible for immune activation in chronic chagasic cardiomyopathy. These cells are activated, via class-I molecules of the major histocompatibility complex (MHC), by macrophages containing remnants of *Try. cruzi*. The absence of a $CD4^+$ T-cell response in the presence of *Try. cruzi* antigens indicates that the presentation of these antigens via class-II MHC molecules is inhibited (Reis *et al.*, 1993; Tostes *et al.*, 1994).

Ultrastructural observations show several degrees of regressive change in the myocardiocytes, such as mitochondrial swelling with disruption of the cristae, and accumulation of glycogen particles, as well as thickening of the basement membrane around the cells and in the myocardial capillaries. These changes may interfere with the metabolism and diffusion of nutrients to the contractile fibres.

Amastigote forms of *Try. cruzi* are rarely found in sections when standard histological techniques are used. Immunochemistry, however, greatly improves the level of detection of intact intracellular amastigotes. Using this technique or molecular methods such as PCR and in-situ hybridization, intramyocardial parasites can often be demonstrated in biopsies taken from cases of chronic Chagas disease (Jones *et al.*, 1992; Higuchi *et al.*, 1997; Añez *et al.* 1999; Vago *et al.*, 2000).

Digestive Form

The histological study of different segments of the digestive tract from patients with the digestive form of chronic Chagas disease usually reveals focal myositis and unevenly distributed lesions of the intramural plexuses (mainly the myenteric plexus). The common anatomical abnormality underlying this form of the disease is the destruction of the parasympathetic ganglion cells that are associated with the muscular layers of the dilated organs. Inflammation and neuronal depletion in the myenteric plexuses of the oesophagus is associated with myositis and fibrosis in the *muscularis propria*. Parasites are only rarely detectable in routine sections of these tissues but molecular tests for the kinetoplast DNA (kDNA) of *Try. cruzi* often give positive results, indicating that the parasites are probably directly involved in the development of the chronic lesions seen in the digestive form of Chagas disease (Vago, 1996).

PATHOGENESIS OF CHRONIC LESIONS

Two primary hypotheses have been formulated to explain the pathogenesis of Chagas disease: (1) *Try. cruzi* infection induces immune responses in the host that are targeted at the host's own tissues and are independent of the persistence of the parasite (the 'auto-immunity hypothesis'); or (2) the persistence of the parasite at specific sites in the tissues of the host results in chronic inflammatory reactivity (the 'parasite-persistence hypothesis'). In both cases, the immune-based pathology results in the cumulative, focal destruction of tissues, and the signs and symptoms of the clinical disease.

For a long time, the prevailing hypothesis was that Chagas disease had an auto-immune aetiology, with chronic *Try. cruzi* infection inducing either a loss of immuno-tolerance to self-antigens or antiparasite antibodies that cross-react with host components. The first reports on the existence of antigenic epitopes that are shared by *Try. cruzi* and mammalian cells, and their role in the pathogenesis of Chagas disease, appeared in the 1970s. Infection can produce antibodies that react with the host's endothelial cells, vascular endothelium and the interstitium of the heart (Kierszenbaum, 1999).

The introduction of new techniques such as immunohistochemistry, PCR and in-situ hybridization, however, provided indisputable evidence of parasites persisting in the tissues of patients in the chronic phase of the disease. Using an immunoperoxidase technique, for example, Higuchi *et al.* (1993) detected *Try. cruzi* antigens in the hearts of patients with chronic chagasic cardiomyopathy. Using a PCR-based assay, Jones *et al.* (1992) consistently detected *Try. cruzi* DNA in heart samples from patients with chronic chagasic cardiomyopathy but did not find such DNA in heart samples from seropositive cadavers with no evidence of chagasic cardiopathy. Similarly, Vago (1996) found *Try. cruzi* DNA in oesophageal tissue from seropositive patients with mega-oesophagus but not in the corresponding samples from patients who had died of chronic chagasic cardiomyopathy without mega-oesophagus.

The different clinical and pathological manifestations of Chagas disease appear related to variations in the efficiency of the human host's immune response, with efficient immune responses controlling the level of parasitaemia and limiting tissue damage. Inefficient immune responses fail to control the parasite burden adequately, promoting more persistent inflammatory reactions and more severe disease. It has been suggested that it is the immune responses during the acute phase that play a fundamental role in the chronic manifestations. The strain of *Try. cruzi* involved also appears to be an important factor in the pathogenesis of the disease.

Recent experimental, histological and clinical observations indicate that Chagas disease should be considered primarily as a parasitic infection, rather than (at least exclusively) as an auto-immune disease. In theory, therefore, it should always be possible to induce a favourable outcome in infected patients by the administration of an effective parasiticide.

LABORATORY DIAGNOSIS

Parasitological Diagnosis

During the acute phase of Chagas disease there are so many parasites in the peripheral blood that infection can usually be detected by the microscopical examination of wet or dry bloodsmears or by parasitological tests such as xenodiagnosis or blood culture. Tests based on PCR can give detection sensitivities that are much higher than those of xenodiagnosis or culture but such tests are relatively complex and require more specialised equipment. The variable region of the minicircle kDNA and a 195-bp reiterated DNA sequence of the parasite

have proven to be useful target sequences for diagnosis by PCR (Russomando *et al.*, 1998)

Immunodiagnosis

Diagnosis is often based on the results of immunological testing because individuals in the chronic phase of Chagas disease develop antibodies, predominantly of the IgG class, against a complex mixture of parasitic antigens. In the acute phase, IgM antibodies are more frequently found than IgG.

Several immunodiagnostic tests are available. Some are classified as conventional and have been extensively validated, are commercially available, and are used in most laboratories, whereas others, which may have better specificity and operational advantages, are still in the testing phase (Moncayo and Luquetti, 1990). The conventional tests that are most widely used are based on indirect haemagglutination (IHA), IFAT or ELISA, and it has been recommended that a definitive diagnosis of *Try. cruzi* infection should be based on a positive result in tests of at least two of these three types.

In most conventional tests, a complex mixture of parasite antigens (IHA and ELISA) or whole parasites (IFAT) are employed. The use of multiple antigens, rather than a single purified or recombinant antigen, gives a better sensitivity but increases the chances of false-positive results because of cross-reactivity, especially in the many endemic areas for *Try. cruzi* where *Leishmania* spp. and/or *Try. rangeli* are present.

The IHA test allows results to be obtained in about 2 h and needs no sophisticated equipment or specialised technical skills. The sensitivity of this test, however, though reasonable (96%–98%), tends to be lower than that seen with IFAT or ELISA. The latter tests often give excellent sensitivity but have to be performed by a skilled technician and may takes several hours to produce a result. ELISA have two main advantages over IFAT: ELISA results have to be read with a spectrophotometer (which avoids subjectivity and permits automation) and ELISA can be used for the simultaneous screening of many samples. When tested by ELISA, IFAT and IHA, 95% of sera give concordant results with all three tests. Discordance between two of the tests may indicate a technical error or the presence of an unusual serum.

TREATMENT

Trypanocidal Treatment

Nifurtimox (a nitrofuran derivative) and benznidazole (a nitroimidazole) have been recommended, almost exclusively, for the treatment of the acute phase of Chagas disease and of congenital infections with *Try. cruzi*. When seropositive schoolchildren in the chronic phase of the disease were given benznidazole, 60% of them became seronegative and all were less likely to develop heart damage than untreated controls (Andrade *et al.*, 1996; Sosa Estani *et al.*, 1998). The results of a follow-up study of 131 individuals with chronic cardiomyopathy, who were examined nearly a decade after treatment with benznidazole, are particularly impressive (Viotti *et al.*, 1994). Compared with the untreated controls, the treated patients showed significantly less clinical deterioration and lower titres of anti-*Try. cruzi* antibodies, the latter indicative of parasitic cure. Although side-effects develop in most adult patients given benznidazole (children have a better tolerance to the drug), they usually disappear when treatment is discontinued. The general benefits of treatment with benznidazole in the acute and chronic phases of Chagas disease indicate that treatment with this drug should be recommended to any individual found seropositive for *Try. cruzi* (PAHO, 1999).

Drug Development

Although benznidazole is usually effective in the treatment Chagas disease, new drugs

that are at least as effective when given over shorter periods and cause fewer or no side-effects are still required.

The costs of drug discovery and development in the pharmaceutical industry have escalated over the past few years. In consequence, pharmaceutical companies have focused their research and development efforts on areas where the returns are likely to be commensurate with the investments. Sadly, as most tropical diseases, including Chagas disease, mainly affect the poor, the major companies have largely abandoned attempts to discover and develop new drugs for their treatment.

Some sterol-biosynthesis inhibitors which might be useful for the treatment of Chagas disease are under clinical development by one pharmaceutical company, albeit for non-trypanosomal illnesses (e.g. mycoses). 'Fourth-generation' azole derivatives such as D0870 and SCH 56592 (posaconazole) are capable of inducing parasitological cure in murine models of both acute and chronic Chagas disease (Liendo *et al.*, 1998). *In vitro*, the bis-triazole derivative D0870 has been found to be a powerful growth inhibitor of both epimastigotes and intracellular amastigotes. In infected mice, this drug has been found to be 30- to 50-fold more powerful than nifurtimox, causing parasitological cure in >60% of the treated animals (Urbina, 1999). Unfortunately, the development of this compound was discontinued in 1997.

PARASITOLOGY

Trypanosoma cruzi belongs to the order Kinetoplastida, which comprises flagellated organisms with a kinetoplast — an organelle in the mitochondrion that contains a fibrous network of DNA.

Biological Characteristics

Strain-dependent variations in the distribution of the intracellular amastigote forms in human tissues have been reported. Some strains display a preferential tropism for macrophages in the spleen, liver and bone marrow, whereas others are very scarce in these organs. Variable patterns of virulence, course of parasitaemia, and mortality have also been described (Andrade, 1974). Even a single parasite strain may behave differently in different lineages of mice.

Genetic Characteristics

Considerable advances have been made towards an understanding of the genetic composition of *Try. cruzi* and of the processes involved in the control of the parasite's gene expression. Many genes coding for structural components, metabolic enzymes, molecules involved in cell penetration and immunodominant antigens have been cloned and sequenced. Recombinant proteins bearing immunodominant domains have been expressed and used as reagents for serodiagnosis. The development of cloning vectors for transfection studies has allowed some genes to be knocked out and others to be super-expressed, to elucidate the role of each gene in the biology of the parasite.

Genetic molecular markers of *Try. cruzi* have been sought, to try to correlate the different strains with their distinct biological properties, clinical manifestations and epidemiological characteristics. Early studies on the population genetics of *Try. cruzi* revealed substantial iso-enzymic variability between isolates, with three major groups or zymodemes (Z1, Z2 and Z3) identified (Miles *et al.*, 1980). Z2 was associated with the domestic transmission cycle whereas Z1 and Z3 were predominant in the sylvatic cycle. Further analysis of 15 enzymatic gene loci disclosed a greater heterogeneity, with 121 *Try. cruzi* isolates from the American continent assigned to 43 zymodemes that could not be grouped into a few natural clusters (Tibayrenc, 1995). It appears that the population structure of *Try. cruzi* is clonal rather than sexual and that the

genetic and biological variability observed today is the result of the independent evolution of clonal lines (Tibayrenc, 1995).

Analysis of restriction-fragment-length polymorphisms (RFLP) in the kDNA of *Try. cruzi* revealed very heterogeneous patterns and allowed parasite isolates to be split into several groups, named schizodemes (Morel *et al.*, 1980). In contrast to the wide polymorphism seen in the parasites' iso-enzymes and kDNA restriction fragments, analysis of the ribosomal RNA (rRNA) gene and intergenic mini-exon sequences has simply revealed a dimorphism. The PCR-based amplification of a specific region of the 24S alpha rRNA gene, for example, produced fragments of either 125 or 110 bp, defining two major groups of strains (Souto *et al.*, 1996). These groups were subsequently confirmed by an analysis, based on the random amplification of polymorphic DNA (RAPD), of 50–60 polymorphic loci (Zingales *et al.*, 1998).

Within the last few years there has been an attempt to standardize the nomenclature of the two groups of *Try. cruzi* (WHO, 2002). Given the group equivalences proposed (Table 1), rapid PCR-based methods are now generally considered preferable to zymodeme typing, since the latter requires very large numbers of parasite cells and culture that may promote the selection of a particular population of the original strain.

The epidemiological distribution of the two groups of strains was investigated. *Try. cruzi* isolates were obtained from mammalian reservoirs, humans and triatomines from several regions of Brazil, Bolivia and Colombia and were typed by PCR as belonging to *Try. cruzi* groups I and II. It was found that there is a strong association of *Try. cruzi* II with the domestic cycle, while *Try. cruzi* I was preferentially encountered in the sylvatic environment (Fernandes *et al.*, 1998).

Since all the parasites isolated from seropositive individuals in endemic regions belong to *Try. cruzi* II, this group appears to have properties that favour human infection and high parasitaemias.

Parasite Genome

The *Trypanosoma cruzi* Genome Project was launched by the World Health Organization's Special Programme for Research and Training in Tropical Diseases (TDR) in 1994. The sequencing of the genome of the CL Brener strain of *Try. cruzi* (and those of *Try. brucei* and *L. major*) was completed, by a network of research groups from South America, the U.S.A. and Europe, in 2005 (Henriksson *et al.*, 1995; Zingales *et al.*, 1997; Di Noia *et al.*, 1998; Hanke *et al.*, 1998; Morel *et al.*, 2005; El-Sayed *et al.*, 2005). The haploid genome contains 12,000 genes that code for approximately 22,570 proteins that are functionally important in the various processes of the parasite's life-cycle, its relationship with the immune system of its mammalian hosts and in the pathogenesis of chronic lesions of Chagas disease. *Trypanosoma cruzi* of the CL Brener strain was used for the genomic sequencing because this strain has been well characterized experimentally. Although this strain belongs to subgroup IIe, it was found to have sequences associated with subgroup I, supporting the role of multiple progenitors

TABLE 1.   *Standardization of the nomenclature of the two principal groups of* Trypanosoma cruzi *(WHO, 2002)*

	Equivalents:					
	Zymodeme	Lineage	Group	Discrete typing unit	Type	Ribodeme
Trypanosoma cruzi I	1	2	1	1	III	II and III
Trypanosoma cruzi II	2 and A	1	2	2	II	I

in the evolution of the *Try. cruzi* strains circulating today.

In the exploration of the genome, the 'shotgun' technique, which consists in fragmentation of the genome and the subsequent sequencing of the fragments, was used. The reconstruction of the complete genome was then carried out using specially designed software.

An interesting finding was the high level of diversity seen in the myosin genes and the fact that such genes share sequences with some host cells; this might explain why auto-immunity appears to play some role in the development of the lesions seen in chronic Chagas disease. Multiple genes were found to code for the surface molecules that permit parasites to penetrate into host cells and obtain, for their metabolism, sialic acid from the host cells.

It is hoped that the sequencing and analysis of the *Try. cruzi* genome will lead to the identification of new targets for drug development and a better understanding of the host–parasite relationship and the mechanisms involved in pathogenesis.

VECTORS

The triatomine bugs considered to be of primary epidemiological importance are those that are highly anthropophilic and form permanent colonies in houses. They are mostly found in the cracks of walls, under loose plaster, in packing cases, and behind pictures and wall ornaments. The bugs that are of secondary importance are those that can produce small, more transitory, intradomiciliary colonies, especially in the absence of the primary vectors. The distribution of the vectors is typically focal, with a population density that correlates with the availability of food.

Chemical Control
Currently, the main method used to control the vectors of *Try. cruzi* is the spraying of infested dwellings with pyrethroid insecticides. After an initial round of such spraying, it is important to continue entomological surveillance so that any new infestations can be detected and selectively retreated. Although the re-appearance of vectors in a sprayed house is usually the result of bugs moving from another, unsprayed building nearby, sometimes bugs move from sylvatic foci into houses, and re-infestations also occasionally arise from bugs that have survived the initial insecticide treatment.

Entomological research, to determine the species of triatomine bug present, their dispersion, and the percentage of houses they infest, should precede the initial spray rounds. A geographical/demographic survey should be undertaken at the same time, with registration of the human population and the identification of the local services and resources that may be useful in the control operation and/or any subsequent entomological surveillance. For effective, long-term surveillance, the participation of the communities at risk will usually be necessary.

All actions after the initial attack phase, which is usually based on two cycles of spraying, must be a function of the type of vector prevalent (the species and whether it is introduced or native in the area) and the operational indicators proposed for the intervention.

Monitoring of Vector Resistance to Insecticides
In 1994, a standard protocol for bio-assays to assess the susceptibility of triatomines bugs to insecticides was developed (WHO, 1994). The results produced, in different laboratories, by following this protocol, which allows both direct and residual insecticidal activity to be explored, can be validly compared. The main application of the protocol was the measurement of the median lethal doses (LD_{50}) of the most important pyrethroid compounds used in the vector-control programmes in Latin

America. The protocol is still used as the basis of an extensive programme of resistance monitoring in Latin America.

EPIDEMIOLOGY

Chagas disease is a zoonosis caused by parasites that are transmitted in natural foci or ecological units, within a well-defined geographical range (from the southern border of the U.S.A. to southern Argentina). The normal life-cycle involves sylvatic or domestic mammals and sylvatic triatomine bugs, continuous transmission often being assured with or without the involvement of human beings.

Measurement of Epidemiological Trends

The prevalence and incidence of Chagas disease and the associated mortality are constantly changing as a consequence of the impact of control programmes, rural–urban human migration, and changes in the socio–economic status of the at-risk communities.

Data on the prevalence and distribution of Chagas disease markedly improved in quality during the 1980s, as a result of research carried out in countries where no accurate information was available. In 1979, a group of experts met in Brasilia and devised standard protocols for carrying out country-wide studies on the prevalence of human *Try. cruzi* infection and house infestation by triatomine bugs. During the 1980s, these protocols were followed in cross-sectional studies (supported by the national Ministries of Health) in Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Panama, Paraguay, Peru, Uruguay and Venezuela. The accurate information obtained has since made it easier for individual countries to plan and evaluate the effectiveness of national control programmes (WHO, 2002).

The results of the country-wide cross-sectional surveys indicated that, in the 18 endemic countries in 1980, there were approximately 17 million people infected with *Try. cruzi* and about 100 million people (25% of all the people in Latin American) at risk of such infection. Total incidence was estimated at 700,000–800,000 new cases/year, and the annual number of deaths from the cardiac form of Chagas disease was estimated at 45,000 (WHO, 1991).By comparing the prevalence of infection in a given age-group in 1980–1985 with the prevalences recorded in the same age-group, in the same country, in 1997–2000, it is possible to illustrate the beneficial effects of approximately 15 years of vector control on the incidence of new cases of human infection (Table 2).

Transmission through Blood Transfusion

In Latin American cities between 1980 and 1989, 1.3%–51.0% of blood units intended for transfusion were found infected with *Try. cruzi*, with far fewer infected with a hepatitis virus or HIV (Schmunis, 1991). The transmission of *Try. cruzi* via blood transfusion is a threat even in countries where there is no vectorial transmission, such as the U.S.A. and Canada, where transfusion-attributable cases of acute Chagas disease have been documented (Kirchkoff *et al.*, 1987; Grant *et al.*, 1989). The level of parasitaemia in the blood donor, the number and volume of transfusions received, the time between blood collection and transfusion, and the immunological status of the recipient all affect the risk of a transfused patient developing symptomatic trypanosomiasis. The general risk of parasite transmission via the transfusion of a single 500-ml unit of whole blood from an infected donor varies from 12% to 20%. *Trypanosoma cruzi* can also be transmitted by the transfusion of plasma or erythrocytic concentrates.

INITIATIVE OF THE SOUTHERN-CONE COUNTRIES

In Brasilia in June 1991, the relevant Ministers of Health launched the Initiative for the Elimination of Transmission of Chagas Disease from the countries of the Southern Cone: Argentina, Brazil, Bolivia, Chile, Paraguay and Uruguay (Anon., 1991). At the time, these countries harboured about 11 million people infected with *Try. cruzi* (i.e. about 62% of all the infected people in the Americas). The main vector in these countries is *Triatoma infestans*, which is intradomiciliary. The local control programmes are based on the spraying of houses with residual insecticides and subsequent entomological surveillance to detect re-infestation. That Chagas disease is recognized as an important public-health problem in the Southern Cone is demonstrated by the money invested in the control initiative (>U.S.$500 million). If transmission of *Try. cruzi* to humans was entirely prevented in this region, the global incidence of Chagas disease would be reduced by 70%, even if control initiatives elsewhere had no affect. The Southern Cone initiative has already been very successful. Recent data on the disinfestation of houses, the results of blood-bank screening, and the incidence of infection in young children (aged <5 years) indicate that transmission of *Try. cruzi* to humans, by vectors or via transfusion, was interrupted in Uruguay in 1997, in Chile in 1999 and in Brazil in 2005 (WHO, 1998*b*, 1999, 2000; www.paho.org/default_spa.htm).

Argentina

The primary vector in Argentina is *Tri. infestans*. The seroprevalences of *Try. cruzi* infection in children aged 0–4 and 0–14 years are now only 0.9% and 1.9%, respectively. Among 18-year-old males, seroprevalence fell from 5.8% in 1981 to 1.0% in 1993 and 0.5% in 2002. Vectorial transmission has been interrupted in 10 of the 13 endemic provinces of the country.

Bolivia

There are no data on the epidemiological impact of the control programme that was initiated in Bolivia in 1998.

Brazil

The main vector in Brazil was *Tri. infestans*, with *Tri. brasiliensis* and *Panstrongylus megistus* of secondary importance. The prevalence of *Try. cruzi* infection in children aged 7–14 years fell by 99.8% between 1980 and 1999, from 18.5% to just 0.04%. When none of the young children (aged 0–4 years) investigated in Brazil in 1999 was found seropositive for *Try. cruzi*, this was taken as evidence of the interruption of the vectorial transmission of the parasite, to humans, in the country. Based on the recent epidemiological and entomological data, an international commission certified Brazil free of

TABLE 2. *Human infection by* Trypanosoma cruzi: *prevalences and the reduction in incidence in the areas covered by the Southern Cone Initiative, 1980–2000 (WHO, 2002)*

Country	Age of subjects (years)	Prevalence (%) in:		Reduction in incidence (%)
		1980–1985	2000	
Argentina	18	5.8	1.20	80
Brazil	0–4	5.0	0.12	98
Chile	0–10	5.4	0.38	94
Paraguay	18	9.3	3.90	60
Uruguay	6–12	2.5	0.06	99

transmission in 2005 (www.paho.org/default_spa.htm).

Chile

The bugs responsible for *Try. cruzi* transmission to humans in Chile was *Tri. infestans*. This species has been successfully eliminated from human dwellings, and hence vectorial transmission has been interrupted. The percentage of houses found infected with *Tri. infestans* fell from 3.2% in 1994 to just 0.14% in 1999. Seroprevalence in children aged 0–4 years fell from 1.12% in 1995 to 0.016% in 1999 — a reduction of 98.5%. An independent commission visited the endemic areas of the country in November 1999 and certified the interruption of vectorial transmission (WHO, 1999, 2000).

Paraguay

In a serological survey carried out in Paraguay in 2000, among a representative sample of youngsters aged 14–21 years, a seroprevalence of 3.9% was recorded. This represents a decrease of 60% since 1972, when a seroprevalence of 9.3% was recorded in the same age-group (PAHO, 1999).

Uruguay

Triatoma infestans was the only intradomiciliary vector in Uruguay. Since 1997 this species has been eliminated from houses throughout the whole country, with house infestation 'rates' dropping from 5.65% in 1983 to 0.30% in 1997. There is 100% screening in blood banks. After the incidence of infection in children aged 0–12 years was found to be zero, the interruption of vectorial and transfusional transmission of *Try. cruzi* in Uruguay was confirmed in 1997 (WHO, 1998*b*).

INITIATIVE OF THE ANDEAN COUNTRIES

Colombia, Ecuador, Peru and Venezuela together hold 5 million infected individuals (27% of all infected individuals globally) and 25 million people at risk of contracting the infection. As the bugs that carry *Try. cruzi* to humans in these Andean countries are not strictly domiciliated, vector-control strategies tend to be more varied than those in the Southern Cone and, to be effective, have to be tailored to the local entomological conditions.

Unfortunately, there are no recent data from Colombia, Ecuador or Peru that might indicate recent trends in the epidemiology of Chagas disease in these countries. In Venezuela, however, there is encouraging evidence of the beneficial effects of several decades of control (Aché and Matos, 2001). Seroprevalence of *Try. cruzi* infection in children under 10 years of age, for example, decreased steadily from 20.5% in 1958–1968 to 3.9% in 1969–1979, 1.1% in 1980–1989, and 0.8% in 1990–1999. In young Venezuelan children (aged 0–4 years), seroprevalence was reduced by 90% between 1990 to 1999, to <1.0%, and in the blood banks the frequency of infection with *Try. cruzi* in the blood units intended for transfusion fell from 1.16% in 1993 to 0.78% in 1998. The transmission of *Try. cruzi* to humans in Venezuela now appears to be restricted to the states of Portuguesa, Barinas and Lara.

INITIATIVE OF THE CENTRAL AMERICAN COUNTRIES

In the countries of Central America (Belize, Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, and Panama) there are 2 million infected individuals (11% of all infected individuals globally) and 26 million at risk of infection. As the vectors of *Try. cruzi* to humans in this region, *Tri. dimidiata* and *Rhodnius prolixus*, are not strictly domiciliated, vector-control strategies have to be adapted to suit the local entomological conditions. All but one country in the region have issued legislation for the compulsory screening of blood units in

blood banks for *Try. cruzi*. Unfortunately, there are no recent seroprevalence data that might allow the epidemiological trends, since the launch of the regional control initiative in 1997, to be determined.

AMAZON INITIATIVE

A programme for the epidemiological and entomological surveillance of Chagas disease across Amazonia was launched in 2002. In Brazilian Amazonia, only 252 acute and 27 chronic cases of Chagas disease are currently recognized. There are no epidemiological data from the bordering countries of Guyana, Surinam, French Guyana, Ecuador, Peru, Bolivia and Colombia that permit the distribution of human infection with *Try. cruzi* or house infestation by potential vectors to be elucidated in this vast region (Guhl and Schofield, 2004).

EPIDEMIOLOGICAL AND ECONOMIC IMPACT

As shown in Table 2, the reduction in the incidence of human infections between 1990 and 2000 that resulted from the control and eventual interruption of transmission of *Try. cruzi* in Brazil, Chile and Uruguay has produced important epidemiological and economic results. Compared with the situation in 1990, 325,000 new

cases of human infection by *Try. cruzi* and 127,000 cases of cardiomyopathy and sudden death are now been prevented each year. From the economic point of view, these countries have saved >U.S.$1140 million in healthcare expenditure and social-security costs. The control efforts in the Southern Cone have cut the incidence of Chagas disease in the whole of Latin America by 67%, from an estimated 700,000 new cases/year in 1983 to <200,000 cases/year in 2000 (Table 3).

At present, the major challenge is to ensure the sustainability of all the control initiatives, both in an epidemiological context (with very low prevalences of human infection with *Try. cruzi*) and in the context of health-sector reform (where the decentralization of operations may result in the control activities losing priority). The successful model implemented in the Southern Cone has already been adapted for use the Andean countries and Central America.

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TABLE 3. *Changes in some epidemiological parameters for Chagas disease between 1990 and 2000, as the result of the reduction of transmission (WHO, 2002)*

Parameter	Year:	
	1990	2000
Annual no. of deaths	>45,000	21000
Annual no. of new cases	700000	200000
No. of countries with disease	18	16*

*Transmission was interrupted in Uruguay in 1997 and in Chile in 1999 (and, more recently, in Brazil in 2005; www.paho.org/default_spa.htm).

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