Acta Tropica 115 (2010) 44–54

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Research needs for Chagas disease prevention

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ARTICLE INFO

Article history:
Available online 19 March 2010

Keywords:
Chagas disease
Prevention
Patient care
Triatominae
Control
Research

ABSTRACT

We present an overview of the two main strategies for the primary (vector control) and secondary (patient care) prevention of Chagas disease (CD). We identify major advances, knowledge gaps, and key research needs in both areas. Improved specific chemotherapy, including more practical formulations (e.g., paediatric) or combinations of existing drugs, and a better understanding of pathogenesis, including the relative weights of parasite and host genetic makeup, are clearly needed. Regarding CD vectors, we find that only about 10–20% of published papers on triatomines deal directly with disease control. We pinpoint the pitfalls of the current consensus on triatomine systematics, particularly within the Triatomini, and suggest how some straightforward sampling and analytical strategies would improve research on vector ecology, naturally leading to sounder control-surveillance schemes. We conclude that sustained research on CD prevention is still crucial. In the past, it provided not only the know-how, but also the critical mass of scientists needed to foster and consolidate CD prevention programmes; in the future, both patient care and long-term vector control would nonetheless benefit from more sharply focused, problem-oriented research.

1. Introduction

Research relevant to Chagas disease (American trypanosomiasis) began well before the outstanding work of Carlos Chagas at the beginning of the last century (1909–1934); Chagas described the disease, its causative agent (*Trypanosoma cruzi*, Kinetoplastida, Trypanosomatidae), and its transmission by insect vectors (Hemiptera, Reduviidae, Triatominae). The first description of a trypanosome (from a Swiss trout), published by Valentin (1841), paved the way for the descriptions of African (Bruce, 1895; Castellani, 1902; Dutton, 1902) and American trypanosomes (Chagas, 1909; Tejera, 1920). The large blood-sucking bugs that were to become notorious as the vectors of Chagas disease had been noted (with some acerbity) by Spanish and Portuguese chroniclers since the 16th century, with the first formal description attributed to De Geer (1773). The trypanosome parasites and their insect vectors have fascinated biologists ever since, with both serving in their different ways as model organisms to help understand basic cell biology and infection dynamics (e.g., Savino et al., 2002; McNeil and Kirchhausen, 2005) and insect physiology and behaviour (e.g., Wigglesworth, 1972). More recent studies have revealed how and when the trypanosome parasites and their insect vectors may have evolved (e.g., Stevens et al., 2001; Gaunt and Miles, 2000, 2002; Miles et al., 2009; Schofield and Galvão, 2009), and how their attributes continue to change (e.g., Gaunt et al., 2003; Tibayrenc, 2003; Dujardin et al., 2009).

But since the work of Carlos Chagas and his notable disciples (Emmanuel Dias, José Pellegrino, Arthur Neiva, Eurico Villela, Belisário Penna, and colleagues), research on Chagas disease took on a more operational urgency, stimulated by progressive recognition of the medical and social impact of the disease throughout Latin America, and the consequent need to help those afflicted with the infection—both by adequate diagnosis and patient care, and by measures to halt further transmission. In turn, these operational needs stimulated a range of epidemiological research initiatives, aimed primarily at assessing the full scale of the problem and so provide adequate justification for the control measures proposed, and also at developing tools to monitor and evaluate progress in their implementation (Dias and Schofield, 1999; Coura and Dias, 2009).

By the early 1960s, the first World Health Organisation (WHO) Expert Committee on Chagas disease 'guessed' that perhaps seven million people were infected with *T. cruzi*, with a further 35 million considered at risk. As data from serological surveys accrued, these estimates were progressively corrected upwards to a peak estimate of 24 million infected and 100 million at risk (Walsh, 1984); the World Bank (1993) ranked Chagas disease as the most severe parasitic disease of the Americas in terms of socioeconomic impact, measured as DALYs—disability-adjusted life years lost. Since then, burden estimates have been reduced in line with a

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doi:10.1016/j.actatropica.2010.03.002

series of multinational control initiatives beginning with the Southern Cone countries in 1991. Current data suggest that ~7.5 million people are infected (OPS, 2006). In parallel with these epidemiological trends, the dreadful and virulent disease seen by Carlos Chagas and colleagues in Brazil, Salvador Mazza and colleagues in Argentina, Rodolpho Tálice and colleagues in Uruguay, and others working during the first half of the 20th century, now seems to be abating. Epidemiological evidence and clinical observations (Dias et al., 2002), supported by experimental studies in murine models (e.g., Bustamante et al., 2002), suggest that the virulence of the infection is declining—possibly due in part to the reduced likelihood of repeated infection as domestic populations of the insect vectors are progressively eliminated.

So what now remain as the key challenges in Chagas disease research? The parasites and vectors will continue to attract academic interest, not least because of their appeal as biological models of evolutionary processes (e.g., Gaunt et al., 2003; Tibayrenc, 2003; Conn and Mirabello, 2007; Dujardin et al., 2009). But of equal concern must be the need to progress further towards the target of eliminating Chagas disease as a major public health problem and impediment to the well-being of millions. Here, we try to assess practical research needs, not simply as questions that could be usefully answered, but as genuine contributions to enhancing the progress of Chagas disease control and prevention.

2. Patient care: secondary prevention of Chagas disease

From a medical standpoint, Chagas disease can be seen as the result of infection with *T. cruzi*—prompting three over-riding concerns: confirmatory diagnosis; specific therapy to remove the infection and supportive treatment to alleviate the disease; and prognosis of disease evolution. Methods, materials and procedures are available for each of these, even if not entirely satisfactory (cf. Prata, 2001; Coura, 2007).

2.1. Diagnosis

Clinical-epidemiological diagnosis, followed by parasitological confirmation for acute cases or serological confirmation for chronic infections, is widely practised and generally satisfactory in the vast majority of cases. For epidemiological studies designed to assess transmission in a particular area, several immunological 'rapid tests' are now available (e.g., Ponce et al., 2005; Schofield et al., 2006) whose results can be confirmed by ELISA (enzyme-linked immunosorbent assay). Again, a wide range of ELISA tests are currently available, both commercial and 'in-house'; a systematic validation programme is being organised by the WHO to provide standardised comparisons of these tests. ELISA tests are also the primary diagnostic tool for individual patient diagnosis, with a range of additional tests available for confirmation if required (e.g., IFI, IHA, RIPA) (WHO, 2002; Luquetti et al., 2009). Inconsistent serological results (e.g., a positive and a negative result from two tests) are infrequent and best judged in the clinical and epidemiological context by the specialist concerned. At present, however, there may be some doubt as to the relevance of such concerns in clinical practice, because a discordant serological result will only involve a chronic infection; in these cases, the benefits of specific therapy have only been confirmed for patients up to 14–15 years old. Resolution of this aspect may become of greater importance in the near future, as several clinicians now advocate specific treatment for asymptomatic chronic cases up to 55–60 years old (e.g., Viotti et al., 2006). Research aimed at improving diagnosis should focus on developing better serological tests (e.g., Cooley et al., 2008) and practical means to directly characterise *T. cruzi* strains in clinical samples (e.g., Freitas et al., 2005; Valadares et al., 2008) (see comments on pathogenesis below).

2.2. Specific treatment

Two drugs are available for the specific treatment of *T. cruzi* infection (Coura and de Castro, 2002). Nifurtimox, a nitrofuran developed by Bayer in 1967 and marketed as Lampit®, acts by reduction of the nitro group to give nitro-anions that then react with molecular oxygen to produce toxic superoxide and peroxide radicals. Benznidazole, a nitroimidazol developed by Roche in 1972 and formerly marketed as Rochagan® or Radanil® (but now produced by the Laboratório Farmacêutico do Estado de Pernambuco, Brazil; www.lafepe.pe.gov.br) appears to act differently, producing metabolites that react with macromolecules such as DNA, RNA, proteins, and possibly some lipids. In both cases, the antiparasitic activity is intimately linked with the inherent toxicity of the drug, such that adverse side effects during treatment are quite common, especially amongst older patients. Both drugs can induce malaise, headaches, and loss of concentration; Nifurtimox frequently causes loss of appetite, weight-loss and, in some cases, anorexia, while Benznidazole is more frequently associated with allergic dermatitis and sometimes peripheral neuritis. In a few cases, the reactions can be severe enough to require suspension of treatment, although intolerance to one drug is rarely associated with intolerance to the other.

Research towards improved therapy thus seems an important goal, and several new compounds are currently in development for the treatment of *T. cruzi* infections. These include sterol inhibitors such as Posaconazol and Ravuconazol, originally developed as fungicides; cysteine-protease ('cruzipain') inhibitors; trypanothione inhibitors; and pyrophosphate inhibitors (see Urbina and Docampo, 2003). However, none of them is expected to become available for public health use for several years (cf. Ribeiro et al., 2009). The problem is not the lack of candidate compounds (e.g., McKerrow et al., 2009), but rather the costs of development, testing, and registration of such new molecules. Even optimists speak of 5–10 years development at costs of several hundred thousand US dollars, and this for a potential market of possibly no more than 30,000 treatments per year – and declining as transmission control continues to reduce the number of newly infected people. As a result, the end-user cost of these potential new treatments is expected to be very high; estimates of up to US$ 3000 per treatment course of Posaconazol have been suggested. For the short term at least, research towards straightforward improvements in available therapies – e.g., improved presentations (including paediatric formulations) of Nifurtimox and Benznidazole, or combined therapies – may have greater impact (cf. Coura, 2009).

Evidence for patient benefit from specific treatment of acute cases seems uncontroversial, with cure rates well in excess of 70%—especially if treatment is started quickly after the initial infection. By contrast, the question of specific treatment for chronic infections has been amply debated (e.g., de Andrade et al., 1996). In essence, it was widely held that Chagasic chronic pathology was largely due to an autoimmune response triggered by the parasite during the acute phase; persistent tissue infection by *T. cruzi* would thus have little or no role in the development of chronic lesions. Specific therapy in the chronic phase would then be of little relevance since, in a sense, the damage had already been done. This theory, combined with observations of serious side effects of the available drugs, tended to inhibit the idea of specific treatment of chronic infections. This view is now being challenged both by a different model of pathogenesis (with pathology attributed mainly to progressive tissue destruction by parasites; e.g., Tarleton and Zhang, 1999; Tarleton, 2001) and by results of cohort studies of chronic infections followed up for several years after treatment

Table 1
Trypanosoma cruzi Discrete Typing Units (DTUs): geographical distribution and hosts.

DTU	Distribution records	Hosts (genera in parentheses)	Notes
TcI	USA, Mexico, Central America, Venezuela, Colombia, French Guiana, Ecuador, Peru, Bolivia, Brazil, Chile, Argentina	Humans (*Homo*), marsupials (*Caluromys*, *Didelphis*, *Gracilianus*, *Marmosa*, *Metachirus*, *Monodelphis*, *Philander*), rodents (*Akodon*, *Cavia*, *Clyomys*, *Coendou*, *Dasyprocta*, *Echimys*, *Holochilus*, *Nectomys*, *Octodon*, *Oecomys*, *Oligoryzomys*, *Oryzomys*, *Phyllotis*, *Proechimys*, *Rattus*, *Sciurus*, *Thrichomys*, *Tylomys*), monkeys (*Aotus*, *Callicebus*, *Callithrix*, *Cebuella*, *Cebus*, *Chiropotes*, *Saguinus*, *Saimiri*), bats (*Carollia*, *Molossus*, *Thyroptera*), sloths (*Bradypus*), armadillos (*Dasypus*), anteaters (*Cyclopes*), carnivores (*Canis*, *Potos*, *Procyon*), triatomines (*Mepraia*, *Panstrongylus*, *Rhodnius*, *Triatoma*)	Formerly TcI. Widely distributed; dominant north of and within Amazonia, where it causes Chagas disease (CD), and common in sylvatic cycles throughout the Americas. A newly described genotype from Brazilian *Myotis* and *Noctilio* bats is more closely related to TcI than to any other DTU
TcII	Colombia, Peru, Brazil, Bolivia, Paraguay, Chile	Humans (*Homo*), marsupials (*Didelphis*, *Philander*), armadillos (*Euphractus*), carnivores (*Felis*), triatomines (*Panstrongylus*, *Triatoma*)	Formerly TcIIb. The main cause of human CD in Brazil
TcIII	Colombia, Venezuela, Peru, Brazil, Bolivia, Paraguay, Argentina	Humans (*Homo*), marsupials (*Monodelphis*, *Philander*), rodents (*Oryzomys*, *Oxymycterus*, *Proechimys*), carnivores (*Canis*, *Conepatus*), armadillos (*Chaetophractus*, *Dasypus*, *Euphractus*), triatomines (*Panstrongylus*, *Rhodnius*, *Triatoma*)	Formerly TcIIc
TcIV	USA, Colombia, Venezuela, Ecuador, Bolivia, Brazil	Humans (*Homo*), marsupials (*Didelphis*, *Monodelphis*), rodents (*Dasyprocta*, *Proechimys*, *Rattus*, *Sciurus*), carnivores (*Canis*, *Nasua*, *Procyon*, *Mephitis*), monkeys (*Aotus*, *Cebus*, *Saguinus*, *Saimiri*), armadillos (*Dasypus*), bats (*Molossus*), triatomines (*Panstrongylus*, *Rhodnius*, *Triatoma*)	Formerly TcIIa. Causes CD in Venezuela, Ecuador, and Amazonian Brazil. North and South American strains moderately divergent
TcV	Ecuador, Peru, Brazil, Bolivia, Paraguay, Chile, Argentina	Humans (*Homo*), rodents (*Cavia*, *Octodon*, *Octodontomys*), armadillos (*Dasypus*, *Euphractus*), carnivores (*Nasua*), triatomines (*Triatoma*, *Rhodnius*, *Eratyrus*)	Formerly TcIId (possibly a hybrid). Causes CD in the Southern Cone and probably in Ecuador
TcVI	Colombia, Brazil, Bolivia, Paraguay, Chile, Argentina	Humans (*Homo*), marsupials (*Didelphis*), carnivores (*Canis*, *Felis*), triatomines (*Triatoma*)	Formerly TcIIe (possibly a hybrid). Causes CD in the Southern Cone

Sources: Chapman et al. (1984), Travi et al. (1994), Brenière et al. (1995), Murta et al. (1998), Barnabé et al. (2000), Diosque et al. (2003), Yeo et al. (2005), Lisboa et al. (2006), Cardinal et al. (2008), Roellig et al. (2008), Zafra et al. (2008), Llewellyn et al. (2009a,b), Marcili et al. (2009a,b), Miles et al. (2009), Zingales et al. (2009), Sturm and Campbell (2010).

(e.g., Viotti et al., 2006). A large trial (named BENEFIT) is currently ongoing to determine the long-term effects of aetiological treatment in chronically infected patients (Marin-Neto et al., 2009).

2.3. Pathogenesis and the genetic diversity of parasites and hosts

Improved understanding of Chagas disease pathogenesis is a major research need, influencing both specific and supportive treatment of patients. The question of pathogenesis is also vital to solving the long-standing riddle of patient prognosis. Why is it that some chronic infections appear to remain asymptomatic for life, while a proportion (generally estimated at 20–30%) will develop serious chronic lesions such as cardiopathy and, in some cases, intestinal mega-syndromes? Can these developments be predicted with any confidence? And, are such observations stable over time, or are there real changes in parasite virulence (and, if so, to what are they attributable)?

Since the pioneering studies of MA Miles and colleagues (e.g., Miles et al., 1977, 1978) it has been established that *T. cruzi* is far from being a homogeneous parasite. In 1999, a consensus classification recognised two broad lineages, *T. cruzi* I (TcI) and *T. cruzi* II (TcII) (Anon, 1999); later on, TcII was subdivided into six biochemically and genetically defined forms (Brisse et al., 2000, 2001; Miles et al., 2009). In August 2009, an expert committee extensively revised *T. cruzi* classification and nomenclature (Zingales et al., 2009). By consensus, it was decided that all known *T. cruzi* lineages and strains should be classified into six major Discrete Typing Units (DTUs) named TcI–VI (Table 1). TcI is widespread throughout the Americas and appears to be an ancestral lineage primitively associated with marsupials (Llewellyn et al., 2009a; Sturm and Campbell, 2010); the genetic diversity within TcI and its possible ecological and epidemiological correlates are a focus of recent interest (e.g., Herrera et al., 2007, 2009; O'Connor et al., 2007; Falla et al., 2009). A newly described *T. cruzi* lineage from bats is more closely related to TcI than to any other DTU (Marcili et al., 2009a). TcII also seems to be an ancient form, but its host associations are yet to be satisfactorily ascertained (Miles et al., 2009). TcIII and TcIV might represent old TcI/TcII hybrids, while TcV and TcVI might have originated from back-hybridisation between TcI/TcII hybrids and a parental TcII form (Sturm and Campbell, 2010). The striking diversity of *T. cruzi* strains and lineages may have developed as ancestral forms were progressively vectored into new hosts, with recombination and genetic exchange also contributing new variants (Schofield, 2000; Yeo et al., 2005; El-Sayed et al., 2005; Freitas et al., 2006). In humans, parasites of any lineage can be associated with Chagasic cardiopathy, but it seems that intestinal mega-syndromes are rarely–if ever–associated with infection by TcI (Miles et al., 2003; Tibayrenc, 2003). It also seems that TcI tends to be a less virulent form of the parasite, particularly in chronic infections, and it is claimed by some clinicians to be somewhat easier to treat. All these points are coherent and perhaps begin to show some relationship between parasite genetics and disease evolution and prognosis (e.g., Tibayrenc, 2003; Campbell et al., 2004; Macedo et al., 2004), but they merit confirmation and leave much to be explained about why some infections develop to clinical disease but others do not (Prata, 2001). The possible roles of reinfection (Bustamante et al., 2002, 2007) and multi-strain infection (Tibayrenc, 2003) in promoting pathology remain to be more fully explored—could it be,

Table 2
General bibliometrics of publications on triatomines and triatomine control in three major databases.

Database	Period	Search terms[a]		Proportion[b]
		Triatominae	Control	
PubMed	1947–2008	1890	192	0.102
ISI	1963–2008	933	203	0.218
Scopus	1963–2008	1907	280	0.147

[a] See text for details on search terms used in each database query.
[b] Calculated by dividing values in the "Control" column by those in the "Triatominae" column.

for instance, that the 20–30% of infected patients that develop serious chronic lesions corresponds to those that have remained exposed to repeated infection? In addition, the question of host background remains largely unexplored. Host genetics may play a more important role than previously thought, such that some genotypes predispose to distinct clinical disease outcomes (Macedo et al., 2004).

3. Biology and control of Triatominae: primary prevention of Chagas disease

From an epidemiological standpoint, Chagas disease can be seen as a major public health problem for the Americas (Hotez et al., 2008). It mainly affects the poorer rural communities, where substandard housing favours the establishment of domestic and peridomestic vector populations. Vector-mediated transmission is the main source of new cases, and vector control is therefore the cornerstone of Chagas disease primary prevention, together with serological screening of blood and organ donations. In this section, we emphasise the achievements and future challenges of scientific research related to vector control. We first ask what fraction of published papers dealing with triatomines directly relates to the control of Chagas disease. Then we provide an overview of major advances on our understanding of the systematics, ecology, and evolution of the Triatominae, and identify some of the main challenges for effectively translating scientific advances into enhanced control strategies.

3.1. An outline of the production of scientific knowledge on the Triatominae

We explored whether published knowledge on the biology of triatomines has patent links with the prevention of Chagas disease. We retrieved data from PubMed (www.ncbi.nlm.nih.gov/pubmed), ISI Web of Knowledge (www.isiknowledge.com), and Scopus (www.scopus.com). We first performed a general search using the major query term "Triatomin*". We then narrowed down our search using an additive term ("Triatomin* AND control") in the queries. The proportion of papers bearing the term "control" was calculated for each year from 1970 to 2009. Thus, three metrics were considered: number of papers on triatomines, number of papers on disease/vector control, and the proportion of the latter. The time trends of this proportion metric were explored using linear regression, including only years with ≥10 published papers on triatomines. These exploratory analyses were conducted separately for each database.

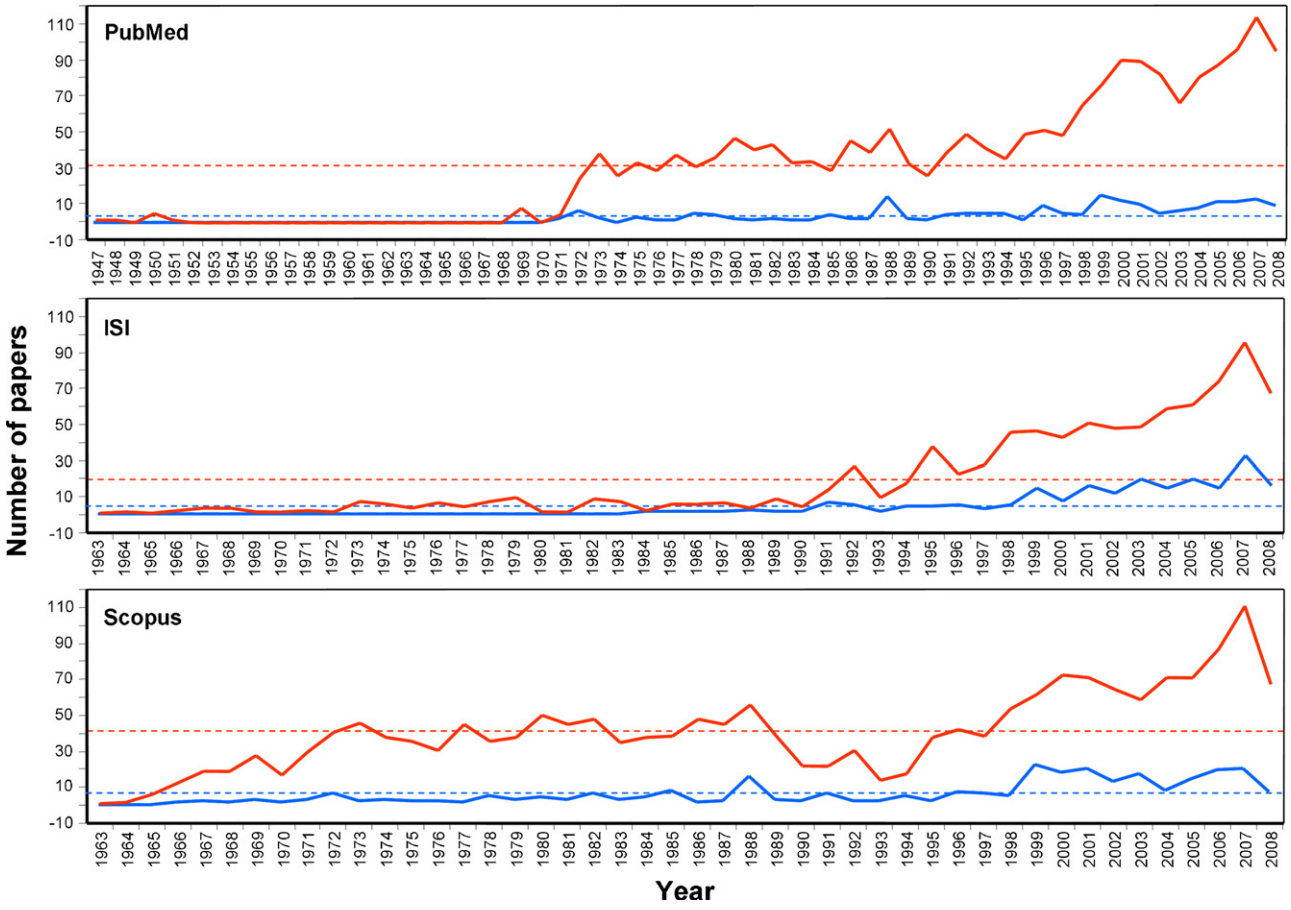

Fig. 1. Number of publications on Triatominae (red lines) and on the control of triatomines (blue lines) retrieved from three major bibliographic databases (PubMed, ISI, and Scopus). See text for details on query terms. Dashed lines indicate overall mean values across years. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

Table 3
Decadal bibliometric trends in the proportion of publications on triatomines that included the term "control" as a major keyword.

Period	Database		
	PubMed	ISI	Scopus
Pre-1970	0	0	0.08
1970–1979	0.097	0	0.078
1980–1989	0.079	0.125	0.113
1990–1999	0.113	0.188	0.173
2000–2009	0.108	0.273	0.193

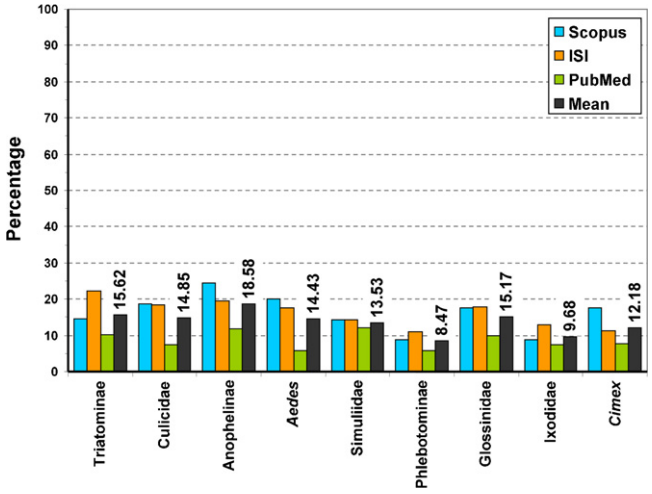

Fig. 2. Overall percentage of publications on several haematophagous arthropod groups that included the term "control" as a major keyword across three major bibliographic databases (from left to right: Scopus, ISI, PubMed; searches up to 15 June 2009). We scaled the percentage axis to 100% to provide an unbiased glimpse of the actual values.

General results (excluding the incomplete 2009 count data) are presented in Table 2; surprisingly, only between 10.2% (PubMed) and 21.8% (ISI) of publications satisfied our "Triatomin* AND control" query. Fig. 1 presents the annual number of publications retrieved in each database search. A general growing trend is evident in all cases, as is the relatively small contribution of papers bearing the term "control". The decadal time trends of this small fraction can be seen in Table 3. While PubMed-indexed publications maintain proportions of about 10%, both ISI and Scopus data show a steadily rising trend up to 19–27% in the current decade. Table 4 shows annual summary statistics for the three bibliographic metrics we analysed.

Linear regression confirmed the general trend of slow growth for the proportion of papers on Triatominae that also deal with disease control. This trend was not statistically significant for PubMed papers (as measured for 38 years with ≥10 papers, $R^2 = 0.025$), but involved a mean annual increase of 0.3% for Scopus papers (44 years, $R^2 = 0.25$; $P = 0.0006$) and of 0.8% for ISI papers (20 years, $R^2 = 0.27$; $P = 0.018$).

Fig. 2 compares the overall percentage of papers dealing generally with several major arthropod vector groups and specifically with their control. The mean percentage across groups is just 13.5%, with a maximum of 18.6% for the Anophelinae and a minimum of 8.5% for the Phlebotominae; the Triatominae come in second place with a 15.6% average across databases.

Assessing these trends in detail is out of the scope of the present paper; we may advance, however, that examination of >850 titles and abstracts of triatomine-related publications revealed that only 13% actually describe control/surveillance interventions (FA-F, unpublished). These results suggest that, for triatomines and for other major vectors, research efforts tend to have little bearing on disease control, which is arguably at odds with the expectations of most funding agencies.

3.2. Systematics of the Triatominae

3.2.1. Relationships with other reduviids and inter-tribe affinities

The Triatominae are closely related to other predator reduviids; in fact, many of them could be viewed as predator bugs that can feed on vertebrate blood, invertebrate haemolymph, or both (Barrett, 1991). Even if largely irrelevant for disease vector control, the question of how many times has haematophagy evolved in the Reduviidae remains unanswered, fuelling debate on whether the Triatominae represent a monophyletic group (e.g., Schaefer, 2003; Schofield and Galvão, 2009). This will only be resolved by suitable phylogenetic analyses, for which the two main issues are taxon sampling and character sampling. To date, studies on this topic suffer from either insufficient taxon coverage, poor character coverage, or both.

The Rhodniini seem to be relatively distant relatives of the Triatomini (Gaunt and Miles, 2002; de Paula et al., 2005), but the phylogenetic affinities of the Bolboderini, Cavernicolini, and Alberproseniini remain to be ascertained. In general, the taxon and marker coverage considerations above apply here too, because monophyly of the subfamily is dubious.

3.2.2. Intra-tribe relationships

There are two main lineages within the Rhodniini—the 'pictipes' lineage including the trans-Andean species, and the 'robustus' lineage including *Psammolestes* (Abad-Franch et al., 2009). Molecular phylogeographical analyses revealed several cryptic clades within the *prolixus-robustus* group (Monteiro et al., 2003), and provided indications that cryptic diversification probably also occurred in other morphospecies (Abad-Franch et al., 2009).

The two major genera within the Triatomini, *Triatoma* and *Panstrongylus*, are not reciprocally monophyletic (Marcilla et al., 2002). Instead, there seems to be a basic split between north- and south-American species, with most *Panstrongylus* species falling within the northern clade; *Dipetalogaster*, *Eratyrus*, *Paratriatoma*, and *Linshcosteus* also appear to belong to this northern group (Hypša et al., 2002; Marcilla et al., 2001, 2002; Schofield and Galvão, 2009). The systematics of the Triatomini are therefore still unclear; defining genera on the basis of monophyly, not phenotypic characters prone to homoplasy or fast divergence (e.g., Patterson et al., 2009), would help, but practical needs must also be considered (cf. Schofield and Galvão, 2009). The relationships of most *Panstrongylus* species and of poorly known *Triatoma* species groups, such as the *Triatoma dispar* complex, are yet to be established (Schofield and Galvão, 2009).

Table 4
Bibliometrics of publications on triatomines and triatomine control in three major databases: summary statistics of annual data (up to May 2009).

Database	Triatominae			Control			Proportion		
	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
PubMed	31.4	31.5	0–114	3.2	1	0–15	0.07	0.04	0–0.5
ISI	20.3	8	1–96	4.4	1	0–32	0.13	0.11	0–0.5
Scopus	41.5	39	1–111	6.1	3	0–22	0.12	0.1	0–0.4

3.2.3. Intra-specific relationships: subspecies and populations

Intra-specific phenotypic diversity can be striking in triatomines; phenotype variants have been characterised in several species of the main genera, including *T. infestans*, *T. dimidiata*, *R. ecuadoriensis* or *P. geniculatus*. Some formerly recognised species or subspecies are probably just phenotypic variants, while specific or subspecific status suitably depicts biological variability for other taxa (e.g., Monteiro et al., 1999, 2004; Bargues et al., 2008; Mas-Coma and Bargues, 2009). Another field of interest has been the characterisation of geographic populations, with studies often stimulated by interest in understanding the dispersal history of important disease vectors (e.g., Dujardin et al., 1998; Bargues et al., 2006, 2008; Piccinali et al., 2009). The geographical coverage of sampling is one common difficulty in these studies, and the results have been diverse. In some cases, geographically distant populations were barely distinguishable (e.g., in *R. pictipes* or *P. megistus*); in others, some populations were seen to represent different species or subspecies (e.g., in *T. dimidiata*, *R. robustus* or *T. brasiliensis*) (Barbosa et al., 2003; Monteiro et al., 2003, 2004; Mas-Coma and Bargues, 2009; Pavan, 2009). The role of macro- and micro-evolutionary processes in shaping these contrasting patterns of diversity outlines a fertile area of research that can find application in the context of regional- and landscape-scale surveillance systems (e.g., Abad-Franch et al., 2009), which can be further enhanced by fast-evolving Remote Sensing-Geographical Information System technologies (e.g., Gorla, 2002; Kitron et al., 2006).

Generally speaking, the study of intra-specific variation in Triatominae would benefit from a more consistent theoretical framework so that comparisons are performed in order to test some meaningful hypothesis. Perhaps the most important question here is *why* only one or a few populations within a given recent, monophyletic lineage (i.e., a species or a species group) have the capacity to successfully colonise human environments (Abad-Franch and Monteiro, 2007). An additional issue is whether this capacity is likely to be inherited by all the descendants of the ancestor within which it first arose, or, conversely, likely to be lost in most of them. Another, more basic question is why ecological/geographic differences seem to promote speciation in some groups but not in others (Dujardin et al., 2009). New technologies, such as high-throughput DNA sequencing (e.g., Rokas and Abbott, 2009) and transcriptome analysis (e.g., Toth et al., 2007), could massively contribute to advancing our understanding of this kind of problems.

3.3. Ecology and evolution of the Triatominae

3.3.1. The ecology of vector-borne Chagas disease

Disease transmission by triatomine bugs is an ecological process: it depends on how different organisms (trypanosomes, triatomines, and their hosts including humans plus their ecotopes, pathogens, predators, and prey) interact with each other in an ecological context. Because transmission of *T. cruzi* to humans requires close contact between infected triatomines and people, and this usually happens within human residences, household infestation by vectors is widely recognised as the main risk factor for human Chagas disease transmission and the focus of most research efforts. In terms of both entomological surveillance and academic enquiry, the key question therefore involves understanding of which silvatic populations of Triatominae are most likely to successfully colonise a human dwelling. Clearly, adults of many different species and populations may enter a house, but relatively few succeed in establishing a viable new colony.

Three major ecological processes determine the invasion and colonisation of households by triatomines. First, the vectors must leave their natural ecotopes to reach a domestic or peridomestic structure (immigration); second, one or more females must give birth to viable offspring so that a colony is founded (establishment); finally, the colony must remain viable until the reproduction cycle is complete, then grow (colonisation). We will briefly examine all these steps.

Immigration: We do not know why triatomines migrate from their silvatic or peridomestic ecotopes into houses—or indeed to any new habitat. Passive accidental carriage by vertebrates is apparent for some domestic-peridomestic bug populations (e.g., Schofield et al., 2009), and there is evidence that active, flight-mediated dispersal can be triggered by starvation (e.g., Lehane et al., 1992), probably when a silvatic host dies or leaves its nest or burrow. However, the relative importance of passive and active mechanisms in triatomine dispersal is far from clear. Our understanding of how actively dispersing bugs orientate – either to a host or to a new habitat – is also fragmentary. It is often assumed that house lights, warmth, and odours may play a role. But electric light is clearly not involved in bug movement between silvatic habitats, and although laboratory studies have indicated that radiant heat, ammonia, carbon dioxide, fatty acids, or pheromones may act as orientation cues (cf. Lazzari and Lorenzo, 2009), we have no unequivocal evidence of their roles in host and habitat finding under natural conditions. From a practical viewpoint, it is clear that whenever suitable ecotopes (such as palms or rocky outcrops) occur near houses and are used as refuges by triatomines and *T. cruzi* reservoirs (e.g., marsupials or rodents), then the chances that infected adult bugs fly into houses will rise–and particularly when bug density is high (e.g., Lehane et al., 1992; Ceballos et al., 2005). This may result in *T. cruzi* transmission either by direct contact between adventitious adult bugs and humans or by the contamination of food or food-processing equipment (Aguilar et al., 2007). In a few cases, triatomines may establish a colony within the household.

Establishment: An immigrating female bug that reaches a household may lay fertilised eggs there. Whether or not colonisation ensues depends on (1) adequate substrate for egg-laying, (2) adequate micro-climatic conditions for egg maturation, egg hatching and, later, nymphal ecdysis, and (3) adequate food supply for the offspring. Of course, stochastic mortality will also play a role, and repeated immigration from a primary focus probably favours the establishment of nearby secondary colonies. However, even with repeated immigration only a fraction of households near a focus becomes infested, suggesting that some factors can limit establishment immediately after invasion. Microclimate adaptations have been suggested as one such factor (Abad-Franch and Monteiro, 2007). This could explain, for instance, why in the Orinoco basin both *R. prolixus* and its sister taxon, *R. robustus*, repeatedly invade households, but only the former succeeds in establishing domestic colonies (Fitzpatrick et al., 2008). Further examples are *P. lignarius*, strictly silvatic throughout the Amazon, and the population known as *P. herreri*, which colonises houses in the drier Marañón valley, or geographical populations of *R. ecuadoriensis*, which seem capable of breeding within houses only in seasonally dry areas (Abad-Franch et al., 2001; Cuba Cuba et al., 2002). More generally, the well-known fact that triatomines successfully infest houses in 'open' (i.e., *drier*) ecoregions (*sensu* Olson et al., 2001), but only very rarely in humid forest ecoregions, matches the predictions of this 'microclimate adaptation hypothesis', which merits further research (Abad-Franch and Monteiro, 2007)

Another factor that appears to merit further investigation is the possible role of salivary interaction with the domestic hosts. In some cases, silvatic bugs that enter houses provoke serious bite reactions in their new domestic hosts–sometimes appearing as immediate toxic reactions (e.g., Valente et al., 1998) but more usually suggestive of an acquired immune reaction (e.g., Ramírez-Sierra et al., 2010). If such reactions were to significantly impede further blood-feeding on that host, then this may help explain why some bugs fail to establish new domestic colonies–bugs whose

saliva provoked little or no reaction, or that were able to vary their salivary antigen presentation, would more successfully colonise the habitat of a new host, compared to bugs whose saliva provoked significant reactions.

Colonisation: Household colonisation by triatomines depends on ecotope structural traits, resource supply, and control activities; all these factors have a bearing on major demographic parameters of the colony by influencing birth or mortality rates. The structural traits of peridomestic and domestic constructions have been shown to be associated with the odds of infestation. Structures that mimic the natural ecotopes of a particular species are more likely to become infested by that species (Lent and Wygodzinsky, 1979; Barrett, 1991). Thus, palm-frond or mud-and-stick roofs favour infestation by *R. prolixus* and *T. infestans*, respectively; mud walls, particularly when cracked and/or unplastered, have similar effects, and earthen floors can favour ground-dwelling species such as *T. dimidiata*. In the peridomestic area, wood, brick or tile piles, wooden fences and henhouses, or stone corral walls have all been linked to higher probability of infestation by different triatomine species. Conversely, structure traits can be protective against infestation: plastered brick walls, tin roofs or wire fences can reduce the odds of infestation. Housing improvements along these lines can sharply and durably reduce Chagas disease transmission risk. Measuring the effects of the Brazilian National Housing Programme (BNHP; Law 11.997, July 2009) on domestic triatomine populations could provide a convincing assessment of this benefit. The BNHP aims at building one million houses for low-income families, with committed investments of over 287 million US$ for rural houses and over 1.4 billion US$ for urban houses.

The key role of nutritional resources in household infestation by triatomines is well established (e.g., Cohen and Gürtler, 2001). These resources are represented by humans and their domestic animals, with a possible role for synanthropic opportunistic vertebrates such as rodents, marsupials, and perhaps some birds. The importance of each food source depends on its biomass and on the feeding habits of each bug species. Eclectic bug species that can make use of different resources, such as birds and mammals, depending on availability, tend to be more dangerous. Some of the structural traits discussed above can favour infestation also by providing shelter to opportunistic animals such as rats, mice, opossums, or birds.

Control interventions aim at increasing bug mortality. Whether institutional (implemented by vector control services) or spontaneous (applied by householders), the expected effect is protective. They should decrease the likelihood of colonisation and, if insecticides have a repellent effect, also of invasion of houses by triatomine bugs. The effectiveness of institutional chemical control (see Section 3.4. below) has been demonstrated by impressively successful control programmes, but unsupervised spontaneous interventions seem to be of limited value (Gürtler et al., 2007). Alternative strategies, such as insecticide-impregnated bednets, curtains, or window screens, are yet to be thoroughly tested (e.g., Kroeger et al., 1999; Herber and Kroeger, 2003; Barbu et al., 2009).

3.3.2. Improving ecological research on Triatominae

The determinants of invasion-colonisation of households by triatomine bugs are only superficially understood, and explanations of the underlying ecological drivers remain largely speculative. Here we briefly present several research tools that could significantly improve our understanding of these key aspects of triatomine ecology.

Study design I: *false absences*. Vector occurrence is the primary variable of interest in most studies on triatomines. However, triatomines searches often yield false-negative results—no bugs detected in an infested household or silvatic ecotope. Better detection techniques are more expensive (in any measure of effort) and tend to be impractical. When the aim of a survey is ascertaining infestation for control purposes, this extra effort is important. But when the aim is to study bug ecology, imperfect detection can be dealt with by explicitly incorporating detection failure into the analyses (e.g., MacKenzie et al., 2002). This approach requires repeated sampling of every unit of analysis (usually a household or a given kind of silvatic ecotope) within a short time period, with the results of each sampling event recorded separately. Assuming that the population is 'closed' (i.e., immigration and local extinction during the sampling period can be ruled out), this information is then used to estimate detection probabilities for the particular study setting and to simultaneously derive an unbiased estimate of the parameter of interest–infestation rate. A recent application of this approach to research on triatomine ecology is Abad-Franch et al. (2010)

Study design II: *sampling rare, clustered populations*. Triatomine populations tend to be small and non-randomly distributed in space. However, most sampling designs assume they are large and evenly distributed, and this threatens both sampling efficiency and the inferences drawn from study results. Sampling theory provides several ways out these problems. Adaptive sampling allows for denser sampling of rare, clustered organisms while retaining the probabilistic properties that will allow meaningful extrapolations (e.g., Brown et al., 2008; Conroy et al., 2008). The initial sample can be stratified following some importance measure so that units within higher-interest sub-areas have a higher probability of being sampled (see Thompson, 2004)

Data analysis. Bivariate null hypothesis-testing and stepwise multivariate regression analysis are often used in triatomine ecology research. Null hypothesis-testing tells us how unlikely the observed (or more extreme) data are, given the presumably false null hypothesis (i.e., the *P*-value); what we want to know, however, is how likely our *hypotheses* are, given the observed data (Cohen, 1994). In multivariate regression analyses, stepwise techniques lead to biased parameter estimates and to overreliance on a 'minimal adequate model' for prediction (Whittingham et al., 2006). These serious problems are suitably overcome by multimodel (Burnham and Anderson, 2002, 2004) or Bayesian inference (e.g., Ellison, 1996), which should therefore be preferred.

These methodological developments allow researchers to derive more reliable estimates of vector population parameters. This information may in turn be used to design sounder management schemes, including adaptive strategies (e.g., Walters and Holling, 1990). When alternative control approaches are tested, adaptive management helps spot the best one for the situation at hand; when a local situation is managed over time, it helps managers adapt their strategies to changing circumstances—the learning by doing approach (Walters and Holling, 1990; Shea et al., 2002).

3.3.3. The (possible) evolutionary consequences of domestication

One long-standing question on triatomine biology is whether 'domestication' somehow modified the evolutionary fate of strongly synanthropic triatomine species; that is, is there any evidence that 'domestic' species or populations differ significantly from their 'silvatic' ancestors or closest relatives in terms of their genetic makeup? The overall answer to this question seems to be 'no' (Barrett, 1991), which is what should be expected within the timeframe of human presence in the Americas and in the absence of strong directional selection (cf. Abad-Franch and Monteiro, 2007). The only exception to this statement (in terms of selection pressure) could be represented by insecticide-resistant domestic populations of *R. prolixus* or *T. infestans*. To our knowledge, cladogenesis related to the establishment of domestic populations has never been identified; without exception, strictly domestic populations are shown to be part of a larger gene pool including silvatic relatives (e.g.,

Bargues et al., 2006, 2008; Fitzpatrick et al., 2008). On the other hand, sharply different allele/gene frequencies can result from the founder effects and subsequent genetic drift involved in the colonisation of human households by a limited subset of the silvatic gene pool (cf. Schofield et al., 1999). This could eventually lead to speciation only if (a) domestic populations remained out of the natural range of the species and with no re-introductions or (b) large genetic rearrangements promoted the rapid establishment of reproductive barriers between silvatic and domestic populations (Dujardin et al., 2009). None of these seems very likely.

3.4. Elimination of domestic and peridomestic Triatominae

A primary goal in the control of Chagas disease is the elimination of domestic triatomines. They not only transmit *T. cruzi*, but are also unpleasant and can contribute significantly to chronic iron-deficiency anaemia (Schofield, 1981). Since the advent of synthetic insecticides, especially pyrethroids, it has been demonstrated that elimination of any domestic population of Triatominae is technically and economically feasible (e.g., Dias et al., 2002; Gürtler et al., 2007; Vázquez-Prokopec et al., 2009). The problem then is how to maintain treated households free of bugs in the long run—the so-called 'reinvasion problem'.

The technical response, widely promoted in the 1980s, was to modify insecticide formulations to give a prolonged residual effect, which would offer lasting protection against any newly invading bugs. While this approach did produce some improvements, especially through the use of wettable powder, suspension concentrate and microencapsulated formulations, no treatment could protect a house forever. The strategic response to the reinvasion problem became thus focused on extended geographic coverage, designed to eliminate source populations of reinvading bugs. This was a key component of the rationale behind the multinational control initiatives; for instance, the Southern Cone Initiative was designed to eliminate all *T. infestans* domestic populations from its entire range in South America (Schofield and Dias, 1991). This approach yielded excellent results in places where *T. infestans* was exclusively domestic, and the species was eliminated from most of its former (pre-control) range (Dias et al., 2002). However, problems arose in areas where extensive peridomestic populations remained as sources for reinvasion of treated houses–especially in the Chaco of northwestern Argentina, Bolivia, and Paraguay. Insecticide formulations that were highly successful indoors proved much less useful in peridomestic habitats, whose extent and physical complexity (e.g., of brushwood corrals) led to poor insecticidal coverage and penetration. In addition, superficial insecticide deposits suffer greater insolation, leading to breakdown of the active components, and often become rapidly covered by dust and animal excreta. Similar problems have been apparent for the control of peridomestic populations of *T. brasiliensis* in north-eastern Brazil, of *R. prolixus* in Venezuela, and of *T. dimidiata* and other members of the *T. phyllosoma* complex in parts of Central America and Mexico.

As before, there is a technical response to these problems, involving alternative approaches to the control of peridomestic Triatominae. Some authors report better results using a double-dose spray of pyrethroids (e.g., Cecere et al., 2006) or slow-release polymer formulations (e.g., Dias and Jemio, 2008; Amelotti et al., 2009a). Others prefer physical modifications to the peridomestic habitat–for example, replacing piled brushwood in goat corrals in the Argentine Chaco can greatly reduce the habitat available for peridomestic *T. infestans* and *T. guasayana* (e.g., Gorla et al., 2007). Other approaches involve the concept of 'xenointoxication'–treating domestic animals with a pour-on or powder formulation of insecticide, in order to kill any bugs that may attempt to feed on them (e.g., Amelotti et al., 2009b). Insecticide-impregnated dog collars have been used for a similar purpose (Reithinger et al., 2005, 2006) and it seems likely that further technical developments will lead to improved ways to control peridomestic Triatominae.This will be critical for *T. infestans* populations in the greater Chaco, where domestic *T. cruzi* transmission soon re-emerges after reinfestation of peridomestic structures (e.g., Gürtler et al., 2007) and where other problems, such as focal resistance to insecticides (e.g., Picollo et al., 2005), further threaten long-term disease control. However, a strategic response can also be considered for the many triatomine species that colonise peridomiciles across their native ranges without re-establishing endemic transmission. Ignoring their possible effects on animal productivity (a barely examined but potentially important research question), it could be argued that the relevance of these peridomestic triatomine populations rests primarily with their potential to reinvade houses. In this sense they are more similar to silvatic populations, which can also act as reinfestation sources. When seen in this light, the strategy changes. It is both impractical and ecologically unacceptable to contemplate large-scale interventions against silvatic populations of Triatominae. It is also irrelevant in terms of transmission control. Only by coming into contact with humans – for example by entering a house – does a silvatic bug assume possible epidemiological significance, either by causing direct transmission or by establishing a new domestic colony. But a newly established domestic colony can be eliminated, and a transmission event can be treated. Perhaps peridomestic and silvatic populations of these native, less dangerous species can be considered similarly—focusing on the vectors only when incipient domestic colonisation is apparent, but otherwise stressing diagnosis and treatment of possible new cases of infection (Schofield, in press).

4. Conclusions

In the course of this review, we have focused on research aimed specifically at improving Chagas disease prevention. We examined what has been done and what needs to be done, assessing and discussing potential improvements. Questions of research interest are abundant; still, we have been able to identify relatively few real knowledge gaps that seriously undermine the possibility of controlling Chagas disease. Primary prevention still depends on reducing the frequency of contact between infected triatomine vectors and susceptible humans. Once the infection is established, secondary prevention of chronic heart, digestive, and neurological lesions depends on adequate diagnosis and specific treatment. Diagnosis of infection seems broadly satisfactory, and the primary need is for standardisation–a question currently being addressed by the WHO together with the relevant authorities. While patient treatment is still far from ideal, the real needs are to optimise current regimes, reach international consensus on who should be treated, and ensure adequate access to the treatments that are available. Patient prognosis remains extremely difficult, suggesting an outstanding research need to clearly understand pathogenesis and the factors that could help predict the outcome of chronic infection. Even this question would nonetheless become of lesser relevance if a larger proportion of chronically infected patients were granted access to specific treatment.

It is also clear that the basic approaches to reducing transmission are broadly adequate. Vector-borne transmission can be substantially reduced with available techniques for eliminating domestic vector populations and monitoring reinfestation. But we do question whether or not this available experience is sufficiently utilised. The multinational Chagas disease control initiatives clearly demonstrate what can be achieved. However, while major reductions in apparent incidence and prevalence are encouraging (e.g., OPS, 2006), it is utterly disappointing that active domestic transmis-

sion persists in many areas where control campaigns have not been installed or maintained.

Finally, it is worth underscoring that the profound impact that a modest number of key research findings have had on Chagas disease prevention was often mediated by the direct involvement of practising scientists–rather than politicians or bureaucrats–in the promotion and effecting of control programmes. Thus, even if many technical or academic questions have already been resolved, there is a real need for Chagas disease researchers. Declining burden figures and waning political priority both indicate that the involvement of the scientific community will be crucial in the long run. However, decision-makers will only seriously consider technical recommendations if they are "...perceived [...] as scientifically accurate and legitimate, and if [they] are communicated intelligibly and meaningfully" (Ellison, 1996, p. 1036). Researchers should thus be encouraged to play a greater role in epidemiological monitoring – of both infections and infestations – to provide yet more high-quality data and stimulate additional control interventions (cf. Dujardin and Schofield, 2007).

The insights we have presented underscore the need for sustained scientific activity on Chagas disease prevention; they also suggest that there is still room for improvement: more sharply focused, problem-oriented research should be promoted if we are to meet the ambitious goals set by the World Health Organisation (WHO, 2007) and by the multinational control initiatives.

Acknowledgements

We thank Michael A. Miles and Martin S. Llewellyn for comments on *T. cruzi* genetics and hosts, and Gonçalo Ferraz for helpful discussion on ecological research methods. This work benefited from international collaboration through the ECLAT Network. Funding by the UNICEF/UNDP/World Bank/WHO TDR Special Programme (ID # A20441) and the Fiocruz-FAPEAM and Fiocruz-CNPq agreements (Brazil) is gratefully acknowledged. Input by FA-F and WSS stems from activities within the Research Programme on Infectious Disease Ecology in the Amazon (RP-IDEA) of the Instituto Leônidas e Maria Deane; this paper is, in this sense, contribution number 10 of the RP-IDEA.

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