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REPORT OF A MEETING ON THE FEASIBILITY OF
 ANALYTICAL EPIDEMIOLOGICAL STUDIES ON CHAGAS' DISEASE:
 GUIDELINES FOR A STANDARD PROTOCOL

CONTENTS

	<u>Page</u>
1. PARASITE CHARACTERIZATION	2
1.1 Isolation	2
1.1.1 Acute phase of Chagas' disease	2
1.1.2 Chronic phase of Chagas' disease	2
1.2 Laboratory Preservation and Amplification	2
1.3 Biological and Biochemical Characterization	3
2. DISEASE CLASSIFICATION	3
2.1 Clinical Forms	3
3. EPIDEMIOLOGY	4
4. METHODOLOGY	4
4.1 Selection of Study Area	4
4.2 Data Handling and Processing	4
5. RECOMMENDATIONS	5
5.1 <i>T. cruzi</i> Characterization	5
5.2 Clinical Chagas' Disease	5
5.3 Epidemiology	5
5.4 Final Recommendation	6
6. PARTICIPANTS	6

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Ce rapport exprime les vues collectives d'un groupe international d'experts réuni par le PROGRAMME SPECIAL PNUD/BANQUE MONDIALE/OMS DE RECHERCHE ET DE FORMATION CONCERNANT LES MALADIES TROPICALES (TDR). Il ne représente pas nécessairement les vues du TDR/OMS et, en vue d'une diffusion accélérée, il n'a pas été l'objet d'une mise en forme particulièrement soignée. En outre, les noms géographiques utilisés dans le présent rapport n'impliquent, de la part du TDR ou de l'OMS, aucune prise de position quant au statut juridique de tel ou tel pays, territoire, ville ou zone, ou de ses autorités, ni quant au tracé de ses frontières.

Meeting in Geneva from 16 to 19 July 1985 under the auspices of the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR), the Steering Committee on the Epidemiology and Vector Biology and Control of Chagas' Disease recommended that multicentre analytical epidemiological studies be carried out to detect host/parasite-associated risk factors responsible for the clinical and geographic varieties of Chagas' disease.

The participants agreed that scientists and centres interested in this subject should be convened at a meeting in Belo Horizonte, Brazil, to agree upon a standard protocol for these analytical studies. This meeting was held from 28 February to 2 March 1986, and the following aspects were discussed and agreed upon.

1. PARASITE CHARACTERIZATION

To study T. cruzi characteristics, it is necessary: (1) to isolate the parasite, (2) to preserve it under laboratory conditions and (3) to amplify it for biological and biochemical characterization.

1.1 Isolation

1.1.1 Acute phase of Chagas' disease

Existing parasitological techniques can be used. In some situations it is even possible to cryopreserve the strains directly without using a laboratory host.

1.1.2 Chronic phase of Chagas' disease

Haemoculture and xenodiagnosis are commonly available techniques; better results have been obtained with haemoculture. However, there are indications that early dissection of the Triatoma host (15-20 days after a blood-meal) would allow detection of transient infection with certain T. cruzi strains. Microtechniques (e.g., DNA probes) being developed may become valuable in the near future not only for diagnosis but also for the detection of mixed infections.

1.2 Laboratory Preservation and Amplification

The isolated strains are routinely transferred to mice and/or acellular culture in order to be preserved in the laboratory. A large number of strains produce low parasitaemias in laboratory animals and do not grow well in culture media. Population selection also seems to take place, not only in the process of isolation but also in the preservation and amplification phases, depending on the culture media, the intermediate host species and the vertebrate host species.

Other factors that may under certain circumstances select or even eliminate some populations during the preservation and amplification phases are:

- (a) time (of culture or infection) when transfers are made;
- (b) stage of the parasite's life cycle;
- (c) temperature of the culture media;
- (d) parasite characteristics, such as duplication time.

Cloning should be performed shortly before or after the first isolation, as well as at different times during these two phases.

However, it has been shown that if a standard protocol is used during the isolation and preservation phases, nonclonal strains will retain stable biological characteristics. The most probable explanation is that the strains were originally homogeneous or, if originally heterogeneous, a selection process had taken place; alternatively, the proportion of different populations that constituted the original strain had somehow remained constant. These strains, once established in the laboratory, do not change their behaviour patterns when transferred to different vertebrate or invertebrate hosts.

Standard protocols should be established and followed in isolating, preserving and amplifying T. cruzi strains. Questions as to which and how many triatomine species should be used in xenodiagnosis have to be addressed. Problems such as the different requirements of different triatomine species and the tendency shown by some species to degenerate after a few generations of laboratory breeding have to be taken into consideration. The isolation of the parasites by haemoculture and the cultivation of parasites from positive xenodiagnosis have to be carried out in parallel. Species of laboratory vertebrate hosts other than the mouse (such as the rat) may be used.

1.3 Biological and Biochemical Characterization

The main aspects of the parasite to be studied are:

- (a) virulence (incubation period of infection, parasitaemia, mortality in laboratory animals). There are indications that more virulent strains (acute phase) would induce more severe cardiopathy (chronic phase);
- (b) morphology;
- (c) clearance time (bloodstream);
- (d) pathogenicity;
- (e) tissue tropism;
- (f) histopathology;
- (g) antigenic constitution;
- (h) effects of immune sera;
- (i) cell interaction at different temperatures;
- (j) superficial electrical charges;
- (k) isoenzyme profile;
- (l) schizodeme profile;
- (m) development in the invertebrate host;
- (n) duplication time; epimastigotes and amastigotes in acellular culture media; amastigotes in cellular culture media; epimastigotes in the invertebrate host.

It has been shown that T. cruzi strains can be grouped with respect to certain biological and biochemical characteristics and that strains with similar characteristics are more frequently isolated from a specific endemic area. Although a correlation between these characteristics and the parasite's behaviour pattern (in laboratory animals) has already been established, it has not been possible up to now to associate them with different clinical forms of Chagas' disease.

2. DISEASE CLASSIFICATION

2.1 Clinical Forms

The following classification of the different clinical forms of Chagas' disease should be adopted:

A. Acute phase:

- A.1 without symptoms (with T. cruzi parasitaemia);
- A.2 with symptoms:

- benign form;
- malignant form (with meningoencephalitis and myocardopathy).

B. Chronic phase:

- B.1 subacute form (severe and fatal disease);
- B.2 indeterminate form;
- B.3 cardiac form:

- with ECG alterations (older patients);
- severe (younger patients);

B.4 digestive form:

- oesophagopathy;
- colopathy;
- oesophagopathy plus colopathy;

- B.5 mixed form (cardiac and digestive forms);
- B.6 nervous form.

Most of the criteria to be used in classifying the different clinical forms of Chagas' disease are already well established. However, some basic definitions need further elaboration, e.g., interpretation of ECG tracings, in order to develop a standard clinical protocol to be used in analytical epidemiological studies.

3. EPIDEMIOLOGY

Descriptive studies have already been conducted in virtually all the Latin American countries where the disease is endemic. These studies have produced a great amount of information and have been fundamental in improving current knowledge of Chagas' disease epidemiology. The next step should be the implementation of analytical epidemiological studies to further elucidate the aetiology and the clinical and epidemiological variability of the disease.

4. METHODOLOGY

Since Chagas' disease runs a long, chronic course, a case-control (retrospective) strategy would be the method of choice for analytical epidemiological studies. In some areas, especially those where follow-up clinical studies have been carried out for decades and patients known to be in an acute phase of the disease have been identified and followed up, the historical (nonconcurrent prospective) cohort strategy would be the most suitable.

4.1 Selection of Study Area

A participating area should: (1) be representative of the different clinical forms of Chagas' disease ("polar" area) and (2) possess reliable medical records and administrative facilities.

4.2 Data Handling and Processing

In view of current human and hardware resources, standardization of

(1) essential equipment ("hardware"), such as CPUs, disk-drives, printers and modems and (2) essential programs ("software"), such as operating systems, data-entry, statistical analysis packages, etc., will be required.

Integration of the system should be achieved by (1) initial local processing of data collected at different participating centres and (2) the subsequent transmission of data from peripheral centres to a main centre, where final analysis and integration would be carried out with a mainframe computer.

The results would be fed back to the participating centres in the form of tables, files, data, graphs, etc. Linkage between local and main centres would be ensured by circulating compatible floppy disks with the collected data.

5. RECOMMENDATIONS

5.1 T. cruzi Characterization

It was decided to conduct a pilot project in order to standardize methods for (1) isolating, (2) preserving and (3) amplifying T. cruzi strains. Standardization will minimize the risks of selecting clones of parasite subpopulations. In the isolation phase, simultaneous haemoculture and xenodiagnosis should be used; xenodiagnosis should be performed with three different triatomine species and haemoculture with LIT culture medium. The parasites obtained from positive xenodiagnosis should be directly inoculated into LIT medium. Part of the material (triatomine faeces and positive haemoculture) should be kept in liquid nitrogen.

The following biological characteristics should be studied in the pilot project:

- (a) incubation period of infection in laboratory animals;
- (b) parasitaemia;
- (c) morphology of bloodstream parasites;
- (d) mortality of infected laboratory animals;
- (e) tissue distribution of parasites in infected animals.

The following methods should be used to study the biochemical characteristics of the parasite:

- (a) isozyme profile;
- (b) kDNA analysis.

5.2 Clinical Chagas' Disease

A standard clinical protocol detailing all definitions, procedures and techniques is needed for: (1) uniform classification of all Chagas' disease patients included in the study according to the clinical forms already established; and (2) final comparative analysis of the results obtained at different centres.

5.3 Epidemiology

It was decided that a case-control or historical cohort protocol (depending on the area to be studied) will be developed, addressing such questions as:

- (a) criteria for inclusion in the case or control groups;
- (b) selection of participants;
- (c) sample size;

- (d) identification of pertinent risk factors (environmental, genetic and socioeconomic);
- (e) study implementation;
- (f) data analysis.

5.4 Final Recommendation

Development of the proposed protocols will require one year at least. The groups will meet again, in November 1986, at Caxambú (basic research) and Araxá (clinical and epidemiological research) to evaluate progress and discuss further steps.

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