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In Search of the Vaccine Against Chagas Disease: A Tedious Road of More Than 100 Years

Domínguez-Guillén A[1,2], López-Domínguez J[2,3], Ochoa-Martínez P[2], López-Monteon A[2,4] and Ramos-Ligonio A[2,4]*

[1]Doctorado en Ciencias Biomédicas, Xalapa, Veracruz, México

[2]LADISER, Inmunología y Biología Molecular, Facultad de Ciencias Químicas, Universidad Veracruzana, Orizaba, Veracruz, México

[3]Universidad Politécnica de Huatusco, Ingienería en Biotecnología, Huatusco De Chicuellar, Veracruz, México

[4]Asociación Chagas con Ciencia y Conocimiento A.C. Orizaba, Veracruz, México

***Corresponding author:** Angel Ramos-Ligonio, LADISER, Inmunología y Biología Molecular, Facultad de Ciencias Químicas, Edificio D, Universidad Veracruzana, Orizaba, Veracruz, México

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Abstract

Chagas disease is a neglected and widely distributed parasitic disease in America, caused by *Trypanosoma cruzi* parasites. Currently, there are 6 to 7 million infected people and between 60 to 80 million people remain at risk of infection in endemic areas. Normally the infection does not manifest itself in the acute phase or it does so in a mild and nonspecific way, but several years later infected people suffer from heart or digestive system problems with varying degrees of disability and even death. In the acute stage of the infection, there are treatments with antiparasitic drugs that are effective and that are why it is very important to treat children who are born infected. During the chronic phase, on the other hand, the effectiveness of the treatment has been much debated by experts, and recent multicenter studies carried out throughout Latin America showed that, although drugs eliminate the parasite, they are not effective in preventing the development of the illness. Therefore, it is an urgent need to have new strategies to control the infection and the development of the disease, therefore, the objective of achieving a vaccine that not only prevents primary infection (when the parasite comes into contact with the body) but also controls the progression of the disease in infected people and reverses the damage associated with the infection by that obtaining a vaccine is imperative. This work aims to highlight the efforts, progress and show the different approaches in the development of the vaccine against ChD.

Keywords: *Trypanosoma cruzi*; Vaccine; Neglected tropical disease; Parasite; Trypanosomiasis

Abbreviations

ChD: Chagas disease; T. cruzi: *Trypanosoma cruzi*; NFX: Nifurtimox; BZN: Benznidazole; MASP: Mucin-Associated Surface Proteins; Tc24, Tc52, TcG1, TcG2, TcG4, T. cruzi antigens; TSA-1: Trypomastigote Surface Antigen 1; DTU's: Discrete Typing Units; TcI-TcVI: DTU's of T. cruzi; GPI: Glycosylphosphatidylinositol; IL-12: Interleukin-12; IL-10, Interleukin-10; GM-CSF: Granulocyte and Monocyte Colony Stimulating Factor; TS: Transialidase; ASP2: Amastigote Surface Protein-2; SARS-CoV-2: Type 2 Coronavirus causing Severe Acute Respiratory Syndrome; VLP: Virus-Like -Particles; TLR9: Toll-Like Receptor-9; TGF-β: Transforming Growth Factor Beta; TLR2: Toll-Like Receptor-2; CD40: Cluster of Differentiation 40; CD4: Cluster of Differentiation 4; CD8: Cluster of Differentiation 8; DNA: Deoxyribonucleic Acid; mRNA: Messenger Ribonucleic Acid; cDNA: Complementary Deoxyribonucleic Acid; CpG: Cytosine And Guanine Rich DNA Regions; WHO: World Health Organization

Background

More than 110 years after its discovery, Chagas Disease (ChD) or American Trypanosomiasis is considered by the World Health Organization (WHO) as one of the neglected tropical diseases. This disease affects about 10 million people worldwide [1], most of those affected are poor and marginalized people living in rural areas of developing countries [2]. It is caused by the *Trypanosoma cruzi* parasite that is transmitted mainly by triatomine bites. Skin bite lesions, or permissive mucosal and conjunctival surfaces, are in contact with triatomine feces that contain the parasite in its trypomastigote form, infecting neighboring cells, entering the bloodstream, and leading to systemic complications, such as cardiomyopathies and enteropathies [3,4]. Other forms of transmission of the parasite can be by blood transfusion [5], orally [6], transplacental route from infected mothers [7], by transplantation of infected organs, and accidentally [8]. Recently, several studies suggest that *T. cruzi* can be spread through sexual transmission [9-11]. This pathology can appear in its acute and/or chronic form, which can be symptomatic or asymptomatic, mainly affecting the heart and digestive system and its importance lies in the fact that it produces disability and sudden death in apparently healthy people. It is estimated that each year between 10,000 and 12,500 people die from this disease, with cardiac complications the main cause of these deaths [12]. Initially, ChD was endemic to the American continent, but due to the various forms of transmission and social phenomena such as the migration of infected people, it has spread to continents such as Europe, Asia and Oceania; becoming a major health problem worldwide [13,14]. However, although there is a treatment for this disease, the available drugs have low efficacy and very serious side effects. These drugs are very effective in the acute stage of ChD infection, but less effective in the chronic stage [15]. In addition, many patients do not present symptoms immediately after infection, so the years may go by and they are only diagnosed when they already have heart and/or digestive disorders, and it is at this time that the drugs lose their effectiveness [12,16-18]. Vaccination is the cheapest strategy to prevent infectious diseases and a commercial

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vaccine against ChD is not yet available. Multiple vaccine prototypes have been tested and have shown to be good candidates in terms of protection and with great advantages for their production and application [19-24]. During infection, the immune response that is generated against the parasite is not enough to neutralize and/or eliminate it, this being the main factor in the persistence of the parasite as well as the development of the chronic phase of the disease; where the parasite load and the unbalance of oxidative stress generate the damage in the host tissue [25], therefore, the control of *T. cruzi* by activating the immune system during the acute phase of the disease would prevent the development of the pathology [26]. This work aims to highlight the efforts, progress and show the different approaches in the development of the vaccine against ChD.

Overview of Vaccinology in Chagas disease

Current control measures for protozoal infection rely solely on chemotherapy to improve the disease. Vector control is also helping to reduce the transmission of infections by this type of parasite. To date, no reliable vaccines are available against these infections, and there is also an alarming increase in drug resistance [27]. As is the case of the ChD [28]. Developing a vaccine is a long and complex process that often takes 10-15 years, and involves the combined participation of public and private organizations. Vaccine development and testing follow a standardized set of steps. The early stages are exploratory. Regulation and oversight increase as the candidate vaccine progresses through the process [29,30]. Initially, the development of a vaccine for ChD was slow and cautious due to considerations regarding autoimmunity as a potential cause of pathogenesis [31], however, it has recently been suggested that the persistence of the parasite in the host plays a key role in disease progression and pathogenesis [32,33]. These studies have shown that in animal models control of *T. cruzi* can be achieved during the disease [31] and these advances have increased and accelerated the search for a vaccine as a treatment for ChD [34].

Complete Parasites (Inactivated, Live, or Attenuated)

For various pathogens, one of the most common and traditional strategies in vaccines is the use of inactivated or attenuated pathogens [35], for the case of *T. cruzi* some have been tested with a certain margin of success [36,37], vaccines that used whole parasites to combat ChD have been evaluated in initial studies with mixed results, but these types of vaccines are difficult to implement [38,39].

Subunit Vaccines (Recombinant Proteins)

Many recombinant antigens derived from *T. cruzi* have been generated using various prokaryotic and eukaryotic hosts [21,40,41]. Antigens such as TSA1, Tc24, TcG1, TcG2, TcG4, Tc52, Cruzipain, ASP2, Trans-Sialidase (TS), are examples of genes used for the development of recombinant proteins and that have been used as vaccine candidates [21,42-47]. A key challenge for the development of not only vaccines with recombinant proteins against *T. cruzi* is the activation of $CD8^+$ cells and $CD4^+$ cells of the Th1 subpopulation [48-50]. Therefore, multiple adjuvants have been tried to bias the immune response towards such a cellular response. Among the advantages of this type of vaccine candidate is the easy production, however, some disadvantages such as performance, the formation of inclusion bodies during their purification, the lack of adequate post-translational modifications [51], and contamination with endotoxins due to the use of bacteria for their production are some points to take into account and that must be resolved so that these vaccine candidates have better availability and safety [52,53].

DNA Vaccines

DNA vaccines have been used in mouse and dog models that carry genes encoding *T. cruzi* antigens and have been shown to reduce parasitemia and increase survival rates [42-45]. However, despite the ease of construction and production of vectors, the stability of DNA, the easy production and administration of vaccines, and the ability to enhance the immune response through the co-administration of genes encoding cytokines such as IL-12, GM-CSF, or costimulation molecules such as CD40 [54-56]. CpG motifs have also been used as adjuvants that can activate the production of Th1 cytokines through the TLR9 pathway [57-59]. Some preclinical studies have highlighted the importance of its administration being combined with recombinant proteins to improve immunogenicity [42]. Most importantly, the administration of antigens by DNA vaccination has been shown to be effective in inducing the production of antibodies, Th1 cytokines, and $CD8^+$ T cell-mediated immune responses [54,55].

Synthetic Peptides

The identification of epitopes in proteins recognized by medically relevant antibodies is useful primarily for the development of diagnostic tests [59-62], however, some peptides have been used as vaccine candidates. *T. cruzi* is coated by a thick layer of Glycosylphosphatidylinositol (GPI) -anchored glycoproteins, such as mucins, Mucin-Associated Surface Proteins (MASP), and Trans-Sialidase (TS)/gp85 glycoproteins. MASP is the second largest gene family, accounting for approximately 6% of the *T. cruzi* genome [63,64]. Overlapping B and T cell epitopes of this protein by using synthetic peptides were able to control *T. cruzi* infection in mice by inducing humoral and cellular immunity [65].

Reverse Vaccination

Previously, in the development of conventional vaccines, the process began with the cultivation of the microorganism, which could be complicated according to the pathogen as well as the biological risks during process, Subsequently, the components or structures capable of generating an immune response were identified, the process could be long and tedious since it is based on trial and error until the candidates for the vaccine are found [66]. An alternative strategy that seeks to solve the difficult implementation of vaccines for inactivated or attenuated pathogens, or for vaccine candidates made up of subunits (recombinant proteins or cDNAs) is based on the section of antigens [67]. What makes the identification of antigens a key point in the development of effective vaccines, previously it was one of the processes in which more time was invested, but now the approach of reverse vaccinology with the help of bioinformatics and genomic analyzes and proteomics of *T. cruzi* [63,68] have facilitated, accelerated and diversified the number of candidate antigens [69]. In this sense, the development of vaccines from the knowledge of the genome of pathogens has increasingly gained ground due to the advantages that this represents. A clear example of the use of reverse vaccinology is its use in the development of the vaccine against SARS-

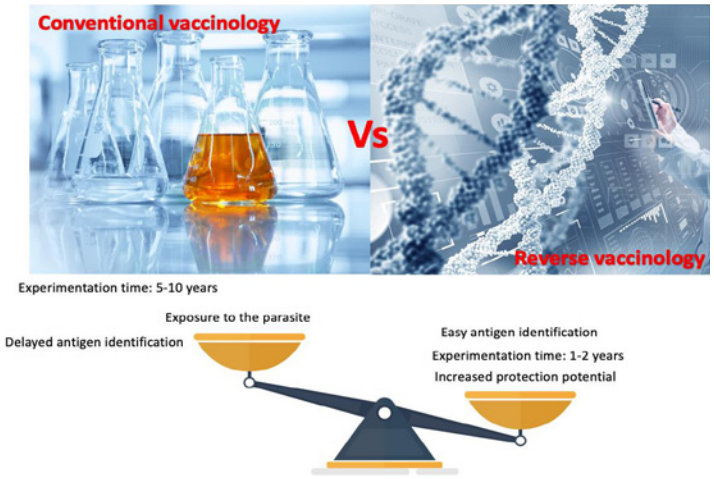

Figure 1: The use of reverse vaccinology in the development of the vaccine for Chagas disease. In conventional vaccinology, the microorganism is cultivated, the next step is the identification and selection of conserved antigens, which takes a great deal of time and the experimental part is trial and error. On the other hand, reverse vaccinology starts with the sequencing of the genome and proteome, later, through bioinformatic and immunomic analyzes, potential antigens are identified, once the candidates are selected, they are cloned and expressed to make way for the studies immunogenicity tests in animal models to evaluate safety and efficacy (preclinical phase) and then in humans (clinical phase). The implementation of new technologies in reverse vaccinology considerably reduces the time in the development of the vaccine.

CoV-2, wherefrom genome sequencing and *in silico* analysis using computational methods for the prediction and design of vaccines [70], a large number of vaccine candidates was generated, developing (because of the critical situation), a vaccine approved and applied in less than 1 year for this virus [71], demonstrating the potential of this approach having a significant impact on reducing the time required for vaccine development and minimizing adverse effects [67,69,72,73]. Now with reverse vaccinology, it is no longer necessary to culture the parasite, from the information of the genome and proteome in databases, the analysis and selection of antigens are made using different prediction tools [74,75], later they are synthesized to be used in experimental animal models and to evaluate the capacity to activate the immune response [76]. The objective of this type of approach is the identification of the minimum essential information for the prediction of antigens with the greatest protection potential against the pathogen in question, with the optimization of this new approach, the time could be reduced by up to one or two years of experimentation (Figure 1) [77].

Other Vaccine Alternatives

The development of vaccines for the control of ChD is an urgent need and the main challenge lies in the biological complexity of the parasite, characterized mainly by the stages of its life cycle, which is why the search for antigenic candidates for the design of vaccines represent a big problem. Therefore, the application of technology including Virus-Like -Particles (VLP) has become an interesting tool for the development of vaccines [78]. The structural conformation of VLPs mimics the morphology and structures of viral particles. One of their main advantages as vaccine prototypes is safety since the particles do not count the virus genome and, therefore, are not infectious viral particles, and the ability to induce a robust immune response [79-82]. In addition, there are several ways to produce recombinant VLPs through the expression of genes in systems of bacteria, fungi, insects, mammalian cells, among others, but this will depend on the biology of the viral particle [83,84]. On the other hand, from a biotechnological point of view, plants have opened up new low-cost strategies to develop affordable vaccines against parasitic diseases, such as malaria, leishmaniasis, toxoplasmosis, among others [85]. In this sense, carrots, papaya, lettuce, and tobacco have been used to express antigens derived from parasites in the nucleus or the chloroplast of cells [86]. The low cost of plant-based vaccine technology represents a great opportunity for governments in poor or middle-income countries that have serious parasitic disease problems but are not served by the pharmaceutical industry (Figure 2).

What Should be Taken into Account, Where are we and where are we Going?

The remarkable advance in DNA sequencing has provided more and more extensive information on the genome of various strains of *T. cruzi*, improving the ability to identify antigens [31,86-89]. This type of information is essential since it is well known that one of the main characteristics of *T. cruzi* is its wide genetic and antigenic variability. It is currently classified by molecular techniques into 7 lineages called Discrete Typing Units (DTU's) TcI-TcVI and TcBat [90, 91]. Large amounts of experimental data show great heterogeneity between strains of *T. cruzi* in various parameters: biological, biochemical, parasitemia, virulence, tissue tropism, drug susceptibility, and immune response [92]. However, there is still controversy between the possible association that has been suggested of this genetic diversity with the heterogeneity in various parameters with the evolution of the infection, clinical manifestations, and treatment during ChD [93]. Overlooking genetic variability during drug development, evaluation, and optimization is not recommended [94]. This same recommendation is suggested during the antigen search (antigen conservation) because all DTU's have been reported in human infections with some genotypes less frequently than others. Therefore, a vaccine must seek to protect against a wide variety of genotypes [30].

The objective of the ChD vaccine should be to act on the two

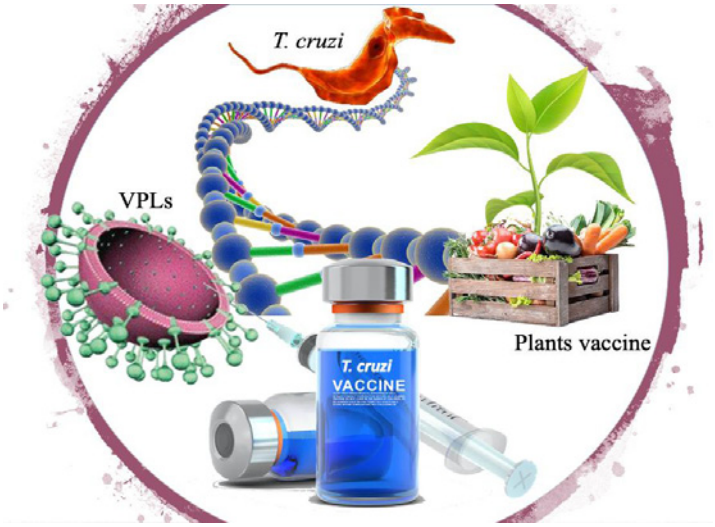

Figure 2: New technological alternatives for the development of vaccines for Chagas disease. In addition to being potential vaccines against the viruses from which they are derived, VLPs can also be used to present individual epitopes of other organisms to the immune system. This can be done by gene fusion and subsequent expression of the resulting recombinant protein or by chemical conjugation between the target antigen and the VLPs-generating structural protein. On the other hand, the low cost of plant-based vaccine technology represents a great opportunity that together with VLPs offer new perspectives in vaccine development.

different phases of the parasite, trypomastigotes (infective phase) and amastigote (intracellular replicative phase), managing to prevent infection and the spread of *T. cruzi* to control parasitemia [95]. For the development of vaccines against *T. cruzi*, VLPs represent a good alternative to generate good immune responses in a targeted way, since the resistance and susceptibility mechanisms of these infections are related to the polarization of the immune response that occurs during a natural infection, specifically during the acute phase. With this type of vaccines, a greater amount of the selected antigen could be expressed compared to that expressed naturally by the parasite, in addition to the fact that they can be coupled to other components that help develop immunogenicity and allow a much more robust immune response [96].

Therapeutic or Prophylactic?

There are two ways to approach ChD vaccine development from a prophylactic and therapeutic perspective. It is difficult to decide which is the best approach to address ChD due to the solutions involved. The area's most affected by ChD are poor areas and the majority of those infected never receive a diagnosis or treatment [85]. Therefore, in this scenario, the benefits that a prophylactic vaccine could provide stand out. In this scenario, economic studies project that a prophylactic vaccine to prevent or reduce heart disorders could represent only 20% of the costs of treatment annually. In the context of a disease associated with socioeconomic indicators such as poverty, a low-cost prophylactic vaccine is considered the best option [86,97-99]. However, more studies are needed because socioeconomic indicators suggest that affected populations have less access to basic and general health services; In this sense, how valid is it to take as a reference the costs of cardiac treatments to which only some patients have access?. In addition to this, it is a reality that these indicators are also associated with cultural factors that do not allow approval of the use of vaccines by the most affected populations [100-103].

On the other hand, the therapeutic approach [104] has proposed the application of vaccines alone or in conjunction with chemotherapy. Vaccine-chemotherapy treatment is one of the most promising strategies to counteract the deficiencies posed by drugs for the treatment of ChD [99]. In the context of a disease that has treatments with efficacy delimited by the phase of the disease [105], long treatments and associated with serious adverse effects that end up impacting non-compliance with the treatments [17] a prophylactic vaccine combined with chemotherapy suggests a quite feasible solution [4,106]. Pre-clinical studies in mice have shown decreased parasitic load in heart fibrosis and cardiac pathologies [18,105-107]. In addition to this, it is suggested that this strategy could reduce the duration and dosage of the medications currently used, bringing as a benefit the reduction of adverse events, favoring the rates of completed treatments [33]; likewise, indirectly delaying the appearance of strains with resistance induced by exposure to Nifurtimox (NFX) and Benznidazole (BNZ). However, to dimension this approach, two questions must be raised to consider: Regardless of a possible vaccine-chemotherapy strategy, the affected populations have little access to treatment and, on the other hand, the application of the therapeutic approach implies a huge advance in diagnostic deficiencies that are still presented.

During the acute phase of *T. cruzi* infection, the parasite replicates extensively and releases immunomodulatory molecules that delay or polarize specific parasite responses mediated by effector T cells. This avoidance mechanism allows the parasite to spread in the host. In the chronic phase, the TGF-β signaling pathway involved in tissue regeneration is affected. As a consequence, the death and replication rates of the parasites are very similar to what occurs during the acute phase of infection. *T. cruzi* is adapted to coexist with a vigorous immune response mounted by $CD8^+$ T cells, calling into question the efficacy of conventional vaccines [108]. Early work shows that *T. cruzi* suppresses lymphocyte activation [109], an effect that depends on the density of the parasites. Therefore, the suppression induced by parasite molecules is more relevant in the acute phase, when the

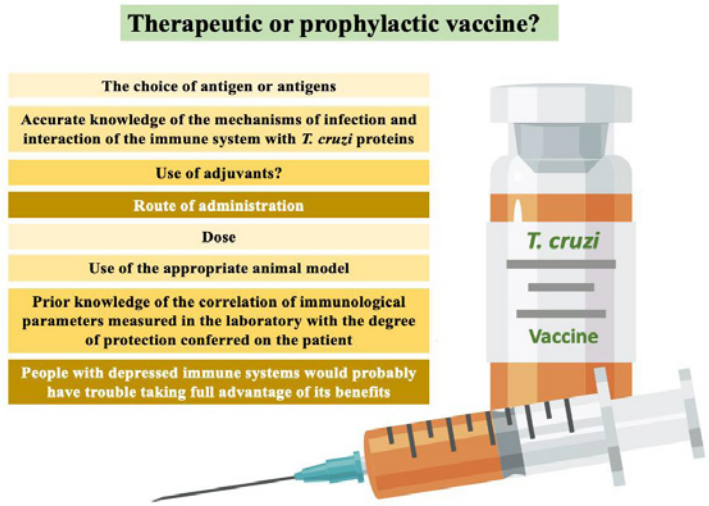

Figure 3: What should be taken into account for the development of the vaccine against Chagas disease.

concentration of such molecules can be quite high. Furthermore, T cell depletion can be observed at the peak of parasitemia [110]. In this sense, for example, cruzipain can induce the secretion of IL-10 and TGF-β, as well as the expression of arginase by macrophages, which leads to an increase in the intracellular replication of *T. cruzi*. Tc52 induces an inflammatory response because it is through TLR2 and only confers protection when combined with adjuvants. In the absence of adjuvants, Tc52 increases IL-10 mRNA in macrophages, which is a macrophage-inactivating cytokine. Furthermore, the expression of Tc52 appears to be necessary for optimal replication of *T. cruzi* in the host [110-112]. Other molecules such as mucins, including the trans-sialidase families, which are abundant in the parasite membrane, induce a state of immunosuppression in the host specifically in T cells, due to the absence of IL-2 and cause dysfunctional functioning in dendritic cells [113]. These behaviors should be taken into account for the use of these proteins as candidates in therapeutic cows. On the other hand, in most cases, the specific immune response generated against *T. cruzi* does not eliminate the parasite efficiently and its persistence is generated in the host, this being a factor for the appearance of the pathology in the chronic phase, this is why the elimination of the parasite in the acute phase would be of importance in preventing the survival of the parasite and preventing the development of chagasic pathologies. In this sense, prophylactic vaccination would be more appropriate [25].

It should be noted that a limitation in a large part of the vaccine candidates evaluated is that they have been mainly tested in murine models, so it is unknown whether this observed efficacy is similar in humans. This leads to the use of other animal models, such as dogs [115], non-human primates [25], as well as the cellular response in chagasic patients [20], and mixed therapy trials have been carried out where low-dose chemotherapeutic treatment is combined with the application of vaccines [4], which in some cases it reduces heart disease [21] in others oxidative damage is controlled, observing a cardioprotective effect [116-118].

Finally, vaccines are developed, tested, and regulated in much the same way as other drugs. In general, vaccines have more meticulous testing than drugs because, in general, there are more humans in clinical trials of vaccines, so do not underestimate the efforts made by different consortia and initiatives that develop candidate's vaccines, since these only aim to reduce the transmission and socioeconomic impact of ChD (Figure 3) [119-121].

Conclusion

Recent efforts have been made to evaluate the T cell epitope response of defined parasites to examine the molecular basis of the immune reaction elicited during infection with protozoan parasites. The severity of the infection is a function of the infecting species together with the consequent inflammatory and immune responses and the genetics of the host. This has been observed with the use of animal models. Studies in computational models suggest that the vaccine against *T. cruzi* would be economically viable, reducing costs in therapies used to combat Chagas' cardiomyopathy, as well as in the prevention of congenital transmission, thus, the development of a vaccine will contribute to prolonging the life of patients with higher quality, by halting the progression of the disease and for its distribution in low and middle-income countries where this disease is endemic and above all, by having a positive impact on health systems that invest large amounts of resources in your attention. One reason for the lack of parasitic vaccines could be low profitability for the pharmaceutical industry. Consequently, research is crucial to generate inexpensive vaccines since the development of new technologies could propose different approaches as well as new and diverse vaccine candidates to address the development of the vaccine against ChD.

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