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REVIEW

Vaccine development against neglected tropical diseases

O desenvolvimento de vacinas contra as doenças tropicais negligenciadas

Desarrollo de vacunas contra enfermedades tropicales desatendidas

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Abstract

Neglected tropical diseases constitute a heterogeneous group of diseases that have as a common characteristic to affect poor and unassisted populations with little vocalization capacity and political power. As a result, they receive little attention from the pharmaceutical industry and academia. The present study aimed to summarize the state of the art regarding vaccine development for three relevant neglected tropical diseases in Brazil: Chagas disease, schistosomiasis (Schistosoma mansoni)*, and leishmaniasis. To this end, we conducted a narrative review of the scientific literature, including publications that allowed us to outline a current overview on the vaccine development for the three diseases. Vaccines against the three diseases are in different stages of development. Vaccine development projects against American trypanosomiasis have yet to reach the clinical evaluation phase. For schistosomiasis, we have candidates for the vaccine in the advanced phase of clinical evaluation. For leishmaniasis, there are already licensed veterinary vaccines, and product candidates for human vaccine in the intermediate stage of clinical evaluation. The reduced funding for these projects has contributed to slow product development.*

Vaccines; Neglected Diseases; Chagas Disease; Schistosomiasis Mansoni; Leishmaniasis

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The neglected tropical diseases (NTD) constitute a heterogeneous group of diseases that have some common characteristics: (a) occurs in poor and unassisted populations, concentrating in these population groups a greater disease burden, leading to loss of productivity and contributing to greater poverty; (b) affects populations with low visibility, small vocalization capacity and political power; (c) most of them do not spread widely, and; (d) many of them cause stigma and discrimination to those affected, contributing to aggravate the scenario of poverty and dismay [1].

NTDs have an important impact on morbidity. It is estimated that 1.59 billion people have at least one of them, which corresponds to 20% of the world's population [2]. Due to their distribution, affecting mainly poor populations with a small capacity for political mobilization, NTDs receive little attention from the pharmaceutical industry, resulting in low investment for developing new drugs, vaccines and diagnostic tests. NTDs have also been relatively neglected by academia, limiting investment for studies focused on NTDs, both in basic and applied research.

In its latest report, the World Health Organization (WHO) included 18 diseases on its NTD list [2]. Of these, twelve have recognized occurrence in Brazil. Some have a record of focal occurrence in the country (onchocerciasis, lymphatic filariasis, hydatidosis), while others have wider dissemination (dengue fever and other endemic arboviruses, leprosy, soil-transmitted helminthiases, taeniasis/cysticercosis, Chagas disease, trachoma, schistosomiasis, leishmaniasis, rabies) [3].

Considering the extent and diversity of NTDs relevant for public health in Brazil, trying to include them all would render the text too extensive. Thus, we decided to limit our study to three of the NTDs we consider of greater relevance for the country: schistosomiasis, Chagas disease, and leishmaniasis.

The number of people with schistosomiasis in the world is estimated at 240 million [2]. Endemic transmission is recorded in 78 countries [4]. In Brazil, schistosomiasis mansoni is considered endemic in much of the Northeast region and in the state of Minas Gerais, and of focal occurrence in almost all other states. It is estimated that there are 1.5 million carriers in the country [5]. A meta-analysis of prevalence studies on these helminthiases estimated the aggregate prevalence in 18.3% (95%CI: 14.7-22.7), with most studies conducted in recognized endemic areas [6]. A nationwide prevalence survey, in a random sample of more than 197,000 schoolchildren between 7 and 17 years of age, conducted between 2010 and 2015, observed a prevalence of 0.99% (95%CI: 0.20-1.78) [7].

It is estimated in 7 million the number of Chagas disease carriers worldwide. Of these, between 1.3 and 3.2 million would be in Brazil. The area considered endemic includes 21 countries from the American Continent [8,9,10]. In 2006, Brazil obtained certification by the Pan American Health Organization (PAHO) for interrupting household vector transmission of American trypanosomiasis by its main vector species in the country, *Triatoma infestans*. While celebrating this important achievement, the country recognized the occurrence of acute foodborne Chagas disease, especially in the Amazon region. Between 2007 and 2019 the country registered more than 3,000 acute cases of Chagas disease, 95% of them in the northern states. Vector transmission still occurs in the country, possibly related to the sylvatic cycle of the parasite [9].

The WHO estimates the occurrence of 60 to 90,000 cases of visceral leishmaniasis annually and 1 million cases of cutaneous leishmaniasis worldwide [11]. Endemic transmission is recorded in 92 countries, of which 25 are classified as high disease burden, among them Brazil [2]. Between 1999 and 2018, the country registered more than 68,000 cases of visceral leishmaniasis, an average of 3,403 cases per year, with an 8% case-fatality rate [5,12]. Transmission takes place in 23 states. In the same period, the country recorded more than 460,000 cases of cutaneous leishmaniasis, an average of 23,121 cases per year, with transmission registered throughout the country [13].

The epidemiological situation of each of the three diseases in the country is distinct. Vector control and screening of blood and organ donors were effective in interrupting the main forms of *Trypanosoma cruzi* transmission in Brazil. However, controlling food transmission has been challenging.

Prevalence, morbidity and mortality have been greatly reduced since the implementation of the schistosomiasis control program in the 1970s. More than 15 million Brazilians were treated [7]. However, the precariousness of basic sanitation, of sanitary sewage collection, its treatment and final destination, provide the necessary conditions for maintaining the schistosomiasis transmission cycle. The average prevalence of 1% among Brazilian schoolchildren hides hyperendemic pockets, not only capable of maintaining transmission, but also generating severe cases.

In turn, the area of leishmaniasis transmission in Brazil has been expanding in the last three decades. From the traditional endemic areas, located in the rural area of some northeastern states, visceral leishmaniasis became urban, and advanced to the south and west, reaching most of the national territory, as well as some neighboring countries. Although there has not been a significant increase in the number of cases, the geographical expansion of the transmission area allows to characterize visceral leishmaniasis as a reemerging disease.

Given this situation, we understand that having vaccines against these diseases could represent another alternative for their control. This article aims to present the current state of research on vaccine development against these three neglected tropical diseases. Therefore, we conducted a narrative review of the scientific literature, including those publications that, in the authors' opinion, contributed to describing this trajectory.

Vaccine development against *Schistosoma mansoni*

There is currently no commercial vaccine of *Schistosoma mansoni* for humans and the drug Praziquantel is the only one currently available for treating schistosomiasis, yet unable to kill helminth larvae and without preventing reinfections. Projects of possible vaccine candidates aim at preventing the migration of schistosomiasis parasites and their maturation into adult worms. Attempts over time have included the use of attenuated parasites as well as the search for antigens that are exposed to the host's immune system and essential for the survival of the parasite, which can be used as vaccines [14]. Experiments with mice have been conducted in the last 50 years, some with good results, but even with protective immune responses to *Schistosoma* infection being similar in humans and mice, some defense mechanisms are different and their premature use in human trials could lead to unwanted effects [15].

Vaccines based on *S. mansoni* cercariae attenuated by heat, chemical treatment, ultraviolet or ionizing radiation have been tested to assess protection against the *S. mansoni* challenge in different mammalian species. Studies conducted with UV-attenuated cercariae in mice showed a significant reduction in the number of worms and eggs in the liver and intestine of vaccinated animals, as well as tegumental changes in adult worms. A recent systematic review and meta-analysis of studies with mice indicated that vaccine with irradiated cercariae can potentially achieve protection of up to 78% with a single dose. These studies also showed that the protection generated decreased, but remained elevated for at least eight months after vaccination. However, despite the potential of an attenuated vaccine against cercariae, there is probably a very high risk of side effects or partially or non-attenuated parasites reaching the mesenteric veins and becoming viable [14].

The main candidates for *Schistosoma* vaccines for humans are based on the use of recombinant proteins and are in different stages of clinical trials. The vaccine composed of the recombinant protein 28-kDa glutathione S-transferase of *Schistosoma haematobium* (rSh28GST) with Allydrogel is produced in *Saccharomyces cerevisiae* and has the trade name Bilhvax. In several preclinical experimental models, Sh28GST has been shown to induce partial protection regarding the reduction of worm proliferation, inhibiting the fecundity of female parasites and lower egg viability. In a randomized Phase 1 clinical study, it was shown to be safe and immunogenic, inducing high titers of specific total IgG, IgG1 and IgG3 and generating Th2-type immune response in healthy adult men. A randomized Phase 2 clinical trial conducted in Senegal showed that using Bilhvax together with Praziquantel is safe. A Phase 3 clinical trial also conducted in Senegal, between 2009 and 2012 with 250 children, showed that Bilhvax is immunogenic and well tolerated, but it was incapable of producing adequate levels of protection and there was no significant reduction in schistosomiasis occurrence between the vaccinated and placebo groups. Despite these results, the authors believe that by modifying the study design or using a different adjuvant, it would be possible to improve the effectiveness of rSh28GST [14,15,16,17].

Recombinant rSm14, made from a fatty acid binding protein (FABP) expressed in *Pichia pastoris*, was tested as a vaccine candidate with glucopyranosyl lipid adjuvant. In experiments with mice, recombinant Sm14 provided up to 67% protection with regard to reducing the parasitic burden of *S. mansoni* without using an adjuvant and no autoimmune response. In addition, it showed cross-protection for *S. mansoni* and *Fasciola hepatica*. A Phase 1 clinical trial tested the vaccine candidate

on 20 male volunteers from a non-endemic area of schistosomiasis in Rio de Janeiro. The study observed no serious adverse event. Although the vaccine was immunogenic, it did not generate any IgE-specific response. Another Phase 1 trial assessed rSm14 safety and immunogenicity on 10 healthy women. After being completed in 2012, the study entered Phase 2 with 30 adult men living in a highly endemic area both of *S. mansoni* and *S. haematobium* in the Senegal River Basin. This trial confirmed the safety and strong long-term immunogenicity of the rSm14 vaccine. Based on these results, a second Phase 2 study was developed in 2018 with 95 Senegalese children aged 7 to 11 years living in the same endemic area for two *Schistosoma* species. At present, Brazil is planning new Phase 2 studies and Senegal new Phase 3 studies [14,15,17].

Sm-TSP-1 and Sm-TSP-2 antigens are important *S. mansoni* tetraspanins, a protein group continuously exposed to the host's immune system and abundant in the tegumentary membrane when the protozoan is in the mammal. In experiments with mice, Sm-TSP-2 provided a high level of protection with corresponding IgG antibodies and was chosen to be part of a recombinant vaccine candidate, 9kDa Sm-TSP-2/Allydrogel using GLA-SE as an adjuvant and produced in *Pichia pink*. This vaccine underwent toxicology studies and showed good preclinical results, with a 40% reduction in the number of adult worms and 65% in the proliferation of eggs in mice submitted to infection by *S. mansoni*. There is a Phase 2 study in the United States testing its safety and immunogenicity and another Phase 1 taking pace in an endemic area in Brazil, testing its safety and immunogenicity in healthy adults who may have previously been exposed to schistosomiasis. Other field trials are also being planned in Uganda [15,16,17].

Vaccine candidate Sm-p80 is made from a large *S. mansoni* subunit, calpain, a protease. It plays a fundamental role in the biogenesis and renewal of the superficial membrane, a mechanism used by helminths to prevent the host's hostile immune response. It had its efficacy tested in different formulations and approaches. In addition, it has been shown to generate significant protection against *S. mansoni* infection challenges with cross-protection in *S. japonicum* and *S. haematobium* species. Experiments using Sm-p80 have proved a prophylactic efficacy against intestinal/hepatic schistosomiasis, reducing the anatomical-pathological changes induced by eggs, both in rodents and baboons, a post-exposure therapeutic effect, with the death of adult worms in chronic infections, cross-protection against Asian vesical schistosomiasis, immune response longevity of 60 weeks in mice and of 5 to 8 years in baboons, and placental transfer of specific Spm-p80 antibodies in baboons. Sm-p80 has also been shown to be effective against various stages of the parasite's life cycle, including eggs, schistosomula and adult worms. The experiments found no Sm-p80-specific IgE in infected populations in Africa and South America, which potentially minimizes the risk of hypersensitivity after vaccination. GLA-SE-associated Sm-p80 is now being prepared, under the name SchistoShield, to enter Phase 1 and 2 trials [15,18].

Another vaccine candidate antigen is Paramyosin (Pmy), protein associated with the muscles of invertebrates, which, despite not being very promising against *S. mansoni*, has been shown to be an interesting model for protection against *S. japonicum* cercariae. The complete isolated paramyosin cDNA, Sj97, can recognize the 97kDa surface molecule of *S. japonicum* and trials of a bovine vaccine against *S. japonicum* showed a reduction in the number of worms in vaccinated Chinese buffaloes compared with controls [19].

Vaccine development against *Trypanosoma cruzi* and Chagas disease

The search for a successful vaccine for *T. cruzi* is in the preclinical phase, where tests have been carried out on mice, dogs and non-human primates. The idea is for the vaccine to contain target antigens in all stages of the parasite and be used both as a prophylactic and therapeutic vaccine.

The first vaccine attempts for Chagas disease used parasites killed by chemical, physical and irradiation methods in several animal models. These products provided no protection against the challenge of lethal infection, but a few experiments generated some degree of resistance to *T. cruzi* and a high proportion of immunized animals survived the challenge of the acute experimental infection. Other studies then started to use *T. cruzi* attenuated by "knockout" technique with one or more genetically modified virulence genes in live virus vaccines and showed high effectiveness in controlling the

infection challenge, with vaccinated animals showing less parasitemia and increasing survival rates compared with non-immunized animals. However, there is concern that the use of vaccines using live attenuated parasites may lead to complete infection or manifestation of the disease in immunocompromised individuals [20]. Another vaccine proposal uses protozoa with antigens similar to *T. cruzi*, but that are not pathogens to humans. Such is the case of *Phytomonas serpens*, a tomato plant parasitic that after being used as a prophylactic vaccine in mice, significantly reduced parasitemia and increased survival when challenged with lethal *T. cruzi* infection. Another experiment used the epimastigote form of *Trypanosoma rangeli*, which infects some non-human mammals, in mice infected with the *T. cruzi* strain. After 2 or 3 doses, the experiment observed a reduced parasitemia and increased survival [21].

Other vaccine attempts sought more immunogenic parts of the parasite that could induce a protective immune response through subcellular fractions of *T. cruzi*. One of these studies used fractions of the epimastigote form of *T. cruzi* to immunize mice and the flagellar portion resulted in partial protection against the development of myocarditis, but immunizations with this portion even without infection caused lesions similar to those of the control animals. Later, it was verified that the rod paraflagellar protein (PRF) purified from of *T. cruzi* epimastigotes reduced parasitemia and showed a 100% survival after the challenge. Other trials also used PRF as an adjuvant, in which it was observed a greater survival of vaccinated animals [21].

Studies were also conducted to verify which purified proteins from the parasite induced immune response and protection against infection: the 90kDa surface glycoprotein, found in all stages of *T. cruzi*, provided protection against trypomastigotes; 72kDa glycoprotein, found only in insect-derived stages protected only against some metacyclic trypomastigotes forms; 45 and 68kDa antigens purified from the *T. cruzi* epimastigote cell membrane generated a strong cellular and humoral response, protecting 100% of the animals challenged with trypomastigotes in the blood. An antigenic preparation obtained from a strain of *T. cruzi* Y lysate that generated a band of 72kDa induced high levels of IgG antibodies, significantly reduced parasitemia and decreased CD4/CD8 rate in mice; antigens excreted/secreted from trypomastigotes reduced acute parasitemia and generated 60% and 100% protection in BALB/c mice and Fisher rats, respectively [21].

Vaccine experiments based on recombinant proteins highlight the trans-sialidase (TS) superfamily, recognized by its antibodies and CD8+ T lymphocytes response in mice; the mucins that have proved to be a powerful inducer of polyclonal B cells, cytokines and inflammatory macrophages; the superficial mucin-associated proteins (MASPs); and the 63s glycoprotein (GP63s). Other antigens that do not belong to large families and that have been tested for their antigenic potential include the complementary regulatory protein (CRP or gp160); the lysosomal cysteine proteinase called cruzipain (60kDa); the calcium flagellar binding proteins FCaBP or Tc24 (24kDa) and GP82 (82kDa); the kinetoplastids membrane proteins KMP-11 (11kDa) and LYT1 (61kDa), and the paraflagellar rod proteins (70-86kDa) and TC52 (52kDa). Along with vaccine candidates, multiple adjuvants, administration routes, concentration and number of doses, and prime boost strategies are also being tested to change immune responses induced by the Th1 vaccine and improve long-term protective efficacy [20,22].

DNA vaccines are being developed both for prevention and treatment of Chagas disease. As an example of this type of vaccine, we have the TcSP gene, which encodes a member of the trans-sialidase superfamily, and its respective recombinant protein rTcSP, which generate a mixed Th1/Th2 immune response. The TcSSP4 gene, which encodes the amastigote-specific surface protein, showed an increase in IFN-γ, suggesting a Th1-type response. For both TcSP and TcSSP4, only mice immunized with DNA showed a significant reduction in the peak of parasitemia and survival to the lethal challenge. Studies with these two genes showed a different protective immune response induction from its recombinant protein counterpart. An assay in dogs with Chagas disease used both of these genes and found that the dominant antibody was IgG2 immunoglobulin and induced IFN-γ lymphoproliferation and production, in addition to reducing electrocardiographic abnormalities and preventing the progression of further cardiovascular disorders [21,23].

TcG1, TcG2 and TcG4 antigens, present in the plasma membrane of trypomastigotes and amastigotes, were selected as potential candidates for a vaccine model for being expressed in the *T. cruzi* stage in mammals, secreted in the cytoplasm of host cells during parasite differentiation and consisting of epitopes present in MHC allele genes of mice, dogs and humans. An experiment using mice with a plasmid expressing these three antigens found and association of IgG2/IgG1 isotopes

with parasite burden control between 50-90% in the acute phase and with an undetectable level of parasites during the chronic phase. Those who were vaccinated also showed decrease heart inflammatory infiltrate and IFN-γ levels and tumor necrosis factor (TNF) [22,23]. The TcG2 and TcG4 genes, considered well-preserved, were selected as components of the TcVac3 vaccine, which in tests with mice induced a Th1-type response and proliferation of CD4/CD8-type T cells. In the chronic phase, the immunized rodents showed decreased pro-inflammatory phenotype, prevalence of immunoregulatory T cells IL-10+/CD4+ T and IL-10+/CD8+ T and showed parasitism, inflammatory infiltrate and tissue fibrosis almost undetectable. The TcVac2 vaccine, on the other hand, with TcG1, TcG2, and TcG4 antigens, showed an increase in lytic antibodies and CD8+T-type 1 cells after the infection challenge, in addition to promoting less parasite and myocarditis expansion in infected rodents. The results also suggested that TcVac2 controls chronic myocarditis due to the antiproliferative and anti-inflammatory responses of macrophages [21].

Another third generation vaccine candidate uses amastigote surface protein-2 (ASP-2), with the yellow fever vaccine 17DD virus as a vector. An experiment with mice observed a reduction in mortality, an increase in survival time, and a reduction in the peak of parasitemia. Another experiment with the ASP-2 gene used an adenovirus as vector in mice that suffered an infection challenge, finding an increase in the frequency of CD8 + T cells in the spleen. Another study found an increase in the immune-protection provided by Cruzipain if co-administered with strains of *Salmonella* containing plasmids with thiol-transferase (Tc52) and calcium-biding flagellar protein 24kDa (Tc24). Tests with this multi-component found a strong humoral and cellular immune response, providing protection against *T. cruzi* infection in mice. The Tc24 antigen together with the Trans-sialidase TSA-1 family gene were targeted by another DNA vaccine tested in dogs both prophylactically and therapeutically, both reducing parasitemia, cardiac inflammation and the development of cardiac arrhythmia. The humoral response was weak, but there was an increase in the IFN-γ levels in immunized dogs [21].

With regard to therapeutic vaccines, the main candidate antigens are TSA-1, TS and ASP-2 from the Trans-sialidase family, the S-transferases Tc52 glutathione and the Ca2+-bound protein, Tc24. Studies with a DNA vaccine containing Tc52, TSA-1 and Tc24 found a decrease in parasitemia and mortality from infection, which was associated to the rapid increase in the number of CD4+ and CD8+ cells. Another study showed that ASP-2 and TS, both individually and combined, did not limit parasitemia or increase the survival rate in infected mice despite exhibiting excellent efficacy in prophylactic vaccines. The Tc24 protein, on the other hand, showed and immune response with an increase in IFN-γ, IgG2A, and CD8+, with a notable reduction in the inflammatory cellular reaction and parasite burden in the tissue. Together with adjuvant E6020, Tc24 showed that 60% of therapeutically vaccinated mice had undetectable levels of parasite and decreased cardiac fibrosis. Greater efficiency was also observed in the therapeutic vaccine TG2/TcG4 in infected animals that overexpressed glutathione peroxidase and that were able to control oxidative damage responses [20,22,24].

Vaccine development for *Leishmania*

First generation vaccines

The first generation leishmaniasis vaccines, which used dead or attenuated parasites, were inexpensive and had some success in animal models, but none of them were validated for commercial use in humans by the WHO. Leishvaccine, which is composed by dead *Leishmania amazonensis* promastigotes and BCG adjuvant, made the most progress in this direction. It was used for the prophylactic treatment of canine visceral leishmaniasis, inducing a significant increase in the pattern of mixed cytokine, including IFN-γ and IL-4 and stimulating innate immunity, mainly of neutrophils, eosinophils, and B, CD4+T and CD8 +T active cells. Phase I and Phase II clinical trials performed in humans attested its safety and immunogenicity, but Leishvaccine did not achieve satisfactory results in Phase III randomized clinical trials [25,26]. Another vaccine using the same principle used *Leishmania mexicana* killed by autoclaving associated to BCG. Its efficacy has been shown for both prophylactic and immunotherapeutic use. A clinical trial conducted in Ecuador used two dead *L. mexicana* and

L. amazonensis species associated with BCG, conferring protection of 73% in a sample of healthy volunteers, with almost no side effects [25].

The first generation vaccines also featured the live form of *Leishmania infantum* attenuated by gentamicin, which was tested in dogs not exposed to *Leishmania* infection in an endemic area and monitored for 24 months. At the end of the trial, 32% of the dogs in the control group had a wild *Leishmania* antigen and no positive ones among the vaccinated, with 29% of the controls showing clinical signs of the disease compared to 2.2% in the vaccinated. Another study attesting its safety and protective effects was conducted, in which healthy dogs were immunized with these strains attenuated with gentamicin and the IFN-γ level was higher than the controls [25,27]. Another attempt at a vaccine with live parasite consisted of *L. donovani* excluded from the centrin gene, which specifically affects the amastigote phase of the parasite within the macrophages. After 15 days, the assay observed that the vaccinated group exhibited higher antibody titer than the group exposed to another vaccine, Leishmune, with greater proliferation of T and B cells and increased TNF-α and IL-12, suggesting immunogenic and protective effects against canine visceral leishmaniasis [27,28].

Second generation vaccines

Four second-generation vaccines obtained commercial versions for veterinary use after field studies results, indicated both to protect dogs and to reduce the transmission of canine leishmaniasis to humans by phlebotomine bites. They are Leishmune and Canileish, based on fractionated antigens; Leish-Tec and Letifend, composed of recombinant proteins.

Leishmune was the first licensed vaccine for canine leishmaniasis registered in Brazil in 2004. It consists of fucose-mannose ligand (FML) of *L. donovani* promastigotes, which has been shown to be suitable for serodiagnosis in dogs and humans, and a saponin adjuvant. Its efficacy was tested in two Phase 3 trials in a visceral leishmaniasis endemic area. The first study used the Riedel de Haën saponin as an adjuvant in 117 dogs and the vaccinated animals showed vaccine-specific seroconversion and positive reaction in delayed-type hypersensitivity skin tests (DTH). There were 4 deaths in the control group and none in the vaccine group; the vaccine efficacy was 76% and the protection against the disease 92%. However, the lack of randomization in the sample and other methodological deficiencies hindered the full validation of these results. The second trial used the adjuvant QuilA saponin and the vaccinated dogs showed specific seroconversion and positive DTH. After 3 and a half years, 8 of the 41 control dogs and 1 of the 44 vaccinated dogs were diagnosed with canine leishmaniasis, with a vaccine efficacy of 80% and protection against the clinical disease of 95% [29].

From then on, under trade name Leishmune, it underwent a field trial with 600 dogs in two endemic areas for canine leishmaniasis and was shown to be safe and well tolerated, without serious adverse reactions. An immunogenicity study monitored a subgroup of 550 vaccinated dogs for two years. In addition to vaccine-specific seroconversion and positive DTH, blood samples collected 18 months after vaccination showed a sustained CD4+ lymphocytes response and an increase in CD8+ and CD21+ populations when compared with a group of healthy unvaccinated controls from a different endemic area. The results of the study revealed 98.8% of asymptomatic dogs at the end of the first year and 99% of healthy survivors at the end of the second year among vaccinated dogs, while the unvaccinated cohort had 79.4% of asymptomatic dogs and 61% survivors. However, these comparisons between the vaccine and control groups were questioned by possible differences in infection pressure at both sites, as well as by the different criteria used to diagnose infection in dogs of both groups. The authors of the study still claimed a 66.1% and 80.2% reduction ($p < 0.005$) in the incidence of leishmaniasis among vaccinated dogs in the two study areas, when compared with the overall incidence of the disease in the same regions [29].

Leishmune entered the market as a transmission-blocking vaccine, based on the assumption that vaccinated dogs could not become infectious to phlebotomine. To support this, a study observed that the FML-induced antibodies present in the serum of dogs could inhibit *L. donovani* and *L. chagasi* promastigotes from biding itself to the intestine of *Lutzomyia longipalpis*. Leishmune was also promoted as able to reduce the incidence of human leishmaniasis as a result of decreased canine leishmaniasis in endemic areas, and that an increase in vaccination coverage could prove more effective in controlling infection than slaughtering dogs. One study compared cases of canine and human infection

before and after the introduction of Leishmune in regions with different vaccination coverage rates and found an inverse correlation between the number of vaccinated dogs and the number of cases of canine leishmaniasis. This study also included the results of a serological screening for *L. chagasi*, showing that from a population of 5,860 vaccinated dogs, only 1.3% were seropositive. Of these, none were positive in a confirmatory test or showed visible parasites in lymph nodes or bone marrow. However, in a subsequent evaluation that found sustained seropositivity up to six months after vaccination in dogs immunized by Leishmune, the diagnostic tests could not distinguish between the vaccinated dogs from the naturally infected ones. In 2014, the Brazilian Ministry of Agriculture canceled the license of Leishmune due to the lack of evidence of its effectiveness in the Phase 3 trials [29].

The LiESP/QA-21 or CaniLeish vaccine, produced from *L. infantum* excreted/secreted protein extract (LiESP) and with *Quilaja saponaria* (QA-21) as adjuvant, was licensed in Europe in 2011. The first study conducted with CaniLeish measured the effect of the vaccine against markers of humoral and cellular immunity in dogs kept indoors under controlled conditions. Results showed that only vaccinated dogs produced antibodies to LiESP and parasite surface antigen (PSA). Vaccination also induced cellular immunity, presenting specific T-cell response in vaccinated animals, with production of IFN-γ when exposed to soluble *Leishmania* antigens (SLA). One year after vaccination, these dogs were challenged with *L. infantum* promastigotes, showing significantly higher results for CMLA (Canine Macrophage Leishmanicidal Assay) index, iNOS activity, NO2 and IFN-γ production in the vaccinated group. Seroconversion after exposure to *L. infantum* antigens was 100% in the vaccinated group, while in the control group only the actively infected animals showed positive titers. Both groups exhibited only mild clinical signs. At the end of the study, three vaccinated dogs and seven controls were considered actively infected. Two vaccinated dogs, with positive results for *L. infantum* in previous parasitological evaluations, were considered reverted to a parasite-free status [29].

A randomized efficacy study prior to CaniLeish licensing included 90 dogs in two endemic areas of canine leishmaniasis in Italy and Spain monitored for 2 years. The only adverse effects observed were local edema and crust followed by alopecia in the region, all with spontaneous resolution. The humoral profile in response to vaccination followed the same trends observed earlier. Results showed a significant difference between the frequency of dogs with active infection ($p = 0.025$) and the number of symptomatic cases ($p = 0.046$). However, there was no significant difference in the proportion of dogs that had a positive PCR result, confirming that the vaccine did not prevent infection of these animals. Some dogs returned to the *Leishmania*-free status, being more frequent in the vaccinated group ($p = 0.039$). Of the animals that died due to severe leishmaniasis, five were from the control group and none were from the vaccinated group ($p < 0.0001$). From these results, the efficacy of CaniLeish in preventing clinical signs was considered to be 68.4% and the level of protection of the vaccine was 92.7% [29].

Another study conducted in four kennels in Italy, which compared the individual efficacy of collars with insecticide and the CaniLeish vaccine in preventing canine leishmaniasis in highly endemic areas, found no statistically significant differences in the number of positive animals in the vaccinated and control groups one year after vaccination ($p = 0.417$). There were also no differences in the development of active symptomatic infections that were measured by cytology results, PCR tests and indirect immunofluorescence and lymph node enlargement, between the groups ($p = 0.495$). A study in Spain with 177 dogs, where the proportion of active *L. infantum* infections was similar in vaccinated (5.6%) and in controls (5.4%), showed similar results. In this study, vaccine-induced cell-mediated immunity (CMI) was short-lived, implying an apparent lack of CaniLeish efficacy in protecting against *L. infantum* [29].

Leish-Tec is a vaccine composed of recombinant protein A2 of *L. donovani* amastigotes with saponin as adjuvant licensed in 2007 in Brazil and is the only canine vaccine authorized in the country currently. Preclinical experiments showed that immunization with recombinant protein A2 conferred a high degree of protection to challenged mice. The humoral response caused by the vaccine was highly specific, showed cell-mediated immunity classified as mixed Th1-Th2, and led to a significant increase in IFN-γ levels. In a later study, Leish-Tec induced partial protective immunity against *L. chagasi* infection and prevented a greater severity of the disease. Immunized dogs produced increased levels of anti-A2 IgG2 after vaccination, with a significantly higher production of IFN-γ among vaccinated when stimulated with A2 antigen or *L. chagasi* total protein extract [29].

Leish-Tec was also tested for the infectivity of dogs to phlebotomine, showed by the xenodiagnosis. A comparative study between Leishmune and Leish-Tec found no significant differences between vaccines in humoral response or infection and transmission rates for phlebotomine; the only difference detected was the higher rate of adverse reactions in the Leish-Tec group. A Leish-Tec field study included more than 500 dogs and observed a significant reduction in the number of cases of canine leishmaniasis in the vaccinated group. Vaccine efficacy was 71.4% when evaluated by parasitological tests, 58.1% in parasitological tests when associated with xenodiagnosis and 80.8% in A2 seroconversion. This study failed to show a reduction of the infectivity of vaccinated dogs, as it found no statistically significant differences in the prevalence of positive pools of phlebotomine feeding from each of the study groups. Another Leish-Tec efficacy study reported significant difference in the infection rate between vaccinated (27%) and controls (42%). However, it observed a twice higher proportion of sick dogs among immunized seropositive animals (44%), when compared with the control group (21.2%). This study suggested that Leish-Tec was ineffective in the field and that its use would have no impact on the incidence of canine leishmaniasis in areas of high transmission. A conclusion similar to that of a systematic review conducted to evaluate the effectiveness of prophylactic control measures for canine leishmaniasis, which found an apparent lack of evidence of the efficacy of the Leish-Tec vaccine. The efficacy of Leish-Tec as an immunotherapeutic vaccine was also tested in a randomized, double-blind field study with 557 asymptomatic dogs seropositive for *L. infantum*. After nine months, the study measured the risk of clinical progression (RR = 1.33; p = 0.045) and all-cause mortality (RR = 3.19; p = 0.0245), being considered higher in controls than in vaccinated animals [29].

Another recently commercially produced recombinant protein vaccine is LetiFend, licensed in Europe in 2016. It consists of a chimeric protein "Q" with five antigenic fragments of four different *L. infantum* ribosomal proteins (LiP2a, LiP2b and LiP0 and histone H2A), without adding adjuvant. Preliminary studies in mice showed the potential of protein Q when associated with adjuvant BCG, which after experimental infection with *L. infantum*, prevented the establishment of parasites in mice and dogs [29]. Other tests found that immunization with protein Q without adjuvants (which corresponds to the current commercial formula of LetiFend) alone was able to generate a protective effect in vaccinated dogs. The Phase 3 pre-licensing trial of LetiFend included 549 dogs exposed to natural infection in two endemic areas of canine leishmaniasis in France and Spain for two years. At the end of the study, 4.7% of vaccinated dogs and 10.2% of controls developed canine leishmaniasis, a difference considered statistically significant (p = 0.048). According to this study, LetiFend showed vaccination efficacy of 72% in preventing clinical signs of canine leishmaniasis, and reduced the likelihood of confirmed cases of canine leishmaniasis by 5 times and the development of clinical signs in vaccinated dogs by 9.8 times in relation to controls. No significant adverse effects were observed following administration of LetiFend during laboratory or field studies [29].

Third generation vaccines

Many third generation vaccine candidates against leishmaniasis are being evaluated: the vaccine with the gene KH, using the ChAd63 adenovirus as vector [25]; the vaccine LJM19, which used a plasmid DNA that codes a salivary protein of *L. longipalpis*; the therapeutic vaccine that uses acidic ribosomal protein P0 and nucleosomal histones of *L. infantum*; the DNA vaccine for visceral leishmaniasis developed from a gene of the acidic ribosomal protein of *L. donovani* (LdP1) with bacterial plasmids pQE or pVAX as vectors; the vaccine that used lipophospohglycan 3 (LPG3), member of the heat shock protein HSP90 equivalent to protein GRP94 of *Leishmania* [26]; the DNA vaccine that express surface glycoprotein gp63, present in amastigotes and promastigotes. We highlight in this group the vaccine based on the prime-boost strategy composed of DNA/vaccinia Ankara, expressing the recombinant protein TRYP of *Leishmania*. It was considered safe and immunogenic in dogs, with antigen-specific type 1 responses and immune response of cellular memory, thus showing itself to be a potential protective vaccine. Finally, LEISHDNAVAX, a DNA vaccine composed of five vectors of different antigens for *Leishmania* (KMP11, CPA, CPB, P74 or TSA), proved to be immunogenic, with prophylactic efficacy of almost 90% in animals vaccinated in preclinical studies. Safety tests in mice and rats were favorable to the use of LEISHDNAVAX in both uninfected and infected animals, which allowed to begin clinical trials for preventive and therapeutic applications of the vaccine in humans [30,31].

Leishmania vaccines in humans

There is still no commercial vaccine for human leishmaniasis. Some candidate products have been evaluated in clinical trials. A first generation autoclaved *Leishmania* vaccine precipitated by Alum, tested with BCG in a Phase 2 study with children in Sudan, proved to be immunogenic, safe and had positive conversion on the *Leishmania* skin test. Safety and immunogenicity were observed in 76% of human volunteers who produced IFN-γ in response to *Leishmania* lysate [32].

One of the first second generation recombinant protein vaccines tested in humans was LEISH-F1, which reached Phase 2 clinical trials. It consists of a thiol-specific antioxidant of *L. major* (TSA) homologue, the stress-inducing protein-1 of *L. major* (LmSTI1), the inhibition and elongation factor of *L. braziliensis* (LeIF) and associated with adjuvant MPL-SE. In its trials, the LEISH-F1+MPL-SE was effective as a therapy in patients with cutaneous leishmaniasis or mucosal leishmaniasis, as well as being able to induce protective immunity in healthy volunteers. Its successor was the LEISH-F2 vaccine, more similar to the "natural" protein of wild species. After safety and immunogenicity were approved, it was evaluated in a Phase 2 trial. With partial results considered unsatisfactory regarding efficacy, the Phase 2 study was discontinued [25,33]. LEISH-F3 is another multicomponent vaccine consisting of nucleoside hydrolase (NH36) from *L. donovani* and sterol 24-c-methyltransferase (SMT) of *L. infantum* with adjuvant GLA-SE (glucopyranosyl lipid A), which was tested in a Phase 1 trial with healthy humans in the United States and showed a robust immune response against visceral leishmaniasis. The vaccine was safe and showed increased secretion of cytokines IFN-γ, TNF-α, IL-2, IL-5 and IL-10. In addition, a study conducted in Bangladesh also found a strong cytokine response for each vaccine component in patients with visceral leishmaniasis. They exhibited Th1-type CD4 cell responses to NH36 and SMT, with secretion of IFN-γ, TNF-α and IL-2 and also IL-5 and IL-10 in whole blood assays [25,32].

Other groups such as the Sabin Institute are exploring prototypes of vaccine combinations that comprise the recombinant NH36 of *L. donovani*, but this study is at its earliest stage of development. On the other hand, the Melevaclin group (European Multivalent Vaccine for Human Visceral Leishmaniasis) is working on vaccines based on both recombinant proteins and DNA vaccines for visceral leishmaniasis in preclinical studies [34].

Final considerations

The vaccine development against the three NTDs discussed in this article is at different stages. Vaccines against *T. cruzi* have not yet reached the clinical evaluation stage, although there are promising candidate products. Regarding leishmaniasis, we identified two approaches. The first consists in developing canine vaccines, which aims to block the transmission of the canine reservoir to vector insects and human hosts. The second is represented by human vaccines, both preventive and therapeutic. Developing vaccines against leishmaniasis faces an additional limitation: the existence of several species of the protozoan capable of infecting the human host and producing the disease. Some canine vaccines have already completed all phases of preclinical and clinical evaluation, and have reached the market. However, they have not yet been incorporated into public health programs. Perhaps evidence regarding its efficacy, effectiveness and efficiency has yet to be considered sufficient for its implementation. Regarding human vaccines, some candidates are already in the clinical evaluation phase, without an established deadline to finalize the trials. Some candidate products for schistosomiasis vaccine are in advanced stage of clinical evaluation. Similar to leishmaniasis, the existence of several trematode species capable of infecting the human host renders vaccine development more complex.

There is still no vaccine against human helminth infections, and only a single human vaccine against protozoa has been licensed for use to date. This is the vaccine RTS,S/AS01 for malaria falciparum [35]. It has recently been licensed and is in use in some pilot programs in Africa. It has modest efficacy, but has been considered an additional tool for disease control in hyperendemic areas.

It is also important to highlight the role of Brazilian science in the vaccine development processes for the three NTDs discussed here. National and other developing countries universities and research institutes have played a prominent role in these processes. Limitations, especially regarding funding, slows this development.

Contributors

E. J. A. Luna contributed to the study design, data analysis, and manuscript preparation. S. R. S. L. C. Campos contributed to the data extraction, data analysis, manuscript editing and revision.

Additonal informations

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Resumo

As doenças tropicais negligenciadas constituem um grupo heterogêneo de enfermidades que apresentam como característica comum afetarem populações pobres e desassistidas, com pouca capacidade de vocalização e de poder político. Em consequência, recebem pouca atenção da indústria farmacêutica e da academia. O presente estudo teve como propósito resumir o estado da arte quanto ao desenvolvimento de vacinas para três doenças tropicais negligenciadas de relevância no Brasil, a doença de Chagas, a esquistossomose mansoni e as leishmanioses. Para tanto, realizou-se uma revisão narrativa da literatura científica, na qual foram incluídas publicações que permitiram traçar um panorama atual do desenvolvimento de vacinas para as três doenças. Essas vacinas estão em estágios distintos de desenvolvimento. Os projetos de desenvolvimento de vacinas contra a tripanossomíase americana ainda não chegaram à fase clínica de avaliação. Já para a esquistossomose há candidatos à vacina em fase avançada de avaliação clínica. Para as leishmanioses já existem vacinas veterinárias licenciadas e produtos candidatos à vacina humana em etapa intermediária de avaliação clínica. O reduzido financiamento para esses projetos tem contribuído para retardar o desenvolvimento dos produtos.

Vacinas; Doenças Negligenciadas; Doença de Chagas; Esquistossomose Mansoni; Leishmaniose

Resumen

Las enfermedades tropicales desatendidas constituyen un grupo heterogéneo de enfermedades, que presentan como característica común el hecho de que afectan a poblaciones pobres y desasistidas, con poca capacidad de interlocución y poder político. En consecuencia, reciben poca atención de la industria farmacéutica, así como de la academia. Este estudio tuvo como propósito resumir el estado de la cuestión, respecto al desarrollo de vacunas para tres enfermedades tropicales desatendidas relevantes en Brasil como son: la enfermedad de Chagas, la esquistosomiasis y leishmaniosis. Para ello, se realizó una revisión narrativa de la literatura científica, en la que se incluyeron publicaciones que permitieron trazar un panorama actual del desarrollo de vacunas para las tres enfermedades. Estas vacunas están en estadios distintos de desarrollo. Los proyectos de desarrollo de vacunas contra la tripanosomiasis americana todavía no llegaron a la fase clínica de evaluación. Ya en el caso de la esquistosomiasis hay candidatos a la vacuna en fase avanzada de evaluación clínica. Para las leishmaniosis ya existen vacunas veterinarias licenciadas y productos candidatos a la vacuna humana en etapa intermedia de evaluación clínica. La reducida financiación para esos proyectos ha contribuido al retraso en el desarrollo de los productos.

Vacunas; Enfermedades Desatendidas; Enfermedad de Chagas; Esquistosomiasis Mansoni; Leishmaniasis

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