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RECENT ADVANCES ON RECOMBINANT ANTIGENS FOR CONTROLLING PARASITIC PROTOZOA: DIAGNOSIS AND VACCINE DESIGN

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ABSTRACT
Parasitic protozoa cause important diseases with a worldwide distribution. Although significant advancements in recognition of etiology of parasitic diseases have been made, there are still obstacles in their control. Hence, prevention and diagnosis are of major importance in such serious pathogenic diseases. Most of the methods for control of parasitic protozoa are based on lysate antigens. Many studies also show that recombinant antigenic proteins may be very useful alternatives for serodiagnosis and vaccine design of parasitic protozoa. In this review, current and recent studies on usefulness of parasite recombinant antigens in diagnosis tests and vaccine development for human are described.

KEYWORDS: Recombinant antigens, Diagnosis, Vaccine, Parasitic protozoa.

1. INTRODUCTION
Human health is almost exclusively dependent on the field of relationship with the environment, where is usually occupied by a variety of contaminations and infectious biological agents. The high inclusiveness and potential effects of these harmful agents have grown increasingly as a problematic issue in global concerns. In the meantime, identification and detection of those factors that endangering human health and well-being are still a fundamental obstacle to preventing and eradicating of caused discomfort and disease. The methodologies are still ambiguities in some cases. The full recognition of the agents as well as the correct diagnosis and prognosis is therefore of a special importance and is a necessity for resolving the barriers and improving the quality of life. Pathogenic microorganisms are specialized to invade and infect body tissues in which they multiply and cause damage. The detrimental interaction between host and pathogen usually involves various molecular factors that ultimately may lead to an infectious disease. The infection is inflicted either by virulence factors of microorganism, the factors distinguishing their pathogenicity from nonpathogens microorganism, or through host immune responses.[1]

Among them, parasites are the microorganisms, which live and feed on another organism and cause severe harms in a wide spectrum of species. They have unique morphological and physiological properties and due to their ultimate variety, indwell the main proportion of the eukaryotic tree of life.[2] According to the division of pathogens to opportunistic, facultative and obligate, the human disease-causing protozoan parasites are capable of creating different diseases via various mechanisms. Among them, four protozoan plasmodium, trypanosoma, leishmania and toxoplasma have major roles in developing diseases causing by protozoan parasites around the world (Fig. 1). The microorganism at the first must orient towards outer surfaces of the host cells or tissues for adhering through biophysical interactions and often for entering or residing within cells by means of its own potential factors. For instance, the resulting of the intracellular entrance is varied ranging from the cytosol of the infected cell (e.g. *Plasmodium*) to enclosed in a parasitophorous vacuole (e.g. *Toxoplasma*) or a phagolysosome (e.g. *Leishmania*). Once inside into the cells, the parasitic protozoa feed on host with its organelles, such as micropore in *plasmodium spp* and divide several times by which thousands of parasites is produced and then may be distributed in the body.[2,3] The outcome of these behaviors can be a decrease in the survival of the hosts and even a decline in the fecundity.

2. Control of parasite diseases
The necessity of parasites diseases control through various ways is very important (Fig. 2). During the infection, the parasite employs diverse virulence factors for propelling towards adverse conditions. Many of these

factors are recognized as an antigen and attacked by the immune system. In the traditional division, antigens are subdivided into somatic antigens and exoantigens or metabolic antigens. The materials of parasite body are the type of somatic and those that may be secreted or excreted are marked as exoantigens, which might be produced during certain periods of the life cycle. In the studies of serology, special attention is drawn over surface antigens of the parasite, because the fact that the induced antibodies in the serum should be easily detected and differentiated by its own antigens from specific infections.[3] Immunodiagnostic tests and some of the vaccine-based approaches are designed based on these antigen-antibody interactions and prospering in prevention and detection strategies of parasite diseases is dependent on recognition of serological responses as well as applying an ideal antigen.

Notwithstanding that, the pure and crude antigenic substances have the tangible ability in stimulating the immune system, producing well-defined and specific antigens from various parasitic organisms are employing to make better immune responses. With the advent of genetic engineering techniques in parasitology, efficient production of proteins in a controlled manner arose, which led to the development of many new previously unavailable proteins lacking problems of nature proteins such as stability. In diagnostic assays, however, the cross-reactivity from host proteins and background interference is a possibly encountered issue in using of recombinant antigens, which can be in part overcome by especially recent advances.[4] Furthermore, recombinant DNA technologies have been successfully employed for producing antigenic proteins from parasites in strategies of subunit vaccine design. Undoubtedly, such an approach should try to improve the efficiency and versatility of materials being used in the vaccine and thereby the purpose is designing the systems with high capability in inducing immunity. On the other hand, the choice of antigen for designing an ideal vaccine is determined on grounds of safety, immunogenicity, costly and time-consuming. In this regards, in comparison with other approaches of development of pathogen-derived vaccines, recombinant antigens due to be expressed in host cells, therefore will not be infected which offering a safe route to generation of the immunologic subunits. They also present less unwanted properties such as unwanted immune response, high reactogenicity, risk of reversion and weaker immunogenicity responses seen in the some of the approaches like whole-pathogen approach.[5,6]

3. Recombinant antigens in diagnosis

Recombinant antigens have brought about many studies in the detection of important pathogenic protozoan parasites or those that there are challenges in their diagnosis (Table 1).

3.1 Leishmania

With the development of molecular and serological assays such as enzyme linked immunosorbent assay (ELISA), indirect Immunofluorescence test (IFAT), indirect hemagglutination (IHA) test and latex agglutination test, significant progress has been observed in the diagnosis of parasites including *Leishmania* spp. However, these immunodiagnostic tests need to be improved in view of sensitivity and the specificity in respect of solubility of antigens. For this, recombinant antigens are being alternative candidates for soluble antigens.[7-9]

It has been shown that *Leishmania* genomes bear sequences for 8200 distinct proteins, which is a complex of nearly 3×10^5 MHC class I, epitopes. The proteins are accessible for the presentation either as surface exposure or through parasite lysis and directed release.[10] K39, a protein with 39-amino-acid repeats encoded by a kinesin-like protein gene, is most promising antigen in diagnosis of leishmania that has been evaluated widely.[11] This antigen also has been widely used for comparison of serodiagnosis techniques of leishmaniasis. In a systematic review with meta-analysis of the literature, rK39 strip-test has shown more sensitive and specific than the IFAT and ELISA with a promastigote antigen preparation (p-ELISA). The authors suggest considering two rK39 strip-test and Direct Aglutination Test (DAT) in endemic areas in which the parasite is not visible in bone marrow or spleen aspirate biopsies.[12] The other kinesin-related proteins such as K28, K26, K9, KE16 and KRP42 also have been successfully tested for their serodiagnosis of leishmania.[11] rK9 antigen has exhibited that may be used as an auxiliary antigen to rK30 antigen or as an alternative in the absence of rK39 antigen in accurate diagnosis of visceral leishmaniasis.[13]

In addition, leishmania heat shock proteins such as HSP70 and HSP83 and nuclear proteins such as lepp12, paplee22 H2A and H2B as recombinant antigens have been used with very high sensitivity and specificity for the diagnosis of visceral leishmaniasis. Souza *et al.* employed L. infantum-chagasi derived histones for serological diagnosis of human tegumentary leishmaniasis. The proteins after cloning into the pQE30 prokaryotic expression vector and expression and purification were tested against a panel of sera from cutaneous leishmaniasis and mucosal leishmaniasis patients and then ELISA was performed to evaluate sensitivity. Among the recombinant histone proteins, rHSP70 and rH2A showed the best reactivity with human sera obtained from endemic areas of tegumentary leishmaniasis.[14]

3.2 Toxoplasma

Toxoplasmosis in adult humans is usually asymptomatic. However, there is a possibility of occurring serious disease in congenital infections and immunocompromised individuals. Due to problem of specificities and sensitivities of serology tests in

diagnostic antigens, lately, many investigators have attempted to do use of recombinant antigenic proteins of *Toxoplasma gondii* as a useful alternative source in the serodiagnosis of toxoplasmosis.

Several dozen genes encoding *T. gondii* proteins like surface antigens (SAGs), dense granule antigens (GRAs), rhoptry antigens (ROPs) and other genes have been cloned into bacterial and eukaryotic expression systems for diagnosis of *T. gondii*, for example, detection of the parasite-specific antibodies in human serum samples.[14-16] Recombinant SAGs show good sensitivity and specificity of for detection of anti-Toxoplasma IgG and IgM and other parts of immunity systems.[17] In a study, SAG1, SAG2 and SAG3 showed sensitivities of 93.6%, 100.0%, 95.4% and specificities of 92.9%, 89.4% and 91.2%, respectively.[18] In a recent study, one of the recombinant granule antigens (rGRA7) has indicated that can be as a potential immunogenic antigen for immunodiagnosis of toxoplasmosis in patients with cancer. In this study, a newly DNA amplification technique, loop mediated isothermal amplification (LAMP) with extremely high sensitivity and specificity was used to verify the accuracy and validity of the results obtained by ELISA. The results of the ELISA had significant conformance with the results obtained by LAMP technique.[19]

Many studies employed various combinations of two or three toxoplasma proteins as coated on ELISA plates with higher sensitivity to antibodies than each protein alone. For example, MIC1 (microneme protein 1)-MAG1 (matrix antigen 1) recombinant chimeric antigen can be tested instead of the TLA (Toxoplasma lysate antigen) in the serodiagnosis of toxoplasmosis.[20] Cocktails of recombinant proteins with His tag domain such as rMAG1+rSAG1+rGRA5, rGRA2+rSAG1+rGRA5 and rROP1+rSAG1+rGRA5 showed sensitivities of 92.6%, 93.1% and 94.2%, respectively for IgG ELISA. This study also revealed that cocktails without rGRA5 antigen have lower potential in detection of anti-*T. gondii* IgG antibodies.[21] Chemeric antigens and multiepitope peptides that contain different immunoreactive epitopes of antigens with a high immunogenicity are also considered a kind of diagnostic tool for the detection of anti-*T. gondii* antibodies.[14,20] The results of study of Maksimov group suggest that predicting epitopes using bioinformatic approaches along with peptide microarray can be a powerful method for the selection of *T. gondii* epitopes in serum-based diagnosis.[22]

3.3 Plasmodium

Due to distinguishing characteristics of plasmodium species, the microscopic examination of blood samples is a surefire and reliable method for detection of malaria. However, in the regions of affording to implement molecular techniques, antigen tests can certainly represent more accurate results than microscopy. Furthermore, with production of commercial molecular tests for detection these tests do not have much experience and also lead to saving time. Hence, rapid diagnostic tests can be ideal alternative of light microscopy for diagnosis malarial infections especially in remote areas.

One of the popular proteins which has been used for detecting malaria parasites, is merozoite surface protein 1 (MSP-1), a 195 kDa glycoprotein. This surface antigen after two proteolytic processings release membrane bound MSP-1_{19} fragment. MSP-1_{19} is highly immunogenic and because it has been shown that parasite growth in vitro is inhibited by antibodies against MSP-1_{19}, therefore, MSP-1_{19} can be a potential serodiagnostic indicator for malaria diseases.[23] In the study of Sonaimuthu *et al.* after cloning P. knowlesi MSP-1_{19} in pRSET B vector and expression and purification, the rMSP-1_{19} could react with 95.5% of the P. knowlesi samples, 75.0% of the P. falciparum samples, 85.7% of the P. vivax samples and 100% of the P. ovale samples obtained from sera of infected patient.[23]

Mirahmadi *et al.* recently have successfully served MSP-1_{42}kDa, which contains both the 33 and 19kDa fragments, for the development of a sensitive serological test in diagnosis of P. vivax malaria.[24] Kattenberg *et al.* employed three plasmodium antigens, glutamate rich protein (GLURP), dihydrofolate reductase-thymidylate synthase (DHFR-TS) and heme detoxification protein (HDP) to immunize mice. The produced antibodies were screened for specificity against P. falciparum and P. vivax and the most optimal antibody couples were selected based on antibody affinity. The antibodies presented in this study showed good potential in diagnostic assays.[25]

3.4 Trypanosoma

Trypanosomiasis is readily diagnosed via direct demonstration of parasites by examination of the blood using light microscopy which is the most easily applied method. Some trypanosomes are nevertheless rarely found in body fluids including blood and so need to immunodiagnostic techniques is understandable because of high sensitivity of serology and its major role in diagnosis foreign antigens. Many recombinant trypanosome proteins have been tested in diagnosis researches with usually gold standard tests ELISA, IFA and IHA. Recombinant proteins also may be used individually or in the better method, in combinations of two or more.[26,27]

Of the proteins employed as recombinant in clinical uses, cytoplasmic protein CRA, cytoskeleton-associated protein FRA, trypomastigote surface protein B13, microtubule-associated protein Ag36, ribosomal proteins TcE and JL5 can be named.[28] In a study, recombinant proteins with demonstrated sensitivity and specificity in humans namely FRA (flagellar repetitive antigen), SAPA (shed acute phase antigen), CP1, Ag1 and a SAPA/TSSA (trypomastigote small surface antigen) V1 mixture of *T. cruzi* was analyzed in an ELISA format to diagnose

infection in infected dogs as main mammal reservoir of *T. cruzi*. The mixture had highest performance with sensitivity of 94% and among individual antigens, SAPA yielded the highest sensitivity (86%).[29]

Duthie *et al.* have used multi-epitope proteins for improved detection of *T .cruzi* in serological tests. It has been previously found that tandem repeat (TR) proteins from *T. cruzi* could be useful as diagnostic indicators in which serving as targets of the antibody response. In this study, the recombinant fusion proteins produced from TRs for detecting the parasites. The results indicated that TcF43 and TcF26 derived from the fusion of TR proteins could cause significant improvements in overall sensitivity along with developing a good distinction among negative and positive sera.[30]

4. Recombinant antigens in vaccine design

Parasitic diseases are responsible for some of the most devastating and common diseases of humans and also cause disease in animals that are widely associated with significant economic loss. Therefore, the control strategies such as vaccine development for parasitic diseases is necessary.[31] There are various strategies for designing and developing vaccine against parasites. Killed or attenuated pathogens, are first generation vaccines to induce immunity systems against parasites. Advances in immunology, molecular biology, biochemistry, genomics, and proteomics as well as our understanding of antigen presentation, including single proteins or synthetic peptides containing many B- and T-cell epitopes led to creation of next generation of vaccine (second generation). Moreover, novel molecular-based strategies applied for vaccine development such as DNA vaccines such viral vector-based vaccines established vaccine third generation.[32]

Conventional vaccine methods against parasitic infections are relatively unsuccessful in eliminating the pathogen because parasitic infections tend to be chronic in nature. This is the result of several factors including inappropriate and ineffective immune responses in the host, various immune evasion strategies such as antigenic variation, molecular mimicry and complex lifecycles and other biological characteristics.[33]

Vaccines based on recombinant proteins have several benefits over traditional vaccines, including safety and production cost. Nevertheless, they generate weakly immunogenic response when given alone due to dilution, degradation and elimination of vaccine by the host. As a result, use of adjuvants is necessary for eliciting a protective and long-lasting immune response[34] and increasing innate immune responses or lengthening their half-life.[35] Adjuvants should be selected in accordance with desired immune response for a particular vaccine. Different adjuvant formulations used in providing vaccines can induce very different levels of protective responses.[36]

Immunogenic surface antigens, which are expressed in almost every stage of the parasite lifecycle, are the most common antigens used for the construction of protein-based vaccines. Moreover, synthetic peptides are promising candidate vaccines for controlling parasitic diseases because they are highly immunogenic, safer and less inexpensive.[37]

4.1 Leishmania

World Health Organization (WHO) reported that Leishmaniasis is among the category-1 diseases described as emerging and uncontrolled diseases. Development of potential vaccine candidates is one of the prevention mechanisms.[38] There have been many attempts to develop a vaccine against leishmaniasis. Leishmanization is an effective protozoa vaccination strategy that has been used in humans for a long time. This strategy that consists of injecting viable parasites has capability of producing a controlled lesion in a nonvisible area in the skin. The major challenges in the development of vaccine against leishmaniasis are including the antigenic complexity, variability of the species Leishmania, wide range of responses among different hosts and cost associated with the development.[39] One way for the development of more innovative control methods is through using reverse genetic engineering on important enzymes, proteins and macromolecules. For example, Almani *et al.* showed that by generating a null mutant using remove of two alleles of GlcNAc-PI-de-N-acetylase (GPI12), might produce a mutant leishmania without any damaging to the host.[40] One of the major limitations in providing vaccine for leishmaniasis is the requirement of combining two or more antigens to conserve antigenic properties for various Leishmania species as well amastigote and promastigote phases of parasite.[41] Recombinant antigens studied to development of vaccines against visceral leishmaniasis include P0, CPI and CPII, GP63, FML, GP36, LiESAp, rLdccys1, SLA, rLdp45, rLelF-2, rLdPDI, rA2, KMP-11, rLeish-111f, Ldp27, pSP, rSMT, Histone H1, HSP70 and HSP83, LBSap, GP63 and HSP70, rLiHyp. The important parameters for designing anti-leishmanial vaccine are identification of suitable antigen, antigen delivery and induction of strong Th1 type immune response. Leishmune® as a second generation vaccine, has been shown good results for controlling canine visceral leishmaniasis.[42]

4.2 Toxoplasma

Toxoplasma gondii, the etiological agent of toxoplasmosis, which has a worldwide distribution, can induce abortion or considerable morbidity of fetuses. However, T. gondii infection in adults is usually asymptomatic or associated with self-limited symptoms (e.g., fever, malaise, lymphadenopathy). Vaccination against toxoplasma should be including of prevention of infection in human or that of clinical disease animals rose for human consumption, thereby preventing transmission also contamination of the environment by oocysts. Therefore, development of an effective vaccine

against toxoplasmosis that can be used in animals or humans can be valuable. To date, with the exception of Toxovax, a unique and live vaccine for sheep, there are no effective vaccines for controlling toxoplasmosis in human or animal.

The effective recombinant vaccines have been shown that have ability to act against both sexual and asexual stages of the parasite. Using recombinant proteins as vaccine antigens against toxoplasmosis has become popular after 1990s. At the first, SAG1 which is protein presented on the surface of the parasite and GRA1 (a dense granule protein) were evaluated as recombinant proteins in vaccination. The studies on mice vaccination against toxoplasma based on the selection of ideal protective antigens and recently more attention on combined proteins from membrane associated surface antigen, excreted-secreted dense granule proteins, rhoptry proteins and micronemal proteins.[43]

GRA7, ROP2, SAG2, SAG3, SRS1, HSP70, HSP30 (BAG1), MIC1, MIC2, MIC3, MIC4, MIC6, GRA2, GRA4, GRA5, GRA6, AMA1, MAG1 ROP4 MIC8, MIC11 and MIC13, ROP1, ROP5, ROP8, ROP9, ROP 13, ROP16, ROP17, ROP18 and ROP38, RON2, RON4, SAG2CDX, SAG5D, SRS4 and SRS9 from SRS, PLP1 (perforin-like protein 1), IMP1, ROM1, CDPK3, eIF4A, eIF2α, CyP, cathepsin B and L like, ACT (Actin, CDPK5, DPA, RACK-1, GST are the proteins that have been used for developing toxoplasma vaccines. Most of these vaccine antigens were chosen randomly without taking into account of *T. gondii*'s multistage property and consequently did not confer the desired immune responses.

The multivalent vaccine design for different life cycle stages of *T. gondii* show more effect that is efficient. For example, two proteins BAG1 and GRA1 with important roles in parasite's life cycle, one known as expressed by the bradyzoite form of the parasite (BAG1) inducing dendritic cells and activating cellular immune response and other expressed by tachyzoite, bradyzoite and sporozoite forms (GRA1) excreting to parasitophorous vacuole during the invasion of host cell. GRA1 and BAG1 genes can be used as vaccine candidate antigens in recombinant protein-based vaccination studies. The selection of appropriate model is also a basic principle in protective approach, whereas infection usually occurs via ingestion of cysts and oocyst in animals[44].

4.3 Plasmodium

Malaria as the most devastating parasitic disease is an infectious disease caused by five species of parasites belonging to the genus Plasmodium. This parasite in the multiple stages of lifecycle displays various antigens at the cell surface that can be used to develop vaccines.[45] Recombinant malaria antigens progressed to clinical testing include PfCSP; PfTRAP; PfCelTOS; PfAMA1; PfLSA1; PfLSA3; PfMSP1; PfMSP2; PfMSP3; PfGLURP; PfRESA; Pf27A; Pf11.1; PfEBA175; PfSERA5; Pfs230; Pfs25; PvCSP; Pvs25; [PfRH5; VAR2CSA; Pfs48/45; PvDBP].[46] For example, recombinant proteins made from fusion of PfGLURP and PfMSP3 expressed in *Lactococcus lactis* (a generally recognized as safe or 'GRAS' organism) is a type of antigens served in preventing the disease. This vaccine was targeted for a sexual blood-stage and aimed at reducing the parasite load in order to confer protection against clinical malaria.

According to the multistage life cycle of malaria parasites therefore, multivalent vaccines would be required to provide sufficient protection against plasmodium. One approach is the design of vaccine that incorporates several antigens. Synthetic peptides containing defined B and T-cell epitopes of different antigens expressed in various stages that can effectively target multiple stages of the parasite cycle. For example, Tamborrini *et al.* have described the immunological characterization of linear and cyclized synthetic peptides comprising amino acids 211-237 of *P. falciparum* merozoite surface protein (MSP-3).[47]

4.4 Trypanosoma

Trypanosomes are eukaryotic, flagellated hemo-protozoan parasites. Different species of trypanosomes infect a wide host range, including animals and humans. Since eradication of entire parasite reservoir of endemic areas is impassible and trypanotolerance is occurred in many mammals endemic, therefore the ultimate goal in the fight against this disease is to develop an effective vaccine.[48]

Tc24 is an excretory/secretory antigen which localizes into the plasma membrane throughout all life stages. Tc24 is produced by T. cruzi which, is a B-cell superantigen. Immune response to a Tc24 recombinant protein was evaluated by Meagan A. et al. In their study, a poly(lactic-co-glycolic acid) nanoparticle delivery system combined with CpG motif-containing oligodeoxynucleotides as an immunomodulatory adjuvant for elicitation cell-mediated immune response in a BALB/c mouse model. Result of this work demonstrated efficacy and immunogenicity of a therapeutic Chagas vaccine using a nanoparticle delivery system.[49]

However, so far development of a vaccine against trypanosomiasis do not have significant success because this parasite have potential of antigenic variation and thereby ability to escape the host immune response.[50] Accordingly, it is necessary to develop a vaccine based on the other potential target proteins of trypanosome. For example, San-Qiang Li et al. repoted that the recombinant T. evansiactin induces protective immunity against T.equiperdum, T. evansi and T. brucei infection.[51]

Table 1. Some recent studies on diagnosis of parasite by recombinant antigens.

Parasite	Species	Antigen(s)	Test sera	Diagnostic test	Result	Reference
Leishmania	L. infantum	Cytochrome c oxidase and IgE-dependent histamine-releasing factor	Human, dog	ELISA	100% sensitivity and specificity	[52]
	L. infantum	K28	Dog	ELISA	100% sensitivity and specificity	[53]
	L. chagasi	K9, K26 and K39	Human	ELISA	78% 38%, 100% sensitivity and 84%, 80%, 72% specificity, respectively	[13]
Toxoplasma	T. gondii	GRA7	Human	ELISA, LAMP	92% sensitivity and specificity 94% for ELISA, 96% for LAMP	[19]
	T. gondii	SAG1, SAG2, and SAG3	Human	ELISA	Sensitivities and specificities: SAG1 (93.6% and 92.9%), SAG2 (100.0% and 89.4%), and SAG3 (95.4% and 91.2%)	[18]
	T. gondii	ROP1, SAG2 and GRA6	Human	ELISA	Combinatorial cases with higher sensitivity than antigens alone	[54]
Plasmodium	P. vivax	MSA-1_{42}	Human	ELISA	86.9% sensitivity, 94.05% specificity	[24]
	P. knowlesi	MSP-1_{19}	Human	Rapid diagnostic kit, PCR, commercial ELISA and microscopy	95.5% sensitivity and 100% specificity	[23]
Trypanosome	T. cruzi	TcF43 and TcF26	Human	ELISA	Yielded strong responses against sera with high sensitivity and specificity	[30]
	T. congolense and/or T. vivax	TeGM6-4r	Cattle (buffaloes)	Immunochromatographic test (ICT) and ELISA	76.5 % sensitivity and 93.4 % specificity for T. b. brucei and 79.6 % sensitivity and 98.7 specificity for T. congolense	[55]
Others	Entamoeba histolytica	Galactose and N-acetyl-D-galactosamine-inhibitable lectin	Human	Microfluidic	great sensitivity with capability of rapid serodiagnosis of amebiasis with little sample consumption	[56]
	Trichomonas vaginalis	α-actinin and mixed lysate Ag	Human	ELISA	Mixed lysate Ag showed higher sensitivity and specificity than α-actinin. 79.5% and 100% for sensitivity and specificity of the mixed lysate Ag and 52.1% and 96.8% for sensitivity and specificity α-actinin, respectively	[57]

Table 2. Some recent studies on development of parasite vaccines by recombinant antigens

Parasite	Model used for testing	Antigen and strategy	Result	Reference
Leishmania infantum	Dog	Recombinant 14kDa polypeptide of L. infantum nuclear transport factor 2 (Li-ntf2)	Immunogenicity in dogs and protection	[58]
Leishmania donovani	Mouse	An engineered, yeast-expressed *Leishmania donovani* nucleoside hydrolase (LdNH36), mutation of four N-linked glycosylation sites (shown to contribute to heterogeneous high-mannose glycosylation) to glutamine (LdNH36-dg2)	Inhibition of hydrolase activity of the wild-type LdNH36	[59]
Toxoplasma gondii	Mouse	Different recombinant microneme proteins (TgMIC1, TgMIC4, or TgMIC6) or combinations of these proteins (TgMIC1-4 and TgMIC1-4-6)	The protective effect was related to the cellular and humoral immune responses	[60]
Toxoplasma gondii	Mouse	Recombinant TgMIC3 (rTgMIC3) recognized by both T. gondii-reactive sera and hyper-immune serum against N. caninum	Elicit some protection against toxoplasmosis and neosporosis	[61]
Plasmodium vivax	Mouse	Domain II (DII) of Plasmodium vivax AMA-1 (PvAMA-1)	Immunogenic when administered in different adjuvant formulations	[62]
Plasmodium falciparum	Mouse and rabbit	Conserved regions of the two leading malaria vaccine candidates, merozoite surface protein 1 (C-terminal 19-kDa region [PfMSP-119]) and merozoite surface protein 3 (11-kDa conserved region [PfMSP-311]).	Protective immune response	[63]
Plasmodium falciparum	Mouse	Recombinant Plasmodium enolase (rPfeno), testing of a parasite-specific epitope of enolase displayed on novel protein nanoparticles produced by a halophilic Archaeon Halobacterium sp.	Higher antibody titres against rPfeno and gas vesicle nanoparticles	[64]
Trypanosoma cruzi	Mouse	Tc24 calcium binding protein, using of a poly(lactic-co-glycolic acid) nanoparticle delivery system in conjunction with CpG motif-containing oligodeoxynucleotides as an immunomodulatory adjuvant	Significantly reduced systemic parasitemia during peak parasitemia, a significant reduction in cardiac parasite burden and inflammatory cell infiltrate	[49]
Trypanosoma cruzi	Mouse	A glycosylated mutant inactive trans-sialidase (mTS), produced in large quantities and high quality using Pichia pastoris	Protection against Chagas disease	[65]

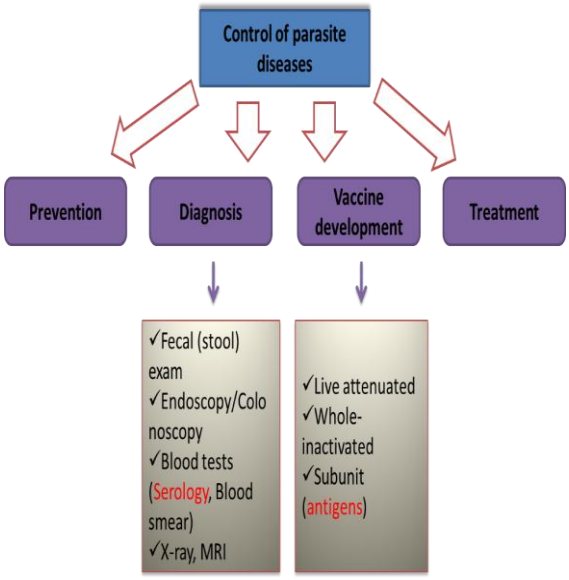

Figure1. Distribution and impact of major human diseases caused by parasitic protozoa.

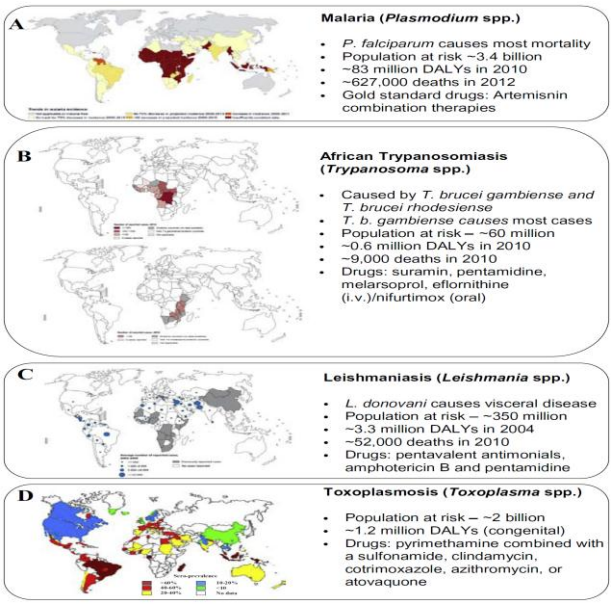

Figure 2. Control and management of diseases caused by parasites. With permission of Elsevier.[66]

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