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Targeting Parasites: Progress and Prospects in Parasitic Vaccine Development

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ABSTRACT: Parasitic diseases pose a substantial health risk and contribute to wide spread morbidity and mortality across the globe particularly in tropical and subtropical regions. To overcome this major cause of morbidity, parasitic illnesses need to be controlled by implementing a multidisciplinary approach including chemotherapy, biological control, host genetic resistance, and parasitic vaccinations. Developing parasitic vaccines a very crucial topic of research. Parasites not only affect humans but also effect the cattle production. Malaria Vaccine RTS S/AS01E (MOSQUIRIX) is the first anti parasitic vaccine for humans. Trypanosomiasis which causes slipping sickness can be controlled by anti- Trypanosomiasis immunization theoretically, but due to its genetic variation its very much difficult. In case of Helminthic infection such as Taeniasis, various antigens are cloned from *T. solium* oncospheres that are used as *S3P* vaccine. For hookworm's vaccines like *Na-ASP- 2* and Ac-APR-1 are still under trials. In Schistosomiasis, *Sm23* a DNA vaccine is used to reduce liver egg number. There are two DNA vaccines for lymphatic filariasis made up of *BmHSP* or *BmALT*. There is no vaccine available for *Fasciola hepatica* but the purified *cathepsin L* proteinase is used as formulation. After many years of work, it is concluded that native recombinant proteins bring about some protection against the target parasite but only few gives degree of effectiveness. Adjuvants of different kind are used to increase efficiency of these vaccines. A lot of challenges remain for the development of vaccine that is safe without any side effect, and offer a lifelong immunity. Parasites' antigenic complexity, various life cycle stages, immune evasion techniques and the utilization of intermediate and reservoir hosts pose challenges in the development of a effective parasitic vaccine. On a biological point of view genetic variation in parasites is the major hindrance in the vaccine development. These researches aren't cheap and unfortunately poor underdeveloped countries with Neglected Tropical Diseases (NTD) are most effected by the parasites. Present paper reviews the developments made in vaccines against human parasites.

Keywords: Parasitic Vaccines, Helminths, Antigen variability, Animal models, Global parasitic impact, multivalent vaccines.

INTRODUCTION

Parasitic infections are very common in developing countries showing high prevalence amongst socioeconomically deprived sections of a community where overcrowding, poor environmental sanitation, lack of education, negligence towards public health by both the community as well as the authorities and lack of access to safe water are prevalent resulting them in a perennial cycle of poverty and destitution (Adamson & Caira, 1994). A parasite is an organism inhabiting other organisms called hosts for its nutrition and shelter needs. Parasitic infections are one of the major causes of morbidity around the world. In less developed countries protozoan and helminthic parasitic diseases is significant cause of morbidity (Higashi, 1988). These diseases are caused by various parasites including protozoa, helminths and ectoparasites. These parasitic infections can manifest in many ways ranging from mild discomfort to severe illness and even deaths.

Parasitic diseases like malaria, chagas disease, leishmaniasis, trypanosomiasis, schistosomiasis are categorised as Neglected Tropical Diseases not only cause significant health issues in man and animals but also have a profound socio-economic impact particularly in low income countries. Parasitic diseases leads to economic instability and social marginalization while affecting the poor most due to malnutrition and repeated infections causing excess morbidity with children being the most vulnerable (Ngui *et al.,* 2011). As per Bradley (Bradley *et al.,* 1992) a high degree of intestinal parasitic infections are found amongst children residing in slums, shanty towns and squatter settlements. Rural migrants to the slums, shanty towns in the urban and semiurban areas of developing countries are most vulnerable. Similar health problems are prevalent amongst poor people of cities in developing countries. Both migrants and long-term slum dwellers bear the burden of parasite infections because the city authorities' limited resources are overburdened, and their services for water supply,

sanitation, waste disposal, health care, and hygiene are poor. For example, (Crompton & Savioli 1993) reported that of the 7 million people of Dhaka, Bangladesh, over 3.5 million live in slums, with only 6% having access to elementary education and 3% to primary health.

Parasitic infections are quite common in certain parts of the world since antiquity. However, it is somewhat managed in developed countries by the help of sanitation, clean water, pesticides and controlling vectors. Still in lesser developed countries (around 130) it is still a major health hazard. According to WHO parasitic infections caused over 25 million cases and more than 5000 deaths each year around the world (Leiby *et al.,* 2019). Intestinal parasitic infection are prevalent in children of poor communities around developing countries (Norhayati *et al.,* 2003).

A multidisciplinary approach encompassing chemotherapy, biological control, host genetic resistance, and parasitic vaccinations are to be implemented in order to prevent a public health disaster (Sharma *et al.,* 2015). Vaccines not only help in preventing the infections but also help in reducing and preventing the spread of diseases. Vaccination becomes even more crucial in areas where other control measures like vector control and sanitation are challenging to implement effectively. Many parasites have sophisticated immune evasion mechanisms, making it challenging to envision the development of effective vaccine. They exhibit complex life cycles and other biological traits, complicating vaccine research against them (Crampton & Vanniasinkam 2007). Also due to parasites' antigenic complexity, various life cycle stages, immune evasion techniques, the utilization of intermediate and reservoir hosts and getting sufficient numbers of parasites, attenuated or otherwise, of constant and acceptable quality to utilize in vaccines is quite difficult causing a hindrance in vaccine development (Mutapi *et al.,* 2013). However ongoing research and development efforts with rapid progress of immunology and the genetic modification of cells offers hope for the future with several promising vaccine candidates in various stages of development for diseases like malaria, Leishmaina and schistosomiasis (Sharma *et al.,* 2015).

Food borne disease are a public health problem worldwide constituting a major part of parasitic infections. To overcome this disastrous problem, the research for parasitic vaccines is the demand of the modern times. The anti-parasitic vaccine is one of the fastest growing sector of animal health market which is over $18 Billion (Rogier, 2007). A number of parasitic vaccine such as mosquirix for malaria are developed for human trials in African counties. Tickgard a vaccine for ectoparasitic ticks is commercially available in Australia since 1997 (Leiby *et al.*, 2019).

GLOBAL IMPACT OF PARASITISM

Parasites have a profound global impact on human health, agriculture, ecosystem, social dynamics and economies. Parasites besides causing wide range of diseases in humans also reduce productivity. Additionally, the cost associated with healthcare, treatment and control measures for parasitic diseases are substantial, diverting resources that could be spent on other essential and developmental activities. Parasitic disease beside humans also majorly effect livestock. Parasites such as ***Haemonchus contortus*** effects large scale sheep/goat farms in tropical and subtropical areas. Food borne infections are a major part of this. Food borne parasitic diseases cause 23.3 Million cases and 4500 deaths annually (Knox & Redmond 2006).

Vaccination is considered to be a sustainable option for controlling parasitic diseases. However, vaccine development against tropical parasites is challenging for both scientific and economic reasons. Vaccines proved crucial in the elimination and reduction of wide variety of infectious diseases such as smallpox in 1980, polio in 1988 post the WHO Global Polio Eradication initiative and other illnesses, such as measles, diphtheria, tetanus, rubella, and mumps. Messenger RNA vaccines use IVT (in vitro Transcribed) mRNA as a template to generate vaccination antigens in a patient. Pathogen-specific antigens can trigger an immune response based on the cell type and immunogenicity of both the mRNA and antigen (Versteeg *et al.*, 2019). The main obstacles to overcome in vaccine development which involve challenges of in culturing of parasites, variability of antigen and lack of animal models (Rogier, 2007). The complexity of life cycle of most parasites along with genetic variability is the major hindrance in the preparation of vaccines. Limiting the impact of parasitism is challenged by the wide spread appearance of drug resistance in animals and man (Kaplan, 2004). In 1980s the experiments to produce recombinant parasitic proteins is considered as a major accomplishment for vaccine development. Yet only few parasitic recombinant vaccines are made for livestock after 43 years (Enea *et al.,* 1984). Blood transfusion is also cause of parasitic infection spread *i.e.,* it poses great risk to blood recipients (Leiby *et al.,* 2019). Even after great efforts for decreasing the impact of malaria approximately 212 million people are still infected each year with more than 429000 deaths each year (WH, 2016).

Ectoparasitic vaccines. Ectoparasites like flees, ticks and lice reside on outer surface of the host. In 1990s first anti-tick vaccine was developed in Australia by Australia's commonwealth scientific and industrial research organization (CSIRO). Proteins from the blood feeding cattle tick ***Boophilus microplus*** were isolated to get (Bm86) antigen (Canales *et al.,* 2009). This antigen is found on the digestive cells of tick gut. ***Boophilus*** **spp.** economically impact cattle production by reducing weight gain and milk production. Two vaccines using BM-86 were registered in Latin America **(GaVac)** and Australia **(tickGARD**) during 1983- 1997 (De la Fuente *et al.,* 2007). The gut proteases of ***H. contortus*** as vaccine components were researched (Knox & Smith, 2001). A vaccine **(Barbax and Wormvax**) which contain two native gut membrane protein **(H11 and H-galQ)** is licensed in Australia in 2007 (Nisbet *et al.,* 2016). With incorporation of genetics, transcriptions, proteomics

and metabolomics will step up the progress of more safe, effective and reliable vaccines (Ehsan *et al.,* 2020).

MALARIA

The *Plasmodium* species of parasites, single- celled organisms with various life stages and multiple hosts required for survival, are the primary cause of malaria (Gardner *et al.,* 2002). In 1897, Ronald Ross discovered the mosquito (vectors) that transmit the disease. 212 million people were infected with malaria infection in 2015, especially in less developed tropical areas such as sub-Saharan Africa causing fatality of 428000 people. 500000 people who succumbed to malaria were with children below age 5. Global efforts have brought down the malarial infection in past decades yet it still threatens millions of children. An effective vaccine will prove as a boon in malaria control strategies. Unfortunately, due to complexity of malaria parasite biology, immune evasion and the intricate nature of the parasites infection cycle, it remains a hinderance (Mahmoudi & Keshavarz 2018).

Plasmodium falciparum, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi*- are the five species of the parasite that cause sickness in humans. The most lethal human strain of *Plasmodium*, *Plasmodium falciparum*, is the focus of the majority of scientific study (Gardner *et al.,* 2002).

The parasite has a complicated lifecycle that begins when infective Female *Anopheles* mosquito inject sporozoites into blood during a blood meal. Three basic approaches to malaria vaccination include to elicit a robust immune response that can stop pre-erythrocytic merozoites from entering the blood. Vaccination that produces immunity that limits the spread of disease and population – based immunization designed to stop further mosquito transmission (Srinivasan *et al.,* 2017).

MALARIA VACCINE

The first vaccination methods developed by Edward Jenner, Louis Pasteur, and Robert Koch established that dead, live-attenuated, or xenogeneic microbes elicited host-protective immunity. Over the next century, the immune system's mechanisms and vaccine-induced protective immunity were gradually understood, and the core concept of vaccination was established. It claims that an immunogen is capable of producing long-lived immunological memory and protective immunity against re-exposure to the same pathogen (Zutshi *et al.,* 2019). Lately, malaria control interventions like artemisinin-based combination therapy (ACT), the antimalarial drug class of choice, and insecticide-treated bed nets (ITNs), as well as other mosquito vector control strategies are being adopted in tropical countries. Yet *P. falciparum* developed resistance to antimalarial drugs, and in Asia to the artemisinin derivatives (Crompton *et al.,* 2010).

The development of a malaria vaccine is difficult because of the complex *Plasmodium* life cycle. Researchers need to decide which stage of the parasite to attack, or whether the vaccine should contain components that attack multiple stages. The possibility of a malaria vaccine is however encouraged by recent discoveries. Researchers has been improved the studies to create variety of prospective malaria vaccines. Most scientists currently employ technology to extract and deliver particular antigens in a vaccine instead of creating a live attenuated vaccination. Additionally, three separate vaccine strategies are being researched because parasite has three distinct life phases (Ann Stewart & Coppel 2009).

PRE-ERYTHROCYTIC VACCINES

Pre Erythrocytic vaccines target the infectious phase and work to either stop sporozoites from infecting liver cells or to kill those that already become infected. The time frame presents the biggest difficulty for pre-erythrocytic vaccine. The sporozoites enter the liver less than an hour after being injected by the mosquito. The immune system only has a short window of opportunity to get rid of the parasite (Ann Stewart & Coppel 2009).

Pre erythrocytic vaccines aim to induce antibodies against surface antigens that remove sporozoites from the skin or circulation or prevent their invasion of hepatocytes (Clyde *et al.,* 1973). Malaria vaccine RTS, S/AS01E (Brand name MOSQUIRIX) received a favorable opinion and most advanced malaria vaccine used for the prevention against *Plasmodium falciparum.* Mosqurix has been developed and deployed in use in certain regions. This is the first and only vaccine for malaria having received regulatory approval for use against malaria. This vaccine targets particularly *P. falciparum* that causes the deadliest form of malaria, cerebral malaria and predominant in Sub Saharan Africa. It works by targeting the pre erythrocytic stages of parasite that occurs in liver before the parasite enters the blood stream and cause symptoms. Mosqurix stimulates host immune system against parasites circumsporozoite protein preventing it from establishing in the liver. To develop the RTS, S vaccine, researchers discovered the protein that was primarily responsible for protection with the irradiated sporozoites. The circumsporozoite protein, or CS protein, was the antigen. Even though this antigen was protective, it did not have a great ability to elicit an immune response on its own. Therefore CS protein antigen was combined with the Hepatitis B virus surface antigen, which is the antigen that confers protection in the Hepatitis B vaccination. The objective was to raise antibody levels to inhibit sporozoites from entering liver cells and to identify specific infected cells (Ann Stewart & Coppel, 2009). Clinical trials have shown that RTS, S/AS01 vaccine provides partial protection in infants and children reducing the risk of severe malaria. The efficacy is also known to vary with age, malaria transmission intensity and other factors. RTS, S/AS01 is a mile stone in malaria vaccine continued research, innovation and investment are needed for development of new and improved vaccines against malaria.

ERYTHROCYTIC or BLOOD STAGE VACCINE

Blood stage vaccine targets the asexual forms of

parasite that undergo repeated multiplication in erythrocytes and causes diseases and death. Blood stage of infection is when symptoms first occur and that is also the most harmful to the patient red blood cells are burst in this stage due to multiplication of parasite. A blood-stage vaccine can only seek to lower the quantity of merozoites infecting red blood cells rather than total blocking their replication because of the enormous number of merozoites produced during this stage. Development of Blood-stage vaccinesPfRH5 and AMA1-RON2 are under clinical trials. **PfRH5 –** Utilizes a viral-vectored prime boost immunogen, *Plasmodium falciparum* reticulocyte-binding protein homolog 5 **(PfRH5),** which binds the crucial red cell receptor and exhibits minimal polymorphism, began clinical trials (Crosnier *et al.,* 2011).

When virulent heterologous parasites were challenged in monkeys, various combinations of PfRH5 viral-vectored and adjuvant protein immunogen induced protective immunity that reduced parasitemia (Douglas *et al.,* 2015). Jenner Institute created human **mAbs** from **PfRH5** vaccine recipients and utilized them in structural investigations to pinpoint the epitopes that neutralizing, non-neutralizing and potentiating antibodies target (Alanine et al., 2019). **AMA1-RON 2 –** AMA1 (Apical MembraneAntigen-1) is a crucial protein for the growth of blood-stage parasite. AMA1 binds to the rhoptry neck protein RON-2 at the merozoite-erythrocyte interface to induce invasion. Stronger anti-invasion antibodies are produced by AMA-1antigen when it forms a compound with the RON 2 peptide than when it is monomeric. When compared to AMA-1 alone, AMA1-RON2 provided significantly higher protection in monkeys against heterologous blood-stage (Srinivasan *et al.,* 2017). Ongoing research is needed for developing more effective vaccines that provide long lasting and broader protection against multiple species of malaria.

TRANSMISSION BLOCKING VACCINE

Vaccines of this kind target the stage of sexual reproduction that takes place in mosquito gut. It aims to eradicate the parasite's vector, the Female *Anopheles* mosquito. This is an indirect method of vaccination since it prevents the parasite from spreading further rather than immediately protecting a person who contracts it. The vaccines which contain *Plasmodium falciparum* surface protein antigens Pfs25 and Pfs28 or their *Plasmodium vivax* homologues are Pvs25 and Pvs28 (Wu *et al.,* 2008). The principle behind this vaccination is that if the body can produce antibodies against the Pfs25 antigen, some of these antibodies will be ingested by a mosquito consuming blood. The antibodies will then come in contact with the antigen there, allowing them to stop the parasite's development and kill it (Leiby *et al.,* 2019).

Trypanosomiasis. Trypanosomiasis is one of the parasitic infection called African sleeping sickness caused by *T brucei, T. gambiense*, transmitted to human by a bite of Tse-Tse fly. With a very high mortality rate there are so many people that are infected every year because there are no drugs. Protozoa of these kind have ability to change their outer antigenic proteins to adapt for their hosts in successive population of their kind (Higashi, 1988). The strong theoretical defense against the disease that alternately inhabits its reservoirs in people and cattle is still anti-trypanosomiasis immunization. Although antigenic variation of the parasite surface coat has been thought to be the main barrier to the creation of a useful vaccine current studies into the biology of B cells have suggested that the issues may extend beyond this (Magez *et al.,* 2010).

African trypanosomiasis are notorious to their capacity to swap the immunodominant variable surface glycoprotein (VSG) coat throughout infection in order to evade immune eradication. Antigenic variation is just one of many ways trypanosomes use to use to influence their hosts immune system. The involvement of hosts factors like **IFN – gamma** as well as parasite factors like Gp1 anchor remains of the shed VSG molecule and discharge of CpG DNA in controlling important feature of innate and acquired immunity during infection (Mansfield & Paulnock, 2005). The expressed VSG gene is a component of a sizable polycistronic transcripton unit that become visible to be a transcribed by a specialized RNA Polymerase and this transcription of the VSG gene only takes place at telomeres (Borst *et al.,* 1996).

The trypanosomes exhibit antigenic diversity with a focus on genetic mechanisms and the expression site of gene encoding variant surface glycoproteins are expressed. In trypanosomes only one expression site is active at a time. By inserting a different VSG gene into an active expression site, trypanosomes can also alter the gene that is expressed. A silent VSG gene is duplicated and transposed into the expression location in this substitution (Horn, 2014).

Taenia solium. *Taenia solium* is a zoonotic parasitic disease that causes cysticercosis, in which the pig act as the intermediate host. The term taeniasis refers as intestinal infection with tapeworms. There are three parasites cause taeniasis in humans, *T. solium*, *T. saginata* and *T. asiatica*. Ingested *T. solium* egg, develop to larvae in various organs of human body. When they enter the central nervous system, they can cause neurological symptoms (neurocysticercosis) including seizures. More than 80% of the world's 50 million people who are with epilepsy live in low and lower middle-income countries. An estimated 2.5 million are infected with *T. solium* and there are 50000 deaths annually due to neurocysticercosis. Vaccination of pigs have been investigated as a measure to control transmission of *Taenia solium* to human (Jayashi *et al.,* 2012; Lightowlers, 2010).

Recently a recombinant vaccine produced in E. coli is highly effective at reducing *T. solium* pig infections under field conditions (Sciutto *et al.,* 2008). Various antigens are cloned from *T. solium* oncospheres that are used as S3P vaccine (Gauci & Lightowlers, 2001) *i.e.,* having recombinant antigens. These oncospheres antigens have provided the highest level of protection. Three different types of protective antigens have cloned from *T. solium* oncosphere there are: TSOL18,TSOL45 (Gauci & Lightowlers 2001) and TSOL16 (Gauci & Lightowlers 2001). There is a small antigen (about 112 AA) and it is possible that whole

antigen can be synthesized as vaccine *i.e.,* TSOL18. The antigen was expressed as a fusion protein Glutathione. S. transferase (GST) via PGEX plasmid vector (Smith & Johnson 1988).

Hookworm. Intestinal hookworms are parasitic, blood-feeding roundworms that lead to helminthiases. Hookworms are known as Soil Transmitted Helminthes. The two primary species of hookworm which causes infection in humans are- *Ancylostoma duodenale* and *Necator americanus.* These are the most common parasitic infection of human in developing countries. Hookworm eggs are transmitted through the stool and grow in the contaminated soil and feces to develop into infective third stage larvae. The larvae bore into human host- typically through bare feet and travel through the blood arteries to the heart and finally to the lungs. Through the pulmonary alveoli they reach the pharynx, and then are swallowed into small intestine's jejunum, where they live and develop into adults. Intestinal blood loss is the main sign of the hookworm infection (Diemert *et al.,* 2008).

Hookworms does not directly contribute to a considerable amount of mortality, but chronic anemia and protein malnutrition are both significant causes of morbidity. Chronic hookworm infection hinders intellectual and physical development of children and also affects pregnant women and their new born. The infection is controlled by the use of anthelmintic drugs-mebendazole and albendazole of class benzimidazole. The regular drug uses necessary to maintain control would probably result in drug resistance. Drug treatment does not, however, prevent reinfection. Consequently, there is a need to create a vaccination that is both reliable and economical (Knox & Redmond, 2006).

Hookworm vaccine. Currently there is no effective vaccine developed for hookworm infection. Vaccines like Na-ASP-2 from *Necator americanus*and Ac-APR-1 from *Ancylostoma caninum* are currently in clinical trials. **Na-ASP-2** is a protein which infective hookworm larvae secrete when they enter their host, they release this 21 kDa protein called Na-ASP-2, also known as *Ancylostoma* Secreted Protein-2 of *Necator americanus*. Recombinant ASP-2 vaccination reduces worm loads and fecundity after challenge infection in hamsters and dogs. Additionally, *in vitro* larvae migration of infectious hookworm through tissue is prevented by sera from vaccinated animals. Antibodies to ASP-2 are linked to a lower chance of getting severe hookworm infections, according to studies of populations living in hookworm endemic areas (Bethony *et al.,* 2005). In laboratory animal models, recombinant Na-ASP-2 produced in *Pichia pastoris has* demonstrated considerable protection, with sera from animals immunized with ASP-2 blocking the migration of infectious larvae through host tissue *in vitro.* According to the latter finding, antibodies against Na-ASP-2 may weaken larvae during tissue migration and prevent them from making it to the human gut where they would otherwise mature into healthy adult hookworms (Bethony *et al.,* 2005).

Ac-APR-1(*Ancylostoma caninum*-Aspartic Proteases-1). Aspartic haemoglobinases Ac-APR-1 and Na-APR-1 from *Necator americanus* were expressed in catalytically active form and cleaved host hemoglobin at numerous different locations, including the hinge region (Williamson *et al.,* 2004).

Recombinant Ac APR-1 vaccination dramatically decreased the number of hookworms and fecal eggs in dogs, and these dogs were also protected from blood loss and did not develop anemia (Loukas *et al.*, 2005). *In vitro* enzyme activity inhibition by IgG from APR-1-vaccinated animals and antibody binding in situ to worms recovered from APR-1-vaccinated dogs suggested that the vaccination inhibits the parasite's capacity to consume blood. This was the first account of a hematophagous parasite-derived recombinant vaccination that considerably decreased both parasite load and blood loss, enabling the creation of APR 1 as a second human hookworm vaccine arm. APR-1 homologue discovered in *Necator americanus* also produced protection against *Ancylostoma caninum* in dogs in a catalytically inactive form. High circulating IgG1 levels to the inactive Na-APR mutant were found in residents of high-transmission areas for *Necator americanus*, suggesting that natural boosting may occur in exposed humans (Pearson *et al.,* 2009).

Schistosomiasis. Schistosomes or blood flukes are parasitic worms responsible for disease schistosomiasis a neglected tropical disease affecting millions of people worldwide. It is the disease of poverty that leads to chronic ill health issues like liver damage, kidney failure, infertility and bladder cancer. Infection is acquired when people come in contact with fresh water infested with the larval forms (cercariae) of parasitic blood flukes *i.e*., schistosomes. Schistosomiasis affects almost 240 million people worldwide and more than 700 million people live in endemic areas. Deaths due to schistosomiasis is about 11792 globally per year. The infection is prevalent in tropical and subtropical areas in poor communities without portable water and adequate sanitation (Barakat, 2013).

Urogenital schistosomiasis is caused by Schistosoma haematobium and intestinal schistosomiasis by any of the organisms *S. intercalatum*, *S. mansoni*, *S. japonicum* etc. The control of schistosomiasis over the last several decades have been centered on the mass drug administration (MDA) of Praziquantal (PZQ) which is the only drug currently available for treatment. PZQ is infective against juvenile schistosomes, but it does not prevent re-infection. Schistosomiasis control have been reached in targeted geographic areas such as Egypt (Barakat, 2013) and China (Gray *et al.,* 2014). Vaccine against schistosomiasis can provide a long-term immunity and significantly reduce transmission. Several vaccine candidates are under trials and in various stages of development including pre-clinical and clinical trials. **Sm-TSP-2**: One of the leading candidates, based on the tetraspanin protein found on the surface of schistosomes. It has shown promise in preclinical studies and is currently in clinical trials (Tran *et al.,* 2006).

Immune response to Schistosomiasis has two distinct components: Immunopathogenesis - resulting from host immune response against antigen released from schistosome eggs trapped in tissues and age dependent,

immune resistance to re infection which leads to protective immunity. Worm elimination is done via coordinated immune response by the host (Cutts & Wilson 1997; Wilson *et al*., 2008).

In case of PZQ there is the production of IgE antibody in host body that is produced at a time of allergic reaction which is associated with aggravating granuloma and fibrosis by egg induced responses (Molehin, 2020). *S. mansoni* 14kD fatty acid binding protein (Sm14/GLA-SE) and tetraspanin SmTSP-2/AL hydrogel. These approaches being used for vaccine development have been reviewed thoroughly (McManus & Loukas 2008).

Wuchereria bancrofti. *Wuchereria bancrofti* cause the lymphatic filariasis. It is estimated that over one billion people are at a risk of getting infected with this disorder whereas over 121million individuals are already infected with the lymphatic filariasis in the world (Organization, 2011). WHO started a program in 2000 Global Programme to Eliminate Lymphatic Filariasis (GPELF) having two goals to prevent the spreading of infection and recommended essential package of care (Hotez, 2009).

Filarial parasites secrete the modulatory molecules by the infected larval stage (L3) such as glutathione-s-transferase, catalase, superoxide dismutase, peroxiredoxins, glutathione peroxidase etc. that are important for their survival in the host body (Dzik, 2006). The *W. bancrofti* infected larval glutathione-s-transferase is critical modulatory molecule essential for the survival of *W. bancrofti* parasite (Veerapathran *et al.,* 2009).

The GST gene (glutathione-s-transferases) was cloned from the third stage of larva of *W. bancrofti* in cDNA libraries and recombinant GST was expressed and purified and analyzed with the serum sample from individuals living in an endemic area for their reactivity with rWbGST (recombinant *Wuchereria bancrofti* glutathione-s transferases). The analysis shows that the normal people of an endemic area carry the significant level of anti

WbGST (*Wuchereria bancrofti* glutathione-s-transferases) IgG antibodies compared to those who are having the symptoms of clinical pathology pre dominance of IgG1 and IgG3 antibodies in individual living in the endemic area. The enzymatic activity of GST is retained by the rWbGST and the antibodies in the individual living endemic area inhibits this enzymatic activity (Veerapathran *et al.,* 2009).

There are two types of vaccines for lymphatic filariasis monovalent and multivalent DNA vaccines. Monovalent vaccine is made up of BmHSP or BmALT-2 in pVAX1 vector (eukaryotic vector). Multivalent vaccine made up of BmHSP and BmALT-2 genes in the same vector pVAX1 vector. Firstly, the BmHSP gene was cloned and then in the same vector BmALT-2 gene was cloned in the same vector. As the result the multivalent and BmALT-2 monovalent vaccine is more effective as compared to the BmHSP monovalent vaccine (Gnanasekar *et al.*, 2004).

Fasciola hepatica. *Fasciola hepatica* is parasitic trematode which causes Fascioliasis in ruminants and humans. *Fasciola hepatica* is highly prevalent in South American countries. Northern Iran and Egypt (Esteban *et al.,* 2003). The endoproteinases are secreted by the mature and immature liver flukes termed as Cysteine proteinases. After the purification they termed as Cathepsin L proteinases (Piacenza *et al*., 1999). There is no vaccine for the fascioliasis. The purified cathepsin L proteinases endoproteinase is used as vaccine formulation. Cathepsin L 5 and Cathepsin L 1 g is purified from S. cerevisiae BJ 3505cells were preceded according to Law *et al.* (2003); Smooker *et al.* (2000) (Law *et al.,* 2003; Smooker *et al.,* 2000). The cocktail vaccine of recombinant proteins provides the higher level of protection in the sheep towards the Teladorsagia infection after the recombinant antigen applied the booster dose was required (Nisbet *et al.,* 2013; Villa-Mancera *et al.,* 2014).

Single or multivalent vaccines. Single or monovalent vaccine is a kind of vaccine prepared to immunize against a single microorganism or single antigen whereas multivalent or polyvalent vaccines are prepared from different strains (serotype/serogroup) of one pathogen in a single vector to immunize against two or more microorganisms. Both of them comes from protein antigen (Kazmi, 2021). The efficacy achieved by parasitic vaccines is often seen to be reduced due to multiple reasons like lack of knowledge between host – parasite relationship, complex life cycle of parasites and the most important reason is antigenic variations. But scientists are still putting their full efforts in the research & developments of various parasitic vaccines.

Over the past few years, a number of parasitic antigens have been notified. The purified native proteins or recombinant proteins, bring about some sort of protection against the target parasite but only a few out of them gave degree of effectiveness to make them as a candidate for single antigen vaccine. Therefore, multiantigen or cocktails vaccines were put forward based on the thinking that as they have combination of different antigen so they will surely show enhanced effect (Knox & Redmond, 2006).

Knox and Redmond (Knox & Redmond 2006) commented that parasites are challenging organisms, and it is probably native to believe that a immune response may be triggered by a vaccine with a solitary protein (Willadsen, 2008) like cattle immunized with a mixture of two *T. saginata* recombinant oncosphere protein were nearly entirely resistant against experimental challenge infection whereas, neither antigen was protective when they test them separately (Lightowlers, 2010). Another example which show antigen pairing may be more efficient are series of test for the immunization of cattle against *F. hepatica* which show that animal immunized with mixture of cathepsin L1 and hemoglobin provided much more protection than that of either antigen alone (Dalton *et al.,* 1996).

By combining the genes that encode different antigens, DNA vaccination offers a means to deliver antigen combination into a single construct. Leishmania (Méndez *et al.,* 2002) and malaria, the constructs targeting more than one parasite stage, are the two diseases for which this strategy is now being investigated. Additionally, immunomodulators like

CpG motifs and cytokines can be included into DNA vaccine constructions to help trigger the proper immune response (Abdulhaqq & Weiner 2008).

A novel class of preventive and therapeutic vaccination method for parasitic illness is in vitro transcribed mRNA vaccines. Three parasite protozoa (*Plasmodium* spp. (Baeza Garcia *et al.,* 2018; Mallory *et al.,* 2021), *Leishmania donovani* (Duthie *et al.,* 2018), and Toxoplasma *gondii* and the black-legged tick, Ixodes scapularis, are the targets of research efforts to date in order to create mRNA vaccines.

Vaccine Adjuvants. Adjuvants can be defined as functional filling materials consisting of diversified group of compounds. They can be classified into Delivery systems or Immuno-stimulators. Some adjuvants possess both properties. The function of Delivery System is to carry the antigen whereas the Immuno-stimulators are particles used to increase the efficacy and enhance body immune response (e.g. Emulsion droplets, Liposomes) (Mohan *et al.,* 2013; Perrie *et al.,* 2008).

There are various type of adjuvants such as TLRs-, nucleotide binding oligomerization domain like receptors (NLRs)-, C type lectin receptors (CLRs) based and some other PRRs agonists, NLR family pyrin domain containing 3 (NLRLR3) activators, formulations including liposomes, adjuvants systems (AS), immune complexes etc (Del Giudice *et al.,* 2018). AS01, AS 02, AS03, AS04 and MF59 etc. have attracted a lot of attention regarding their efficacy (Del Giudice *et al.,* 2018).

ADJUVANTS UNDER CLINICAL EVALUATION

Liposomes - are formed due to flat packing upon scattering or distribution of some amphiphilic molecules in an aqueous buffer (Bangham, 1972; Lasic, 1998). The adjuvanticity of liposomes is specified because of their ability to interact with antigen presenting cells. They also disclose the antigens as well as the immunostimulators to the APCs (Brunner *et al.,* 2010). They are highly expensive and cause pain at the site of injection (Mata *et al*., 2013). The instability of the liposomal vaccines causes the formulation to be blended or amalgamated with vaccine before their administration (for example Mosquirix vaccine) (O'Hagan & Fox 2015).

AS02 – The immune stimulants present in AS02 are QS 21 and MPL (3 deacylated monophosphoryl lipid A). The two immunostimulants are in the squalene O/W emulsion (Mata *et al*., 2013; O'Hagan & Fox 2015). The different malarial antigens like LSA –1 (liver stage antigen) and PfCS102 (a Plasmodium falciparum circumsporozoite protein immunogen) when mixed with AS02 showed an increased Th1 response (Audran *et al.,* 2009). RTS, S/AS02 formulation provided successful cell mediated immune response and an outstanding protection against Sporozoite – challenge Malaria than other AS formulations (Garçon & Di Pasquale 2017; Regules *et al.,* 2011; Stoute *et al.,* 1998).

Emulsion – For the development of Malaria vaccine, no progress was made with MF59 because of very less efficacy (Corradin & Giudice 2005).

Montanides (ISA 720) It is a w/o type emulsion. It mainly consists of Mineral oil and non-Mineral oil and have Mannidee monooleate as an emulsifier (Wu *et al.,* 2008). ISA 720 induces high antibody titer production than cellular immune response and they provide a vigorous immune system response. But the adjuvant causes pain and reaction at the injection site (Aucouturier *et al.,* 2002; Mata *et al.,* 2013). Therefore, O/W emulsions are preferred in human vaccines (Bonam *et al.,* 2021).

Bacillus Calmette Guerin (BCG) – It induces Th1 immune response (Tokunaga *et al*., 1999). It was profitably used along with a combination of killed Leishmania maxicana/Leishmania brazilliensis promastigotes by Convict and colleagues. Therapeutically this vaccination induced effective protection against the diseases even in severe/ extreme cases of Leishmaniasis (Cabrera *et al.,* 2000; Castes *et al.,* 1989).

RIBI - consists of monophosphoryl lipid A, MPL, tetrahalose dicorynomycolate (TDM). This advent causes the cytokines to get activated that further affects the growth of all blood cells and other cells that help the body's immune response and inflammation responses. It further increases the antigen visualisation which stimulates nontoxic antibody mediated immune response and cell, mediated immune response in case of Schistosoma infection (Deeb *et al.,* 1992).

Challenges in developing Anti Parasitic Vaccines. Developing vaccines against parasitic diseases, such as schistosomiasis, malaria, and leishmaniasis, presents several unique and complex challenges. These challenges stem from the complex life cycles of parasites, their ability to evade the host immune system, and the need for long-lasting immunity in endemic regions. Parasites, including protozoans and metazoans, present the host immune system with stage-specific antigens that change with time. Some will trigger protective immunological responses, while others will not. Identifying the necessary proteins for protective immunity is a serious issue for vaccinologists (Knox, 2010) . Main challenges in developing a parasitic vaccine includes complex life cycles of the parasite, immune evasion strategies, antigen selection, inducing of appropriate immune response, safety and efficacy and regulatory and developmental challenges. Overcoming these challenges require multidisciplinary efforts including advances in immunology, molecular biology and biotechnology and funding and international collaboration.

Complexity of life cycle of Parasites - Parasites exists in different stages in different hosts. This is called Complexity of the life cycle. The parasites have developed the capability to evade the immune response of the host (Abath *et al.,* 1998). But how exactly these parasites evade the immune response of the host is not well known (Versteeg *et al.,* 2019).

Impact on poor people - Parasitic infections mainly impact tropical countries. People in tropical countries or countries with weak economy live in poor hygienic conditions. People of these countries have highest performed in contact with insects and other disease-

causing agents/vectors. People have limited healthcare facilities. In developed countries, such infections are not prioritized by the government. "Antipoverty vaccines" describe NTDs vaccines because they mainly impact people's health and economy as well (Hotez, 2018).

Most parasites cause chronic diseases - Most of the parasites mainly cause a chronic diseases and cause disabilities but they do not kill the host. Because there is a co-evolution between parasite and their host (Perry, 2014). Malaria is a noteworthy killer but other parasites mostly do not kill the host. This is the main reason parasitic infections are underestimated even though they have extreme burden (Versteeg *et al.,* 2019).

Limitations of traditional vaccine platforms - Traditional vaccine platforms like Live attenuated vaccines, heat-killed, subunit vaccines, recombining protein vaccines are not always effective due to multi stage life cycle of parasites. Limitation of production and inadequate immune system induction mainly halts the anti-Parasitic vaccine development (Rogier, 2007). Multiple attempts have been made to produce an anti-parasitic vaccine with desirable efficacy. E.g. A preventive live attenuated vaccine was given to persons which provided significant protection but it was stopped because of safety concerns as one person developed lesions after the vaccination (Nadim *et al.,* 1983; Sacks, 2014). MSP-142 was a Recombinant Malaria vaccine. This vaccine was tested on children in Kenya. It induced high production of antibodies but it was fruitless in providing significant protection against the infection (Ogutu *et al.,* 2009; Wykes, 2013). Mosquirixis partly safe guarding. It was witnessed that the service of dendritic cells was changed by the malarial parasite and it also debilitated their capability to hold up memory B cells (Versteeg *et al.,* 2019).

Marginal or no profitable recovery from the market - Third world infection such as malaria Leishmaniasis and Schistosomiasis are unappealing to the pharmaceuticals or Industries. The market does not produce a great deal of profit to recuperate the cost of manufacturing (Vaccines *et al.,* 2011). Anti parasitic and anti- Leishmaniasis vaccine development is on the list of WHO and Bill Gates and Melinda Gates Foundation (Srivastava *et al.,* 2016).

Suitability of Adjuvant - A Vaccine of required efficacy needs proper information about the adjuvant to be used. The adjuvant must be in particular framing in order to make it stable and safe. The adjuvant suitability depends on nature of antigen and route of Management. These things very considerably to develop an efficacious vaccine (Srivastava *et al.,* 2016).

Difference in fluctuation of virulence in parasites. There are two forms of leishmaniasis cutaneous and visceral leishmaniasis. Both the infection very in their symptoms. The variety of infection is mainly because of disparity in the causative species of leishmania for example *Leshmania major* and *Leishmania mexicana* or *Leishmania amazonensis* although they cause cutaneous Leishmaniasis but are still different. *Leishmania* major and Leishmania mexicana or *Leishmania amazonensis* are different from each other phylogenetically. There are different virulence factors among these species and they induce different immune response as well. So these factors halt the production of anti-Leishmania vaccine development (Srivastava *et al.,* 2016).

Genetic variability in parasites. Genetic variability means "Genetic differences". It refers to individuals/organisms which differ in their complete set of genetic material on the contrary to variance influenced by the environment. Because of genetic variability, there are temporarily acquired changes in the phenotype/set of observable characters (Rieger *et al.,* 2012).

In case of parasites, the genetic variability in their genome is mainly co-related with the set of clinical symptoms they cause (Santi & Murta 2022). Parasites have developed an effective survival strategy called antigenic variation or genetic variation that enable them to remain in immunized host Some parasites generate novel antigen by the random mutations during the replication. There is another claim that specific antigens variations can only attack a certain host genotype for instance host differing in their MHC genotype. Antigenic variations can be present due to variant surface glycoproteins which are densely packed on the surface of parasites. As in trypanosome which contain only one VSG gene but can mutate into others. It is a major challenge to find out these variations. genetic variability in trypanosome can be demonstrated on the basis of this protein level (Horn, 2014). They also acquire great genetic variability in multiple species such as Trypanosoma *brucei*, *T. congolense*, *T. simiae*, *T. godfreyi*, *T. suis* and *T. vivax. Trypanosoma brucei* is of special medical importance, as the subspecies *T. b. gambiense* and *T. b. rhodesiense* which makes it genetic variability more complex in a small area. Research on different samples of Leishmania from different areas have shown that genetic variability is crucially or appreciably higher (Santi & Murta 2022).

According to single cell sequencing, there are different karyotypes present within Leishmania clone (Negreira *et al.,* 2022). Within same host and tissues, numerous genotypic infections are illustrated (Negreira *et al.,* 2022).

CONCLUSIONS

Parasitic infections continue to be a global burden and cause of significant morbidity and mortality across the globe especially in tropics and sub-tropical countries. Developing a potent vaccine against parasitic diseases still remains one of the most challenging yet crucial goals in human health. Despite great understanding in parasite biology and immunology several obstacles impede the progress of developing a parasitic vaccine. Complex life cycle along with sophisticated immune evasion mechanisms by the parasites and identification of protective antigens pose a challenge in vaccine development. Recent technological advances and innovations, new adjuvants and new advanced delivery systems are paving way for more effective vaccines. A comprehensive approach involving vaccination with improved sanitation, health education and vector control is crucial for the effective control and

eradication of parasitic diseases. Continued commitment, innovation, and collaboration for global health is the key to overcome the obstacles and in realising the promise of a parasitic vaccine.

FUTURE SCOPE

Parasite vaccines even though are successful but is still rare. Further in-depth understanding of parasitic biology will help in identification of necessary antigens for vaccine development. However, for this aim to become a reality, a need for continuous investment in basic research over the complex relationship between parasite and host is paramount.

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