Plant-Based Vaccines Against Human Parasitic Diseases

8

Anjali Priyadarshani, Mansi Malik, and Cherita Devi Khangembam

Abstract

Historical evidences substantiate the vulnerability and susceptibility of humans to major pandemics. Worldwide, outbreaks of infectious diseases caused increased mortality and morbidity of millions. Despite improved hygiene, medical care, advanced medicines, and sanitary conditions in "modern" era, developing countries witness mortality due to a variety of infectious diseases. Globally, it is extremely difficult to combat the enhanced recurrence of infectious diseases that include avian influenza, Ebola, and Zika. Vaccination is considered one of the best alternatives for control of parasites in the future. To overcome the burden of infectious diseases, individuals are subjected to mass immunization drive with the aim to develop immunity in the community. Occurrence of innumerable variants of the infectious agent necessitates intensive research on vaccines. Success in vaccine development against parasites is severely limited by innumerable unknown, unidentified antigens and complete lack of understanding of the kind of immune response essential for protection. Regardless of these barriers, several vaccines are under different phases of development against several parasitic diseases. Other limitations include high cost of vaccine production, maintenance of vaccine depots, costs involving distribution, and degeneracy. Further, proper management to maintain biosafety and biosecurity is detrimental to the success of vaccines. The past decade has witnessed resurgence in

A. Priyadarshani (✉)
Department of Zoology, Kirori Mal College, University of Delhi, New Delhi, India
e-mail: anjali18kumar@kmc.du.ac.in

M. Malik
Nanobiotech Lab, Kirori Mal College, University of Delhi, New Delhi, India

C. D. Khangembam
Department of Zoology, University of Allahabad, Allahabad, UP, India

A. Singh et al. (eds.), *Natural Product Based Drug Discovery Against Human Parasites*, https://doi.org/10.1007/978-981-19-9605-4_8

developing plant biotechnology that offers innovative approaches for vaccine development based on gene transfer strategies for engineering newer recombinant vaccine in plants with added advantages in the form of improved production, better isolation, purification, and enhanced efficacy with least immunological side effects. The production of plant-based vaccines provides a promising alternative to create affordable biological products. Such recombinant vaccines can irrefutably offer us novel standards and authorized regulations for better approval, licensing, distribution, and marketing of plant-based vaccines. The chapter elaborates on various strategies based on recombinant DNA technology and plant biotechnology for exploiting plant-based vaccine research for therapeutic management of infectious diseases.

Keywords

Plant vaccines · Infectious diseases · Immunization · Vaccine recombinant technologies

8.1 Introduction

Vaccines and vaccination were the initial attempts to prevent infectious diseases in human host. In late eighteenth century, smallpox became a weapon of war. On May 14, 1796, Edward Jenner demonstrated that infection with cowpox could give protection against smallpox infection. In 1803, the term *vaccination* was coined based on the Latin for cow-"*vacca*." This paved the way for vaccine development against dreadful diseases like polio, influenza, cholera, measles, rabies, hepatitis, and many more all over the world and saving innumerable lives globally. The major disadvantages of these vaccines are their high cost of production and purification and maintenance of vaccines in cold storage that requires highly skilled labor. Gradually, plants are being used as vaccine bio-factories for expressing foreign antigens and corresponding antibodies using genetic engineering technologies. There is an inherent advantage in using plants for the production of vaccines as they are cost-effective, with inexpensive upscaling as greenhouses or bioreactors. Plants can express complex antigens that can carry human pathogens or endotoxins inherent to the bacterial, insect, or mammalian cell systems. Plants act as bioreactors wherein larger recombinant proteins could be produced with no contamination from humans or animal pathogens. Their potential low cost, ease to administer, high scalability, and ready acceptance by patients in the form of carrier plants put them at an advantage over the conventional vaccines. Apart from these cited advantages, they can be easily frozen and stored (Egelkrout et al. 2012). Plant genetic engineering technology has given fresh insight on vaccine research via gene transfer technology that can incorporate the desired gene in plants. In 1986, Barta and colleagues reported chimeric gene expression of human growth hormone and nopaline synthase in tobacco plants and sunflower with Ti plasmid (Barta et al. 1986). Different plants such as peanut, tomato, tobacco, maize, lettuce, carrot, rice, and soybean are often

referred to as hosts for addition of preferred gene of interest. Criteria that should be taken into consideration while developing plant-derived vaccines for commercial purposes include identification of the genes to be transfected, high expression of recombinant genes, enhanced stability, and a high shelf life of antigens with zero contamination with live pathogens. Finally, ease of manufacturing and newer targetable antigens against variant pathogen subtypes are desirable.

8.1.1 Production of Plant-Based Vaccines by Recombinant Technologies

Plant-derived vaccine research and technologies necessitate the incorporation of the preferred gene of interest encoding the antigen protein into the plant genome.

The transgene of interest is introduced into the vector for further expression in plants either by stable transformation or via transitory transformation mechanism within plant cells. A steady and ephemeral gene expression can be attained through gene delivery methods. Broadly, it can be categorized as direct gene delivery and indirect gene delivery method (Fig. 8.1).

(a) Direct method for delivery of gene, where the nucleic acids (DNA/RNA) are straight incorporated into the plant cells with biolistic method, where two different kinds of antigen expression within transgenic plants can occur such as nuclear transformation and chloroplast transformation.
(b) Indirect method for delivery of gene provides evidence for more considerable vaccine production as it engages exploitation of plant bacteria, chiefly the *Agrobacterium* species and other plant viruses, which unsurprisingly contaminate the plant cells and are employed on work to amalgamate the desired gene of interest into plant genome.

8.2 Human Parasitic Diseases

The past two decades have witnessed a meteoric rise in infectious diseases that often go undiagnosed and are fatal if left untreated (Fig. 8.2). Parasitic diseases are a great burden in tropical regions where the morbidity index and mortality rate indicate ~1.1 million deaths occurring annually. Major causes of reported deaths are malaria and schistosomiasis. Drugs administered are ineffective, probably due to developed parasitic drug resistance. Hence, there is an urgent need to look for alternative approaches like development of vaccines for the parasitic diseases. Clinical trials for vaccines against malaria and leishmaniasis are also under trials, and vaccines for treatment of schistosomiasis are in their phase trials (I/II) (Shahid and Daniell 2016). "Classic" vaccines are based on attenuated infective stages of protozoan, and helminths like *Coccidiosis*, *Toxoplasmosis*, and *Dictyocaulus* are very unstable and expensive. Recombinant technology has enabled quick processing of protective antigens in bulk amounts. Cultivation, purification, and processing of the

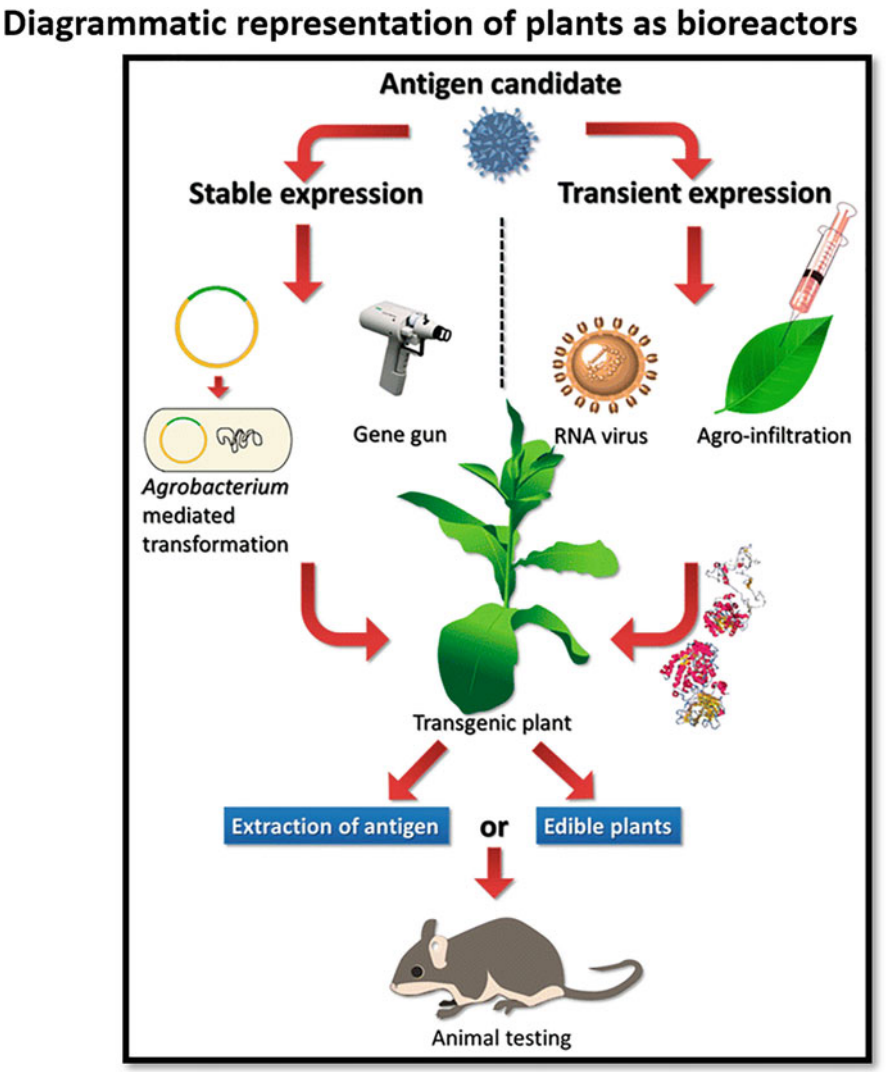

Fig. 8.1 Diagrammatic illustration of plant-based bioreactors

recombinant protein are less expensive than the maintenance of host animals and isolation of protective antigens from harvested parasites. But till date, there are no vaccines that can be used against the life-threatening advanced stages of malaria, leishmaniasis, schistosomiasis, trypanosomiasis, toxoplasmosis, cryptosporidiosis, and many other diseases. At present, TickGARD is the only anti-parasite recombinant protein vaccine that is commercially available (Kumar and Ghosh 2016). Edible vaccines, based on transgenic plants that express the protective parasitic antigens, present alternative approaches in the research for anti-parasitic vaccines and may

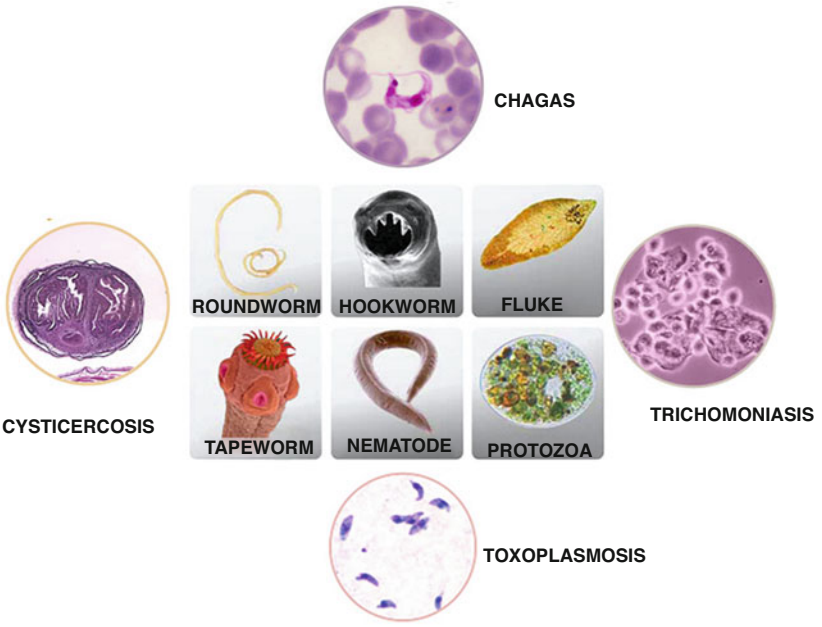

Fig. 8.2 Parasites and parasitic infections

also be important against gastrointestinal parasites (Zahmanova et al. 2022). Such vaccine prototypes have also been assessed at preclinical levels. Predominantly, these plant-based vaccines target various parasitic infections with foremost potential in the form of immunogenicity and protection. Vast number of human parasites that cause infection can be grouped under the following categories:

8.2.1 Protozoa

Protozoan disease occurrence is centered around tropical and subtropical areas of the globe. Protozoans have characteristic life cycles where it is able to switch between active infectious and inactive (cyst) form. Table 8.1 represents key pathogenic protozoa responsible for the occurrence of various diseases. Flagellates, Leishmania, and Trypanosoma are proficient enough in invading the blood and tissue of human host, producing severity. Occurrence of *Trichomonas vaginalis* and *Giardia lamblia* in the reproductive and gastrointestinal tracts does not cause mortality but is surely responsible for moderate morbidity. In contrast, sporozoan produces two most lethal diseases such as malaria and toxoplasmosis (del Yácono et al. 2012). Further, appearance of HIV has provided a new dimension to be looked upon, i.e., "opportunistic" parasitosis. Treatment and prophylaxis of protozoan-inflicted diseases have been reliant upon the type of drugs, many of which have become less effective, thereby imposing urgency in exploration for alternative systems.

Table 8.1 Important pathogenic protozoa and diseases caused by them

Type and location	Species	Disease
Urogenital tract	*Trichomonas vaginalis*	Trichomoniasis
Blood and tissue	*Plasmodium* species *Toxoplasma gondii* *Trypanosoma* species *Leishmania* species *Naegleria* species *Acanthamoeba* species	Malaria Toxoplasmosis Trypanosomiasis Leishmaniasis Amoebic meningoencephalitis Amoebic meningoencephalitis
Intestinal tract	*Entamoeba histolytica* *Giardia lamblia* *Cryptosporidium parvum* *Balantidium coli Isospora belli* *Cyclospora cayetanensis*	Amoebiasis Giardiasis Cryptosporidiosis Balantidiasis Cyclosporiasis

8.2.2 Pathogenic Free-Living Amoebae

Various free-living amoebae live in different habitats such as soil and water habitats. Certain amoebic species including Naegleria, Acanthamoeba, and Balamuthia are also known as facultative parasites for human host. Human infections that are caused due to amoebae are attained with time on exposure to contaminated water; secondly, it can also be inhaled in the form of cysts that are present in dust. *Naegleria fowleri* causes acute primary amoebic meningoencephalitis and brain abscesses in immune-incompetent individuals. Treatment of such free-living amoebic related infections is largely unsuccessful.

8.2.3 Pathogenic Flagellates

8.2.3.1 Trichomonas

The trophozoite of *Trichomonas vaginalis* is mainly present in the urethra and vagina of the female individual, as well as in the urethra and prostate gland of individual men. Their proliferation causes inflammation, and the presence of trophozoites is also found in part of tissues and secretions from the glands. Early signs of vaginal or vulvar pruritus are in the form of sudden discharge during or after menstruation; this also increases vaginal pH. Vaginal secretions are greenish to pale yellowish color, or at times it may be frothy in nature, and with a foul smell. Infections that occur in male are more latent, with negligible symptoms.

8.2.3.2 Leishmania

L. donovani, causative agent of kala-azar ("black sickness") commonly called as dumdum fever, is persistently taking place in diverse regions of Africa and Southeast Asia. Annually, ~12 million suffering individuals are reported to be infected with

three different clinical forms such as visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (MCL). All three of them have separate immunopathogenesis, mortality, and morbidity rate. As parasites invade the host body, it proliferates and infects several cells of different organs such as liver and spleen, resulting in organ enlargement and leading to subsequent weight loss. Its persistence further leads to post-kala-azar dermal leishmaniasis. Untreated visceral leishmaniasis on the other side may be fatal because of the occurrence of secondary infection. Drugs such as pentavalent antimonials (Pentostam™; GSK) and meglumine antimoniate (Glucantime, Aventis), amphotericin B and its lipid formulations, pentamidine, and ketoconazole are considered effective treatments (Sundar and Chakravarty 2013). Diamidine-pentamidine is extremely toxic. The antimonials have erratic efficacy against leishmaniasis. Futile therapeutic regime with antimonial drugs is also known (Lira et al. 1999), while varied results have been observed with ketoconazole treatment (Herwaldt et al. 1992; Ozgoztasi and Baydar 1997). Amphotericin B is effective against *Leishmania donovani* VL. Although there has been an upsurge of innovations in the development of antileishmanial drugs, their progression is restricted because of the differential chemosensitivities of *Leishmania* species (Croft and Coombs 2003).

8.2.3.3 Trypanosoma

Trypanosoma is the causative agent of African sleeping sickness. Around 60 million sub-Saharan Africans are threatened by this infection caused by *Trypanosoma gambiense* alone, and it is responsible for 95% of the cases (Brun et al. 2011; Simarro et al. 2012) (Checchi et al. 2008). *T. b. rhodesiense* affects the central nervous system, and the parasites are able to cross the blood–brain barrier (Grab and Kennedy 2008). Till date, there is no vaccine available for trypanosomiasis. The remedial approach relies on the infecting species and the occurring stage of the disease. Administration of pentamidine or suramin to patients at an early stage of *T. b. gambiense* and *T. b. rhodesiense* infections is more effective (Steverding 2010). With the advancement in disease, the treatment relies on melarsoprol or eflornithine, where the former can cause severe adverse effects including reactive heart failure, encephalopathy, and even death (Balasegaram et al. 2009; Burri and Brun 2003). Eflornithine is less toxic as compared to melarsoprol, but it is not cost-effective and is difficult to administer (de la Cruz et al. 2019). In addition, resistance to a particular drug can also develop in patients. With the serious limitations in current therapies, further research for the advancements of therapeutic management of sleeping sickness is essential and indispensable.

An estimate of ~7 million people worldwide, predominantly in Latin America, are infected with *Trypanosoma cruzi*, a causative agent for Chagas disease. The main transmission route for *T. cruzi* to humans is vector-borne through the insect triatomine bug. Currently, there are no certified DNA vaccines for the management of *T. cruzi* infection in humans due to the inadequate immune responses in the infected host. Nonetheless, recombinant protein vaccines, Tc24 and its variants, are being administered to test their potential in mice where they have shown their

efficacy to elicit a heightened immune response to combat and control the elevated infection (de la Cruz et al. 2019).

8.3 Medically Important Ciliates

8.3.1 Sporozoa

The genus *Plasmodium* is the prototype of this class and causes malaria. It is found in poverty-stricken tropical and subtropical areas worldwide and affects ~3.3 billion individuals causing approximately 1.1 million deaths annually. Six species of *Plasmodium* are accountable for occurrence of malaria in human hosts (Garrido-Cardenas et al. 2019; Sutherland et al. 2010). Amongst them, *P. falciparum, P. vivax*, and *P. falciparum* are the chief sporozoans that invades both young and older erythrocytes. Globally, treating drug-resistant infections caused due to *P. falciparum* presents a unique challenge. While *P. vivax*, *P. ovale*, and *P. malariae* infect only the mature erythrocytes. quinine is the recommended treatment for severe malaria, and artemisinin derivatives are also used (Paddon et al. 2013). Primaquine is the only drug against *P. vivax* infection and is known for its adverse effects in inhabitants with glucose-6-phosphate dehydrogenase insufficiency (Von Seidlein et al. 2013; Krishna and Kremsner 2013). The present drugs for malaria treatment have been allied with acquired drug-resistant parasites (Krishna and Kremsner 2013; Ariey et al. 2014). Thus, there is an urgent requirement for the development of novel antimalarial drugs that are effective against multidrug-resistant (MDR) parasites.

Vaccination has been the key to reduce the adverse effects of many human infectious diseases and has even led to the eradication of few. The WHO has recommended for RTS,S/AS01 malaria vaccine. "Mosquirix" has been developed as a vaccine and is given to children aged between 6 weeks and 17 months to protect against malaria caused by the parasite *P. falciparum*. This vaccine has shown 30% efficacy in severe disease cases. Recent studies from Africa indicated a significant reduction of ~70% in malaria, if the vaccine was given in combination with an antimalarial medication (Cotton 2020).

Advances in vaccine technology can impact transmission and occurrence and enable targeted management of sexual and oocyte stages. Preerythrocytic and erythrocytic vaccines that can aid in reducing transmission rate have been designed. The accelerated costs for a successful immunization plan and long-scale vaccine production method, allocation, and deliverance are the key obstacles in the expansion of subunit malaria vaccines. However, plant-based expression systems offer significantly reduced costs, enhanced efficacy, and amplified scalability. Till now, expression stratagem has been standardized in plants for antigens against *Plasmodium* to elicit a substantial immune response in mice.

Consequently, plant bioreactors present excellent opportunities for developing commercial vaccines. They help to attain a high expression level of recombinant genes, will be able to rapidly design and easily produce new antigens in response to unique pathogen subtypes, and, lastly, identify the genes to be transfected and

warrant the safety of the produced proteins for use in both animals and humans. Additionally, proficient malaria antigen expression in the chloroplast of lettuce and nuclear transformation in tobacco and seeds of *Arabidopsis thaliana* for oral immunization are landmark improvements that permit the oral administration of subunit vaccines in combination with an adjuvant (Laguía-Becher et al. 2010; Lau et al. 2010). These conclusions provide a rationale for the advancement of a plant-derived oral vaccine against infectious malaria.

8.4 Other Coccidian Parasites

Toxoplasma gondii is another protozoan causative agent for toxoplasmosis. Over 25–30% of the global population is affected with it (Baril et al. 1999). It has both definite and intermediate hosts, where the former is cat and the latter host is human. Its vertical transmission can infect the fetus through mother (Lima and Lodoen 2019). Toxoplasmosis can have complications in the immunocompromised individuals, e.g., AIDS, with fatal consequences (Luft and Remington 1992). The global burden indicates a shocking increase in congenital toxoplasmosis (1.2 million DALYs) (Torgerson and Mastroiacovo 2013). The current treatment approach for toxoplasmosis is quite inadequate. Therapy for severe diseased forms may consist of a combination of pyrimethamine and sulfonamide. For prevention of transmission to fetus in a pregnant female, spiramycin and leucovorin are often prescribed. Severe side effects to pyrimethamine combinations accentuate the requirement for developing alternative therapeutic approaches (Farthing et al. 1992).

8.5 Helminthes

Helminthes are common parasitic worms. They have the high global morbidity and mortality as quite often they are the root source of anemia and malnutrition-related ailments in individuals, with few that are life-threatening. Helminthes can penetrate the host body via the skin, mouth, and respiratory tract through inhalation of airborne eggs. The helminthic parasites are categorized into three main classes—Trematodes (flukes), Cestodes (tapeworm), and Nematodes (roundworms).

8.5.1 Flukes

Flukes reside in the alimentary canal, liver, bile duct, ureter, and bladder of craniate animals. Depending on the sites they enter, flukes are categorized into four groups: intestinal flukes, blood flukes, lung flukes, and liver flukes. Blood flukes like *Schistosoma* cause schistosomiasis bilharziasis, liver flukes are commonly known as *Fasciola hepatica*, and also sheep liver fluke is a familiar and globally distributed parasite. Various studies reported that immune response elicited by orally administered plant-based vaccine that expresses the recombinant cysteine protease

against *F. hepatica* metacercaria infection in rats shows significant effects (Kesik-Brodacka et al. 2017).

8.5.2 Nematodes

This class includes the filarial worms—the guinea worm (*Dracunculus medinensis*) and *Trichinella spiralis*. Filariasis is an infectious disease commonly seen in tropical climates. The filarial worms reside in the lymphoid immune system and the subcutaneous tissues of human and can lead to lymphedema (fluid retention) or hydrocele (swelling in the scrotum). The microfilariae form the early stage in the life cycle of nematodes (facultative parasite), and the adults live in the tissue or the circulatory system of vertebrates (definitive hosts) where these develop into filariform larvae forming the infective stages. Humans get infected by *Wuchereria bancrofti*, another filarial worm that resides in the host lymph nodes and lymphatic vessels and leads to lymphatic filariasis. Around 1.5% of the global population is reported to be infected (Katiyar and Singh 2011). According to the WHO, over 880 million people are presently at the threat of acquiring lymphatic filariasis (LF) in over 52 countries worldwide. Though medication can kill the worms, prevent them from spreading the infection to someone else, and reduce the symptoms of filariasis (Chavda et al. 2021), current approaches to control LF are short of the anticipated goal. The complex behavior of this parasite is evident as yet there is no single vaccine for filariasis.

8.6 Cestodes (Tapeworms)

Worldwide prevalence of taeniasis is caused by tapeworms (*Taenia saginata*, *T. solium*, and *T. asiatica*). *T. solium* is frequently reported in underdeveloped communities with deprived sanitation facility, mostly where people have adapted to eating raw food, especially undercooked pork. A disease called cysticercosis occurs due to the ingestion of *T. solium* eggs. The adult tapeworms are generally delivered to host via portal blood supply to the various organs such as lungs, liver, and brain, which causes extraintestinal diseases. In rare cases, it can also lead to intestinal blockage and thinning and shortening of the intestinal ducts like bile duct or pancreatic duct that can be fatal (Gonzales et al. 2016). The frequent management of tapeworm infection involves oral medications including praziquantel (Biltricide), albendazole (Albenza), nitazoxanide (Alinia) that target the adult tapeworm (Lloyd et al. 2014).

Development of effective delivery systems for vaccines is a priority in vaccinology. Transgenic papaya callus lines expressing the components of the S3Pvac vaccine constitute a stable platform to produce an oral vaccine against cysticercosis caused by *T. solium* or *T. crassiceps*. The parasitic disease adversely affects human health and acquires a lethal form when the cysticerci are blocked in the central nervous system of the infected host, causing neurocysticercosis. Here,

pigs are known as the obligate intermediate hosts to complete the parasitic life cycle. Vaccination of pigs significantly amplifies the antibodies and triggers mononuclear cell proliferation that can help in curbing human transmission by reducing cysticercosis in pigs. S3Pvac-papaya vaccine is under evaluation for understanding the cost-benefit of developing a delivery system and also a dose range (Fragoso et al. 2017).

Diseases caused by parasitic protozoans are responsible for ill-health and put an immense social and economic burden particularly in tropical areas of the world. To add to the woes, these parasitic pathogens acquire drug resistance, and they recur with superior virulence. The unavailability of an accredited vaccine for several human parasitic diseases, collectively with a paucity of inexpensive, secure, and operative drugs for some diseases, or challenges posed by parasite drug resistance necessitate steering an exploration for new anti-parasitic agents. Vaccination to control helminthes has been an important part of an integrated veterinary and public health policy. Attempts are being made to recognize more efficient, economical, and effortlessly deliverable mucosal vaccines. One such research area that is presently under development focuses on genetic plant modifications for large-scale production of immune-protective proteins that has progressed dramatically over the last quarter of the century. Edible vaccines offer pertinent solutions for treating known diseases where its therapeutic management is restricted by the intrinsic constraint of traditional vaccines, like cost of production, storage problem, and expensive logistics. Sixteen foods by now are known for producing antigens to counter human and animal diseases. However, using plants for generating drugs against human parasitic diseases will be extremely important. In summary, to minimize the upsurge of infectious and parasitic diseases globally, edible vaccines have the potential to mitigate and avert parasitic diseases in countries where conventional vaccination approach is still inadequate.

8.7 Future Potential

Plant-based vaccines are emerging as a novel alternative to traditional vaccines with greater therapeutic potential to treat infectious and parasitic diseases. A transient and stable gene expression has been achieved with the aid of genetic engineering. Chloroplast transformation via particle bombardment gene delivery method or through biolistic has been deliberated upon as a promising alternative for improving the production of plant-based vaccines. Nevertheless, efficient and optimum vaccine production requires continued development and improvement of suitable gene delivery methods. The bioethical issues arising from the production of plant-based vaccines include the risk of transferring the allergens from transgenic plants to humans and animals. As virus and bacteria are used as the vectors to produce plant-based vaccines, the pathogens might be reverted to its pathogenic form and infect other organisms. The benefits of plant-based vaccines will overwhelm the challenges faced by this interesting biological product. Thus, it is anticipated that regulatory approvals will be granted ultimately to help in the global disease control. There is an urgent need to generate bio-pharmed vaccines that can respond to the

sudden outbreaks of emerging parasitic diseases. Increasingly, the generation of plant-based "bio-betters" opens novel pathways to facilitate biopharming that is rapid, safe, and easily scaled up to manufacture high-value biopharmaceuticals. In view of the enhanced development of plant-derived vaccines, regulatory agencies must advance their knowledge about the latest advanced emerging technology and adapt accordingly.

References

Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois A-C, Khim N et al (2014) A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature 505(7481):50–55

Balasegaram M, Young H, Chappuis F, Priotto G, Raguenaud M-E, Checchi F et al (2009) Effectiveness of melarsoprol and eflornithine as first-line regimens for gambiense sleeping sickness in nine Médecins Sans Frontières programmes. Trans R Soc Trop Med Hyg 103(3): 280–290

Baril L, Ancelle T, Goulet V, Thulliez P, Tirard-Fleury V, Carme B (1999) Risk factors for Toxoplasma infection in pregnancy: a case-control study in France. Scand J Infect Dis 31(3): 305–309

Barta A, Sommergruber K, Thompson D, Hartmuth K, Matzke MA, Matzke AJ (1986) The expression of a nopaline synthase—human growth hormone chimaeric gene in transformed tobacco and sunflower callus tissue. Plant Mol Biol 6(5):347–357

Brun R, Don R, Jacobs RT, Wang MZ, Barrett MP (2011) Development of novel drugs for human African trypanosomiasis. Future Microbiol 6(6):677–691

Burri C, Brun R (2003) Eflornithine for the treatment of human African trypanosomiasis. Parasitol Res 90(1):S49–S52

Chavda VP, Pandya A, Pulakkat S, Soniwala M, Patravale V (2021) Lymphatic filariasis vaccine development: neglected for how long? Expert Rev Vaccines 20(11):1471–1482. https://doi.org/10.1080/14760584.2021.1990760

Checchi F, Filipe JA, Haydon DT, Chandramohan D, Chappuis F (2008) Estimates of the duration of the early and late stage of gambiense sleeping sickness. BMC Infect Dis 8(1):1–10

Cotton MJTD (2020) The Mosquirix (RTS.S) malaria vaccine, vol 50. SAGE Publications, London, pp 107–1072

Croft SL, Coombs GH (2003) Leishmaniasis—current chemotherapy and recent advances in the search for novel drugs. Trends Parasitol 19(11):502–508

de la Cruz JJ, Villanueva-Lizama L, Dzul-Huchim V, Ramírez-Sierra M-J, Martinez-Vega P, Rosado-Vallado M et al (2019) Production of recombinant TSA-1 and evaluation of its potential for the immuno-therapeutic control of *Trypanosoma cruzi* infection in mice. Hum Vaccines Immunother 15(1):210–219

del Yácono LM, Farran I, Becher ML, Sander V, Sanchez VR, Martín V et al (2012) A chloroplast-derived *Toxoplasma gondii* GRA 4 antigen used as an oral vaccine protects against toxoplasmosis in mice. Plant Biotechnol J 10(9):1136–1144

Egelkrout E, Rajan V, Howard JA (2012) Overproduction of recombinant proteins in plants. Plant Sci 184:83–101

Farthing C, Rendel M, Currie B, Seidlin MJT (1992) Azithromycin for cerebral toxoplasmosis. Lancet 339(8790):437–438

Fragoso G, Hernández M, Cervantes-Torres J, Ramírez-Aquino R, Chapula H, Villalobos N, Segura-Velázquez R, Figueroa A, Flores I, Jiménez H, Adalid L, Rosas G, Galvez L, Pezzat E, Monreal-Escalante E, Rosales-Mendoza S, Vazquez LG, Sciutto E (2017) Transgenic papaya: a useful platform for oral vaccines. Planta 245(5):1037–1048. https://doi.org/10.1007/s00425-017-2658-z

Garrido-Cardenas JA, González-Cerón L, Manzano-Agugliaro F, Mesa-Valle C (2019) Plasmodium genomics: an approach for learning about and ending human malaria. Parasitol Res 118(1):1–27

Gonzales I, Rivera JT, Garcia HH (2016) Pathogenesis of *Taenia solium* taeniasis and cysticercosis. Parasite Immunol 38(3):136–146. https://doi.org/10.1111/pim.12307

Grab DJ, Kennedy PG (2008) Traversal of human and animal trypanosomes across the blood–brain barrier. J Neurovirol 14(5):344–351

Herwaldt BL, Arana BA, Navin TR (1992) The natural history of cutaneous leishmaniasis in Guatemala. J Infect Dis 165(3):518–527

Katiyar D, Singh LK (2011) Filariasis: current status, treatment and recent advances in drug development. Curr Med Chem 18(14):2174–2185

Kesik-Brodacka M, Lipiec A, Kozak Ljunggren M, Jedlina L, Miedzinska K, Mikolajczak M et al (2017) Immune response of rats vaccinated orally with various plant-expressed recombinant cysteine proteinase constructs when challenged with *Fasciola hepatica* metacercariae. PLoS Negl Trop Dis 11(3):e0005451

Krishna S, Kremsner PG (2013) Artemisinin resistance needs to be defined rigorously to be understood: response to Dondorp and Ringwald. Trends Parasitol 29(8):361–362

Kumar B, Ghosh S (2016) Laboratory scale production of recombinant Haa86 tick protein in *Pichia pastoris* and in *Escherichia coli* system. In: Vaccine design. Springer, New York, pp 459–482

Laguía-Becher M, Martín V, Kraemer M, Corigliano M, Yacono ML, Goldman A et al (2010) Effect of codon optimization and subcellular targeting on *Toxoplasma gondii* antigen SAG1 expression in tobacco leaves to use in subcutaneous and oral immunization in mice. BMC Biotechnol 10(1):1–14

Lau OS, Ng DWK, Chan WW, Chang SP, Sun SS (2010) Production of the 42-kDa fragment of *Plasmodium falciparum* merozoite surface protein 1, a leading malaria vaccine antigen, in *Arabidopsis thaliana* seeds. Plant Biotechnol J 8(9):994–1004

Lima TS, Lodoen MB (2019) Mechanisms of human innate immune evasion by *Toxoplasma gondii*. Front Cell Infect Microbiol 9:103

Lira R, Sundar S, Makharia A, Kenney R, Gam A, Saraiva E et al (1999) Evidence that the high incidence of treatment failures in Indian kala-azar is due to the emergence of antimony-resistant strains of *Leishmania donovani*. J Infect Dis 180(2):564–567

Lloyd AE, Honey BL, John BM, Condren M (2014) Treatment options and considerations for intestinal helminthic infections. J Pharm Technol 30(4):130–139. https://doi.org/10.1177/8755122514533667

Luft BJ, Remington JS (1992) Toxoplasmic encephalitis in AIDS. Clin Infect Dis 15(2):211–222

Ozgoztasi O, Baydar I (1997) A randomized clinical trial of topical paromomycin versus oral ketoconazole for treating cutaneous leishmaniasis in Turkey. Int J Dermatol 36(1):61–63

Paddon CJ, Westfall P, Pitera DJ, Benjamin K, Fisher K, McPhee D et al (2013) High-level semi-synthetic production of the potent antimalarial artemisinin. Nature 496(7446):528–532

Shahid N, Daniell H (2016) Plant-based oral vaccines against zoonotic and non-zoonotic diseases. Plant Biotechnol J 14(11):2079–2099

Simarro PP, Franco JR, Cecchi G, Paone M, Diarra A, Ruiz Postigo JA et al (2012) Human African trypanosomiasis in non-endemic countries (2000–2010). J Travel Med 19(1):44–53

Steverding D (2010) The development of drugs for treatment of sleeping sickness: a historical review. Parasit Vectors 3(1):1–9

Sundar S, Chakravarty J (2013) Leishmaniasis: an update of current pharmacotherapy. Expert Opin Pharmacother 14(1):53–63

Sutherland CJ, Tanomsing N, Nolder D, Oguike M, Jennison C, Pukrittayakamee S et al (2010) Two nonrecombining sympatric forms of the human malaria parasite *Plasmodium ovale* occur globally. J Infect Dis 201(10):1544–1550

Torgerson PR, Mastroiacovo P (2013) The global burden of congenital toxoplasmosis: a systematic review. Bull World Health Organ 91:501–508

Von Seidlein L, Auburn S, Espino F, Shanks D, Cheng Q, McCarthy J et al (2013) Review of key knowledge gaps in glucose-6-phosphate dehydrogenase deficiency detection with regard to the safe clinical deployment of 8-aminoquinoline treatment regimens: a workshop report. Malar J 12(1):1–12

Zahmanova G, Takova K, Valkova R, Toneva V, Minkov I, Andonov A et al (2022) Plant-derived recombinant vaccines against zoonotic viruses. Life (Basel) 12(2):156