CONSTRUCTION OF DNA VACCINE EXPRESSING MSP-1C OF *PLASMODIUM FALCIPARUM*

by

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CERTIFICATE

This is to certify that the dissertation entitled "**Construction of DNA Vaccine against MSP-1C of *Plasmodium falciparum***" is the bonafide record of research work done by Miss Noraini binti Mat Yunus during the period from July 2008 to October 2008 under my supervision.

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LIST OF ABBREVIATIONS

ADCC	Antibody-dependent cell-mediated cytotoxicity
APC	Antigen presenting cell
bp	Base pair
C'	Complement
CTL	Cytotoxic T Lymphocyte
ddH_2O	Deionised distilled water
dH_2O	Distilled water
DCs	Dendritic cells
DNA	Deoxyribonucleic acid
mAbs	Monoclonal antibodies
MCS	Multiple cloning site
MHC	Major compatibility complex
MSP-1C	C-terminal region of merozoite surface protein
kbp	Kilo base pair
LB	Luria Bertani
LA	Luria Bertani agar
OD	Optical density
PCR	Polymerase chain reaction
RBC	Red blood cell
RE	Restriction site
SERA	Serine repeat antigen
UV	Ultraviolet
WHO	World Health Organization

ABSTRACT

Malaria is one of the most threatening parasitic diseases, being endemic in most of the tropical and subtropical countries. This problem becomes severe with the rapid spread of drug resistant *Plasmodium falciparum*, development of resistance to insecticides by Anopheles mosquito, poor hygienic care, socioeconomic problems and transition of people. Therefore an effective and universal malaria vaccine against *Plasmodium falciparum* should be developed. A great deal effort of researchers nowadays has been focused on vaccines targeting at the asexual blood stage due to prolong life cycle within the blood and it can directly reduce morbidity and mortality associated with malarial disease in human. One of the vaccine candidates from asexual blood stage is a 19 kDa C-terminal region of merozoite surface protein 1 (MSP-1C). MSP-1C protein has been considered as a good candidate for malaria vaccine development due to their obvious accessibility by host's immune response. The purpose of this project is to construct a DNA vaccine expressing MSP-1C of *P. falciparum.* In order to develop this vaccine, MSP-1C fragment was amplified from plasmid pNMN016 by using polymerase chain reaction (PCR). The MSP-1C fragment was ligated into pVAX1, a plasmid vector that is designed for use in the development of DNA vaccines. Hopefully, the development of DNA vaccine against *P. falciparum* would decline the number of malaria cases worldwide.

ABSTRAK

Malaria merupakan salah satu penyakit merbahaya yang disebabkan oleh parasit dan biasanya ditemui di kawasan tropika dan subtropika. Masalah ini menjadi semakin parah apabila meningkatnya kerintangan parasit *Plasmodium falciparum* terhadap drug anti-malaria, kerintangan nyamuk Anopheles terhadap insektisid, penjagaan kebersihan yang terabai, masalah sosioekonomi dan penghijrahan penduduk berlaku secara mendadak. Oleh itu, vaksin yang lebih efektif dan universal harus dibangunkan. Pada hari ini, para saintis telah berusaha keras dalam membangunkan vaksin malaria yang mempunyai sasaran pada fasa eritrositik disebabkan oleh kitar hidup parasit dalam darah adalah lebih lama berbanding fasa pra-eritrositik dan pembinaan vaksin pada fasa ini secara langsung boleh mengurangkan kes morbiditi dan mortaliti penyakit malaria dalam manusia. Salah satu calon vaksin pada fasa eritrositik adalah 19 kDa C-terminal region daripada protein permukaan merozoit 1 (MSP-1C). MSP-1C telah dikenalpasti sebagai calon vaksin yang berpotensi untuk penyakit malaria kerana protein ini terdedah secara langsung kepada sistem imun perumah. Tujuan projek ini dijalankan adalah untuk membangunkan vaksin DNA yang mengekspreskan MSP-1C yang terdapat pada *P. falciparum*. Dalam membangunkan vaksin ini, fragmen MSP-1C diamplifikasi daripada plasmid pNMN016 dengan menggunakan tindakbalas rantai polimerase (PCR). Fragmen MSP-1C kemudiannya diligasi ke dalam vektor pVAX1 iaitu plasmid vektor yang direkabentuk khas untuk pembangunan vaksin DNA. Diharapkan pembangunan vaksin DNA yang mengekspreskan antigen MSP-1C pada *P. faciparum* akan dapat menurunkan jumlah kes malaria sedunia.

CHAPTER 1

LITERATURE REVIEW

1.1 Malaria disease

Malaria is still one of a major cause of disease and death in many regions of the world. More than 500 million individuals contact malaria annually and approximately 1 million die, most of them are infants, young children and pregnant women (World Health Organization, 2007). Malaria can be transmitted to people of all ages (WHO, 2007). The common first symptoms of malaria are fever, headache, chills and vomiting (Boon et al., 2006; WHO, 2007). These symptoms would appear 10 to 15 days after a person is infected. If not treated promptly with effective medicines, malaria can cause severe illness that is usually fatal (WHO, 2007).

Malaria is caused by the *Plasmodium* parasites, transmitted to vertebrates by the bite of a female Anopheles mosquito. The parasite's asexual blood forms (merozoites and schizonts) are those life cycle stages responsible for plasmodial morbidity and mortality in the vertebrate host (Phillips, 2001). There are four species of humans malaria parasites; *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium vivax* and *Plasmodium ovale*. The majority of deaths from malaria are due to *P. falciparum*, the disease's most aggressive causative agent (WHO, 2007).

Most of malaria cases and deaths are in sub-Saharan Africa. However, Asia, Latin America, the Middle East and parts of Europe are also affected (WHO, 2007). Following WHO-sponsored campaigns focusing on prevention and effective treatment, the incidence of malaria was greatly reduced between 1950 and 1960, but since1970 there

has been resurgence. According to Boon et. al (2006), this problem has been reemergence due to resistance *P. falciparum* to chloroquine notably in Asia and Africa. Increasing in travel and neglect of chemoprophylaxis cause over 2000 malaria cases imported annually into Britain. Most of the cases are due to *P. falciparum,* usually from Africa, and 1% of them died due to late diagnosis. Immigrants returning home after a long residence in United Kingdom are particularly at risk, they have lost their partial immunity and do not realize that they should be taking malaria prophylaxis. A few people living near airports in Europe have acquired malaria from accidentally imported mosquitoes (Boon et al., 2006). The endemic area of malaria and *P. falciparum* parasites in human blood are depicted in figure 1.1 and 1.2, respectively.

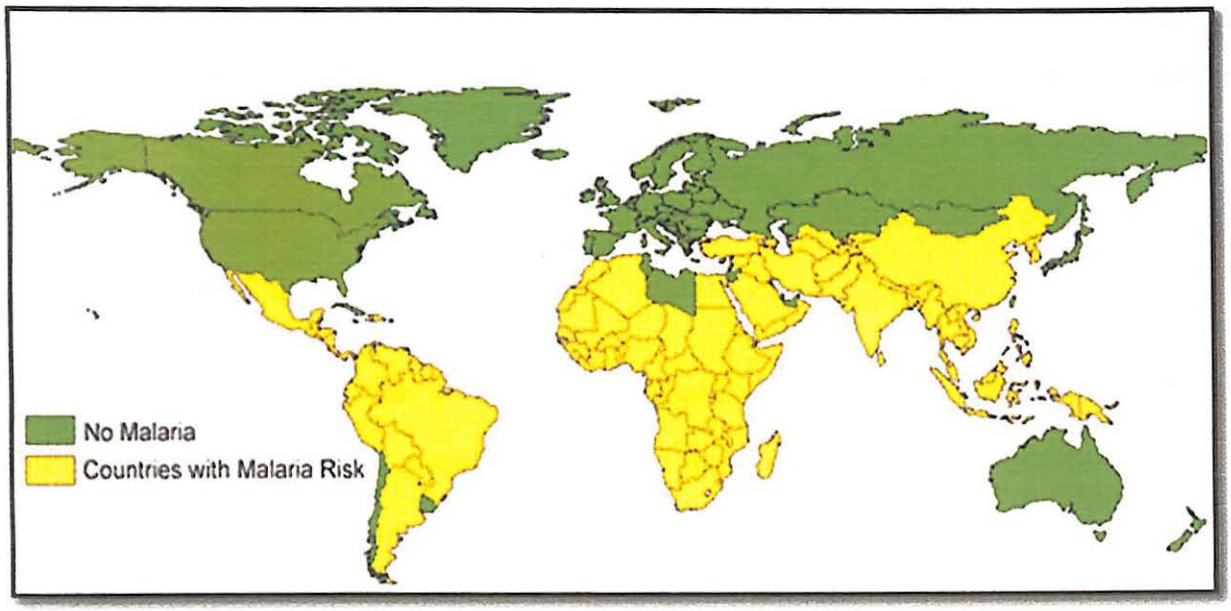

Figure 1.1: Areas of the world where malaria is endemic (WHO, 2005)

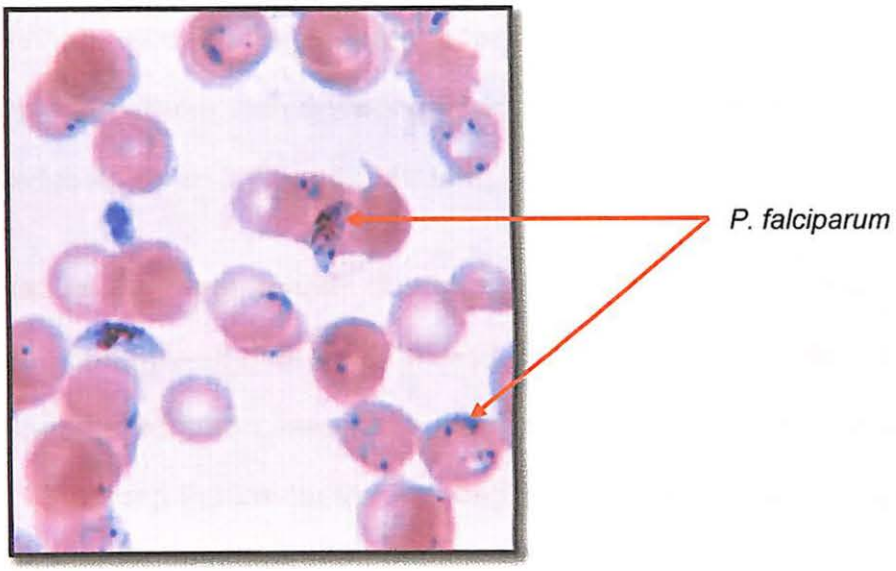

Figure 1.2: *P. falciparum* in human blood (Britton, 2001)

1.2 Malaria in Malaysia

According to WHO (2005), malaria remains the most common vector-borne parasitic disease in Malaysia despite a decline in the annual number of cases (Table 1.1). *P. falciparum* is the most predominant species in Malaysia followed by *P. vivax* and *P. malariae* (Singh et al., 2001).

The malaria prevalence data for Malaysia is based on microscopy-confirmed cases, and the majority of malaria cases was detected when individuals who were ill sought treatment at hospitals or rural health clinics (Ministry of Health Malaysia, 1999; Singh et al., 2001). However, the prevalence of *Plasmodium* species in Malaysia is probably underestimated because self-medicated individuals and sub-clinical or microscopy-negative infections were not included in malaria case reports (Singh et al., 2001).

Table 1.2 shows the reported malaria cases by age and gender in Malaysia from year 2000 until year 2003 (WHO, 2005). Among gender, malaria cases for male are higher than female; while among age, more than 5 years old individuals are higher contact in malaria cases compared to less than 5 years old children.

Malaysia can be divided into 3 geographically distinct regions with respect to malaria transmission: Peninsular Malaysia, Sabah and Sarawak. According to the table 1.3, Sarawak shows the majority reported cases (41%), followed by Peninsular Malaysia (31%), and Sabah (28%). Among Peninsular Malaysia region, Pahang has been shown the highest malaria cases compared to the other regions (WHO, 2005).

Year	Malaria cases
1990	50500
1991	39189
1992	36853
1993	39890
1994	58958
1995	59208
1996	52060
1997	26651
1998	13491
1999	11106
2000	12705
2001	12780
2002	11019
2003	6338

Table 1.1: Reported malaria cases in Malaysia (WHO, 2005)

Group	Subgroup	2000	2001	2002	2003	%
	Total	12 705	12 780	11 019	6 338	100
Gender	Male	8 633	8 817	7 527	4 483	71
	Female	4 072	3 963	3 492	1 855	29
Age	<5 years	1 795	1 723	1 486	607	10
	5> years	10 910	11 057	9 533	5 731	90

Table 1.2: Reported malaria cases by age and gender in Malaysia (WHO, 2005)

15 of 15 areas	2000	2001	2002	2003	%
Sarawak	3 011	3 145	2 496	2 615	41
Sabah	5 776	6 050	5 096	1 770	28
Pahang	1 301	1 544	1 563	850	13
Johor	710	671	579	284	4
Perak	852	470	280	276	4
Selangor	271	172	159	119	2
Pulau Pinang	209	197	76	106	2
Kelantan	386	184	333	99	2
Kedah	12	26	82	92	1
Terengganu	94	76	140	47	1
Negeri Sembilan	37	205	180	45	1
W.P.Kuala Lumpur	27	20	15	20	<1
W.P.Labuan				7	<1
Melaka	18	15	16	7	<1
Perlis	1	5	4	1	<1

Table 1.3: Reported malaria cases by selected sub national area in Malaysia (WHO, 2005)

1.3 Pathogenesis of *P. falciparum*

1.3.1 Life cycle of *P. falciparum*

The life cycle of *P. falciparum* is complex. It involves a mosquito vector in which sexual stage takes place and human hosts in which asexual stage takes place (figure 1.3). The female Anopheline mosquito becomes infected when it feeds on human blood containing gametocytes (Boon et al., 2006). In the mosquito, the malarial parasite undergoes sexual reproduction to produce the infectious stage parasite, called sporozoite (Greenbaum, 2008). Development of the parasites in the mosquito normally takes about 7-20 days (Boon et al., 2006). When the mosquito takes a bloodmeal, it will inject sporozoites into the human host (Greenbaum, 2008). Immediately after these sporozoites enter the circulatory system, they will invade the liver cells within 30 to 60 minutes (Boon et al., 2006). After invading the liver cell for some days, the parasite undergoes asexual reproduction, followed by differentiation into thousands of merozoite stage parasites, which flow into the blood stream to begin the erythrocytic cycle (Greenbaum, 2008).

Merozoites released from infected liver cells into the blood stream and invade red blood cells (RBCs). Again, asexual reproduction occurs with as many as 36 merozoites being produced in one RBC. In the erythrocyte, the merozoite goes through ring, trophozoite and schizont stages. The stage of merozoite within erythrocyte is illustrated in figure 1.4. The merozoites spill into the blood once again when the schizont ruptures. This RBC cycle is chronic and cycles every 48 hours. During this stage, the malaria associate morbidity and mortality occur. At some frequency during erythrocytic cycle sexual forms, gametocytes are produced. The gametocytes are then taken up by another mosquito during a blood meal. The sexual between a macro-gametocyte and

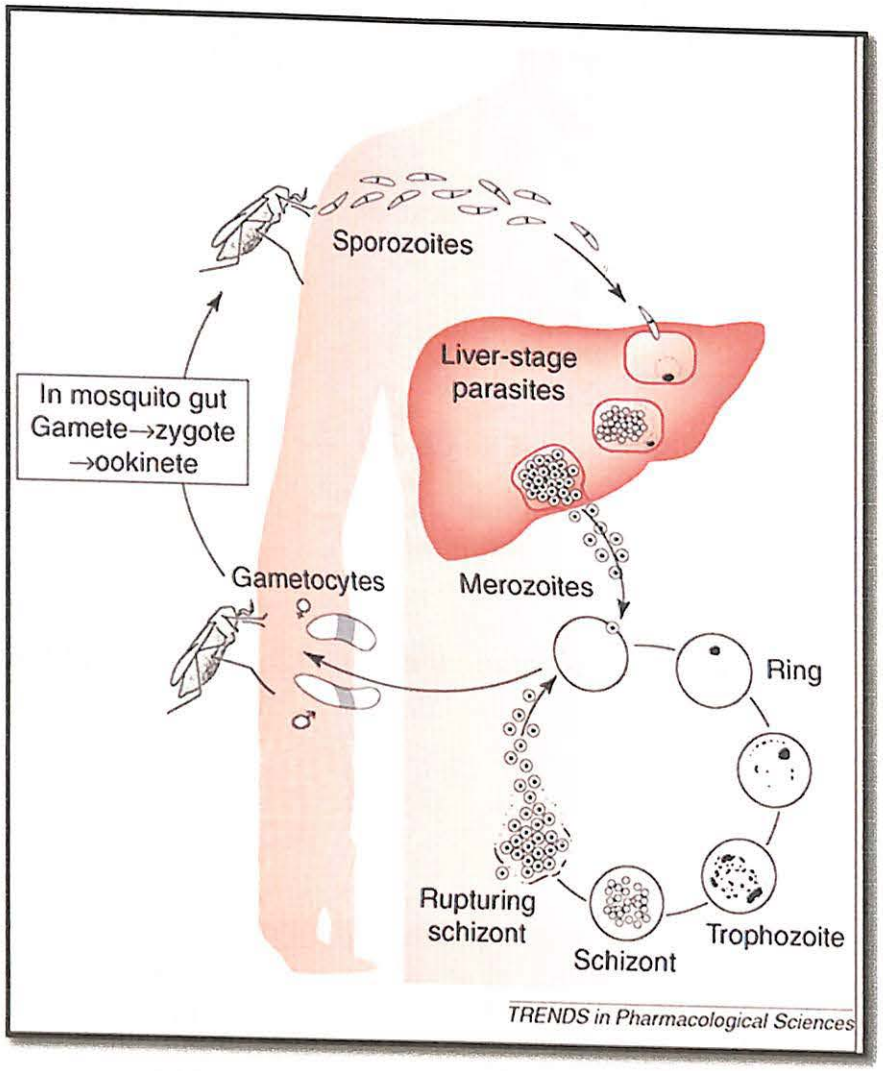

Figure 1.3: Life cycle of *P. falciparum* parasites (adapted from Greenbaum, 2008)

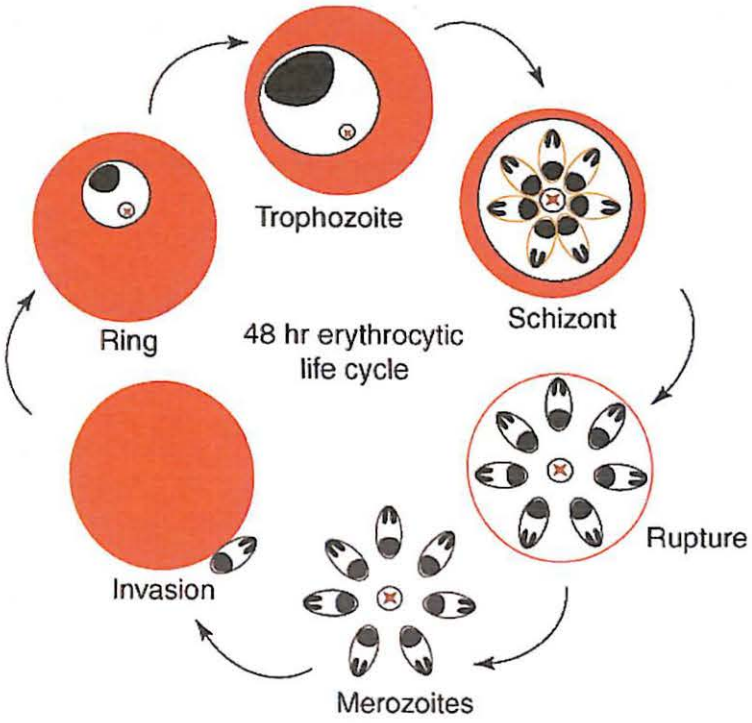

Figure 1.4: Life cycle of *P. falciparum* parasites in erythrocyte (adapted from Greenbaum, 2008)

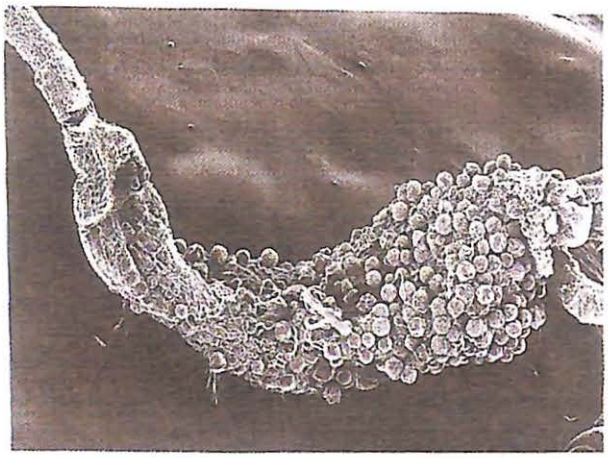

Figure 1.5: Scanning electron micrograph of *P. falciparum* oocysts lining the Anopheles mosquito (adapted from Boon et al., 2006)

micro-gametocyte would be ingests by mosquito from a malaria-infected individual. The gametocytes combine and differentiate into sporozoites, which are eventually secreted into the blood stream of another human when the mosquito takes its next blood meal (Greenbaum, 2008). The figure 1.5 in the page 9 shows the scanning electron micrograph of *P. falciparum* oocysts lining the Anopheles mosquito.

1.3.2 Pathology of malaria

According to Boon et. al (2006), the pathology in malaria is due to haemolysis of infected RBC and adherence of infected RBC to capillaries. In *P. falciparum* malaria disease, RBC containing schizonts adhere to capillary endothelium in brain, kidney, liver, lungs, and gut. The blood vessels become congested and the organs anoxic. Rupture of schizonts liberates toxic and antigenic substances that may cause further damage. The onset of infection is often insidious, with malaise, headache, cough, diarrhea and vomiting. Jaundice is also common in malaria cases due haemolysis and hepatic dysfunction. A patient with *P. falciparum,* apparently not seriously ill, may develop dangerous complication such as coma, hyperpyrexia, convulsion, hypoglycemia and acute renal failure.

1.4 Management of malaria

1.4.1 Diagnosis

The most common method used to diagnose malaria is via Giemsa stain of thick and thin blood film. The thick blood film is essential to check low level of parasitaemias while the thin blood film is essential to identify the species of parasite and essential to quantify the parasite load by counting the percentage of infected RBC (Boon et al., 2006). Nowadays, immunochromatographic dipstick test are marketed and provide a useful non-microscopic diagnosis (Boon et al., 2006). According to K. Erdman and C. Kain (2008), nucleic acid based tests such as Polymerase Chain Reaction (PCR), Loop-mediated isothermal amplification (LAMP), Microarrays and Quantitative nucleic acid sequence-based amplification (QT-NASBA) are also introduced to provide more efficient result in diagnosis malaria disease. Nucleic acid based technique is very specific and sensitive tools but not suitable to apply as universal diagnosis tools because they are very expensive and require the expert to do the diagnosis.

1.4.2 Treatment

According to Centers for Disease Control and Prevention (2007), treatment against malaria should be guided by three main factors;

1. The infecting *Plasmodium* species
2. The clinical status of the patient
3. The drug susceptibility of the infecting parasites

For *P. falciparum* infections acquired in areas without chloroquine-resistant strains, patients should be treated with oral chloroquine. For *P. falciparum* infections acquired in areas with chloroquine-resistant strains, three treatment options are available. The first

two treatment options are quinine sulfate plus doxycycline, tetracycline, or clindamycin; or atovaquone-proguanil (Malarone). The third option, mefloquine, is associated with a higher rate of severe neuropsychiatric reactions when used at treatment doses. The third option treatment is recommended only when the quinine sulfate combination or atovaquone-proguanil options cannot be used. For pediatric patients, the treatment options are the same as for adults except the drug dose is adjusted by patient weight. The pediatric dose should never be exceeding the recommended adult dose.

1.5 Rationale of vaccines development

The global resurgence of malaria today are due to the rapid spread of drug resistant *P. falciparum,* development of resistance to insecticides by anopheles mosquitoes, movement of population for seeking jobs, deterioration of socioeconomic conditions and environmental change (Krogstad, 1996; Marsh, 1998; Boon et al., 2006). Among reliable strategies to combat these prevailing problems involve the development of new drugs (WHO, 2007), restructuring of social economic condition (Singh et al., 2001), as well as the development of suitable candidate for vaccine.

Although several vaccine candidate antigens of *P. falciparum* have been identified and characterized, the development of a universal malaria vaccine against *P. falciparum* is still a difficult challenge due to the antigenic variation of different geographic isolates of the parasite (Akram et al., 2007), and the difficulty in diagnosing clinical malaria (O'Meara et al., 2007). Thus, the urgent need for the development of an effective vaccine and other malaria control strategies would eradicate the prevalence of malaria disease to a more manageable level.

There are several vaccines that have been developed against malaria disease, such as, irradiated sporozoites vaccines, SPff subunit vaccines, RTS,S recombinant vaccines, [NANP]19-5.1 recombinant vaccines and etceteras (Rapeah, 2003). The failures of these vaccines to curb the disease are due to antigenic variation in different geographic isolates of the parasite, impractical method to produce, expensive, short period immunity and its instability (Rapeah, 2003). Therefore, safer, easy to produce, relatively more stable, lower production cost as well as having a long term persistence of immunogens vaccines need to be developed.