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**Abstract:** Parasites cause some of the most devastating and prevalent diseases in humans and animals. Moreover, parasitic infections increase mortality rates of other serious non-parasitic infections caused by pathogens such as HIV-1. The impact of parasitic diseases in both industrialised and developing countries is further exacerbated by the resistance of some parasites to anti-parasitic drugs and the absence of efficacious parasite vaccines. Despite years of research, much remains to be done to develop effective vaccines against parasites. This review focuses on the more recent vaccine strategies such as DNA and viral vector-based vaccines that are currently being used to develop vaccines against parasites. Obstacles yet to be overcome and possible advantages and disadvantages of these vaccine modalities are also discussed.

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## **Parasite vaccines: The new generation**

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## **Abstract**

Parasites cause some of the most devastating and prevalent diseases in humans and animals. Moreover, parasitic infections increase mortality rates of other serious non-parasitic infections caused by pathogens such as HIV-1. The impact of parasitic diseases in both industrialised and developing countries is further exacerbated by the resistance of some parasites to anti-parasitic drugs and the absence of efficacious parasite vaccines. Despite years of research, much remains to be done to develop effective vaccines against parasites. This review focuses on the more recent vaccine strategies such as DNA and viral vector based vaccines that are currently being used to develop vaccines against parasites. Obstacles yet to be overcome and possible advantages and disadvantages of these vaccine modalities are also discussed.

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## Introduction

Infections caused by parasites are responsible for some of the most devastating and prevalent diseases of humans, livestock and companion animals. A single genus of parasite, *Plasmodium*, is responsible for the third most common cause of human mortality globally; malaria (Gardiner et al., 2005). Other similarly devastating parasitic diseases of humans include cysticercosis (including neurocysticercosis), toxoplasmosis, schistosomiasis and leishmaniasis, which cause significant morbidity and mortality (White and Garcia, 1999; Hill et al., 2005; Brooker et al., 2006; Schwartz et al., 2006). While parasitic infections can be found on every continent it is developing countries that suffer most from the multitude of diseases caused. This is further exacerbated by the HIV epidemic that many of them are currently experiencing as opportunistic parasitic infections increase HIV mortality (Losina et al., 2006). Recent research with mice has raised serious concerns regarding the impact of parasitic co-infection and the success of vaccines against serious life threatening diseases such as AIDS (Da'Dara et al., 2006).

In addition to causing diseases in humans, parasites also cause disease in animals. Infections caused by *Toxoplasma* spp. *Schistosoma* spp. and *Neospora* spp. are widely associated with significant economic loss in livestock and associated industries (Nielsen et al., 2006; Zhu et al., 2006; Williams and Trees, 2006). Whilst several cheap and effective vaccines do exist for parasites of livestock, resistance and public concerns about chemical residues in animal products (Vercruysse et al., 2004) mean safer and more effective vaccines are still needed even for those parasites currently under control.

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Vaccination against parasitic infections is relatively unsuccessful despite decades of research and millions of dollars spent. One reason is that parasitic infections, unlike those caused by bacterial or viral pathogens, tend to be chronic in nature. This is primarily because parasites often elicit inappropriate and ineffective immune responses in the host, or dampen the host immune system, thereby preventing a robust and effective immune response by the host (Cox, 1997). Moreover, parasites often exhibit various immune evasion strategies such as antigenic variation, molecular mimicry and sequestration at both the individual and infective population levels. Additionally, they exhibit complex lifecycles and other biological characteristics which complicate vaccine development against them (Good et al., 2004). Nevertheless, in most cases, parasitic infections do confer immunity to subsequent infections by the same parasite in the host, thus demonstrating the potential for a vaccine strategy (Scott, 2005).

Parasite vaccines have been developed utilising a range of strategies from crude whole organism preparations to peptide antigens (Liddell et al., 1999; Woollard et al., 1999). More recently, novel molecular based strategies have been employed. These include DNA vaccines, viral vector based vaccines and combinations of DNA and viral vector based modalities (Da'Dara et al., 2003; Nielsen et al., 2006). For example, a multitude of vaccine modalities have been evaluated against a single pathogen, *Leishmania* spp., (Table 1).

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This review aims to describe the progress made so far in the development and application of the latest generation of vaccine modalities to be employed against parasites; the DNA and viral vector based vaccines.

[Insert Table 1]

### **A brief history of parasite vaccine development**

The early vaccines against parasites were often based upon crude whole organism preparations, either intact irradiated larvae or ground-up parasites of various stages (Sharma et al., 1988; Delgado and McLaren, 1990; Eberl et al., 2001; Ploeger, 2002).

More recently, vaccines to parasites have sometimes been based upon protein, glycoprotein or carbohydrate antigens (Law et al., 2003; Vervelde et al., 2003). Recombinant proteins in particular are popular as vaccine antigens as they are easy to identify and easily produced commercially. Immunogenic surface antigens are the most common antigens used for the construction of protein based vaccines. Tran et al., (2006) reported on the success of their vaccine based upon the

surface antigens tetraspanin (TSP1 and 2). Studies revealed that the presence of antibodies directed towards these

membrane proteins in the human host conferred protection from chronic infection. Recombinant TSP based vaccines were evaluated in mice and TSP-2 in particular was effective in the mouse model reducing worm burdens by 57% and liver egg burdens by 64% in

-challenged mice (Tran et al., 2006). Another example of a protein based vaccine is a commercial vaccine that was developed for use in dogs, the Leishmune® vaccine (Table 1). Approaches involving the use of more than one antigen from the parasite have also been adopted with success, such as the

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(based on the ESAs and TSA-18 antigens) (Lightowlers et al., 1996).

In addition to protein and carbohydrate antigen based vaccine technology many vaccinologists are now focussing on developing genetic and viral vector based vaccines for parasitic diseases. The application of a broad range of technologies to vaccine development is perfectly illustrated by the range of vaccine candidates that have been developed against *Leishmania* spp. with vaccines ranging from purified whole organisms to DNA vaccines and a commercialised protein based vaccine (Leishmune®) (Table 1).

Interestingly, some whole organism vaccines have been found to be at least as effective if not more effective than some of the more recent technologically advanced vaccine formulations (Boulter and Hall, 1999; Matthews et al., 2001; Suo et al., 2006). It has been suggested that whilst the whole organism vaccine and the recombinant vaccine may contain the same key antigens, the process by which the recombinant protein is produced could result in the loss of crucial structural features associated with the protein's immunogenicity in its native state (Hein and Harrison, 2005). Hence the recombinant vaccine may be less effective than the whole organism based vaccine. Consequently, it appears that when it comes to parasites some vaccinologists are moving towards refining the traditional whole parasite based methodologies; for example a whole organism vaccine approach is being pursued to develop an effective vaccine against

*Schistosoma mansoni*

(Kariuki et al., 2006) and a phase III clinical trials of an attenuated parasite based vaccine against leishmania is currently underway in Iran ([www.ClinicalTrials.gov](http://www.ClinicalTrials.gov)). Yet such whole organism vaccines have only been effective for a handful of parasitic

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diseases, hence the continued focus on other vaccine modalities such as genetic and viral vector based vaccines.

### Genetic vaccines for parasites

In the years since Wolff et al., (1990) first demonstrated that naked DNA injected into mammalian muscle cells resulted in protein expression, DNA or genetic vaccines have been developed against a wide range of pathogens including

(Ganley-Leal et al., 2005), Mycobacteria (Kamath et al., 2000), Schistosoma mansoni

(Vanniasinkam et al., 2004), Hepatitis C virus Rhodococcus equi

(Dumonteil et al., 2004) and (Encke et al., 2007), Trypanosoma cruzi

(Guo et al., 2007). The ease of construction, preparation and administration, as well as the potential for its use in remote locations, contribute to the overall attractiveness of this vaccine modality. Furthermore, DNA vaccines can be used to generate either strong Th1 or Th2 type immune responses in the host. This makes them ideal for use against parasitic infections that require specifically tailored immune responses in order to confer protection. For example studies on leishmaniasis reveal that a Th1 type response is protective whilst a Th2 response is detrimental, eventually resulting in death of the host (Campos-Neto, 2005). Conversely, recent findings on schistosomiasis suggest that a Th2 biased cytokine response is essential for resistance to infection (Leenstra et al., 2006). Studies on

have also shown that a Th1/Th2 cytokine balance is critical to determining the course of the infection (Nishikawa et al., 2003). Interestingly, studies on

spp. demonstrate that although Th1 responses in the host are protective, leishmania antigens that typically elicit a Th1 response in the host are not necessarily protective when used as a vaccine. Conversely antigens such as LACK (leishmania homologue of receptors for

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activated C kinase) that usually promote a Th2 type immune response in the host, when manipulated to induce a protective Th1 type response in the host using immunomodulating adjuvants, have been found to elicit a protective response (Campos-Neto, 2005).

That DNA vaccine efficacy is largely dependant upon the route of vaccine application and choice of gene is well known (Li et al., 2004). Studies on *Brugia malayi* and *Plasmodium* spp., underscore the importance of the route of vaccine delivery, with the same antigen administered by two different routes resulting in diverse immune responses (Li et al., 2004; Weiss et al., 2000).

In recent years, large scale screening of parasite genomes is being used extensively to identify genes that may be potential DNA vaccine candidates (Bhatia et al., 2004). However, due to the differences in codon usage between mammals and parasites, the codon preference of the DNA used in the vaccine must first be optimised to ensure maximum efficacy (Gaucher and Chadee, 2002). Genes used in DNA vaccine construction range from those involved in the adhesion and invasion process within the host to genes that encode heat shock proteins, antioxidant enzymes and paramyosins (LoVerde et al., 2004; Beghetto et al., 2005; Solis et al., 2005). Not surprisingly, the majority of genes used in the construction of anti-parasitic vaccines are those which encode immunogenic surface antigens (Da'Dara et al., 2003).

DNA vaccine technology also lends itself to the construction of multi-epitope vaccines. Scorza et al., (2005) have described a multi-epitope malaria vaccine (derived from a

*Plasmodium chabaudi adami* DS DNA expression library) that when used to vaccinate

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BALB/c mice generated opsonizing antibodies and an antigen specific interferon gamma response, importantly the vaccine conferred protection against a heterologous (*P. chabaudi adami*

DK) parasite challenge (Scorza et al., 2005).

On the other hand, some researchers have demonstrated the ineffectiveness of the multivalent DNA vaccine approach. Li et al., (2004) developed DNA vaccines based upon four antigens of *Brugia malayi* (paramyosin (BM5), heat shock protein (BMHSP-70), intermediate filament (BMIF) and an immunogenic antigen (BM14)). Subsequent studies in mice indicated that a mixture of all four vaccines induced a significant antibody response to all antigens. Interestingly, the response to each of the 4 antigens was varied. Importantly, the response to the polyvalent vaccine regimen was less effective than the response induced when each component was administered alone, suggesting that a multi epitope vaccine approach may not be feasible in some cases (Li, et al., 2004)

Many researchers have demonstrated variable success using DNA vaccines. In one study, a vaccine against

*Schistosoma mansoni* (Sm23), that encodes a membrane protein, was developed and trialled in mice as both a DNA vaccine and a recombinant protein vaccine (Da'Dara, et al., 2003). Three weeks after 2 doses of vaccine, administered 21 days apart, the animals were challenged with

cercariae. The results indicated that the best level of protection, which was a 44% reduction in worm burden, was obtained in mice primed and boosted with the DNA vaccine (Da'Dara et al., 2003). Other investigators have developed DNA vaccines based upon antigens expressed during the chronic stage of the parasitic infection; this approach is particularly relevant to diseases such as toxoplasmosis that can occur as relapsing

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chronic conditions. A DNA vaccine based upon the bradyzoite antigens (BAG1 and MAG1) of *Toxoplasma gondii* was administered (Nielsen et al., 2006). These examples further highlight the difficulty in obtaining sterilising immunity using DNA vaccines against parasites.

Overall, DNA vaccines have not been seen as being universally successful parasite vaccines. Despite showing promise in the mouse model, clinical trials of DNA vaccines (parasite and non parasite specific) indicate that overall, these vaccines are not efficacious in large mammals including humans (Smooker et al., 2004; Laddy and Weiner, 2006). Furthermore, some studies have shown that DNA vaccines are not as effective as other vaccine modalities, for instance, a comparison between the schistosomal membrane antigen encoding gene (*sm23*) based DNA vaccine with a vaccine comprising irradiated cercariae showed that although the DNA vaccine induced significant parasite specific immune responses in mice, it did not induce optimal vaccine efficacy (Ganley-Leal et al., 2005). The reasons for the lack of efficacy of DNA vaccines have been discussed in detail in other reviews (Ertl, 2003; Vanniasinkam and Ertl, 2004).

In order to improve overall efficacy, DNA vaccines are now often used as part of prime boost vaccination schedules with either recombinant protein or viral vector based vaccines (Goonetilleka et al., 2006; Liang et al., 2006). However this strategy does not always lead to efficacy levels required of an effective vaccine or vaccine strategy

Iborra et al.(2003) developed a DNA vaccine based upon the immunodominant acidic ribosomal protein P0 (LiP0) (Iborra et al., 2003). The efficacy of this vaccine was

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ascertained in the BALB/c mouse model. Animals were immunized with either DNA vaccine alone or as a prime followed by a recombinant protein (rLiPO) boost. Results indicated that protection was achieved by the DNA vaccine alone and not when used with an rLiPO boost or when mice were immunised with rLiPO alone. In this study the DNA vaccine elicited a protective Th1 type response and the rLiPO vaccine elicited a Th2 response. Consequently the heterologous prime boost protocol resulted in a mixed Th1/Th2 response which was not as protective as the un-mixed Th1 response. Interestingly, other studies have found no significant difference between a recombinant protein based vaccine and its DNA counterpart. For example, Sol

s et al., (2005) constructed a DNA vaccine based on the i

paramyosin gene VW2-1. Mice immunised with the DNA vaccine or recombinant VW2-1 vaccine, were challenged intraperitoneally with

cysticerci. The results of this study revealed that there was no significant difference in the level of protection (43-48%) afforded by the DNA vaccine when compared with the recombinant VW2-1 vaccine. These results were replicated in the porcine model ( Sol

is et al., 2005).

Other attempts to enhance the efficacy of a DNA based vaccine strategy have included co-administering DNA vaccines with cytokine expressing vaccines in order to augment the efficacy of the DNA vaccine and some researchers have found this approach to be reasonably successful. The success of this approach was illustrated in a study which showed that the co-application of IL-12 (as a DNA vaccine) together with a DNA vaccine based upon the

triose-phosphate isomerase gene resulted in approximately a further 30% decrease in liver egg burdens in the porcine model (65.8% reduction in liver

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egg burden) than when the triose-phosphate isomerase-based DNA vaccine was used alone (49.4% reduction in eggs found in the vaccinated animal's liver). (Zhu et al., 2006).

Some researchers have found that DNA vaccines are most effective when administered in a heterologous prime boost protocol comprising of a DNA vaccine prime and viral vector based vaccine boost with both vaccine modalities based upon the same antigen. Pérez-Jiménez et al., (2006) demonstrated a significant immune response (increase in interferon gamma secreting CD8<sup>+</sup> T cells) in mice primed with a LACK-expressing DNA vaccine and boosted with a LACK expressing modified vaccinia Ankara (MVA) (Table 1). Recent studies suggest that the co-administration of cytostatic drugs such as Trichostatin A may help upregulate viral promoter driven DNA vaccine activity thus improving the vaccine's performance. Further studies are required to determine if this tactic will improve DNA vaccine efficacy in large animals (Vanniasinkam et al., 2006).

### **Viral vector based vaccines**

Viral vectors based upon adenovirus and pox virus are being increasingly used in the construction of effective vaccines (Xing et al., 2005; Hanke et al., 2007). Overall, studies have shown them to be far more effective than DNA vaccines (Prieur, 2004).

Viral vector based vaccines are capable of inducing potent, highly effective immune responses in the vaccinated hosts (Pérez –Jiménez et al., 2006; Vanniasinkam and Ertl, 2005). Furthermore, studies have shown that these vaccines may indeed be used to elicit strong Th1 type immune responses which are particularly useful when developing a vaccine against a disease such as leishmaniasis that typically requires a strong Th1 type protective response (Campos-Neto, 2005).

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One disadvantage associated with some viral vaccine vectors (e.g. human adenovirus serotype 5-Ad5) is that they may not work effectively in the host if there is pre-existing immunity to the carrier. This is particularly a concern with some human serotype adenovirus-based vectors as extensive research in this area has revealed that a significant percentage of the population possess neutralising antibodies to some of these viruses (Fitzgerald et al., 2003). Consequently, research involving alternative adenovirus serotypes such as those of simian origin is being undertaken (Roy et al., 2006). Some researchers have focussed their attention on developing replication defective viral vaccine vectors which are considered to be safer than replication competent vectors (Farina et al., 2001).

Some researchers have developed parasite vaccines based upon human serotype adenoviruses that demonstrate relatively low seroprevalence in the target population. Ophorst et al., (2006) used a replication-deficient human serotype adenovirus 35 based vector to construct a vaccine against

. This adenovirus serotype was chosen on the basis of studies that have shown relatively low seroprevalence (20%) to this vector in humans living in regions of Africa with endemic malaria when compared with the human adenovirus serotype 5 vector (85% of the same population exhibited significant levels of neutralizing antibodies to this virus). In this study, a single dose of the vaccine (10

to 10

6

cidum, of recombinant (G6) virus serotype 35 (Ad35) BAP using the Potent CS1-specific T cell and antibody responses were obtained. However, when these results were compared

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to those achieved with the recombinant Ad5 vaccine the immune responses were significantly lower with the Ad35 based vaccine. In addition, both the Ad35 and Ad5 based vaccines significantly reduced parasite liver burdens when mice were challenged with

sporozoites two weeks following immunization, although the Ad5 based vaccines were significantly more effective in reducing parasite burdens. These results illustrate that even though the Ad35 vector based vaccine was not as efficacious as the Ad5 based vaccine, it is still possible to promote effective immune responses using Ad35 based vaccines.

Whilst a range of viruses have been developed as vaccine vectors, research has shown that some viruses are more effective as vaccine vectors than others. In some cases different strains of the same virus have exhibited different levels of vaccine efficacy as seen with pox virus vectors. The FP9 strain of fowlpox virus for example is considered to be more immunogenic than the Webster FPW fowlpox virus strain. The reason for this is postulated to be extensive gene mutations and deletions acquired through multiple tissue culture passages by the FP9 strain (Cottingham et al., 2006). Therefore it appears that the choice of viral vaccine vector is crucial for developing an effective parasite vaccine.

In addition to pox virus and adenoviral vectors, vaccinia virus based vectors have also been used in parasite vaccine development. Nishikawa et al., (2001) developed vaccines against the bovine pathogen

constructed using recombinant vaccinia viruses expressing surface antigens of

In one study immunized mice (mice were immunized on two occasions with  $1 \times 10^7$

plaque forming units of recombinant virus expressing the NcSRS2 antigen) were challenged with  $4 \times 10^7$

*N. caninum*

4

tachyzoites 18 days following the last immunization and found to be protected

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infection 26 days post challenge. In addition, interferon gamma responses of splenocytes from vaccinated mice was significantly higher than that from splenocytes obtained from unvaccinated mice, further confirming the efficacy of the vaccine (Nishikawa et al., 2001).

Some researchers have employed vaccination regimes comprising of multiple viral vector based vaccines in order to enhance vaccine immunogenicity. This approach was adopted in a study conducted by Caetano et al., (2006) in which three recombinant adenovirus vaccine vectors expressing three different *T. gondii* surface antigens (AdSAG1, AdSAG2, and AdSAG3) were used to immunize mice. Following challenge with live *T. gondii* a high level of protection against cyst burden (80% reduction in cysts) was observed when mice were vaccinated with all three vaccines (Caetano et al., 2006). However this vaccination regimen was found to be ineffective against a highly virulent strain of *T. gondii* tachyzoites.

It appears that if multiple viral vector-based vaccines are being used in a prime boost protocol a heterologous prime boost regimen may be more effective than a homologous prime boost regimen. In a series of experiments utilizing a prime boost protocol,

PP010114 Falciparum vaccine development (H3SETH) (Pressing et al., 2004). The vaccines induced potent CD8

T cell responses and the blood levels of vaccinated mice were significantly higher than that obtained in a homologous prime boost schedule (Prieur et al., 2004). This is an observation that has been made by other researchers and may have

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been due to vector specific immune responses interfering with the immune response to the vaccine antigen (Prieur et al., 2004).

Other studies have confirmed that using heterologous prime boost vaccination protocols result in enhanced memory T cell responses (Ophorst et al., 2006).

Furthermore, Bejon et al., (2006a) ascertained that when using multiple viral vector based vaccines in a vaccination regimen, the most immunogenic regimen is an alternating vector immunization of a 3 dose regimen rather than a prime boost protocol. In this study attenuated fowlpox virus (FP9) or modified virus Ankara (MVA) vaccines expressing the multiple epitope thrombospondin-related adhesion protein (ME-TRAP) of *P. falciparum* were administered to human volunteers in a malaria endemic area. The vaccines administered 3 weeks apart elicited a strong immunogenic response when administered in an alternating vaccine vector regimen (e.g. MVA/FP9/MVA). Notably, impressive memory T cell responses were induced (Bejon et al., 2006a).

In order to improve the efficacy of viral vector based vaccines, viral vector vaccines are often applied as part of a prime boost protocol along with either recombinant protein vaccines or DNA vaccines. Studies on *Taenia ovis* have demonstrated that a DNA vaccine prime followed by an ovine adenoviral vector based vaccine boost, elicited a protective immune response, whilst the DNA vaccine or adenoviral vector based vaccines alone did not elicit a significant immune response in the ovine host (Rothel et al., 1997). Studies on *Leishmania infantum* (Table 2) and more recently studies on malaria

Researchers have also used cytokine expressing viral vector vaccines to improve vaccine efficacy and powerful Th1 responses have been obtained to vaccine candidates when co-

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administered with a Th1 promoting cytokine-expressing viral vaccine vector such as an adenovirus vector based IL-12 vaccine (Gabaglia et al., 2004).

Overall viral vectors have been shown to be promising for use in parasite vaccine development. However, an important issue that needs to be addressed before viral vector based vaccines can be more widely used relates to an observation by researchers that there is batch to batch variation in the quality of viral vector based vaccines that can result in inconsistencies in the levels of immunity these vaccines induce (Bejon et al., 2006a). This issue will need to be addressed before viral vector based parasite vaccines can be developed for global immunization programs.

[Insert Table 2]

### **Challenges that remain to be overcome**

Researchers have trialed a plethora of approaches towards developing vaccines against parasites of medical and veterinary significance, however, very few parasite vaccines are commercially available today. Vaccines to helminth pathogens in particular have proven to be difficult to develop. Some commercial vaccines which were found to be very useful initially are not as highly favored anymore. A noteworthy example of this being the vaccine against

(Smith and Zarleng, 2006). This vaccine whilst being effective against infections in cattle has some distinct disadvantages such as instability, labor intensive to manufacture and requiring frequent exposure to natural challenge in order to be effective (Matthews et al., 2001) and there have been reports of a resurgence in

infections in cattle possibly as the result of farmers using antihelminthic treatments in preference to the vaccine (Mawhinney, 1996). Furthermore,

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other hosts is also highly effective in (Johnson et al., 2003) parasite infections in

With some of the other vaccines that have been more successful, such as the EG95 vaccine developed against *Echinococcus granulosus* (Gauci et al., 2005) it is difficult to predict the long term efficacy of such vaccines as scientists have contemplated on the possibility that the large scale, long term use of defined antigen based vaccines, would inevitably encourage the emergence of vaccine resistant strains in a species that is extremely genetically diverse (Lightowlers et al., 2003). Currently there is no evidence to support this hypothesis, although, it is reported that that the EG95 vaccine does not elicit sterilizing immunity in 14% of vaccinated hosts (Lightowlers et al., 1999) which suggests that the widespread use of this vaccine may not be beneficial in the long term.

Some scientists have postulated that host genetic makeup also plays a role in the immune response to parasite vaccines; research is underway to determine genetic traits that predispose animals to parasitic infections (Sonstegard and Gasbarre, 2001). In some cases researchers have considered it more feasible to think in terms of developing vaccines to control levels of parasite infection rather than elicit sterilizing immunity in the host (Geldhof et al., 2004). In this regard, in the future parasite vaccines could be directed more towards control rather than eradication.

### **Concluding remarks**

Tremendous progress has been made in parasite vaccine research in the last decade. Despite this, there are few commercially available vaccines to parasites. Moreover, there

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are no commercially available parasite vaccines for use in humans. There are nevertheless some parasite vaccines currently being evaluated in clinical trials. Most of these vaccines are, not surprisingly, malaria vaccines (Druilhe et al., 2005; Takala et al, 2007). Of the vaccines that have been or are undergoing evaluation in clinical trials at present a number of them have been developed against

and are frequently based upon recombinant protein or synthetic peptide technology (Herrera et al., 2007). Studies on these vaccine candidates have been excellently reviewed recently by Epstein et al. (2007) and Hill (2006). One of the most promising of all malaria vaccines assessed in clinical trials to date is the RTS,S vaccine. The safety of this vaccine has already been established and Glaxo SmithKline Biologicals (GSK Biologicals) is currently conducting several studies to determine the long term efficacy of this vaccine (Malaria Vaccine Initiative) ([www.malariavaccine.org](http://www.malariavaccine.org)). Interestingly, Stewart et al., (2007) have recently demonstrated that the effectiveness of this vaccine could be significantly improved if used in a prime boost protocol with a replication-defective human adenovirus serotype 35 (Ad35) vector based vaccine.

Other parasite vaccines currently being evaluated in clinical trials include a killed leishmania vaccine (*L. major* (ALM) co-administered with the BCG (Bacillus Calmette Guerin) vaccine ([www.clinicaltrials.gov](http://www.clinicaltrials.gov).) and the recombinant larval protein Na-ASP-2 based vaccine against the helminth *Necator americanus* (Fujiwara et al., 2005), (Phase I clinical trial to be conducted in Brazil in 2007) ([clinicaltrials.gov](http://clinicaltrials.gov)).

Few parasite vaccine clinical trials currently being undertaken involve DNA and viral vector based vaccine technologies. These vaccine modalities have been widely applied to

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(Table 2) parasites data available from public clinical studies and Soljerno et al. (2006). Parasite vaccines have sometimes been disappointing. For example in a study conducted on human volunteers immunized with DNA and viral vector based vaccines against

only 1 in 8 subjects was completely protected from *P. falciparum* challenge (Dunachie et al., 2006).

However, recently, DNA and viral vector based vaccines have been included in large scale clinical trials. Two examples of clinical trials on DNA and viral vector based parasite vaccines from which results will be available in the near future include a Phase I randomized study on an adenovirus human serotype 35 based vector encoding the malarial circumsporozoite protein. The primary aim of the study is to generate data relating to safety and efficacy of the candidate vaccine. Participants in this study will receive doses of  $10^8$  to  $10^{10}$  vp/ml vaccine administered at 0, 1 and 6 months (www.clinicaltrials.gov). Another Phase I study has been planned for completion in 2008 and will involve a circumsporozoite protein-expressing MVA (MVA.CSO) and *P. falciparum* circumsporozoite protein-expressing (CSP) DNA vaccine. The vaccination regime will comprise two doses of the DNA vaccine (1 month apart) followed by the MVA vaccine 1 or 6 months after the 2<sup>nd</sup> dose of DNA vaccine. The DNA vaccine will be applied intramuscularly whilst the MVA vaccine will be administered intradermally (www.clinicaltrials.gov). Once again the aim of this trial is to verify safety and efficacy of the vaccines used.

In view of the lack of safe and affordable anti-parasitic drugs for humans, the increasing resistance to anti-parasitic treatments in livestock and humans (Schellenberg et al., 2006;

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Roberts, 2006) and the continuing problems associated with controlling vectors that transmit some parasites (Roberts et al., 2000) it is imperative that efficacious vaccines to parasites, particularly vaccines that are capable of inducing strong immunological memory responses are developed. However, more research especially into immunologically relevant vaccine antigens and vaccine modalities that work against particular parasites, including the use of adjuvants to improve vaccine efficacy, is needed.

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			<b>vaccinated animals</b>	
<p>Table 1</p> <p>A Leishmania based example outlining the wide spectrum of vaccine technologies adopted over the last two decades in the search for effective vaccines against parasites.</p> <p>*studies were conducted on a small numbers of animals</p>	1985	Purified native antigen of <i>L. major</i> promastigotes (glycolipid-parasite receptor for macrophages)	Partial protection in mice following challenge with <i>L. major</i> promastigotes (25% vaccinated mice exhibited lesions 60 days following challenge)	Handman and Mitchell, 1985
	1988	Partially purified antigens from sonicated <i>L. infantum</i> and <i>L. major</i> promastigotes	Neutralizing antibodies in BALB/c mice and protection against challenge with <i>L. major</i> or <i>L. mexicana</i> promastigotes	Frommel et al., 1988
	1995	Live attenuated (auxotrophic gene knockouts) <i>L. major</i>	Protection against cutaneous leishmaniasis in the BALB/c mouse model when challenged with virulent <i>L. major</i>	Titus et al., 1994
	2000	Live bacterial vector based ( <i>L. monocytogenes</i> expressing LACK (leishmania homologue of receptors for activated C kinase) vaccine	Significant Th1 response in vaccinated mice. No protection in BALB/c mice following challenge with <i>L. major</i> .	Soussi et al., 2000
	2003	Fusion protein (Q protein - 5 antigenic determinants of 4 different proteins)	*Antibody responses in dogs (beagles) following immunization, upon challenge with <i>L. infantum</i> promastigotes 90% protection against clinical infection.	Molano et al., 2003
			DNA Vaccine prime with viral (vaccinia-WR strain) boost (both expressing LACK)	*60% protection achieved with DNA/LACK-prime followed by vaccinia(WR)/LACK boost regime upon challenge with <i>L. infantum</i> promastigotes.
	2005	Purified naturally secreted antibodies	* Following challenge with <i>L.</i>	Lemesre et al., 2005

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Table

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Parasite	Vaccine strategy	Antigen	Model used vaccine
<i>Trichinella spiralis</i>	DNA	TspE1 (31 kDa protein)	Mouse (BALB/c)
<i>Clonorchis sinensis</i>	DNA	FABP (fatty acid binding protein)	Rat (Sprague-Dawley)
<i>Toxoplasma gondii</i>	DNA	GRA1 (excreted-secreted granular protein)	Mouse (C3H/HeJ)
<i>Anaplasma marginale</i>	DNA	MSP1b (major surface protein)	Calves (Holstein-Friesian)
<i>Schistosoma japonicum</i>	DNA	SjCTPI (Triose-phosphate isomerase)	Pigs (Chinese Songjiang)*
<i>Plasmodium falciparum</i>	Viral vector Vaccine (Fowlpox virus (FP9) and modified vaccinia virus Ankara (MVA))	ME-TRAP (multiple epitope-thrombospondin-related adhesion protein).	Human* (1-6 year old malaria endemic region)
<i>Leishmania infantum</i>	DNA prime/viral vector (vaccinia-Western Reserve virus (WR) or MVA) boost	LACK (Leishmania homologue of receptors for activated C kinase)	Mice (BALB/c)

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