Vaccination Against Cysticercosis and Hydatid Disease

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Infections with the larval stages of taeniid cestode parasites cause substantial human morbidity as well as economic losses in domestic livestock species. Despite ongoing efforts around the world, few countries have been able substantially to reduce or eradicate these infections through the use of anthelmintics and lifestyle changes. Vaccines offer an additional potential tool to assist with the control of parasite transmission. Here, Marshall Lightowlers and colleagues review the substantial progress that has been made towards developing practical vaccines against hydatid disease in sheep and cysticercosis in sheep and cattle. Recombinant antigens have been used to induce more than 90% protection against challenge infections. Such success in animals encourages investigation of the potential use of vaccines in humans to prevent hydatid disease arising from infection with Echinococcus granulosus *and cysticercosis from infection with* Taenia solium.

Cysticercosis and hydatid disease are caused by cestode parasites belonging to the family Taeniidae. Infection is acquired after ingestion of the parasite's egg, containing an infective oncosphere (Box 1). In a suitable intermediate host species, the parasite develops into an infective metacestode in body tissues. Although humans are rarely involved in perpetuating transmission of the parasites as intermediate hosts, they are suitable hosts for several taeniid species. Parasites of the genus *Echinococcus* cause hydatid disease, whereas *Taenia* species cause cysticercosis.

Echinococcus granulosus is by far the most common aetiological agent of hydatid disease, causing what is known as cystic hydatid disease. It has a worldwide distribution, particularly in countries where pastoral activities are prominent[1]. Dogs and other canids are definitive hosts. Many herbivorous species are susceptible to infection with *E. granulosus,* including wildlife and domesticated animals. Sheep are frequently involved in transmission of the disease and the common use of dogs with sheep flocks provides a close association that promotes transmission of hydatid disease. In animals and humans, hydatid cysts frequently occur in the liver and lungs, although they can occur at any anatomical site. Medical treatment for hydatid disease frequently involves major surgery. Human infections are most common in poor rural populations, especially in parts of Africa and China, where prevalence has been described as high as 2900 in 100 000 (Ref. 2).

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Unlike *E. granulosus*, parasites of the genus *Taenia,* which cause cysticercosis, are typically very host specific for both intermediate and definitive host species. The parasite of major medical importance is *T. solium*. Humans are the only natural definitive host species, with the parasite occurring as a large tapeworm in the small intestine. Pigs are the only intermediate hosts involved in transmission of the parasite. Ingestion by pigs of *T. solium* eggs derived from human faeces leads to the establishment of the metacestode stage (cysticercus) in the striated muscles and brain. Humans become infected with the tapeworm by ingesting insufficiently cooked, infected pig meat. The medical consequences of infection with the adult tapeworm (referred to as taeniasis) are typically trivial. However, the tapeworm eggs are not only infective for pigs, but can also infect humans, maturing into cysticerci that often occur in nervous tissue, causing the medically serious disease neurocysticercosis[3,4]. *Taenia solium* is most prevalent in Central America and the northern countries of South America, and also has a widespread distribution throughout the non-Islamic countries of Asia, India and Africa. *Taenia ovis* and *T. saginata* are economically important species causing cysticercosis in sheep and cattle, respectively. Economic losses are incurred when meat infected with cysticerci is condemned as unfit for human consumption. Dogs are the definitive host for *T. ovis* and humans act as definitive host for *T. saginata*.

Existing measures for parasite control

General improvement in the economic situation of countries or regions is likely to lead to decreased transmission of both cystic hydatid disease and cysticercosis. Improvements in public education about sanitation and the availability and use of prepared dog foods impact on transmission of hydatid disease. Similarly, improvements in public health education and sanitation, the provision of latrines and the industrialization of pig raising decrease transmission of *T. solium*. Such

Box 1. Life Cycle of Taeniidae

Taeniid cestodes have a prey–predator life cycle involving two mammalian hosts. Sexual reproduction occurs in hermaphroditic tapeworms in the small intestine of a carnivorous or omnivorous definitive host. Mature infective eggs are released with the faeces of the definitive host. When ingested by a suitable species of intermediate host, the oncosphere hatches, penetrates the intestinal mucosa and migrates via the circulatory system to a suitable tissue site at which the parasite develops into a mature, infective metacestode, possibly remaining viable for the life of the animal. Further transmission of the parasite occurs when the infected tissues of the intermediate host are eaten by a suitable definitive host species. The larval cestode attaches to the wall of the small intestine and grows into a mature, gravid tapeworm over a period of about 6–8 weeks. Gravid infections can persist in the definitive host for months or years.

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Table 1. Results of vaccine trials against hydatid infection in sheep using the EG95 vaccine[a]

Source of *Echinococcus granulosus* isolate	Number of viable hydatid cysts [mean (range)]		Protection (%)
	In controls	In vaccinates	
New Zealand	156.6 (70–270)	5.8 (0–16)	96
New Zealand	256.0 (32–445)	7.5[b] (0–44)	97
New Zealand	36.8 (0–85)	0.0 (0)	100
Australia	4.7 (0–16)	0.2 (0–1)	96
Argentina	23.1 (2–64)	0.1 (0–1)	99
China	15.4 (6–40)	0.4 (0–2)	97

[a] Data from Refs 14,15; D. Heath, unpublished.
[b] Combination of three different adjuvant groups.

measures, not directly related to *T. solium*, are thought to have been the cause of the decline in neurocysticercosis in western Europe over the past century[5]. Specific measures that have been used for control of these diseases have included education about how to prevent transmission, treatment of the definitive hosts with anthelmintics and, in the case of hydatid disease, other measures to control or reduce the number of the definitive hosts of the parasites.

The need for vaccines

Numerous countries have had active control programmes for hydatid disease for many years, based on public education and dog control. However, few control programmes have had a major impact on disease transmission. It is difficult to change people's behaviour even in affluent, well-educated populations. In dogs, little or no immunity develops to *E. granulosus*, and so to ensure that *E. granulosus* transmission cannot occur, dogs need to be treated with anthelmintic drugs, such as praziquantel, every six weeks. Where the resources are available for control measures to be sustained effectively for many years, and where importation of new disease-carrying hosts can be prevented, hydatid disease can be eradicated. Such measures have been effective in Iceland[6], New Zealand and Tasmania[7], but few other countries have been prepared to invest the resources necessary to achieve a high degree of control of transmission. In addition, many countries have extensive, relatively unprotected borders with neighbouring regions where hydatid disease continues to occur, which provides an ongoing source of new infections. Sylvatic transmission also provides a source of new infections in livestock animals, potentially perpetuating the domestic transmission cycle.

Control measures for prevention of transmission of *T. solium* cysticercosis have not been investigated as extensively as for hydatid disease. Public education[8] and treatment of the human population to remove tapeworm carriers[9–11] have resulted in significant decreases in *T. solium* transmission in experimental trials. The longer-term practicality and effectiveness of these measures have not been assessed. In New Zealand, control of ovine cysticercosis by treatment of the definitive hosts with anthelmintics resulted in the appearance of cysticercosis storms when, occasionally, an infected dog failed to be treated and went on to contaminate a wide area and a large number of stock[12]. The control programme appeared to have reduced the level of immunity to the parasite in the sheep flock such that, when a breakdown in transmission did occur, the level of infection observed was greater than had been the case before the instigation of control measures.

Introduction of new sources of infection from regions outside the parasite control areas remains perhaps the greatest single problem with achieving sustained control of *E. granulosus* or *T. solium* in most parts of the world. This problem could potentially be reduced or eliminated by the prevention of infection in animal intermediate hosts by vaccination. The combined use of public education, control measures in the definitive host and vaccination of animal intermediate hosts would provide the most effective ongoing control of parasite transmission.

Vaccination against hydatid disease

Osborn and Heath[13] showed that sheep could be vaccinated against infection with *E. granulosus* by immunization with non-living antigens obtained from the parasite oncosphere. Recombinant DNA methods provided the necessary tools for these antigens to be produced on a scale suitable for practical application. Oncosphere RNA was cloned and expressed in *Escherichia coli* and the product of one clone, designated EG95, was found to induce host-protective immunity in sheep[14]. Subsequent vaccine trials in New Zealand, Australia, Argentina and China have confirmed the effectiveness of the vaccine[15] (Table 1; Box 2). Further trials are in progress to define the operational characteristics of the vaccine and how it might be used most effectively for the practical control of hydatid transmission. One important outcome of trials

Box 2. Characteristics of the EG95 Vaccine Against Hydatid Disease

The following summarizes the information available about the EG95 antigen and its effectiveness as a vaccine against hydatid disease (Refs 14,15; D. Heath and O. Jensen, unpublished).

EG95 antigen

- Native oncosphere antigen of 24.5 kDa
- cDNA encodes a 153 amino acid protein of 16.5 kDa
- Produced in *Escherichia coli* as a fusion protein with glutathione *S*-transferase

Vaccine trials

- Induces >95% protection against experimental challenge infection in sheep (Table 1) and goats
- Similarly effective in vaccine trials carried out with *Echinococcus granulosus* isolates from New Zealand, Australia, Argentina and China
- Immunity lasts at least one year after two immunizations
- Immunity can be transferred passively to neonates with colostral antibody from a vaccinated dam
- Immunity measured in *in vivo* trials correlates with the ability of serum from vaccinated sheep to induce complement-mediated lysis of *E. granulosus* oncospheres in *in vitro* culture

Table 2. Results of vaccine trials against cysticercosis in sheep and cattle using recombinant antigens[a]

Host	Challenge parasite	Antigen(s)	Total number of cysticerci [mean (range)] In controls	In vaccinates	Protection (%)
Sheep	*Taenia ovis*	45W	29.0 (11–570)	2.0 (0–7)	94
Sheep	*Taenia ovis*	45W B/X	65.4 (19–110)	2.4 (0–10)	96
Sheep	*Taenia ovis*	16K	59.8 (19–104)	4.8 (0–19)	92
Sheep	*Taenia ovis*	18K	59.8 (19–104)	1.0 (0–3)	98
Cattle	*Taenia saginata*	TSA	169.0 (47–265)	11.0 (0–30)	94
Cattle	*Taenia saginata*	TSA	502.0 (327–675)	0.9 (0–6)	99

[a] Data from Refs 18,19,23,39.

completed in both Argentina and China is that the very high level of immunity stimulated by two immunizations with the vaccine lasts for at least one year after the second injection (O. Jensen *et al.*, unpublished). A major mechanism responsible for protection induced by the vaccine is antibody- and complement-mediated lysis of oncospheres[14], and this immunity can be transferred to neonates with colostral antibody from a vaccinated dam (O. Jensen and D. Heath, unpublished).

Vaccination against cysticercosis

Most progress has been made with vaccination against cysticercosis caused by *T. ovis* in sheep and *T. saginata* in cattle. Native oncosphere antigens were identified as a source of host-protective antigens[16,17]. Specific host-protective molecules were identified for *T. ovis* and recombinant DNA techniques were used successfully to clone and express the genes encoding three different antigens, each of which was able to induce almost complete immunity against experimental challenge infection in sheep[18,19]. The vaccine has undergone extensive trials[20,21] and has been registered for commercial use in New Zealand. Regrettably, economic and political factors have prevented the commercial release of the vaccine to date[22].

Identification of three host-protective recombinant antigens from *T. ovis* provided the opportunity to use the DNA sequence information to develop an effective vaccine against infection with the closely related parasite, *T. saginata*, in cattle[23]. Hybridization experiments with *T. saginata* genomic DNA identified the presence of DNA fragments having homology with each of the three host-protective antigens of *T. ovis*. Two of these were cloned from *T. saginata* oncosphere mRNA. In repeated vaccine trials in cattle, neither antigen was able reliably to induce protection against *T. saginata* infection. However, combination of the two recombinant proteins into a single vaccine was highly effective, inducing up to 99% protection (Table 2). The vaccine has potential application for reducing economic losses incurred as a result of contamination of beef meat owing to infection with *T. saginata*. The vaccine would be particularly valuable for the protection of cattle grazing pastures that have been irrigated or fertilized with water or solids recovered from human sewage. These practices are becoming increasingly common in western countries as authorities seek to make practical use of the water and other resources contained in sewage effluent.

The results of vaccination studies against *T. solium* cysticercosis in pigs have paralleled the early work with *T. ovis* and *T. saginata*. Pigs can be protected against challenge infection by vaccination with various non-living extracts from the parasite[24,25], with oncospheral antigens inducing the highest levels of protection[26]. Limitations on the supply and quality control of antigens derived directly from the parasite prevent such vaccines being applied more broadly; however, they have been used successfully in field trials in endemic areas in Mexico[27,28]. The preparation of antigen from other taeniid cestode species that are more easily obtained than *T. solium* is one method that has been evaluated for the supply of vaccine antigens[29]. However, the use of defined recombinant antigens offers the greatest potential for overcoming limitations with supply of antigens. Protection can be achieved against *T. crassiceps* infection in mice by vaccination with either recombinant proteins[30] or peptide epitopes based on these proteins[31]. However, the value of *T. crassiceps* as a model for *T. solium* infection in pigs is unclear. Challenge infections with *T. crassiceps* in mice involve intraperitoneal injection of a proliferative metacestode. In this respect, the protection demonstrated with recombinant antigens against oral infection with eggs of *T. ovis* in sheep and *T. saginata* eggs in cattle might provide more relevant models for immunity against oral infection in pigs or humans with eggs of *T. solium*.

Like *T. saginata*, the *T. solium* genome contains genes that encode protein homologs of each of the three host-protective *T. ovis* antigens. The cloning of the first of these antigens in *T. solium* has been described recently[32]. By analogy with vaccine development for *T. saginata*, these proteins in *T. solium* are highly likely to be effective as a vaccine against *T. solium* infection in pigs. Indeed, the probable success of this approach has been highlighted by the results of a vaccine trial against *T. solium* infection in pigs, which was carried out using the heterologous *T. ovis* antigens[33]. Pigs were immunized twice with a combined vaccine containing the three *T. ovis* recombinant antigens 45WB/X, 16K and 18K. After challenge infection with *T. solium* eggs, the vaccinated pigs developed 93% fewer cysticerci compared with control pigs. More recently, the other *T. solium* gene homologs of the protective *T. ovis* antigens have been cloned and expressed (C. Gauci and M. Lightowlers, unpublished) and the results of vaccine trials with these *T. solium* recombinant proteins are expected in the near future.

Protective oncosphere antigens contain a fibronectin sequence motif

DNA sequence comparisons between the clones that produce the various protective recombinant antigens of *T. ovis*, *T. saginata* and *E. granulosus* do not identify all antigens as having significant homology. However, analysis of the predicted amino acid sequence has

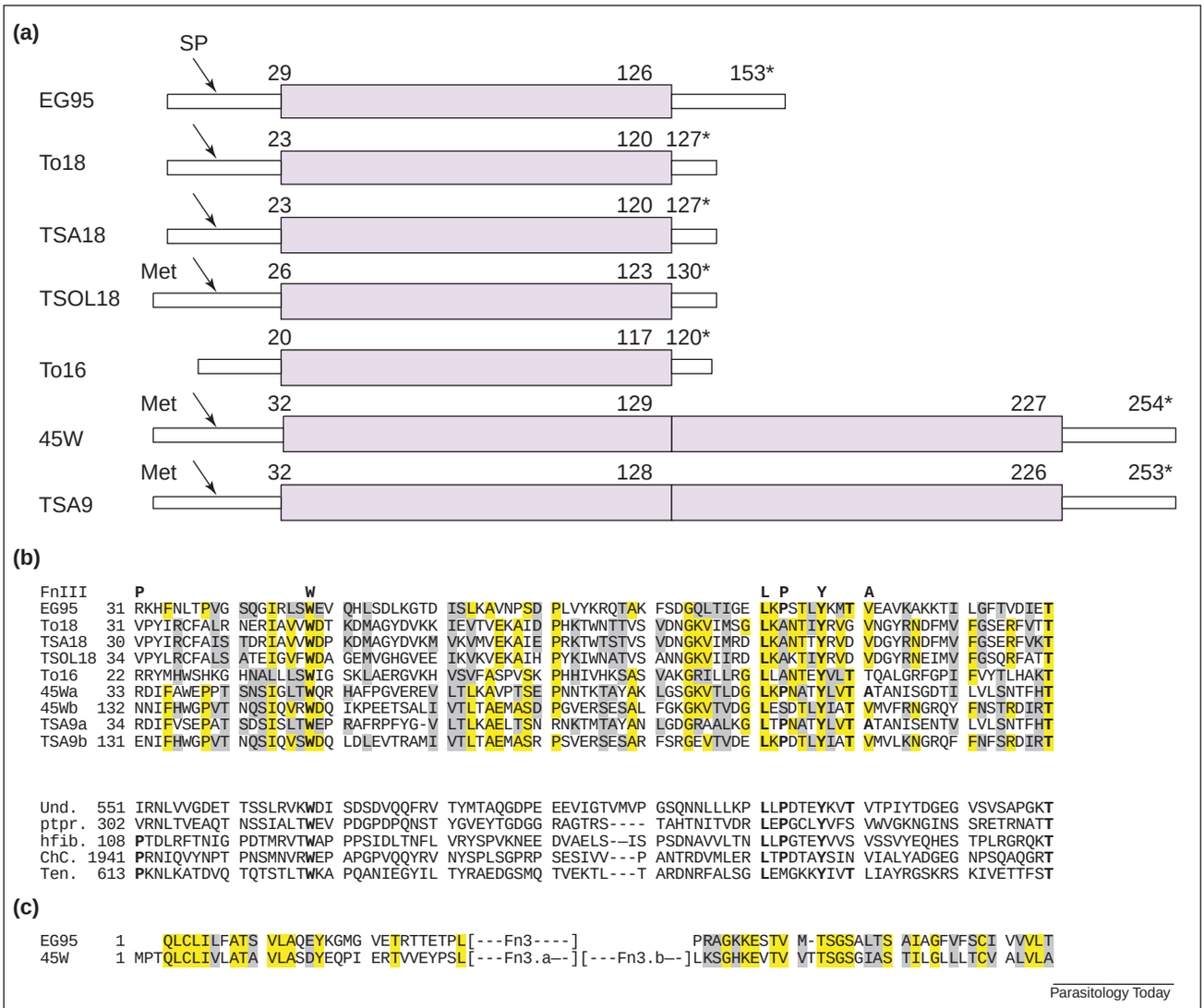

Fig. 1. Amino acid sequence homology among the host-protective vaccine antigens of *Echinococcus granulosus* and *Taenia* spp and homologies with other eukaryotic proteins. This schematic illustration shows the location of fibronectin type III domains (FnIII) in taeniid vaccine antigens (shaded region) (a). Numbers indicate amino acid number in GenBank sequences (see below). SP, putative signal peptide cleavage site; Met, putative initiating methionine; *, stop codon. Amino acid sequence comparison of the FnIII domain in seven taeniid vaccine antigens and a selection of other eukaryotic protein sequences are also shown (b). The sequence row marked FnIII, and the sequence shown in bold, correspond with highly conserved amino acids described by Bork and Doolittle[37]. Also shown are other sequence homologies within the vaccine antigens; yellow shading denotes >50% sequence identity; grey shading denotes conservation of amino acids according to Dayhoff[46]. Amino acid sequence homology up- and downstream from the FnIII domain(s) in EG95 and 45W protein sequences (c). Protein identities (GenBank accession number): EG95 (CAA62433); To18 (AAB49686); TSA18 (CAA67186); TSOL18 (AAD09326); To16 (AAB49687); 45W (CAA33300); TSA9 (CAA67185); Und., human undulin 1 (A40970); ptpr., human protein tyrosine phosphatase receptor type H (NP_002833); hfib., human fragment of fibronectin encompassing type III repeats 7–10 (1421281); ChC., chicken collagen alpha 1(XII) chain precursor (P13944); Ten., zebrafish tenascin-W (CAA04755).

revealed the presence of a conserved motif as part of all the host-protective recombinant antigens described to date (Fig. 1). This relationship exists despite the antigenic clones having been isolated independently and their having no known immunological relatedness. The motif defines a fibronectin type III (FnIII) domain that identifies a group of proteins ubiquitous among eukaryotic organisms and includes proteins associated with extracellular matrix and adhesive functions. Proteins containing FnIII domains include the immunoglobulin superfamily, cell adhesion molecules, cell surface receptors and carbohydrate-binding proteins[34–36].

There is evidence that all of the host-protective oncosphere antigens are secreted by activated oncospheres[13,16–19] but other functional information about these proteins is lacking. Bork and Doolittle[37] recognized the motif in sequences from *T. ovis* and *T. taeniaeformis*, and speculated that the proteins could be involved in parasite attachment to the intestinal wall. The available evidence suggests that a principal host-protective immune response evoked by the recombinant vaccines is antibody- and complement-mediated lysis of the oncosphere. This suggests that, if the antigens do have a role in attachment of oncospheres to the intestine, they remain associated with the surface of the oncosphere, providing a target for host-protective serum antibody in vaccinated animals. Detailed analysis of the expression of the 45W family of genes demonstrated that the proteins continue to be produced by the early developing metacestode[38], at a time when any

role for the proteins in intestinal binding would no longer be required.

Vaccine trials carried out with truncated *T. ovis* 45W[18,39] or EG95 (D. Woollard and M. Lightowlers, unpublished) proteins, in which the fibronectin domain structure is incomplete, have found that the truncated proteins are not protective. Further investigations will be necessary before any relationship can be confirmed between the domain structure of these antigens and their ability to induce a host-protective response. One feature of the FnIII domains in the cestode antigens is that they correspond, where known, to exons in their respective gene structures[38]; antigens with more than one FnIII domain have each domain encoded by a separate exon.

In addition to the sequence homologies within the FnIII domains of EG95 and 45W, these independently isolated oncosphere antigens also share significant homology within their sequence elsewhere (Fig. 1c). This homology is not a feature of all the protective antigens (eg. To16, TSA18), and indicates there is a closer relationship between 45W and EG95 than there is between these proteins and the other oncosphere antigens.

Previous reports of a *T. solium* antigen with fibronectin-like properties, Antigen B[40], are not related to the sequence motif features described here. Antigen B does not have an FnIII domain[41] or other sequence similarities with oncosphere antigens.

Future challenges

The focus of this review has been on the potential for control of the zoonotic infections, cysticercosis and hydatid disease, by vaccination of livestock animals. The benefits to human morbidity and mortality of such an approach are indirect. A more direct approach would be to use protective vaccines against cysticercosis and hydatid disease in humans. Viewed from this perspective, the vaccination trials that have been so successful against hydatid disease in sheep can be regarded as a model for development of a vaccine for humans, where the model is the same pathogen in another natural host species. Following a request from the Ministry for Health in Chubut, Argentina, discussions have begun towards undertaking human clinical trials with the EG95 antigen. Should a similarly effective vaccine be developed against *T. solium* infection in pigs, this could also be considered for use directly in humans.

Cysticercosis caused by *T. solium* has been identified as a potentially eradicable disease[42], and attention has focused on the possibility of achieving this predominantly through the removal of tapeworm carriers by mass treatment of the population in endemic areas with anthelmintics[43,44]. Such a strategy might lead to reduced parasite transmission in the short term, but unless treatment to remove *Taenia* carriers was repeated, transmission would be unlikely to be affected in the longer term. The frequency with which tapeworm carriers would need to be removed from the population to achieve high-level, long-lasting control is not known. Even if the treatments were carried out frequently, the potential for transmission would remain, because a susceptible pig population would be vulnerable to infection from immigration of tapeworm carriers from regions outside the control area. A case has been made for consideration of the potential value of vaccination in pigs as part of an integrated strategy[45]. The combined use of anthelmintics to remove *Taenia* carriers and the prevention of infection in pigs with an effective vaccine might enable the eradication of human cysticercosis to be achieved.

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The Journey of the Malaria Parasite in the Mosquito:
Hopes for the New Century

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In this review, Anil Ghosh, Marten Edwards and Marcelo Jacobs-Lorena follow the journey of the Plasmodium *parasite in the mosquito vector. At each developmental step, they highlight some of the major unanswered questions currently challenging cell and molecular biologists. A more thorough understanding of* Plasmodium*–mosquito interactions might lead to the development of mosquitoes unable to support parasite development.*

Malaria is one of the deadliest diseases of the tropics. It affects 200–300 million people worldwide and kills 1–2 million people every year, mostly African children under the age of five. Despite serious efforts to control this disease, the results have been disappointing. The parasite has become resistant to the best available drugs. Furthermore, an effective vaccine has not been brought to production. This is perhaps not surprising if one considers that the parasite lives in intimate contact with the immune system of the host and has evolved mechanisms to escape it. Among the five most deadly infectious diseases (acute lower respiratory infection, TB, cholera, AIDS and malaria), only malaria requires a vector for transmission. In the past, campaigns to control mosquito populations have resulted in dramatic decreases of malaria incidence. However, insecticide resistance and environmental damage quickly reversed early successes and now complicate vector control. Alternative approaches for malaria control, such as manipulation of vectorial capacity, need to be considered.

Plasmodium parasites undergo a complex developmental program in the mosquito[1]. This includes differentiation into six completely different forms (female and male gametes, zygote, ookinete, oocyst and sporozoite), fertilization, penetration of the extracellular peritrophic matrix (PM) and invasion of two different epithelia (midgut and salivary gland) (Fig. 1). During differentiation in the mosquito, the parasite has to overcome a number of bottlenecks (Table 1)[2–5].

The sexual cycle and exflagellation

Successful development of *Plasmodium* in the mosquito depends on the differentiation of sexual forms in the vertebrate host. Here, most of the parasites divide asexually in the peripheral blood. Concomitantly, a small proportion of the parasites terminally differentiate from merozoites into gametocytes[6]. Little is known about the genes that control the developmental switch to the

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