EXPERT REVIEWS

Vaccination against helminth parasite infections

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Helminth parasites infect over one fourth of the human population and are highly prevalent in livestock worldwide. In model systems, parasites are strongly immunomodulatory, but the immune system can be driven to expel them by prior vaccination. However, no vaccines are currently available for human use. Recent advances in vaccination with recombinant helminth antigens have been successful against cestode infections of livestock and new vaccines are being tested against nematode parasites of animals. Numerous vaccine antigens are being defined for a wide range of helminth parasite species, but greater understanding is needed to define the mechanisms of vaccine-induced immunity, to lay a rational platform for new vaccines and their optimal design. With human trials underway for hookworm and schistosomiasis vaccines, a greater integration between veterinary and human studies will highlight the common molecular and mechanistic pathways, and accelerate progress towards reducing the global health burden of helminth infection.

Keywords: echinococcosis • filariasis • hookworm • schistosomiasis • trichostrongylidiasis

The need for anthelminthic vaccines

Tropical diseases include many poorly controlled and highly prevalent infections across scores of low- and middle-income countries. Among the most 'neglected' of the tropical maladies are a suite of diseases caused by helminth worm parasites, which together infect over 25% of the world's current population. The global burden of disease can be seen to be quite astronomical once the very extensive infestation and debilitation of livestock by helminth parasites is also taken into account. Remarkably, however, no effective vaccines are available to protect humans or animals from these infections, and efforts need to be greatly intensified to break through into a new era of helminth control and elimination [1].

The lack of progress toward vaccination against helminth infections reflects both scientific obstacles, such as the complexity and diversity of the helminth parasites [2], and policy deficiencies that have resulted in helminthiases remaining the most prevalent of the 'neglected tropical diseases' [3]. In this review, we summarize the current status of vaccine development across the range of different helminth diseases, identify the major scientific targets for progression and discuss future innovations that can deliver vaccines against these parasites.

The helminths comprise two distantly related taxa, the roundworm Nematoda and the flatworm Platyhelminthes, with the latter itself divided into Cestodes (tapeworms) and Trematodes (flukes) (Figure 1). Although these groups diverged evolutionarily many hundreds of millions of years ago, their patterns of transmission, infection and pathogenesis are in many ways similar. Likewise the dominant type of immune response to helminths is a type 2 (Th2) profile [4], which in most, but not all, cases is able to mediate immunity to these parasites [5,6]. This is the case even though the worms establish themselves in very different physiological niches (e.g., intestinal lumen, the vasculature, serous body cavities or subcutaneous sites), meaning the combination of host effector molecules and cells responsible for protection necessarily differs between the parasites in question. Despite this, coordinated type 2 immune responses have the potential to be protective against most helminths, with cytokines such as IL-4, IL-5 and IL-13 associated with reduced worm burdens in humans [4,7–9], linked to their ability to promote eosinophilia, alternative activation of macrophages, intestinal epithelial responses and antibody (particularly IgE) production.

In many chronic helminth infections, however, the protective type 2 response appears to

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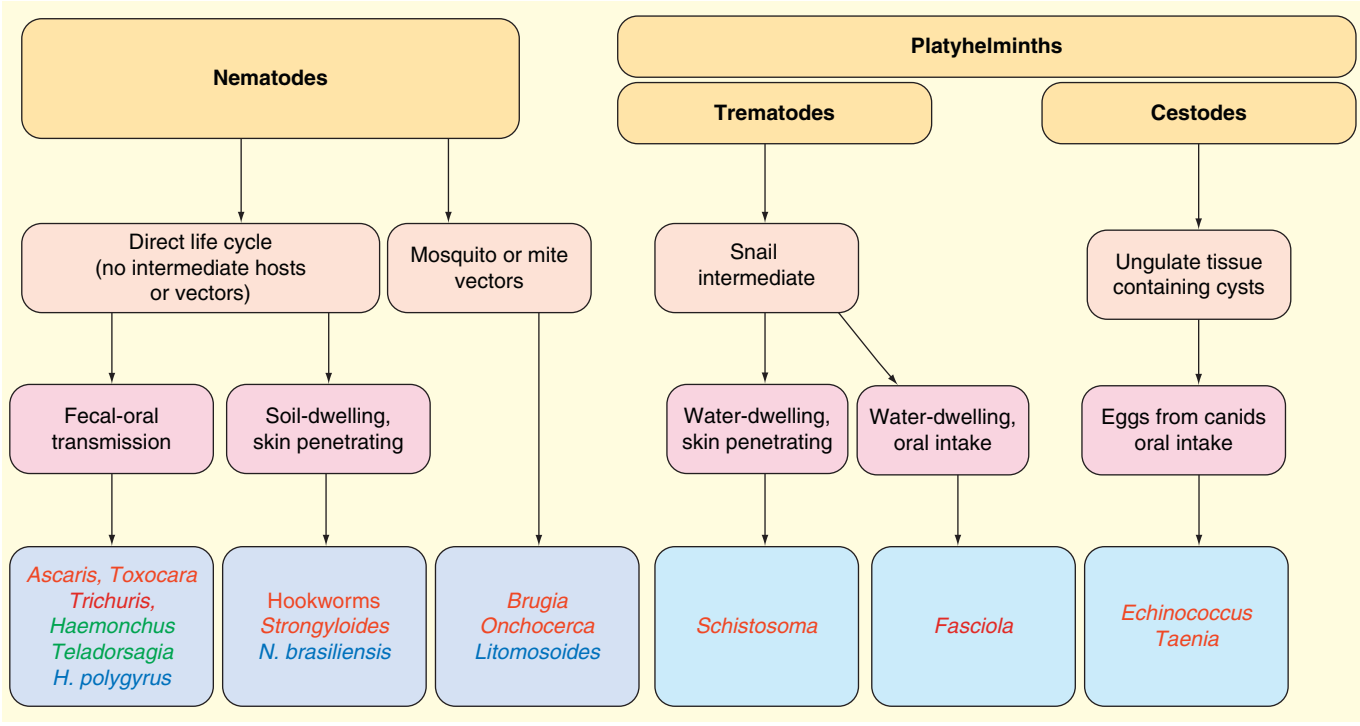

Figure 1. Simplified classification and life history traits of major helminth parasites. Top line: taxonomic grouping; second line: intermediate niche prior to infection of mammals; third line: route of infection; bottom line: parasite species, parasites causing human disease are in RED, veterinary parasites in GREEN and mouse model parasites in BLUE. Not shown: the nematode *Trichinella spiralis* directly transmitted by oral ingestion of infected meat.

be restrained by the body's own immunosuppressive mechanisms, such as regulatory T cells and the down-modulatory cytokines they produce [9–12], which results in ineffective and unresponsive immune cell populations that cannot eliminate the parasite. The ability of helminth parasites to promote host regulatory pathways to block Th2 immunity is equally apparent in human and animal infections [13,14], presenting in both settings a shared challenge of directing new vaccines to select protective and not counter-productive components of the immune system.

One consequence of the immunoregulatory nature of helminth parasites is that most individuals fail to develop protective immunity to infection, despite frequent exposure with infective stage parasites. Even following curative drug treatment, subjects show little resistance to rapid reinfection with soil-transmitted nematodes that establish in the intestinal tract [15]. In the case of blood-dwelling schistosomes, chemotherapy causes systemic release of and sensitization to parasite antigens, boosting immunity to subsequent infection [16], although this is not noted in all settings [3]. Repetitive mass drug administration programs may engender drug resistance, as well as fatigue among pharmaceutical donors and recipient populations alike [17]. However, drug cure and vaccination have clear and complementary roles in reducing overall disease burdens [18], and drug-mediated clearance of adult worms may eliminate parasite-encoded immunomodulatory molecules that would otherwise confound vaccine success [19].

Strategies for new vaccines against helminth infections

The complex developmental cycles of helminth parasites in their hosts, as well as the plethora of antigenic components expressed by each stage, can present an overwhelming number of alternative targets for vaccination against these organisms. In addition, immature and mature life cycle stages often occupy distinct tissue niches with differing immunological environments and pathological consequences. Where possible, vaccines aim to intercept newly invading larval parasites, for example in the skin or intestinal mucosa, in order to minimize duration of infection and prevent migration and dissemination. Vaccines may also aim at blocking transmission or focus primarily on the stages most closely associated with pathogenesis.

For major neglected tropical diseases, new vaccines will be aimed for human application, but require testing in model systems which necessarily differ in key features from the human. Despite this, small animal models (using either the human parasite or a related rodent-adapted species) provide the tools to define the immunological mechanisms of protection, and so lay the basis for rational rather than empirical vaccine design. Vaccines for veterinary use can be developed within the relevant animal species, a setting that may also provide valuable insights into immunity to a related human parasite, representing as it does a natural host–parasite pairing. A further targeting strategy would be to vaccinate reservoir host species, where these are the major source of transmission, such as water buffalo in the

case of *Schistosoma japonicum*, or dogs in the case of Echinococcosis and Toxocariasis.

Vaccine development for helminth infections has progressed through several scientific generations from live, attenuated organisms (using irradiation [20]), biochemical fractions and in the post-genomic era, recombinant proteins (Figure 2). As detailed below, radiation-attenuated larval vaccines successfully induced protective immunity in dogs and cattle, and although they were not widely adopted [21], the high levels of protection they engender have even led to calls for their use in against human helminth infections [22]. Amongst the first attempts at a nonliving vaccine was the use of 'excretory–secretory' (ES) products, which were able to generate significant immunity in animal models of *Trichinella spiralis* infection [23]. Although these early studies did not define the protective immunogens recognized by the host immune systems, in more recent years, a substantial number of helminth products have been characterized and tested, with some excellent results.

A distinction can be drawn among antigens selected as vaccine targets into those targeted by the immune response during the course of a natural infection and those that are classified as 'hidden antigens'. This latter group represents molecules to which the host immune system is not usually exposed, such as those expressed exclusively in the gut of blood-feeding worms, as discussed below. Antibodies generated against 'hidden' gut antigens, ingested in the blood, are posited to neutralize essential parasite enzymes, particularly proteases [24], compromising parasite nutrition and leading to earlier expulsion [25,26].

Intestinal nematodes in model systems

Vaccine development depends on testing in tractable and appropriate model systems. However, most helminth parasites are highly adapted to their definitive host and survive poorly, if at all, in a non-natural host species. This is particularly the case with intestinal nematodes, and laboratory animals are not susceptible to the human-infective species. In this case, it is generally preferable to study a related non-human parasite in its natural rodent host, to understand the mechanistic factors that contribute to vaccine-induced immunity to helminths in the gastrointestinal tract.

For this purpose, a range of intestinal nematode models are available in laboratory mice, with varying abilities to establish a chronic infection. One example is the natural mouse parasite, *Heligmosomoides polygyrus*, which establishes infections for 10 weeks or longer to susceptible strains such as C57BL/6. These mice can be effectively immunized with irradiated larvae [27]. Further, ES antigens from adult [28] or fourth stage larval [29] parasites are able to elicit complete sterile immunity. This is associated with strong antibody reactivity to a venom allergen/ASP-like protein VAL-1, and to a secreted acetylcholinesterase enzyme [29]. As *H. polygyrus* is most closely related to the Trichostrongyle parasites of ruminant livestock, as well as the human hookworms, this system may prove very instructive for vaccine development against a range of veterinary and clinically important pathogens, revealing both potentially protective molecules and immunological mechanisms of immunity.

A similarly valuable model of intestinal helminthiasis is *Trichuris muris*, related to the human pathogen *Trichuris trichiura*. These parasites, alongside (human infective) *T. spiralis*, possess a prominent secretory structure, the stichocyte. Hence, somatic extracts of adult *T. muris* contain the major secretory proteins and can be used to induce protective immunity [30]. Susceptible AKR mice vaccinated with ES antigens expel 97% of worms by day 21, with expulsion complete by day 35 post-infection [31]. Similarly vaccination with *T. spiralis* ES [23], or purified antigens corresponding to major surface or ES products [32–34], greatly reduces worm burdens in mice. More recent investigations have reported significant protection against *Trichinella* infection by vaccination with individual antigens (specifically Ts87 and gp43) expressed in recombinant *Salmonella* and administered orally [35,36].

In contrast to these comprehensive vaccine models, the widely used experimental nematode *Nippostrongylus brasiliensis* is less well-suited for vaccine studies as primary infections with this parasite are naturally expelled from the gut of naïve mice in 6–10 days. However, following skin penetration, this species migrates like human hookworm to the lung, where up to 90% can be eliminated in mice rendered immune by prior infection [37]. Hence, this system may offer a good model for vaccine-induced immunity against migratory immature hookworm larvae. In another laboratory model, immunization with parasite HSP60 in alum (but not Th1/17-inducing complete Freund's adjuvant) confers partial protection against *Strongyloides ratti* challenge [38]. Immunity to the related human pathogen *Strongyloides stercoralis* can be measured by implanting infective larvae within diffusion chambers into mice; survival of larvae is reduced by up to 80% after vaccination with a single immunoreactive proline-rich antigen, Ss-IR, or following transfer of serum from Ss-IR-immunized mice [39].

The human hookworm vaccine project

The human hookworms *Ancylostoma duodenale* and *Necator americanus* have been selected as the first major target for vaccination against gastrointestinal nematode infection, reflecting both their high prevalence and disposition toward anemia and other pathologies [40]. Based on successful trials in dogs [41–43] and hamsters [44], with *Ancylostoma caninum*, several antigens have been trialed in humans, including the VAL family member ASP-2 [45]. In this case, while the antigen evoked strong antibody responses, certain patients in endemic areas carried significant levels of ASP-2-specific IgE, resulting in allergic adverse effects following vaccination [46]. Possible avenues to prevent this include removing the IgE epitopes from the protein [46], and by its expression as a fusion protein with human IgG Fcγ1, promoting ligation of the inhibitory FcRγIIb receptor, thus preventing IgE-mediated basophil degranulation [47]. Nevertheless, as an alternative approach, the focus of human hookworm vaccination has shifted to two additional antigens which do not naturally elicit strong IgE responses, glutathione *S*-transferase (GST-1) and aspartyl protease (APR-1) which are currently being trialed in Brazil [48].

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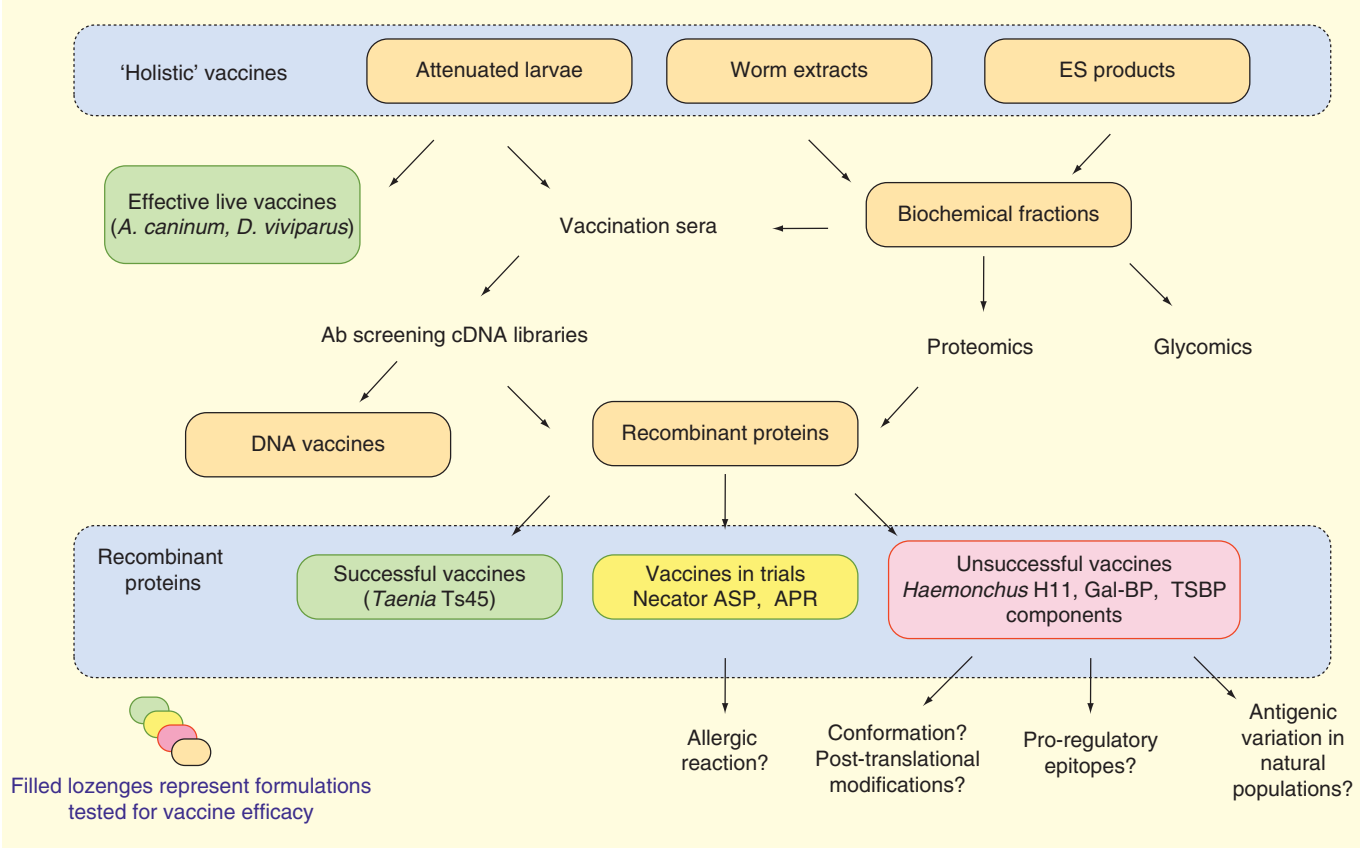

Figure 2. Strategies and pathways for helminth vaccine development.
APR: Aspartyl protease; ASP: Associated secreted protein; ES: Excretory–secretory; TSBP: Thiol Sepharose-Binding Protein.

The APR-1 enzyme is expressed in the hookworm gut to digest hemoglobin ingested with host blood [49], and vaccination is aimed at generating sufficient titers of serum antibody that the enzyme will be neutralized *in situ*, so compromising worm nutrition [43]. For immunization, a mutated form of the protein is used lacking potentially pathogenic protease activity [50]. Parasite GST is also involved in blood meal processing, binding heme in a detoxifying role [51], and is the target of protective antibodies, prompting the development of a bivalent vaccine with APR-1 to stimulate immunity against the adult worm in its intestinal niche [52]. This leads to reduced egg production, as well as a lower degree of blood loss and anemia in infected patients.

Further, hookworm antigens have been tested in dogs, including Ac16, related to prominent antigens in ascariasis (As14) and filariasis (RAL-2, SXP-1, Ov-17) mentioned below [53]. Although Ac16 vaccination only reduced adult worm load by approximately 25%, there was >60% reduction in egg output in vaccinees, who were also protected against anemia [53].

Vaccines against filarial nematodes

Filarial nematodes currently infect over 100 million people, prompting the search for vaccine candidate antigens from the parasites of humans, *Brugia malayi*, *Onchocerca volvulus* and *Wuchereria bancrofti* [54]. Of these, only *B. malayi* can complete its life cycle in rodents, the jird *Meriones unguiculatus* and a multimammate mouse species, *Mastomys coucha*. Irradiated infective mosquito-borne L3 larvae of *B. malayi* provide an effective vaccine in jirds [55], and this host species can also be successfully vaccinated (>70% protection) with the major L3 gene products of the stage, ALT-1 and ALT-2 [56]. In a concerted effort to identify vaccine immunogens in onchocerciasis [57], many key antigens were found to be homologous to candidates from other helminths, including *B. malayi* ALT, hookworm ASP molecules as well as the cystatin CPI-2, all components of a successful bovine vaccine [58] detailed in a subsequent section.

Because none of the human filarial parasites can productively infect laboratory mice, natural rodent filarial systems offer a tractable alternative to study vaccine-induced immunity. The filarial nematode *Litomosoides sigmodontis* completes its life cycle in susceptible mouse strains, and prior vaccination with irradiated larvae elicits protective immunity [59,60], which requires the presence of basophils at the time of immunization [61]. Vaccination with plasmids encoding *L. sigmodontis* antigens (including ALT) has been reported to reduce parasite establishment [62], although responses induced by vaccination were not directly measured against the immunizing antigens. Moreover, development of a transmission-blocking vaccine that

targets the circulating microfilarial stage has been shown to be possible in trials administering intact *L. sigmodontis* microfilariae absorbed to alum adjuvant [63].

Other approaches adopted in recent studies of immunity to filarial worms include immunization of jirds with ES from larval *Brugia pahangi*, a close relative of the human pathogen *B. malayi*, resulting in reduced microfilarial levels without loss of adult worms [64]. Interestingly, a similar outcome was observed by passive immunization with a monoclonal antibody to triose phosphate isomerase, the most abundant ES protein of adult *B. malayi* [65]. In this latter case, vaccination with this glycolytic enzyme did not generate either immunity or antibody capable of neutralizing enzyme activity, but selection of a monoclonal that inhibited the enzyme provided a reagent that prevented microfilarial production *in vivo*. However, in both instances, the ES products appear much less effective at inducing protective immunity to adult worms than has been found for intestinal nematode species.

Vaccine research in filariasis is relatively demanding, as the key parasites vary in their ability to infect model species, and there is still much discussion about the ideal systems for evaluation. Current knowledge and interpretations have very recently been fully analyzed in a systematic review of the field which includes trials with filarial antigens across the full range of models available [54].

Vaccines against schistosome infections

Human schistosome species complete their life cycle in a number of rodent and primate species, offering several animal models for the development of a human vaccine against schistosomiasis [66]. As with the nematode parasites, radiation-attenuated larvae (termed cercariae) provide a strong baseline for vaccine-induced immunity. Thus, multiple exposures to irradiated cercariae elicit high levels of immunity (up to 90%) against challenge in mice [67] and non-human primates [68]. A consensus emerged that challenge larval migration to and through the lung is delayed in immunized mice, although conclusive proof that immunity acts against the lung-stage parasite is still wanting (reviewed in [69]).

Many of the first attempts to test recombinant-based vaccines against helminths were conducted in the mouse model of *Schistosoma mansoni* infection, using proteins identified as most highly immunogenic in infected animals. These often represented abundant somatic constituents, such as cytosolic structural proteins (e.g., paramyosin) and glycolytic enzymes (such as aldolase), which did not generate high levels of immunity [70,71]. In another instance, while *S. mansoni* GST was not strongly protective in mice, the *Schistosoma haematobium* homolog Sh-GST28 reduced egg output by >50% in infected monkeys [72], and has now progressed into human trials as 'Bilhavax' [73,74]. In addition, the fatty acid-binding protein Sm14 [75] is being advocated as a cross-reactive vaccine antigen that may elicit protective responses against both human schistosomiasis and fascioliasis in cattle [76]. As published reports with these antigens show mixed success in animal tests [77,78], it remains to be seen whether they will prove highly effective in human subjects.

Perceived shortcomings of the first generation of defined vaccines prompted a reconsideration of potential vaccine targets, with greater emphasis on surface, secreted and intestinal proteins in both the immature (skin/lung) and adult stages [3,66]. Surface proteins have been released through enzymatic treatment (e.g., cleavage of GPI-anchored proteins [79]) or differential extraction (e.g., organic extraction of worm homogenates [80]), immunization with which results in moderate reductions in worm loads (42% and 27–62%, respectively). These approaches are complemented by proteomic identification of surface proteins (e.g., Sm29, SmCD59-like and Sm200 [81,82]), which can then be expressed in recombinant form to assess vaccine potential. In this regard, immunization with the tegumental protein Sm29 expressed by larval and adult worms induces 51% reduction in worm burdens [83], whereas immunization with the cercarial surface protein SmTOR reduced worms by up to 64% [84].

Two other schistosome surface antigens that are progressing toward clinical trials are Sm-p80 and TSP-2. Immunization with the membrane-associated large subunit of calpain Sm-p80 [85] resulted in up to 70% worm reductions in mice [86] and >50% worm reductions in baboons [87]. p80 from *S. mansoni* can even induce a degree of protection against challenge with another prevalent schistosome species, *S. haematobium* [88]. A mechanistic insight is that Sm-p80-induced immunity is mediated by antibodies, with a 48% reduction in worm load in mice passively immunized with vaccinated baboon sera [89]. This protection appears to be independent of complement [90], perhaps indicative of the importance of FcR-bearing cells, such as macrophages and granulocytes. Most excitingly, Sm-p80 immunization of infected baboons resulted in a reduction of up to one-third of established adult worm numbers [91], which portends well for future applications to human populations.

The schistosome tetraspanin TSP-2 is recognized by serum from putatively immune, but not chronically infected, individuals [92]. Vaccination with recombinant TSP-2 reduces adult worm burdens and liver eggs by >50 and >60% respectively. Although different field isolates of *S. mansoni* show very limited variation at only 4-amino acid positions in the TSP-2 sequence [93], in the related schistosome species *S. japonicum*, TSP-2 shows significant polymorphism (32/215 amino acid positions) detracting from its potential for vaccination against other schistosome species [94]. For *S. mansoni*, however, development of TSP-2 has progressed to focus on a 9-kDa extracellular domain, which can be produced in quantity for human vaccine trials [95]. With the completion of genome sequences for the human-infective schistosomes, an additional spectrum of new vaccine candidates may now emerge [96].

With intense focus on the nature of protective antigens, a critical issue that has remained unresolved is the mode of immunity that should be stimulated by any effective vaccine against schistosomiasis, a consideration which itself determines route of delivery and choice of adjuvants. In mouse models,

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intradermal inoculation of cercarial antigen with bacterial-based adjuvant induced a type 1-associated, nitric oxide-dependent, macrophage-mediate immunity to challenge infection [97,98]. However, mice unable to mount an IL-12-directed Th1 response are protected following schistosome vaccination through a less effective Th2 pathway [99]. Moreover, vaccine protocols entailing repeated immunizations promote antibody-dependent protective responses that can be passively transferred to naive recipients [100], while evidence from human studies link immunity to challenge infection more closely with IgE and the type 2 arm of immunity [7].

A further, and far-reaching, issue with current schistosome vaccination strategies is whether the choice of antigen, adjuvant and route of immunization may be unusually important in generating anti-schistosome immunity, for example by inducing TGFβ responses [101] or more broadly by modulating the balance between effector and Treg responses. For example, administration of *S. japonicum* GST together with the TLR ligands R848 and CpG generated anti-parasite immunity while inhibiting Treg activity [102]. Indeed, it is interesting to speculate whether the inability of current schistosome vaccines (and those against other helminths) to induce complete immunity may be due to the concomitant stimulation of mutually conflicting effector and regulatory pathways. This principle has been demonstrated most clearly in animal models, in which the regulatory cytokine IL-10 prevents the development of immunity to reinfection induced in mice following drug (praziquantel)-mediated adult worm clearance [103]. It will be increasingly important to pursue these issues as human trials of schistosome vaccines progress.

Successful vaccination against cestodes with recombinant proteins

Several cestode species are widespread in livestock and can cause severe disease if transmitted to humans. However, these parasites can be effectively targeted by new recombinant protein-based vaccines.

Infection with the human tapeworm *Taenia solium* occurs when poorly cooked pork with cysticercal larval parasites is ingested [104]. When larvae migrate to and encyst in the CNS of the 'accidental' human host, they cause neurocysticercosis and inflammation. Vaccination of pigs to prevent the development of infective stages would be considerably more cost-effective than vaccination of the much larger pool of potentially exposed humans, and hence candidate vaccine antigens from the larval stages of several *Taenia* species have been tested [105].

Early work showed that native ES material from *Taenia ovis* oncospheres could induce sterile immunity in lambs against challenge infection [106], associated with antibodies binding 16-, 18- and 45-kDa antigens. Immunization of sheep with recombinant forms of these antigens successfully induced high levels of protection against *T. ovis* challenge [107,108]. *Taenia saginata* homologues of these proteins (TSA-18 and TSA-9, homologous to the *To* 45-kDa antigen To45W) similarly protected cattle [109].

More recently, *T. solium* homologues of the same two proteins (TSOL18 and TSOL45) have been cloned [110,111]), alongside a new gene TSOL16 [112], which can induce almost complete (>97%) immunity in pigs against either experimental [113] or field [114] challenge. Both TSOL18 and TSOL45 genes show sequence variation [111] and one splice variant, TSOL45-1B, failed to protect [112], leading some to question these antigens as vaccine candidates [115–117]. However, field trial vaccinations against *T. solium* have been successful [114], and more so if animals are immunized with a mixture of vaccine antigens [116].

Echinococcus is another important cestode genus, particularly as human cystic and alveolar echinococcosis can be fatal if eggs from the canid definitive host are ingested [118]. Vaccination may be targeted to either the final hosts (typically rural dogs and foxes) or intermediate ungulate hosts (sheep and cattle) upon which canids may feed. Protective homologues of the *Taenia* vaccine antigens such as *Echinococcus granulosus* Eg95 [119,120] and Em95 from *Echinococcus multilocularis* [121], both of which are related to To45W and TSA-9, have proven effective in reducing infection levels in sheep and cattle by up to 99% [119,122]. Moreover, dogs vaccinated with adult-stage recombinant EgM proteins show not only lower worm burdens but greatly reduced maturation to egg production, effectively preventing significant transmission following infection [123]. Hence, both intermediate and definitive hosts can be protected from echinococcosis.

Vaccine-induced immunity to parasites of veterinary importance

Over 50 years ago, the first radiation-attenuated larval vaccines were developed, for the bovine lung worm *Dictyocaulus viviparus* [124] and the dog hookworm *Ancylostoma caninum* [125]. These were highly effective, reducing parasite levels on challenge by 88–97% for hookworm, and resulting in worm-free cattle following *D. viviparus* infection. The latter vaccine, launced as 'Dictol', continues to be marketed as 'Huskvac'. Despite their ability to protect animals from intense infection, these vaccines did not necessarily induce sterile immunity and require an intensive logistical chain to produce and deliver [20]. Consequently, most research effort has been channeled into developing recombinant subunit vaccines against these infections.

Vaccines against intestinal nematodes: *Haemonchus contortus*

H. contortus is a blood-feeding nematode of sheep and goats and forms part of a wider family of trichostrongyle parasites that cause high morbidity in livestock throughout the world [126]. Although natural immunity develops with repeat exposure [127], younger animals remain highly susceptible. Irradiated larval vaccination promoted resistance but acted poorly in lambs [128]. A wide range of parasite molecules have been evaluated, as previously reviewed [129], with the primary focus developing on parasite proteases responsible for the digestion of host hemoglobin, as with human hookworms. At least three independent approaches have been taken, each partly successful

in inducing immunity but all confounded by the multiplicity of proteases involved which has so far defied reductionist vaccine design. These three vaccine targets were termed H11, H-Gal-BP and Thiol Sepharose-Binding Protein (TSBP).

H11 is an integral membrane glycoprotein complex obtained from detergent extracts of *H. contortus* adult worms and generates 70–90% reduction in parasite loads [127,130]. Further fractionation identified a 110-kDa H11 component as a microsomal-type aminopeptidase [131], but vaccine trials with recombinant H11 using a baculovirus-derived insect cell homogenate delivered in the absence of adjuvant, rather than a purified protein [127,132], induced disappointingly low level of protection (30%). H-gal-HP is also obtained from detergent extracts of adult *H. contortus*, followed by peanut agglutinin affinity chromatography, which binds to Gal β1,3 GalNAc disaccharide motifs (hence the name '*Haemonchus* galactose-containing glycoprotein'). Vaccination with H-gal-GP results resulted in >70% reduction in adult worm counts [133,134], but the antigen represents approximately 1000 kDa multi-protein complex of at least 12 proteins [135], including metalloproteases MEP-1, -2, -3 and -4, two pepsin-like aspartyl proteases PEP-1 and PEP-2 (which show homology to *Necator americanus* Na-APR-2) and cysteine proteases, as well as a cysteine protease inhibitor and thrombospondin [136]. Determining which of these components, if any single one, is the target of the protective response is biochemically difficult, given the susceptibility of the immunogen to reduction of disulfide bonds [137]. The efficacy of Hp-Gal-BP has yet to be reproduced by candidate proteins, including MEPs, PEP-1 [137], cystatin or galectin (reviewed in [138]), individually or in combination, irrespective of the expression system used. The implication that recombinant proteins do not present the correct post-translational modifications has led some authors to suggest that a peptide mimitope phage display library for anti-Hc-gal-GP reactivity could identify the authentic epitope for an effective vaccine [139]; however the possibility also should be considered that only a multi-targeted, multi-epitope response can be effective, and tests to date have employed too reductionist a setting. In the meantime, it may be feasible to purify sufficient native H-gal-HP for limited vaccine use in the field. The third *H. contortus* gut immunogen is TSBP, which was isolated using a method designed to purify cysteine proteases associated with *H. contortus* gut extracts [140]. Extracts of adult *H. contortus* are first depleted of Hc-gal-GP by lectin binding, then subjected to thiol-sepharose affinity chromatography, to purify proteins with free cysteine residues, including (but not limited to) cysteine proteases. This protease-rich fraction conferred 43–52% protection against challenge [141], with similar efficacy from adult ES products purified in a similar manner [142]. Importantly, TSBP does not react with antisera to H11 or Hc-gal-GP but contains a different range of antigens, including a major glutamate dehydrogenase and minor cathepsin B-like cysteine proteases (hmcp-1, 4 and 6) which are the actual protective targets [143]. However, bacterial expressed recombinant hmcp-1, -4 and -6 did not protect against challenge [143]. This may again suggest the importance of post-translational modifications, or alternatively that the native protease fraction contains a more diverse range of gene products than the individual recombinants. In this instance, minor key co-purifying components may be the true protective targets, while natural sequence variation in the vaccine candidate could mean only a subset of parasites will be targeted, or simply that combinations rather than individual antigens are most effective.

Vaccines against *Ostertagia ostertagi*

O. ostertagi, a major intestinal infection of cattle, differs from *H. contortus* in that it is not a direct blood-feeding nematode, instead (like *Teleodorsagia circumcincta* in sheep) being an abomasal 'mucus-dweller' [144]. The different niche may explain the general lack of success of various vaccine formulations based on approaches that are protective against *H. contortus* [144]. For example, immunization of cattle with the Oo-gal-GP equivalent of Hc-gal-GP results in only a 23–50% reduction in eggs and no change in adult worm burden. Similarly an *O. ostertagi* TSBP equivalent ('ES-thiol') purified from ES induced 60% reduction in eggs but no significant decrease in adult numbers [145]. ES-thiol contains not only cysteine proteases but high levels of two ASP/VAL proteins, homologues to the Na-ASP-2 human hookworm vaccine (see above), and immunizations with different subfractions of ES-thiol containing either proteases or ASPs induced equivalent reduction in egg counts [146]. Cattle immunized with purified ASPs and showing lowered egg numbers are found to upregulate a number of genes associated with protection, including granulysin, secreted together with mucus by goblet cells [147], which may be exerting a direct effect on parasite fitness in the lumen. For reasons discussed above with *H. contortus* (and in common with other trichostrongyle nematode systems), vaccination with single recombinant versions of *O. ostertagia* antigens (which are partly protective in their native state) has failed [148].

Research into veterinary gastrointestinal nematodes is notably focused on adult worm antigens, while in most other helminth systems, the infective larvae are the dominant target of the protective response. However, a number of larval ES products have been identified as potential protective antigens [149], such as OPA, *Ostertagia* polyprotein allergen, a polymer of repeating 14 kDa units. Immunization of calves with an OPA-enriched fraction resulted in a 60% reduction in fecal egg counts, although there was no significant change in worm burdens [150].

Vaccines against other nematodes of veterinary importance

Trichostrongylus colubriformis differs from other ruminant nematode parasites in that, after passing through the abomasum, adults reside in the lumen of the small intestine. Moreover, robust immunity (99% reduction in adult parasites) established by drug-clearance of infection is associated with the induction of anti-larval antibodies that preferentially target a heat-stable glycolipid [151], known as CarLA (carbohydrate larval antigen). While CarLA is an attractive vaccine candidate, its biochemical

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and epitopic structure have yet to be defined, and a recent study showing antigenic variation of CarLA at the population level will need to be taken into account [152].

As discussed above, to date most recombinant protein vaccines have not met with success. However, a recent trial with *T. circumcincta* has shown that lambs immunized with a combination of eight recombinant *T. circumcincta* proteins, including potential immunomodulators (macrophage migration inhibitory factor, *Tci*-MIF; apyrase, *Tci*-APY-1; TGFβ homolog, *Tci*-TGH-2), larval IgA targets (cathepsin F, *Tci*-CF-1; Astacin metalloprotease, *Tci*-MEP-1; a 20 kDa protein of unknown function; VAL/ASP protein, *Tci*-ASP-1) and the homolog of a protective hookworm antigen (*Tci*-SAA-1) resulted in a 75% reduction in adult worms and a 92% reduction in cumulative fecal egg counts [153]. As such, this study represents something of a proof-of-principle of the feasibility of recombinant vaccines against veterinary nematodes.

A common helminths of horses that can cause disease is *Strongylus vulgaris*. Irradiated larval vaccines against *S. vulgaris* in young horses can elicit up to 91% reduction in parasite burden [154].

Ascarid parasites of animals & humans

Ascaris lumbricoides infects more than 1 billion humans, while its close relative *Ascaris suum* is widespread in pigs. Infection is initiated by ingestion of embryonated eggs that hatch in the gastrointestinal tract to release tissue-invasive infective larvae. Hence, vaccines with eggs attenuated by, for example, ultraviolet irradiation has been tested and found to induce up to 94% protection in pigs [155]. Perhaps surprisingly, veterinary vaccine studies appear more advanced than those against human ascariasis, with recombinant protein vaccines against *A. suum* now including As14 and As16, homologues of hookworm Ac16 as well as filarial proteins such as RAL-1; As16-immunized pigs showed a greater than 50% reduction in tissue larvae compared to naïve challenge controls [156]. As14 also showed protective potential in a mouse model [157] and an As14 homologue in *Baylisascaris schroederi*, reportedly the major infectious cause of death of giant pandas, elicits a protective response in mice challenged with this parasite [158]. A further antigen, *A. suum* As24, similarly stimulated significant protective immunity [159]. These antigens may form the basis of a human vaccine, and attempts to generate a multi-valent human vaccine that will be effective against *Ascaris*, *Trichuris* and hookworm may take advantage of shared gene families such as these [160].

Another important nematode of the Ascarid family is *Toxocara canis*, the common intestinal roundworm of dogs which is able, if infective eggs are ingested by humans, to cause visceral and ocular larva migrans. Vaccination with the ES proteins of the tissue-invasive larval stage can significantly reduce infection levels in mice [161], but trials in the definitive canid host have not been reported.

Filarial parasites in dogs & cattle

Heartworm in dogs is often fatal, and although well-controlled by simple drugs, vaccination would provide long-term protection. An irradiated larval vaccine provided over 70% protection in dogs [162].

Cattle in East Africa harbor *Onchocerca* species closely related to the human parasite *Onchocerca volvulus*, and hence provide an excellent 'real-world' model of vaccination and protective immunity against natural transmission. Microfilarial extracts can act as a partially protective vaccine [163], while irradiated L3 larvae induce a higher level of immunity [164]. The transition to a subunit vaccine commenced with the use of a cocktail of recombinant antigens including ALT, RAL2, tropomyosin, CPI-2, FAR1 and others, resulting in over 40% of cattle showing complete resistance to challenge infection [58].

Fascioliasis

Fasciola hepatica is a trematode fluke that resides in the liver of cattle and other livestock and is infective to a range of laboratory animals. Among the antigens tested for vaccine potential are proteases such as the leucine aminopeptidase (LAP) involved in parasite blood digestion, following the same strategy as targeting parasite intestinal enzymes for antibody neutralization. Thus, *Fasciola hepatica* LAP vaccination reduces worm loads in rabbits by >75% [165]. Interestingly, additional vaccine targets are similar to those identified in other helminths including GST, cathepsin proteases [166] and FABP, which in the latter case is sufficiently similar to the schistosome protein Sm14 that cross-protection between the two trematode parasites can be elicited [77,167].

Future of vaccines against helminths

Anti-helminth vaccination is at a crucial juncture with some clear success stories (*Taenia* in veterinary use), some strong candidates for human use (e.g., in the hookworm project) but also some cautionary tales. Because protective mechanisms are very different from immunity to microparasites, and because many helminths are actively suppressing host immunity, finding an effective combination of antigens, adjuvant and route of administration inevitably involves a good deal of trial and error. It is important therefore not to expect instant success, but to progress incrementally while understanding that each parasitic species will have its own vulnerabilities that can be exploited.

The major obstacles to successful immunization against helminth parasites are threefold: identifying the protective antigens (and there is no reason to expect a single protein to be sufficient), identifying the protective mechanisms (to monitor and optimize vaccine success) and developing the appropriate stimuli to mobilize these pathways *in vivo* and overcoming the challenge of vaccinating against a background of continuous exposure to organisms with intrinsic immunosuppressive capacity.

Currently, the first problem is somewhat accentuated by the increasing number of candidate antigens identified through large-scale proteomic, transcriptomic and genomic surveys [96] with screens for efficacy that are often in a suboptimal host species, or using surrogate readouts where laboratory infection with the target species is not possible. A reasonable shortcut may be based on the assumption that host antibodies mediate

protection, which allows the use of large-scale protein [168] and carbohydrate [169] arrays to assess antibody targets in putatively immune hosts. However, this limits selection to natural targets of immunity in the infected host and excludes 'hidden antigens' which may be particularly effective. Such techniques also assess antibody binding at the whole molecule level, which may fail to take into account the importance of binding to particular epitopes which neutralize the active site of enzymes or a particular protein domain.

Recently, rapid progress in genome sequencing projects for most major helminth parasites have defined the full spectrum of potential proteins that could serve as vaccine candidates. While this information is not in itself sufficient rationale for vaccine antigen selection, it will facilitate progress from model systems vaccines into human and veterinary settings, and allow high-throughput screening of parasite protein arrays with antibodies from immune individuals (Figure 2). Nevertheless, it seems inevitable that hypothesis-based and function-led selection of potential targets such as key enzymes or immunomodulatory proteins will remain an indispensible part of vaccine research.

The second obstacle is arguably the most critical. Unless the goal of vaccination is simply the generation of sufficient titers of neutralizing antibody (e.g., against the enzyme active site), it is essential to induce the correct mode of immune responsiveness, which in most helminth settings is the Th2 pathway. The type of response is itself crucially determined by signals from innate cells and tissues, so that the appropriate stimulation of the innate compartment is now recognized as key to the design of new vaccines [170]. However, relatively little is yet understood about how type 2 immunity is selected, other than that it is promoted by certain adjuvants such as alum [171], resulting in few rational strategies currently available to selectively boost strong Th2 responses to helminth infections. Indeed, a recent study has revealed that adjuvant choice is critical for the successful vaccination against *H. contortus*, with diethylaminoethyl (DEAE)-dextran outperforming alum [172].

The final challenge of vaccinating populations experiencing current infection and transmission is common to almost all infectious disease settings. It is likely that once the response to infection is initiated, it is difficult to modulate in mode or volume by vaccination. Moreover, natural infection may generate suppressive regulatory pathways, which block vaccine-induced development of protective immunity. Hence, almost all vaccines are only effective if administered prior to initial infection. A further concern is whether in the epidemiological context of frequent exposure to small number of parasites, vaccinees may develop tolerance and lose their immunity. When this question was modelled in rodent filariasis, mice given repeated injections of small numbers of *L. sigmodontis* larvae retained their vaccine-induced resistance [60]. While this result is reassuring in principle, the question remains of whether it will hold in the many and varied field conditions of helminth transmission.

Recent years have thus seen real and exciting advances in vaccines against helminth parasites, but some substantial obstacles remain to be negotiated. Despite the powerful advocacy for greater prioritization of helminth vaccine development [1], the global vaccine agenda has yet to fully implement this vital component for human health [173,174]. Nevertheless, progress continues to be made across a broad range of helminth disease targets, with excellent examples and many ongoing developments which promise increasing availability and application for anti-helminth vaccines in the coming years.

Expert commentary

In this review, we have emphasized the critical parameters of vaccine development against helminths, and the need for both scientific and organizational breakthroughs if the world is to meet the enormous challenge of eliminating helminth parasites. Scientifically, the first objectives are to define the most effective set of vaccine antigens for each parasite, which will increasingly depend on targeting helminth products with a known role and function in the host–parasite interaction. Determinants that promote immunosuppressive effects should be avoided, unless they can be presented in an inactive form, thus precluding parasite-induced immunomodulation. These antigens should also be relatively invariant in structure within the target species, and may even be shared between species if polyvalent vaccines are to be produced. Second, we need a much greater emphasis on mechanisms of protection, and optimal induction of immunity, which in turn demands a deeper integration of rodent models, human and veterinary research so that each field cross-informs and stimulates the other. Such coherence will lead beyond empirical vaccine testing to a more rational and predictive vaccine design. Finally, while the priority given to helminth vaccines is already at a historical high point, there are still many parasites for which adequate research funding is not yet available. We very much hope that growing success of helminth vaccination programs will encourage more international organizations and funders to support a much broader and more ambitious plan to eradicate the major helminth pathogens from the world.

Five-year view

By 2019, it is likely that both the hookworm and schistosomiasis human trials will have been completed, and one or both moved forward into more general availability. Few other human helminth vaccine projects are close to human trials, but if these pioneering studies prove successful, much greater impetus will be given across the range of other important infections, so that 2019 should see the first candidates coming forward for Trichuriasis and Ascariasis in humans. In parallel, vaccines against the intestinal nematode infections which are currently being targeted in trials in sheep are likely to be scaled up, although separate formulations are likely to be required for each of the different helminth species found in the ruminant intestinal tract. Finally, the proven successful vaccines against cestodes (tapeworms and *Echinococcus*) should over the next 5 years be marketed and by 2019 already making a substantial impact in the field.

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Key issues

- Vaccine antigen selection can be clouded by the immense choice now posed by genomic analyses.
- Focus on functional antigens, for example proteases or immunomodulatory products, is more likely to bear fruit.
- Hidden antigens should be considered in addition to natural targets of antibody responses in infection.
- Helminths may promote regulatory responses through antigens or epitopes which should be modified or omitted from vaccine formulations.
- Helminth immunity is generally type 2-mediated, but better tools to drive type 2 response upon vaccination are needed.

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