

OVERVIEW AND PREFACE

Development and applications of cestode and trematode laboratory models

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INTRODUCTION

The phylum Platyhelminthes comprises important endoparasitic organisms, some of which can cause serious diseases in humans and other mammalian hosts. Cestodes and trematodes are characterized by, sometimes complex, life cycles with distinct larval and adult stages and obligatory changes between intermediate and final hosts.

Most adult cestodes parasitise the intestine of a definitive host, where the sexual development takes place. Self- or cross-fertilisation results in the production of eggs, which contain a zygote eventually forming a first larval stage (oncosphere), and which is then taken up by an intermediate host. Within the intermediate host, the parasite is usually targeted to distinct compartments or organs, and develops into a second larval organism, within which the pre-adult parasites develop. The life cycle is completed when the infected intermediate host tissue is consumed by a definitive host. Depending on the species, cestode larvae can localise in a variety of organs, but often they are targeted to a specific site, causing diverse pathologies which depend on the localisation, the reproductive potential and the size of parasites. For many years, researchers have been studying medically and economically important members of this class. They are represented by several species, most of them included in the family *Taeniidae*, which can cause severe diseases such as echinococcosis and cysticercosis. Others such as *Hymenolepis*, *Mesocestoides*, *Schistocephalus* and *Ligula* have less medical importance, but can still be economically relevant. Due to the fact that they could be relatively easily maintained in the laboratory either *in vitro* or in a laboratory animal host, they represent important model systems for investigating various aspects of cestode biology, some of which are covered in this special issue.

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Digenean trematodes are similar to cestodes, since they also exhibit an amazing variety of life cycles, which include at least one (sometimes more) intermediate and one final host. The former is usually a mollusc, the latter is almost always a vertebrate. As with cestodes, most trematodes are monoecious, with schistosomes being the important exception from that rule. Although over 20,000 trematode species have been described, only few cause important diseases, particularly in the tropics. Clearly the genus *Schistosoma* is foremost among those. Schistosomes have separate sexes, and the final host is infected percutaneously by an actively swimming cercaria. Infections are acquired in freshwater and may lead to serious disease and death by worms located in the blood vessels, urinary tract or intestine, depending on the species. Infections with liver flukes such as *Clonorchis sinensis*, *Opisthorchis felinus* and *O. viverrini* are acquired by eating raw or insufficiently cooked freshwater fish of many species. Serious symptoms such as liver cirrhosis and even death occur. Another liver fluke, *Fasciola hepatica*, is rare in humans and common in sheep and cattle, and symptoms of infection can be serious. Infection is acquired by ingesting metacercariae encysted on vegetation. Metacercariae, attached to aquatic plants, are also the source of infection by the giant intestinal fluke *Fasciolopsis buski* of man and pigs, which is common in many parts of Eastern, Southeastern and Southern Asia, and on Pacific islands.

IMPACT OF CESTODES AND TREMATODE INFECTIONS

The medical and economic impact of diseases caused by cestodes and trematodes has, until recently with few exceptions, constantly been ignored and severely underestimated. With the exceptions of schistosomiasis, which affects around 250 million people, the food-borne trematodiasis and the diseases caused by cestodes, such as echinococcosis and cysticercosis, have made it on the list of the World Health Organization neglected tropical diseases (NTDs) only recently (Hotez *et al.* 2009; Budke *et al.* 2009).

This is sometimes difficult to justify. Global estimates of echinococcosis indicate that in Asia and in Africa 50 million people are at risk of acquiring the disease (Hemphill and Kern, 2008). The global burden (disability-adjusted-life years – DALYs) for human cystic echinococcosis was estimated to be similar or even greater than for several other better known NTDs such as Chagas disease, dengue and African trypanosomiasis, all of which have been the subject of larger scale intervention programmes much earlier (Budke *et al.* 2006, 2009). Thus, the significant impact of echinococcosis has been confirmed, but not adequately recognized by the relevant authorities. There are no figures on DALYs lost due to food-borne trematodiasis, but they are likely to be in a similar range or even higher, since approximately 40 million people are infected worldwide (Keiser and Utzinger, 2007; Budke *et al.* 2009). An earlier study estimated that 400,000 people have symptomatic neurocysticercosis in Latin America alone (Bern *et al.* 1999).

Despite the considerable medical and economic impact, there has been no dedicated drug discovery programme, and basic research on cestodes and trematodes has been notoriously underfunded. Nevertheless, as the reader of this special issue will see, many dedicated scientist have undertaken major efforts to develop the field, despite these difficulties. A major prerequisite for this has been the establishment and constant improvement of *in vitro* and *in vivo* laboratory models for experimental studies on cestodes and trematodes, which has allowed us to study defined life cycle stages, thus aiming towards a better understanding of the host-parasite relationship and parasite physiology. *In vivo* animal models have been mostly used for investigations on pathogenesis and drug efficacy, and on aspects of the host-parasite relationship at the immunological and physiological levels. However, while animal models have proven to be advantageous in many instances, it has been notoriously difficult to draw definite conclusions about the factors modulating parasite differentiation, and investigations on gene expression and respective regulation have often been hampered by the complexity of the host-parasite interplay. There has always been a need for *in vitro* culture models which would enable researchers to dissect specific parasite compartments in more detail. Thus, *in vitro* models have been developed which have found wide acceptance, as they have been used for maintenance and generation of defined parasite stages, for investigations on parasite structure and antigenic composition, for description and functional characterization of defined parasite molecules, for drug screening purposes, and for biochemical, physiological and structural studies. Finally, the major advances have been achieved by the development and application of targeted genetic manipulation of cestodes and trematodes (most notably in

Echinococcus and *Schistosoma*), which now allows us to study in considerable detail the functional aspects and validity of putative vaccine and/or drug targets, and will allow us to answer fundamental questions related to the developmental biology of cestodes and trematodes.

CONTENTS OF THIS SPECIAL ISSUE

This special issue highlights the current state on the use of experimental cestode and trematode laboratory models. It is comprised of 16 reviews and 2 original research papers.

A frequently used experimental model cestode is *Taenia crassiceps*, which affects wild and domestic animals, but only rarely humans. *T. crassiceps* cysticerci proliferate asexually by budding, and thus can be maintained indefinitely by serial injection into the peritoneal cavity of inbred mice. In addition, adult stages can be produced in immunosuppressed hamsters. These properties have rendered *T. crassiceps* ideally suited for studies on various aspects of taeniid biology, which are reviewed here by Willms and Zurbarian. Two contributions in this special issue deal with models specifically dedicated to investigate *T. solium* infection. *T. solium* is the causative agent of neurocysticercosis, the most frequent parasitic disease of the brain. The adult tapeworm that develops in humans is the main risk factor for acquiring this disease. Flisser *et al.* have reviewed the rodent laboratory models for production and maintenance of the adult *T. solium* tapeworms and associated applications such as in immunodiagnosis of taeniosis, intervention measures, immunological responses and vaccination. Neurocysticercosis on the other hand is caused by the larval stage of *T. solium*. There is no *in vivo* laboratory model that would display the infection of *T. solium* cysticerci in the central nervous system. Thus, Alvarez *et al.* provide us with a report on the use of *Mesocestoides corti*, a closely related cestode, whose metacestodes can be injected intracranially into mice. They demonstrate the usefulness, as well as the limitations, achieved by the *M. corti* model to study different aspects of the host-parasite interaction during neurocysticercosis.

An important feature of *M. corti* in terms of its use as a model system is the fact that it has the ability to develop from its larval stage to the adult worm *in vitro* by trypsin and serum stimulation. This offers the possibility to apply this model to investigate cestode development *in vitro*, and this is demonstrated here in a research paper presented by Cabrera *et al.* The authors describe the morphological features, glycogen mobilization and distribution of neutral polysaccharides in different developmental stages of *M. corti* during *in vitro* development, and define cellular territories that are maintained during the developmental process. The authors conclude that degradation of polysaccharides

may be related to energy needs necessary for the cellular processes leading to mature worms.

Another frequently applied cestode model system is *Hymenolepis diminuta*, a tapeworm of rats, which act as definitive hosts. Thus, in contrast to many other cestodes, laboratory rodents can harbour the adult *Hymenolepis*, rendering this stage accessible to experimental laboratory investigations, including biochemical studies, investigations of chemotherapeutic options targeting the adult cestodes, and research on immunological responses to adult cestodes. With respect to the latter, Derek McKay has provided an excellent review on the current findings on the enteric immune responses of mice and rats against *H. diminuta* infection, how these responses are modulated by the adult tapeworms, and he shows that these findings can be translated into novel treatments for inflammatory and autoimmune disorders. Another paper, presented by Fioravanti and Vandock, provides a comprehensive overview on the current knowledge of cestode anaerobic energetics, and especially the findings on transhydrogenase and anaerobic mitochondrial metabolism in *H. diminuta*.

Two other review papers in this special issue deal with pseudophyllidean cestode models that affect fish. *Schistocephalus solidus* plerocercoids are confined to only one definitive host, the three-spined stickleback, whose existence is restricted to the Northern hemisphere, e.g. the margins of the Atlantic and Pacific oceans. Barber and Scharsack describe in their review how *S. solidus* plerocercoids affect their host's growth, reproductive development, fitness and behaviour, and describe more recent laboratory studies on the immune responses of fish towards infection. In contrast to *S. solidus*, *Ligula intestinalis* is a frequently occurring and widespread parasite, whose plerocercoids parasitize the body cavity of a wide range of fish worldwide. The ubiquitous nature of *Ligula* in terms of intermediate fish host and geographical range has rendered this parasite a good model to study speciation and diversity in fish parasites, including topics related to pollution, immunology, ecology, and genetic diversity (reviewed here by Hoole *et al.*). Genetic diversity of parasite populations has also been addressed by the review presented by Knapp *et al.* who consider the question of how another widespread cestode, *E. multilocularis*, has managed to survive and perpetuate the species within different host populations all of which try to resist infection. For this, Knapp *et al.* have applied a recently characterized multilocus microsatellite named EmsB, which is tandemly repeated in the genome, and serves as a molecular tracker to detect transmission of *E. multilocularis* among different hosts and at different geographical scales. As a future application, this genotyping strategy could be used as a complementary tool in health control programmes.

The recent release of the genomes of *S. mansoni* and *S. japonicum* has opened the avenues to apply functional genomics approaches for schistosomes, with the goal to establish transgenic parasites and to elucidate functional aspects of schistosome genes. Among the laboratory models for the maintenance and culture of schistosomes, *S. mansoni* is the most advanced. Mann *et al.* provide an excellent overview of methods for the isolation and culture of developmental stages of *S. mansoni*, including eggs, sporocysts, schistosomules and adults. Methods of genetic manipulation such as RNA interference (RNAi), introduction of binary transposon plasmid constructs by square wave electroporation, and the application of retrovirus virions as transgenic vectors are also described and discussed. Yoshino *et al.* on the other hand review the isolation and culture of larval stages of Schistosomatidae, Fasciolidae and Echinostomatidae for the purpose of whole genome sequencing. Culture-based development of larvae provides the basis for gene expression analyses including transcript and protein profiling, and for transgenic and RNAi approaches, and these topics are also discussed. RNAi in schistosomes was pioneered by Skelly (2003), and the research paper in this special issue by Krautz-Petersen *et al.* coming from that laboratory is focusing on the machinery and methodology of RNAi in adult schistosomes. Although silencing of gene expression by RNAi is a powerful approach, the authors demonstrate that not all genes are suppressable to the same extent. Short interfering RNA (siRNA) can effect suppression when delivered by soaking. They also identified, *in silico*, an important component of the RNAi machinery in schistosomes, which is a homologue of the *C. elegans* RNA-importing protein SID1. This protein is likely to act as a channel to import dsRNA into schistosomes. The authors present a draft of potential RNAi pathways in schistosomes.

The development of functional genomics in schistosomes enables researchers to characterize the functional significance of proteins or protein networks, employing techniques such as *in vitro* culture of different stages, *in silico* gene identification, yeast-two-hybrid analyses, RNAi to silence gene function, and proteomics. This in turn provides important information of potential targets for intervention by chemotherapeutic or immunological means. Grevelding *et al.*, in this special issue, review how post-genomic approaches have increased the understanding of proteins that fulfill crucial roles in the male-female interaction of schistosomes. They summarize the current understanding of gonad signaling molecules and events during the differentiation process of these organisms. These findings are highly important and are likely to yield novel interventions in the pathology of schistosomiasis, since the differentiation of gonads in the female

is a prerequisite for egg production, and eggs are the major cause of pathology during the disease.

Besides schistosomes, the most frequently investigated group of plathyhelminthes are the taeniids. Most notably the larval stages of *Taenia* and *Echinococcus* have a high medical and economic impact and cause severe diseases. However, the developmental biology of these parasites needs to be investigated in much more detail in order to define suitable targets for intervention by chemotherapeutic or immunological means. As outlined in the review by Jabbar *et al.*, it is the first larval stage, the oncosphere, which is the most vulnerable when attacked by the host immune system, and this has been exploited for the development of highly effective vaccines. The authors also describe the ultrastructure of the oncosphere prior- and post-activation, and state that localization of potential vaccine targets at the level of electron microscopy could provide important insights into the mechanisms of vaccine efficacy. Jabbar *et al.* also describe the events occurring during post-oncospherical development and establishment of the second larval stage, the metacestode, citing findings obtained from *T. saginata*, *T. taeniformis*, and *E. granulosus*. It is very interesting to compare their findings with those provided in the review by Klaus Brehm on *E. multilocularis* as an experimental model in stem cell research and molecular host-parasite interactions. Brehm describes the considerable progress that has been made in terms of *E. multilocularis* neoblast (or stem cell) cultures obtained from metacestodes. These neoblasts closely resemble those cells observed during the oncosphere-metacestode transition mentioned by Jabbar *et al.* Besides this aspect, Klaus Brehm also covers recent information on the *E. multilocularis* genome sequencing project, and provides an outlook on how combining these genomic data with genetic manipulation of gene expression and stem cell cultivation will advance the field in the future.

Once *E. multilocularis* metacestodes are established, they can persist within an infected host, most notably in the liver, for extended periods of time. The disease, alveolar echinococcosis (AE), is in many cases fatal if left untreated. The course of human AE closely resembles the disease in the murine host, thus the mouse represents a valuable laboratory model for studies on the immunological host-parasite relationship. Corresponding findings on this topic are reviewed by Mejri *et al.* in this issue and demonstrate how the parasite employs different metacestode surface molecules and excreted/secreted metabolic products to modulate the host responses to his own advantage. These findings could be applied for either prevention of infection and/or disease early during infection or for the elimination of established infection, possibly in combination with appropriate chemotherapeutic measures.

With respect to the latter aspect, Hemphill *et al.* provide a summary on the use of *in vivo* and *in vitro* laboratory models for screening drugs against AE and cystic echinococcosis. The more recent developments and achievements in relation to the *in vitro* culture of *E. multilocularis* metacestodes (also pointed out by Brehm in this issue) and the establishment of simple readouts of drug-induced effects (besides morphological observations) offer the possibility of medium- to high-throughput screens. The genetic manipulation of *E. multilocularis* cells, and the accessibility of the *E. multilocularis* genome and EST sequence information, will contribute to the discovery of suitable drug targets, which could also be potentially relevant in disease-causing trematodes.

In terms of developing novel and improved chemotherapeutic treatment options for pathogenic trematodes, Jenifer Keiser has reviewed the current *in vitro* and *in vivo* models for *S. mansoni*, the liver fluke *F. hepatica*, and the intestinal fluke *Echinostoma caproni*. Phenotypic assessment of drug activity is the major readout for *in vitro* screens, and a big step towards medium- or high-throughput whole organism screening would be the development of more simple readouts such as viability markers or calorimetric analysis.

The reason for producing this special issue has been to provide an overview on the current state of investigations on cestode and trematode laboratory models, including *in vivo* and *in vitro* approaches. The authors are recognized experts in their corresponding research areas and they have provided up-to-date and comprehensive reviews and/or research papers on their respective fields of expertise. I would like to thank all those who have made these contributions for their time and the considerable effort they have put into this. Urgent needs for future studies are also identified, and it is hoped that the advances described herein will stimulate us, and motivate others as well, to ensure further developments for these research topics.

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