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Echinococcus: the model cestode parasite

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Editorial

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Cestodes are important endoparasitic organisms, some of which are responsible for serious zoonotic diseases. The most severe diseases are inflicted by the larval stages (metacestodes) of *Echinococcus multilocularis* and *E. granulosus sensu lato (s. l.)*, which are the causative agents of alveolar (AE) and cystic echinococcosis (CE), respectively. Another member of the Taeniidae family, *Taenia solium*, has also a prominent role as the causative agent of cysticercosis, with a special importance for neurocysticercosis (NC). The adult-stages of these parasites colonialize the intestine of their definitive hosts, which are foxes and dogs for *E. multilocularis*, canines for *E. granulosus* and humans for *T. solium*. In the intestine of the definitive host, self- or cross-fertilization of adults leads to the production of eggs which contain a zygote, eventually forming a pre-larval stage (oncosphere), which is then accidentally ingested by an intermediate host. CE and AE have been historically considered as food- and water-borne infections, with more recent evidence also pointing to the importance of hand-to-mouth transmission, and they are ranked as the most important foodborne parasites worldwide (Tamarozzi *et al.* 2020; Torgerson *et al.* 2020). For *Echinococcus* spp. and *T. solium*, several mammalian species can be infected as intermediate hosts, including humans, which act as dead-end hosts for *E. granulosus* and *E. multilocularis*. Within their intermediate hosts, the parasite larvae are usually targeted to distinct compartments or organs, such as the liver in most cases for *E. multilocularis* infections or liver and lungs for *E. granulosus*. In the case of *T. solium* infections, the most serious consequences occur upon invasion of the central nervous system, which less commonly also can be caused by *Echinococcus* spp. There, development into the larval metacestode (*Echinococccus* spp.) or cysticercus (*T. solium*) stage takes place, which causes disease due to expansion, extensive proliferation and/or inflammatory reactions on part of the host. Besides *Echinococcus* spp. and *Taenia* spp., there are a number of other, albeit less prominent, cestodes that exhibit a considerable zoonotic potential. A more recently, identified cestode causing disease in humans was identified in human cases in North America, causing systemic infection of organs and blood vessels (Deplazes *et al.* 2019). Metacestodes exhibited molecular hallmarks that resembled, but were clearly distinct from, *Vesteria mustelae. Vesteria mustelae* parasitizes weasels and has not been demonstrated to be zoonotic, indicating that previously unrecognized human pathogenic cestodes species with still unknown potential animal hosts exist (Deplazes *et al.* 2019). Other species causing disease in humans include *Hymenolepis* and *Dibothriocephalus*, but also *Bertiella*, *Dipylidium*, *Raillietina*, *Inermicapsifer* and *Mesocestoides*. As indicated in a recently published review, the true clinical significance of these less prominent tapeworms is not well known, as they are often more difficult to identify and there is also a rather limited awareness of their existence (Sapp and Bradbury, 2020).

For the study of NC caused by *T. solium*, the exploration of the cellular and molecular processes underlying disease progression is a difficult task, and *in vitro* models have only a limited value. However, different animal models for NC have been exploited to carry out experimental infections in a controlled environment, and these allow to study disease progression both with and without treatment interventions (reviewed in de Lange *et al.* 2019). In contrast, for *E. multilocularis* several *in vitro* laboratory models have enabled researchers to maintain and propagate metacestodes as well as germinal layer cells (including parasite stem cells) (Spiliotis and Brehm, 2009). These models have opened the way for researchers to elucidate several aspects of the parasite biology, such as the involvement of signalling pathways in differentiation processes during larval stage formation, parasite physiology, metabolomics, proteomics and transcriptomics, and different aspects of the host–parasite relationship, including immunomodulatory mechanisms (Tsai *et al.* 2013; Zheng *et al.* 2013; Nono *et al.*, 2014; Brehm and Koziol, 2017; Monteiro *et al.* 2017; Ritler *et al.* 2019; Zhou *et al.* 2019; Fratini *et al.* 2020). In addition, different rodent models have been applied to investigate the pathogenesis and aspects of the host immune response. Very importantly, these *in vitro* and *in vivo* models provided the basis to establish reliable screening platforms for the assessment of either novel candidate drugs and/or different drug formulations of potential therapeutic value (Lundström-Stadelmann *et al.* 2019).

These advances have placed *E. multilocularis* and *E. granulosus* on the radar as the prime models to study different aspects related to cestode biology and treatment of cestode infections. This is illustrated in a series of seven papers that have been published in *Parasitology* during 2018–2020. These papers deal with different aspects encountered during surgery, establishment of *in vitro* and *in vivo* infection models, and development of novel chemotherapeutical treatment options against *Echinococcus* ssp.

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Surgical treatment of alveolar and cystic echinococcosis

The treatment of AE largely relies on surgery and/or chemotherapy depending on different factors that include metacestode size and location, number of cysts, viability status, the interaction between the expanding parasite and the adjacent host tissue, and bacterial and fungal super-infection. In the case of CE, other options include PAIR (puncture, aspiration, injection and re-aspiration) and 'watch and wait' (Agudelo Higuita *et al.* 2016), and potential complications are related to cyst rupture and spillage of protoscoleces, which can lead to secondary cyst formation. For AE, complete surgical resection is the only real curative treatment, since the established chemotherapy with benzimidazoles [albendazole (ABZ)/mebendazole (MBZ)] does not act parasiticidal. In addition, surgery is always accompanied by peri- and post-operative chemotherapeutical benzimidazole treatment, not only for AE, but also for CE. The paper by Yang *et al.* (2019) summarizes the findings of a study of 178 patients suffering from hepatic AE, which were all treated by definitive radical surgery in the West China Hospital of the Sichuan University, Chengdu, China. Patients were grouped into four categories: A, patients with direct radical hepatic resection; B, patients with percutaneous puncture external drainage followed by radical hepatic resection 2 months later; C, patients undergoing two-step hepatic resection; D, patients undergoing liver transplantation. Following surgery, patients were followed up for 8–72 months, and data on mortality, post-operative complications and recurrence rates were compared. No post-operative mortality was seen in groups B and C, while a fraction of patients in groups A and D (2.29 and 8.62%, respectively) suffered from lethal postoperative complications. For the others, no recurrence was noted. The authors concluded that radical resection of the liver by experienced hepatic surgeons is the preferred treatment of hepatic AE. Akbulut and Sahin (2020) published a comment on that paper and provided some additional explanations and clarifications which are based on the current WHO guidelines (Brunetti *et al.*, 2010). First, *E. multilocularis* metacestodes exhibit the hallmarks of a neoplastic, invasive lesion. Therefore, a clear surgical margin of 10 mm around the lesion can reduce the postoperative recurrence risk. Unfortunately, in many cases, radical surgery cannot be performed as these are diagnosed at the late stage of disease. ABZ therapy, and also MBZ therapies should continue for at least 2 years after surgery. Other anthelmintic drugs such as praziquantel and nitazoxanide have also been used (with no or only limited success), and the anti-fungal compound amphotericin B was applied as a salvage treatment. For post-operative surveillance, a combined radiological and serological follow-up for up to 10 years would be optimal. Inoperable cases of AE must undergo long-term/mostly life-long benzimidazole treatments. Other surgical options beside radical resection include *ex vivo* liver resection and auto-transplantation, and ultimately liver transplantation. However, these are major surgical procedures and are associated with high morbidity and mortality risk.

Overall, these two papers underline the need for improved and more efficacious, preferentially curative, chemotherapeutical treatment options for patients suffering from echinococcosis. To identify and perform pre-clinical studies on such novel options, experimental *in vitro* and *in vivo* models are indispensable tools.

In vitro and *in vivo* models for the maintenance and study of *E. multilocularis* metacestodes

The paper published by Laurimäe *et al.* (2020) highlighted important issues related to *E. multilocularis in vitro* and *in vivo* models, which are also relevant for drug discovery. The traditional method to preserve different *Echinococcus* isolates/strains has been to maintain the proliferative metacestode tissue in rodents, typically by intraperitoneal injection into the highly susceptible *Meriones* spp. or different laboratory mouse strains such as BALB/c or B57BL/6. Due to the tumour-like proliferation of metacestode tissue, animals are typically euthanized after 2–3 months (in most cases prior to protoscolex development), dissected and parasite tissue is transferred to another animal. A major caveat of this procedure is physiological adaptation, meaning that the biological characteristics of the metacestode might change due to adaption to the murine host. Different techniques have been developed that would allow *in vitro* culture of *E. multilocularis* metacestodes (Hemphill and Gottstein, 1995; Jura *et al.*, 1996). However, the major breakthrough that allowed to maintain and propagate metacestodes at a larger scale is the culture method established by Spiliotis and Brehm (2009). This method enables researchers to propagate metacestode vesicles at a high rate, makes it possible to induce protoscolex development *in vitro*, and also allows axenic cultivation and culture of germinal layer cells, and thus the totipotent parasite stem cells. The insight that these stem cells need to be killed to achieve true parasiticidal effects is important when it comes to drug development. While application of these techniques led to a steady increase in our knowledge on the parasite biology, its metabolic requirements and its interaction with the host, adaptation to *in vitro* culture conditions is again likely to provide biased results. In terms of treatment options, there is a clear risk that drug susceptibility properties of rodent-adapted isolates or culture-adapted parasites could be rather different from low-passage-number-isolates obtained from, e.g. human tissue, which would reflect the 'true' parasites.

In their paper, Laurimäe *et al.* provide a potential solution to this problem. They show how cryopreservation of isolates, namely of metacestode-infected tissue blocks and/or protoscoleces, could be valuable alternative to long-term *in vitro*/*in vivo* maintenance. Surprisingly, this paper has revealed that protocols for cryopreservation of *E. multilocularis* had been established and validated already in 1985 (Eckert and Ramp, 1985; Eckert, 1988). By analysing samples that were cryopreserved with this 'forgotten' protocol from 1984 to 1986, the authors showed that some of the metacestodes and protoscoleces were still viable after 35 years of storage in liquid nitrogen. Metacestodes (35%) underwent growth and proliferation *in vitro*, and protoscoleces (76%) were motile and thus viable *in vitro*. The *in vivo* infection rate in a rodent model was at 58%, of which 36% showed abortive lesions. Thus, with the described cryopreservation technique the parasite was able to survive, though at a limited rate. Genetic analysis confirmed that the isolates belonged to European, Asian and North-American clades. It is not clear whether metacestode proliferation after cryopreservation is due to surviving micro-vesicles or due to the stem cells from the germinal layer. Cryopreservation is thus a suitable method for long-term storage of *Echincoccocus* isolates, and this has the potential to (i) reduce the number of experimental animals that would otherwise be needed to propagate the parasite in serial passages, and (ii) to carry out drug-treatment studies without the bias of adaptive processes during *in vitro* culture or *in vivo* maintenance.

Improving treatments by improving the solubility of benzimidazoles

While clinical studies have shown that benzimidazole-based chemotherapy of CE can actually act curative, depending on several factors as outlined above, the situation is different for AE, and benzimidazoles will halt parasite growth but do not act parasiticidal. Nevertheless, it is important to point out that the benzimidazole-based therapy developed by Professor

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Dr Johannes Eckert and colleagues over 40 years ago (Schantz *et al*., 1982) has been a major advance, as it has significantly increased the 10-year survival rate for AE patients that cannot undergo radical surgery from around 20 to 80–85%. Nevertheless, adverse reactions to benzimidazole therapy such as severe hepatotoxicity have been reported frequently, often leading to treatment discontinuation. To avoid such adverse effects, regular monitoring of liver enzymes, drug serum levels and, if necessary, adjustment of the dosage are required. As this is only possible in countries with an advanced health service infrastructure, and most AE and CE cases are found in resource-poor settings, better drugs are needed. Despite these shortcomings, no new drugs, or only few new formulations of already existing lead drugs, have been specifically patented in the last 40 years (Patentscope database, 2020).

The poor bioavailability of ABZ and MBZ is due to inadequate solubility and thus absorbance in the intestine. Thus, high dosages and repeated and/or long-term treatments are required to reach adequate systemic distribution, which expose patients to adverse side-effects and decrease their quality of life. Strategies to improve bioavailability have been pursued, and newly developed formulations of benzimidazoles were developed, such as ABZ complexes with phospholipid-based liposomes, or ABZ emulsions that exhibit increased absorption without increasing the toxicity (reviewed in Hemphill *et al*., 2014, 2019). Another and highly valuable strategy has been introduced by the paper by Vural *et al*. (2020), which reports on the use of novel benzimidazole salt formulations and their use in experimentally induced CE in mice. These formulations were developed within the framework of the HERACLES European funded project (Casulli *et al*., 2020). Salt formulations of ABZ and ABZ-sulfoxide (ricobendazole, RBZ) have been patented for the European and US markets, and include sodium salts of ABZ (ABZ-Na), RBZ (RBZ-Na) and salts of the RBZ enantiomers. These formulations exhibit dramatically increased solubility of the compounds. Vural *et al*. comparatively evaluated oral application by gavage of ABZ, RBZ and RBZ enantiomers and respective salt formulations in BALB/c mice intraperitoneally infected with *E. granulosus* protoscoleces and monitored changes in the pharmacokinetics of RBZ enantiomers and the parasite mass upon treatments. They demonstrated a significant reduction of parasite load in mice treated with most of the salt formulations compared to conventional ABZ treatment. This reduction coincided with improved bioavailability as determined by PK studies, and aberrant structural integrity of both laminated and germinal layer of the parasite. They also found consistently lower drug concentrations in the cyst fluid compared to plasma samples, illustrating the problems to get the drug to the desired site of action, namely the interior of the parasite. Overall, these results are encouraging, since benzimidazole salts can be synthesized in a simple, cheap and rapid manner, they have an improved and more rapid anthelmintic activity than the conventional benzimidazoles, and are prone to cause less side-effects (Vural *et al*. 2020).

Another way to increase solubility of benzimidazoles is to introduce structural modifications, by adding hydrophilic moieties to the benzimidazole scaffold. Recently, Xu *et al*. (2019) introduced an epoxy group to MBZ by a reaction with epichlorohydrin and obtained two isoforms (M-C1 and M-C2), both of which exhibited much higher solubility compared to non-modified MBZ. *In vitro* exposure of *E. multilocularis* protoscoleces to 1 μM M-C2 (1–30 μM) resulted in protoscolex mortality similar to MBZ. In addition, *in vitro* treatments of *E. multilocularis* metacestodes with M-C2 resulted in a high degree of damage. The other derivative, M-C1, did not affect neither protoscoleces nor metacestodes. However, both derivatives showed a markedly reduced cytotoxicity in rat hepatoma cell cultures compared to MTZ. Thus, these modifications on the benzimidazole scaffold improved MBZ solubility, resulting in one compound with higher efficacy, and an isoform with lower anti-parasitic activity. Further studies on *in vivo* pharmacokinetics, pharmacodynamics and toxicity should be carried out to forward such compounds to a suitable *in vivo* model.

Natural products for the treatment of alveolar and cystic echinococcosis

Powerful pharmacological activities, accessibility, relatively low costs and generally low toxicities are the hallmarks of medicinal plants, whose pharmaceutical and anti-parasitic properties are often attributed to essential oils. For instance, essential oils with documented activities against *E. granulosus* protoscoleces and metacestodes have been derived from *Mentha piperita* and *M. pulegium*. The main compound in *M. pulegium* essential oil is piperitone oxide, with suspected protoscolicidal and metacestodicidal effects. Other natural products with significant anti-protoscolex activity are derived from endophytic *Pestalotiopsis* sp., from the circassian walnut *Juglans regia*, *Myrtus communis L.* essential oil and *Nectaroscordum tripedale L.* leaf extract. However, in all these studies, the corresponding active substances have not been determined (Hemphill *et al*., 2019; Lundström-Stadelmann *et al*., 2019).

Of all the natural herb products tested, thymol and carvacrol, two isomers that differ only by the positioning of a hydroxyl group, represent promising options. Thymol and carvacrol are the main components of essential oils of *Thymus vulgaris* and *Origanum vulgare*.

In the study by Hizem *et al*. (2019), the essential oil of *Thymus capitatus*, seven defined fractions (F1–F7) obtained from silica gel chromatography and several pure essential oil components were evaluated with respect to *in vitro* activities against *E. multilocularis* metacestodes and *in vitro* cultured germinal layer cells. Measurements of metacestode viability and transmission electron microscopy demonstrated that exposure to essential oil, F2 and F4 impaired metacestode viability. F2 and F4 exhibited higher toxicity against metacestodes than against mammalian cells, whereas essential oil was as toxic to mammalian cells as to the parasite. However, none of these fractions exhibited notable activity against isolated *E. multilocularis* germinal layer cells. Hizem *et al*. also analysed the essential oil and different fractions by gas chromatography and mass spectrometry and showed that carvacrol was the major component of the essential oil (82.4%), as well as of fractions F3, F4 and F5 (>90%). Other major components of essential oil were β-caryophyllene, limonene, thymol and eugenol. However, exposure of metacestodes to all these individual components, including carvacrol, and a combination of these components, was ineffective. Thus, while the fractions F2 and F4 of *T. capitatus* essential oil contain potent anti-echinococcal compounds, the activities of these two fractions are most likely based on synergistic effects between several major and minor constituents, and not on an individual compound. Nevertheless, these compounds could be exploited for the development of novel treatments once these synergisms are elucidated.

Drug repurposing and nano-encapsulation

Besides exploiting natural compounds, drug repurposing, meaning the use of already existing drugs, is regarded a valid strategy to accelerate the pipeline for drug licensing. Drug repurposing has the potential to decrease the time that is required to reach the market and to reduce costs. *In vitro*, a variety of anti-cancer, anti-fungal and anti-protozoal drugs were efficacious against metacestodes of *E. multilocularis and E. granulosus* (Hemphill

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et al., 2019; Lundström-Stadelmann *et al.*, 2019). A classic example of drug repurposing was reported by Fabbri *et al.* (2019), by introducing the use of dichlorophen (DCP), a halogenated phenolic compound used as bactericide and fungicide in cosmetic product formulations. This compound has been repurposed earlier for a variety of infections by intestinal stages of nematodes and tapeworms, including *Ascaris lumbricoides*, *Taenia saginata* and *Hymenolepis nana*. DCP has a very low solubility in water and is poorly absorbed after oral administration. Thus, to be able to apply this compound for treatment of a systemic infection, solubility must be improved to increase absorption. Nano-encapsulation of drugs is a well-known strategy, and Fabbri *et al.* evaluated the *in vitro* and *in vivo* efficacy of DCP and DCP-loaded silica nanoparticles (DCP-NP) against *E. multilocularis* metacestodes. *In vitro*, NP-DCP (0.1, 0.5 and 1 μg/mL) impaired the survival of protoscoleces in a time- and dose-dependent manner and to a much higher degree than free NP or ABZ, and microscopy demonstrated distinct morphological and structural alterations which indicated parasite death. *In vitro* treatment of *E. multilocularis* metacestodes with 0.5 and 1 μg/mL of NP-DCP strongly altered the germinal layer of parasites and lead to rapid vesicle death. *In vivo* studies in a rodent model demonstrated that the NP-DCP applied at 4 mg/kg had similar efficacy as ABZ applied at 25 mg/kg and was more efficacious than non-encapsulated DCP. Therefore, the repurposing of DCP combined with silica nanoparticles could be an alternative for the treatment of echinococcosis.

Where to go from here…

The examples presented here are just a very minor part of a much larger number of preclinical studies that have been carried out to inform on and/or improve the current treatment options for echinococcosis. The published literature on the subject, when using the keywords 'echinococcosis' and 'therapy', amounts up to >9200 entries in PubMed, and in the last 20 years, 200–280 papers on these topics have been published annually. These publications include case reports, immunological, molecular and biochemical studies, and also reports on compounds that would be potentially interesting as novel treatment options. However, for many compounds or compound formulations, no or very limited *in vivo* studies were published. This could be due to very limited project financing, the lack of specificity and toxicity of the compounds or, and this is unfortunate, the authors wanted to refrain from publishing 'negative' results. The latter is commonly observed, and creates a disturbing lack of knowledge, which hinders advancements in the field. Another striking observation is that most studies which claim to have investigated compounds against AE or CE have applied subjective methods for *in vitro* testing against *Echinococcus* protoscoleces by light microscopy. However, metacestodes, and not protoscoleces, are the disease-causing stages of AE and CE. *In vitro* culture methods for metacestodes and objective measurements of drug-impact are actually available, and researchers in the field should implement these methods more often.

In addition, several substances, even though they looked promising in mouse trials, were not pursued further. This could be due to side-effects and toxicity issues (often also not reported in the literature). Other compounds were not followed up most likely because of financial constraints, which is a commonly encountered problem when it comes to developing novel treatments for diseases with no, or only little, market return. With few exceptions, pre-clinical studies do not take into account the regulatory process. Thus, there is a lack of clear strategy encompassing preclinical and clinical studies aiming at licensing new treatments for the market. Overall, none of the approaches carried out to date have identified alternatives with improved properties compared to the benzimidazoles used to date. Further screening efforts should be undertaken to identify better compounds with increased efficacy and specificity, and improved safety, and novel drug targets should be identified and validated, with the clear aim to overcome the regulatory hurdles leading to clinical application.

Echinococcus multilocularis and *E. granulosus* are highly adapted to a parasitic lifestyle, and crucial genes and entire pathways for the *de novo*-synthesis of pyrimidines, purines and amino acids are absent in the genome, and genes for fatty acid and cholesterol *de novo* synthesis are largely missing. Transcripts coding for respective proteins/enzymes involved in uptake and transport of these essential components are upregulated in the metacestode stage, as has been recently shown for fatty acid binding proteins (Pórfido *et al.*, 2020). These auxotrophies should be targeted and exploited for the development of novel therapeutic options.

References

Agudelo Higuita NI, Brunetti E and McCloskey C (2016) Cystic echinococcosis. *Journal of Clinical Microbiology* **54**, 518–523.

Akbulut S and Sahin TT (2020) Comment on surgical approaches for definitive treatment of hepatic alveolar echinococcosis: results of a survey in 178 patients. *Parasitology* **147**, 1408–1410.

Brehm K and Koziol U (2017) Echinococcus-host interactions at cellular and molecular levels. *Advances in Parasitology* **95**, 147–212.

Brunetti E, Kern P and Vuitton DA; Writing Panel for the WHO-IWGE (2010) Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Tropica* **114**, 1–16.

Casulli A, SilesLucas M, Cretu CM, Vutova K, Akhan O, Vural G, CortésRuiz A, Brunetti E and Tamarozzi F (2020) Achievements of the HERACLES project on cystic echinococcosis. *Trends in Parasitology* **36**, 1–4.

de Lange A, Mahanty S and Raimondo JV (2019) Model systems for investigating disease processes in neurocysticercosis. *Parasitology* **146**, 553–562.

Deplazes P, Eichenberger RM and Grimm F (2019) Wildlife transmitted *Taenia* and *Versteria* cysticercosis and coenurosis in humans and other primates. *International Journal for Parasitology-Parasites and Wildlife* **9**, 342–358.

Eckert J (1988) Cryopreservation of parasites. *Experientia* **44**, 873–877.

Eckert J and Ramp T (1985) Cryopreservation of *Echinococcus multilocularis* metacestodes and subsequent proliferation in rodents (*Meriones*). *Zeitschrift für Parasitenkunde* **71**, 777–787.

Fabbri J, Pensel PE, Albani CM, Arce VB, Mártire DO and Elissondo MC (2019) Drug repurposing for the treatment of alveolar echinococcosis: *in vitro* and *in vivo* effects of silica nanoparticles modified with dichlorophen. *Parasitology* **146**, 1620–1630.

Fratini F, Tamarozzi F, Macchia G, Bertuccini L, Mariconti M, Birago C, Iriarte A, Brunetti E, Cretu CM, Akhan O, Siles-Lucas M, Díaz A and Casulli A (2020) Proteomic analysis of plasma exosomes from cystic echinococcosis patients provides *in vivo* support for distinct immune response profiles in active vs inactive infection and suggests potential biomarkers. *PLoS Neglected Tropical Diseases* **14**, e0008586.

Hemphill A and Gottstein B (1995) Immunology and morphology studies on the proliferation of *in vitro* cultivated *Echinococcus multilocularis* metacestodes. *Parasitology Research* **81**, 605–614.

Hemphill A, Stadelmann B, Rufener R, Spiliotis M, Boubaker G, Müller J, Müller N, Gorgas D and Gottstein B (2014) Treatment of echinococcosis: albendazole and mebendazole – what else? *Parasite* **21**, 70.

Hemphill A, Rufener R, Ritler D, Dick L and Lundström-Stadelmann B (2019) Drug discovery and development for the treatment of echinococcosis, caused by the tapeworms *Echinococcus granulosus* and *Echinococcus multilocularis*. In Swinney DC, Pollastri M (eds), *Neglected Tropical Diseases: Drug Discovery and Development*. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA, pp. 251–286.

Hizem A, Lundström-Stadelmann B, M'rad S, Souiai S, Ben Jannet H, Flamini G, Ascrizzi R, Ghedira K, Babba H and Hemphill A (2019) Activity of *Thymus capitatus* essential oil components against *in vitro* cultured *Echinococcus multilocularis* metacestodes and germinal layer cells. *Parasitology* **146**, 956–967.

https://doi.org/10.1017/S003118202100113X Published online by Cambridge University Press

Jura H, Bader A, Hartmann M, Maschek H and Frosch M (1996) Hepatic tissue culture model for study of host-parasite interactions in alveolar echinococcosis. *Infection and Immunity* **64**, 3484–3490.

Laurimäe T, Kronenberg PA, Alvarez Rojas CA, Ramp TW, Eckert J and Deplazes P (2020) Long-term (35 years) cryopreservation of *Echinococcus multilocularis* metacestodes. *Parasitology* **147**, 1048–1054.

Lundström-Stadelmann B, Rufener R, Ritler D, Zurbriggen R and Hemphill A (2019) The importance of being parasiticidal… an update on drug development for the treatment of alveolar echinococcosis. *Food and Waterborne Parasitology* **15**, e00040.

Monteiro KM, Lorenzatto KR, de Lima JC, dos Santos GB, Förster S, Paludo GP, Carvalho PC, Brehm K and Ferreira HB (2017) Comparative proteomics of hydatid fluids from two *Echinococcus multilocularis* isolates. *Journal of Proteomics* **162**, 40–51.

Nono JK, Lutz MB and Brehm K (2014) EmTIP, a T-cell immunomodulatory protein secreted by the tapeworm *Echinococcus multilocularis* is important for early metacestode development. *PLoS Neglected Tropical Diseases* **8**, e2632.

Patentscope database (2020) Available at https://www.wipo.int/patentscope/en/ (Accessed 15 June 2020).

Pórfido JL, Herz M, Kiss F, Kamenetzky L, Brehm K, Rosenzvit MC, Córsico B and Franchini GR (2020) Fatty acid-binding proteins in *Echinococcus* spp.: the family has grown. *Parasitology Research* **119**, 1401–1408.

Ritler D, Rufener R, Li JV, Kämpfer U, Müller J, Bühr C, Schürch S and Lundström-Stadelmann B (2019) In vitro metabolomic footprint of the *Echinococcus multilocularis* metacestode. *Scientific Reports* **9**, 19438.

Sapp SGH and Bradbury RS (2020) The forgotten exotic tapeworms: a review of uncommon zoonotic Cyclophyllidea. *Parasitology* **147**, 533–558.

Schantz PM, Van den Bossche H and Eckert J (1982) Chemotherapy for larval echinococcosis in animals and humans: report of a workshop. *Zeitschrift für Parasitenkunde* **67**, 5–26.

Spiliotis M and Brehm K (2009) Axenic in vitro cultivation of *Echinococcus multilocularis* metacestode vesicles and the generation of primary cell cultures. *Methods in Molecular Biology* **470**, 245–262.

Tamarozzi F, Deplazes P and Casulli A (2020) Reinventing the wheel of *Echinococcus granulosus sensu lato* transmission to humans. *Trends in Parasitology* **36**, 427–434.

Torgerson PR, Robertson LJ, Enemark HL, Foehr J, van der Giessen JWB, Kapel CMO, Klun I and Trevisan C (2020) Source attribution of human echinococcosis: a systematic review and meta-analysis. *PLoS Neglected Tropical Diseases* **14**, e0008382.

Tsai IJ, Zarowiecki M, Holroyd N, Garciarrubio A, Sánchez-Flores A, Brooks KL, Tracey A, Bobes RJ, Fragoso G, Sciutto E, Aslett M, Beasley H, Bennett HM, Cai X, Camicia F, Clark R, Cucher M, De Silva N, Day TA, Deplazes P, Estrada K, Fernández C, Holland PWH, Hou J, Hu S, Huckvale T, Hung SS, Kamenetzky L, Keane JA, Kiss F, Koziol U, Lambert O, Liu K, Luo X, Luo Y, Macchiaroli N, Nichol S, Paps J, Parkinson J, Pouchkina-Stantcheva N, Riddiford N, Rosenzvit M, Salinas G, Wasmuth JD, Zamanian M, Zheng Y; *Taenia solium* Genome Consortium, Cai J, Soberón X, Olson PD, Laclette JP, Brehm K and Berriman M (2013) The genomes of four tapeworm species reveal adaptations to parasitism. Nature 496, 57–63.

Vural G, Yardimci M, Kocak M, Yasar TÖ, Kurt A, Harem IS, Carradori S, Sciamanna I, Siles-Lucas M, Fabiani M, Hemphill A, Lundström-Stadelmann B, Cirilli R and Casulli A (2020) Efficacy of novel albendazole salt formulations against secondary cystic echinococcosis in experimentally infected mice. *Parasitology* **147**, 1425–1143.

Xu S, Duan L, Zhang H, Xu B, Chen J, Hu W, Gui W, Huang F, Wang X, Dang Z and Zhao Y (2019) *In vitro* efficacies of solubility-improved mebendazole derivatives against *Echinococcus multilocularis*. *Parasitology* **146**, 1256–1262.

Yang C, He J, Yang X and Wang W (2019) Surgical approaches for definitive treatment of hepatic alveolar echinococcosis: results of a survey in 178 patients. *Parasitology* **146**, 1414–1420.

Zheng H, Zhang W, Zhang L, Zhang Z, Li J, Lu G, Zhu Y, Wang Y, Huang Y, Liu J, Kang H, Chen J, Wang L, Chen A, Yu S, Gao Z, Jin L, Gu W, Wang Z, Zhao L, Shi B, Wen H, Lin R, Jones MK, Brejova B, Vinar T, Zhao G, McManus DP, Chen Z, Zhou Y and Wang S (2013) The genome of the hydatid tapeworm *Echinococcus granulosus*. *Nature Genetics* **45**, 1168–1175.

Zhou X, Wang W, Cui F, Shi C, Ma Y, Yu Y, Zhao W and Zhao J (2019) Extracellular vesicles derived from *Echinococcus granulosus* hydatid cyst fluid from patients: isolation, characterization and evaluation of immunomodulatory functions on T cells. *International Journal for Parasitology* **49**, 1029–1037.

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