Reflections on the biochemistry of *Echinococcus*: past, present and future

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SUMMARY

This review discusses 5 of my earliest papers on the biochemistry of larval *Echinococcus* published in *Parasitology* in the 1970s and 1980s. Two of the publications consider aspects of the basic biochemistry, intermediary metabolism and the regulation of respiratory pathways in *E. granulosus* and *E. multilocularis*, and emphasize the existence of inter- and intra-species variation in their general metabolism. The third reports on the detailed biochemical analysis of the tegumental surface of the protoscolex of *E. granulosus*, and the final 2 papers describe the genomic cloning of *Echinococcus* DNA fragments and their use, along with other DNA markers, in molecular identification of *E. granulosus* isolates collected worldwide from areas endemic for hydatid disease. A number of years have elapsed since these publications in *Parasitology* and, in this Centenary Issue article, I reflect briefly on some of the subsequent studies undertaken in these research areas that have advanced the field. As well, I provide brief insight on new research directions, emphasizing the impact of molecular biology and associated techniques on future studies of *Echinococcus* and hydatid disease.

Key words: *Echinococcus granulosus*, *Echinococcus multilocularis*, biochemistry.

INTRODUCTION

Echinococcosis (hydatidosis; hydatid disease) is caused mainly by infection with the larvae of the dog/fox tapeworms *Echinococcus granulosus* and *E. multilocularis*. This brief review reflects on several of my papers published on the biochemistry of these fascinating parasites in *Parasitology* in the 1970s and 1980s, and briefly discusses where the field has advanced since then, and where it might lead to.

Two of the publications (McManus and Smyth, 1978, 1982) discuss strain variation and metabolic differences in *Echinococcus*, and are some of the most detailed studies carried out on the biochemistry and intermediary metabolism of these parasites. The third paper (McManus and Barrett, 1985) discusses detailed biochemical analysis of the tegumental surface of the protoscolex of *E. granulosus*. It provided the first insight into the range of proteins and enzymes present in this complex structure, and revealed novel information on the membrane-bound proteins, many exhibiting enzyme activity associated with the tegumental brush border of this larval stage. The final two publications arose from my earlier report of the first successful isolation of functional nucleic acids from *Echinococcus* spp. (McManus *et al.* 1985). One describes the first genomic cloning of *Echinococcus* DNA fragments (Rishi and McManus, 1987) and the other their use, along with other DNA markers, in molecular identification of *E. granulosus* isolates collected worldwide from areas endemic for hydatid disease (McManus and Rishi, 1989).

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STRAIN VARIATION AND METABOLISM IN *ECHINOCOCCUS GRANULOSUS*

A key feature of *E. granulosus* that fascinated me early in my career, and still does, is the fact that this parasite comprises a number of forms that exhibit considerable genetic variation. This extensive strain variation appears to influence life-cycle patterns, host specificity, development rate, antigenicity, transmission dynamics, sensitivity to chemotherapeutic agents and pathology of *E. granulosus* (Thompson and McManus, 2002; McManus and Thompson, 2003). It is now recognized that this variability has potentially important implications for the design and development of vaccines, diagnostic reagents and drugs impacting on the epidemiology and control of echinococcosis (McManus *et al.* 2003; Craig *et al.* 2007).

In a paper published in *Parasitology*, Des and Mim Smyth (Smyth and Smyth, 1964), first pointed out that the *Echinococcus* organisms have a mode of reproduction which favours the expression of mutants, with the result that new parasite variants or strains can readily arise. However, the key in our understanding of this phenomenon of strain variation came in the early 1970s with the first demonstration of

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physiological differences between *E. granulosus* from British sheep and horses in another paper co-authored by Des Smyth (Smyth and Davies, 1974); in identical culture media, the parasite of sheep origin developed *in vitro* to sexual maturity whereas that from horses did not. In addition to these differences in *in vitro* growth characteristics, epidemiological data published at the time (Hatch, 1975) suggested that the horse-dog form may be a different strain with possibly no, or only low infectivity to humans. Indeed, all subsequent studies have reinforced this view and, indeed, separate species status for the two strains has been proposed with important consequences for the epidemiology of hydatid disease and public health in the UK and elsewhere (Thompson and McManus, 2002; McManus and Thompson, 2003).

Although minor morphological differences had long been recognized between the two forms of *E. granulosus*, possible biochemical differences had not been investigated. In my first paper published in *Parasitology*, Des Smyth and I (McManus and Smyth, 1978) compared the basic biochemical composition and carbohydrate metabolism of protoscolex larvae of the two strains of *E. granulosus* and between *E. multilocularis*. The biochemical differences were marked and provided a striking example of a phenomenon described for other helminths such as *Hymenolepis diminuta* and *Haemonchus contortus* that also exhibit intra-species variation in metabolism (Bryant, 1983). Protoscoleces of the horse strain contained less protein and RNA and more lipid than those of the sheep strain. *E. multilocularis* had more lipid and DNA than either, but otherwise it occupied an intermediate position. Of perhaps greater interest were the differences in respiratory parameters. Under aerobic conditions, the sheep strain used more oxygen and glycogen and produced more succinic and acetic acids but less lactic acid than the horse strain. Anaerobically, there was little difference in glycogen utilization, but the sheep strain produced less succinic and lactic acids, and more acetic acid and ethanol. In other words, both aerobically and anaerobically, the major end-products of the sheep strain were mainly acetic acid with some succinic acid, whereas those of the horse strain were mainly lactic acid with some succinic acid. *E. multilocularis* showed an entirely different pattern in each case which was not surprising in view of its separate species status.

A subsequent, wider ranging study I carried out on *E. granulosus* from Kenya involving protoscoleces from 5 different host species (sheep, goats, camels, cattle and humans) and adults, also indicated metabolic variability (McManus, 1981) although the significance of the variability was, at the time, unclear. Subsequent isoenzymatic (Macpherson and McManus, 1983) and DNA (Wachira *et al.* 1993) studies of Kenyan isolates indicated the presence of 2 distinct strains; a sheep-dog strain, infective to humans and a camel-dog strain which is not infective to humans. This work led to later, more extensive studies by my group and others investigating further the molecular basis for this variation.

REGULATION OF RESPIRATORY PATHWAYS IN *ECHINOCOCCUS*

In the 1980s the emphasis in studies of parasite biochemistry changed from the elucidation of pathways, typified by the classic studies of Agosin (1968) on *E. granulosus*, to an understanding of how they are regulated. Somewhat surprisingly, although there are many examples of such regulatory studies in the parasite literature, to date, only one, published in *Parasitology* (McManus and Smyth, 1982) has been carried out with *E. granulosus*, where the activities of glycolytic and associated enzymes, and the concentrations of their substrates in the protoscoleces of the horse and sheep strains and in *E. multilocularis* were measured. By assaying each of the component enzymes, the presence of a complete glycolytic pathway was demonstrated. Furthermore, by comparing the observed mass action ratios of these enzymes with their theoretical equilibrium constants, phosphorylase, hexokinase, phosphofructokinase and pyruvate kinase were shown to be regulators of carbon flow in glycolysis, an observation similar to other organisms.

Pyruvate kinases from the two species of *Echinococcus* were stimulated by fructose bisphosphate but were not inhibited by alanine, a common invertebrate allosteric effector. Phosphoenolpyruvate carboxykinase was more specific for inosine diphosphate (IDP) than adenosine diphosphate (ADP) but was activated to a greater extent by Mg^{2+} than Mn^{2+}. This conflicted with observations made earlier on *E. granulosus* protoscoleces from South American sheep by Agosin and Repetto (1965) – possibly reflecting a strain difference – , and in other parasites (Bryant, 1975), where the enzyme was shown to be more sensitive to Mn^{2+}.

The horse and sheep strains of *E. granulosus* and *E. multilocularis* were shown to possess the complete suite of tricarboxylic acid cycle enzymes with relatively high activities and the levels of intermediates were of the same order as those reported in tissues with an active cycle (McManus and Smyth, 1982). On the other hand, fumarate reductase, which catalyses the reverse reaction to succinic dehydrogenase, had a low activity, although the assay method used would have seriously under-estimated the activity, because of its instability and because it is notoriously difficult to measure. The precise role of the tricarboxylic acid cycle is difficult to assess in parasitic helminths generally because a respiratory end product, succinate, is also an intermediate in the cycle (Fig. 1) and, along with partly oxidized metabolites,

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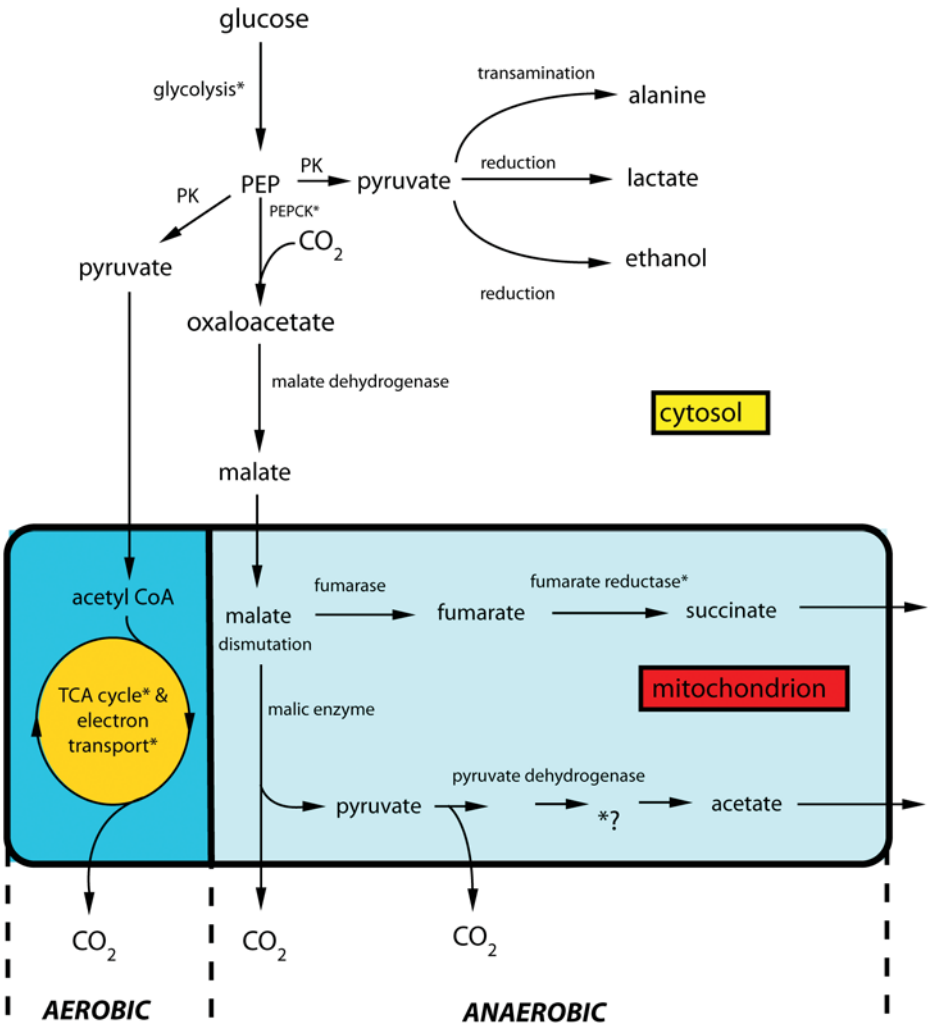


Fig. 1. Respiratory metabolism in *Echinococcus granulosus* (after McManus and Bryant, 1995). *Presumed sites of ATP synthesis; PK, pyruvate kinase; PEPCK, phosphoenolpyruvate carboxykinase; TCA, tricarboxylic acid. The reactions which take place outside the box occur in the cytosol; those inside the box occur in the mitochondria. Almost certainly, aerobic and anaerobic reactions do not occur simultaneously. Further, it is probable that 2 distinct types of mitochondria exist.

is produced even under aerobic conditions. Nevertheless, in our earlier study (McManus and Smyth, 1978) we had shown marked differences in oxygen uptake between the sheep and horse strains of *E. granulosus*, again reinforcing the existence of biochemical variants with greater or lesser dependence of tricarboxylic acid cycle activity (McManus and Bryant, 1995).

Our end-product analysis showed that, while the tricarboxylic acid cycle is functional at least in part in *E. granulosus*, fermentative pathways yielding acetic, succinic and lactic acids occur even under aerobic conditions (McManus and Smyth, 1982). We (McManus and Bryant, 1995) presumed that succinic and acetic acids are formed in the mitochondria, as is the case with other helminths, but whether this occurs in the same mitochondria which house the tricarboxylic acid cycle is unknown. In the pathway illustrated in Fig. 1, NAD(P)H, produced from the glyceraldehyde-3-phosphate dehydrogenase step in glycolysis and by the activity of cytoplasmic malic enzyme, can be re-oxidized by the reduction of pyruvate to lactate or ethanol and of oxaloacetate to malate. In the mitochondrion, reducing equivalents generated on the acetate arm are used to drive the fumarate reductase reaction in the generation of succinate. The adenine nucleotide contents of the protoscoleces of *E. granulosus* and *E. multilocularis* were comparable with those of other parasites; the adenylate energy charge: ((ATP)+(ADP))/((ATP)+(ADP)+(AMP)) in the range 0·86–0·92 was, however, the highest recorded for a parasite. ATP: AMP ratios were also very high and probably reflect the low demand for ATP by a fully developed protoscolex in a hydatid cyst.

BIOCHEMICAL CHARACTERIZATION OF THE *ECHINOCOCCUS GRANULOSUS* TEGUMENT

Cestodes lack a gut and the host/parasite interface is represented by the tegument that is bounded on the external surface by a series of spine-like

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processes, the microtriches. These structures morphologically resemble the brush border microvilli of many vertebrate and invertebrate cells and they are covered with a single outer plasma membrane overlain by a glycocalyx composed of mucopolysaccharide and glycoproteins (Smyth and McManus, 1989). The tegument plays a vital role in the physiology of cestodes, being involved in nutrient uptake, defence against enzyme and immunological attack by the host, in excretion and ionic exchange, and in metabolic transfer (Smyth and McManus, 1989).

In the 1970s and 1980s, cognisant of the obvious importance of the cestode tegument, a series of studies were aimed at achieving a greater understanding of its biochemical nature. The majority of these considered the adult stage of *Hymenolepis microstoma* or *H. diminuta* (e.g. Oaks *et al.* 1977; Pappas and Narcisi, 1982). Our study published in *Parasitology* was different in that it involved the protoscolex of *E. granulosus* (McManus and Barrett, 1985). Being a tissue-dwelling larval stage enclosed within several discrete coverings (brood capsule wall and the germinal, laminated and pericyst layers of the hydatid cyst), the protoscolex encounters different physiological challenges than those of an adult tapeworm. As with others at the time, we utilized the unusual syncytial nature of the cestode tegument to lift off the distal cytoplasm and brush border by physical and/or chemical means, thereby leaving the remainder of the syncytium including the basement membrane. Several approaches were adopted for the disruption and removal of the tegumental surface from protoscoleces. The effectiveness of each method and the purity of subsequent microthrix-enriched fractions obtained by differential centrifugation were evaluated by electron microscopy, by the amount of protein released and by the degree of enrichment of surface plasma membrane marker enzymes. Incubation in saponin for 10 min produced the purest microtriche preparation, but in low yield; freeze/thawing, incubation in Triton X-100 for 10 min or in saponin for 20 min produced fractions containing significant amounts of relatively pure microtriches, but mild homogenization was a poor method for surface disruption and subsequent isolation of microtriches. Phosphodiesterase, adenosine triphosphatase (ATPase) (total and ouabain-inhibited), leucine aminopeptidase and glutamyltransferase were active in the protoscoleces but none were enriched in any of the microthrix fractions. In contrast, alkaline phosphatase, acid phosphatase, 5′ nucleotidase and maltase were enriched significantly in all of the isolated microtriche preparations, which suggested that these enzymes are predominantly surface-membrane bound. The protein profiles of the microthrix-enriched fractions, following SDS-PAGE, were basically similar, although there were some qualitative and quantitative differences in the proteins released by each isolation procedure. Three major PAS-staining components were present in all the preparations and these probably originated from the glycocalyx. One of these PAS-positive components, with an approximate molecular weight of 110 kDa was a glycoprotein, likely of glycocalyx origin, and putatively specific to the horse strain of *E. granulosus*. Its identity has still not been established.

CLONING OF *ECHINOCOCCUS GRANULOSUS* DNA

In the 1980's it had become apparent that the new techniques in molecular biology offered great potential as tools for parasite detection and identification, and would provide a new approach to studies on the taxonomy, genetics and population biology of parasites. The sensitivity and specificity of DNA analysis was ideal for addressing important problems in diagnostic parasitology and achieving two of my research objectives of producing a simple field test for distinguishing *Echinococcus* eggs from those of other taeniid cestodes, and ascertaining the extent of strain variation in *E. granulosus*.

Andrew Simpson and I had used cloned DNA fragments of the ribosomal RNA gene of *Schistosoma mansoni* to discriminate between isolates of *E. granulosus* from UK horses and sheep as well as *E. granulosus* from *E. multilocularis* (McManus and Simpson, 1985). However, I reasoned it would be necessary to use cloned DNA markers, constructed from *E. granulosus* DNA, to provide increased powers of resolution for identification purposes. Accordingly, Arun Rishi and I described in *Parasitology* the construction of a small, size selected (0·5–5·0 kbp) genomic DNA library in a plasmid vector (pAT153) of *Escherichia coli* using total DNA isolated from *E. granulosus* (Fig. 2) (Rishi and McManus, 1987). A panel of taeniid cestode DNAs was then used in conjunction with hybridization and restriction-enzyme analysis to identify in the library 2 recombinant plasmids with *Echinococcus*-specific inserts and a single recombinant plasmid (coded pEG18) with a DNA fragment unique for *E. granulosus*. These, and other recombinant plasmids with *E. granulosus* DNA inserts, were then used in restriction endonuclease, Southern transfer and hybridization analysis to independently and reproducibly discriminate between the UK horse and sheep strains of *E. granulosus*; these probes had the potential for characterizing isolates from other endemic areas. The recombinant insert (approximately 2·3 kbp in size) of pEG18 had a low copy number – estimated at approximately 26 – and it proved later to be insufficiently sensitive for practical use as a DNA probe in the identification of small numbers (ideally, a single egg) of *E. granulosus* eggs by molecular hybridization. Success in this area was only achieved later after the advent of the polymerase

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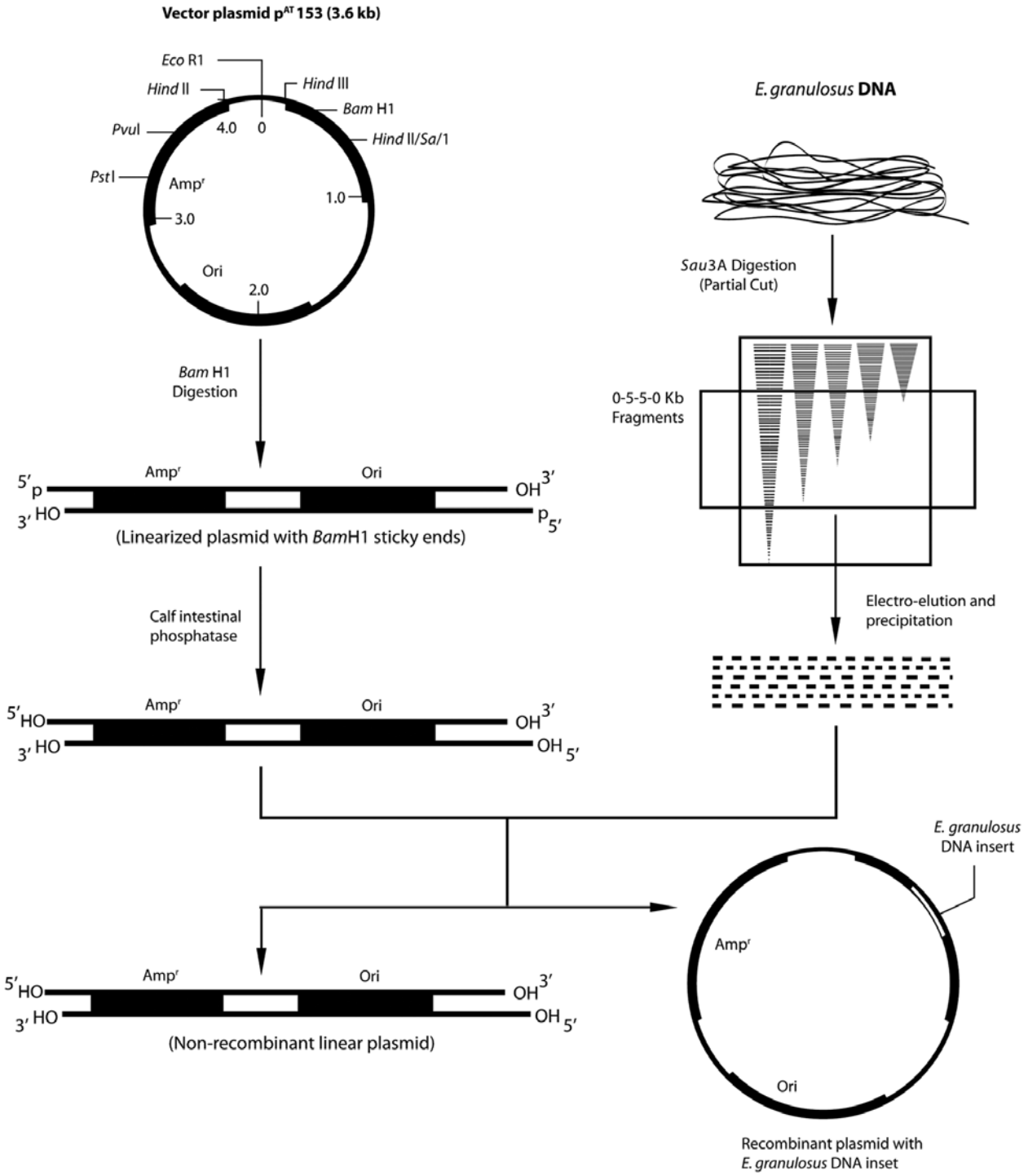


Fig. 2. Cloning of *Echinococcus granulosus* genomic DNA using the plasmid vector, $P^{AT}153$. kb, 10^3 base pairs (after Rishi and McManus, 1987).

chain reaction (PCR) and its application in research on *Echinococcus*.

We used a similar procedure, described in a paper published the following year in *Parasitology* to clone specific DNA fragments from *Taenia solium* that we used to detect intra-specific genetic variation in cysticerci of *T. solium* from India, Mexico and Zimbabwe, and to distinguish *T. solium* from *T. saginata* in a simple dot-blot assay (Rishi and McManus, 1988).

GENETIC HETEROGENEITY WITHIN *ECHINOCOCCUS GRANULOSUS* CHARACTERIZED WITH DNA PROBES

In a paper published in *Parasitology* in 1989, we used the *E. granulosus*- and *S. mansoni*-derived DNA probes in restriction endonuclease fragment length (RFLP) and Southern blot analysis to distinguish between isolates of larval and adult *E. granulosus* from different host species (sheep, goats, cattle,

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camels, humans, buffalo, horses, donkeys, pigs and dogs from various geographical areas (United Kingdom, Kenya–Masailand and Turkana-, Sudan, Somalia, Uruguay, Argentina, Italy, Turkey, Spain, Lebanon, Jordan, Holland, India, Ireland, Yugosalvia and Poland); a total of 251 larval isolates, 16 individual adult worms and 3 pools of adult worms were analysed (McManus and Rishi, 1989). The study did not demonstrate any significant genetic variation within the United Kingdom horse/dog or sheep/dog strains but confirmed the distinctiveness of the two strains shown in previous studies. The sheep/dog strain was shown to be cosmopolitan in its distribution and fertile bovine material originating from the United Kingdom, Kenya, Spain and India conformed to this strain by DNA hybridization. In contrast, cattle isolates from Holland produced markedly different DNA hybridization banding profiles indicating that cattle can harbour more than 1 strain of *E. granulosus*. Similarly, it was shown that goats can harbour 2 different strains of *E. granulosus*, the sheep/dog strain and a form which infects camels. The analysis showed that the strain of *E. granulosus* infecting equines in Spain and Ireland was genetically identical to that infecting horses in the United Kingdom. There was also a different strain infecting pigs in Poland and Yugoslavia. This pig/dog strain appeared to be very similar genetically to the forms of *E. granulosus* which use camels and goats as intermediate hosts and was similar, though not identical, to the variant infecting Dutch cattle. The study also showed that *E. granulosus* material, fixed for a prolonged period in ethanol, or lyophilized, was amenable to DNA analysis and that it was possible to characterize the DNA of a single adult worm.

QUO VADIT?

A number of years have elapsed since these early studies on the biochemistry of *Echinococcus* published in *Parasitology* and, in this Centenary Issue article, it is timely to reflect briefly on some of the subsequent work that has been undertaken in this area, highlighting some of the significant contributions made, and providing some insight into possible future research.

Metabolism of Echinococcus *larvae*

There have been, surprisingly, few recent studies investigating the basic metabolism or the regulation of respiratory pathways in *Echinococcus*. One area of interest that has been pursued has involved determining the effects of anti-hydatid drugs on the activity of enzymes of carbohydrate metabolism in the cyst wall of *E. granulosus*, where glycolysis was shown to be the main respiratory pathway operating (Xiao *et al*. 1994, 1995). These studies suggested that pyruvate kinase, phosphoenolpyruvate carboxykinase and ATPase might be key targets for mebendazole and albenzdazole but not praziquantel, which appears to act by interfering with calcium flux across membranes. On this theme, Bonilla *et al*. (2008) demonstrated the existence of functional linked thioredoxin-glutathione systems in the cytosolic and mitochondrial compartments of *E. granulosus* through a single thioredoxin-glutathione reductase (TGR) enzyme, a promising drug target, and indeed, auranofin, a known TGR inhibitor, killed larval worms *in vitro*.

Studies by Matsumoto *et al*. (2008) suggest that the mitochondrial respiratory system of *E. multilocularis* is also a good target for the development of novel selective anti-echinococcal compounds, as demonstrated previously for other helminths. Their most notable finding was that *E. multilocularis* protoscoleces possess a unique mitochondrial respiratory system that is highly adapted to anaerobic conditions. Specifically, the predominant enzymatic activity in an enriched mitochondrial fraction prepared from the protoscoleces was NADH-fumarate reductase, which does not normally function in the aerobic respiratory chain of mammals, compared with that of NADH oxidase, an enzyme involved in aerobic respiration in aerobic organisms. The high-performance liquid chromatography-mass spectrometry that they carried out revealed that the primary quinone of the parasite mitochondria was rhodoquinone-10, which is commonly used as an electron mediator in anaerobic respiration by the NADH-fumarate reductase system of other eukaryotes. This also suggested that the mitochondria of *E. multilocularis* protoscoleces possess an anaerobic respiratory chain in which complex II of the parasite functions as a rhodoquinol-fumarate reductase. Furthermore, *in vitro* treatment assays using respiratory chain inhibitors against the NADH-quinone reductase activity of mitochondrial complex I demonstrated that they had a potent ability to kill protoscoleces, emphasizing the promise of the mitochondrial respiratory chain of the parasite as a target for chemotherapy of alveolar echinococcosis.

In our earlier study, we had observed that *E. granulosus* protoscoleces cultured under anaerobic conditions produced more succinate than parasites kept under aerobic conditions, suggesting their survival under anaerobic conditions by utilizing the NADH-fumarate reductase system (McManus and Smyth, 1978). We further reported that the specific activity of fumarate reductase in *Echinococcus* protoscoleces was lower than those of enzymes involved in the tricarboxylic acid cycle (McManus and Smyth, 1982). These results, however, did not establish the importance of NADH-fumarate reductase activity in the mitochondrial respiratory system of the

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parasites because the other enzyme activities were not analysed. Clearly, experiments on *E. granulosus*, along the lines undertaken by Matsumoto *et al.* (2008) on *E. multilocularis* would be highly informative and indicate the potential for developing specific inhibitors of the mitochondrial respiratory chain as lead compounds for the chemotherapy of cystic echinococcosis.

Proteins of the Echinococcus granulosus *tegument*

Some recent proteomics analysis of *E. granulosus* has been undertaken with 15 prominent proteins including actins, tropomyosin, paramyosin, thioredoxin reductase, antigen P-29, cyclophilin, and the heat shock proteins hsp70 and hsp20 shown to be present in protoscoleces (Chemale *et al.* 2003). As with studies on schistosomes, a combination of proteomics with differential extraction procedures (Braschi *et al.* 2006) or biotinylation (Braschi and Wilson, 2006) of the tegument would likely reveal very useful information on the nature of the echinococcal parasite surface and its membrane-associated proteins. Notwithstanding, there are some recent reports on tegumental enzymes and proteins from larval *E. granulosus*. A fatty acid-binding protein was shown to be present in the tegument of protoscoleces (Esteves *et al.* 1993; Jakobsson *et al.* 2003), paramyosin (Mühlschlegel *et al.* 1993) and molecules with both Fc-binding and proteolytic activity are present on the surface, likely playing a role in the host-parasite interaction (Baz *et al.* 1998). A novel 29 kDa antigen (immunologically related to antigen 5, one of the 2 major antigenic components of hydatid cyst fluid) has been shown to be distributed in the tegument and rostellum (González *et al.* 2000), and the antioxidant protein thioredoxin peroxidase is expressed in the tegument and subtegument as well as calcareous corpuscle forming cells in protoscoleces of *E. granulosus* (Hou *et al.* 2007).

Following a study on schistosomes (Smyth *et al.* 2003), we used the signal sequence trap technique to identify genes coding for secreted and membrane, including tegumental surface membrane, bound proteins from *E. granulosus* (Rosenzvit *et al.* 2006*b*). Many of these proteins are likely to be involved in host-parasite interactions, and because of their potential exposure to the host immune system, such components are candidates for improved diagnostic tests, as well as being new putative drug and vaccine targets. Several of the clones obtained shared between 30 and 40% amino acid identity with amino acid transporters belonging to members of the neutral and excitatory amino acid transporters of the Dicarboxylate/Amino Acid Na+ and/or H+Cation Symporter family (DAACS) (TC 2.A.23).

In a subsequent study, Camicia *et al.* (2008) confirmed one of these clones (termed *egat1*) as a DAACS member and bioinformatics analysis showed the presence of 9–10 transmembrane domains, consensus sequences for N-glycosylation between the third and fourth transmembrane domain, a highly similar hydropathy profile with ASCT1 (a known member of DAACS family), high score with SDF (sodium dicarboxylate family) and similar motifs with EDTRANSPORT, a fingerprint of excitatory amino acid transporters. *In situ* hybridization indicated distribution of *egat1* mRNA throughout the tegument and immunofluorescence showed localization of the EgAT1 protein in the subtegument of protoscoleces particularly around suckers and rostellum. The close proximity to the surface of the larva, its expression in all life-cycle stages (Rosenzvit *et al.* 2006*a*), and particularly the intimate association with the suckers and rostellum, which are of major importance for the survival in the final host, could make EgAT1 a particularly amenable target for drug development. The presence of a dicarboxylate amino acid (Na+ or H+) symporter (DAACS) member in protoscoleces of *E. granulosus* is supported by the concomitant presence of the active Na+/K+-ATPase that we had described earlier (McManus and Barrett, 1985).

Genetic variation in Echinococcus

In the 1990s, it had become clear that application of the powerful polymerase chain reaction (PCR) would increase considerably our capabilities for studying the molecular genetics of *Echinococcus*. In combination with direct sequencing approaches and PCR, we were able to sequence and compare 2 regions (cytochrome oxidase subunit 1 (*cox1*) and NADH dehydrogenase (*nad1*)) of the mitochondrial genome of a large number of hydatid isolates (Bowles *et al.* 1992; Bowles and McManus, 1993). As a result, we were able to distinguish the 4 recognized species of *Echinococcus* and group *E. granulosus* into 7 genotypically different forms of genoptypes. To date, 10 distinct genotypes within *E. granulosus* have now been identified, and this categorization follows very closely the pattern of strain variation emerging based on published biological characteristics (McManus, 2002, 2006).

Complete sequences for the mitochondrial genomes of *E. granulosus* (horse and sheep strains) and *E. multilocularis* have been obtained as well as sequences for variable mitochondrial genes from additional genotypes of *E. granulosus*, from *E. vogeli* and *E. oligarthrus* (Le *et al.* 2002). Based on this and considerable biological information, a re-evaluation of the taxonomy and phylogeny of *E. granulosus* has been advocated (Thompson and McManus, 2002; McManus and Thompson, 2003; McManus, 2006, Nakao *et al.* 2007). Indeed, mitochondrial DNA-based detection of *Echinococcus* species has been shown generally to be an excellent tool for analysis of strain/genotypic variation in the genus, determining

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phylogenetic relationships, and informing taxonomic species questions (McManus and Thompson, 2003; McManus, 2006, Nakao *et al*. 2007; Xiao *et al*. 2005). As well, several stool-based PCR (copro-PCR) tests have been developed that allow rapid identification of *Echinococcus* DNA from eggs or from adult parasites, thereby providing a highly complementary approach for positive and highly specific diagnosis of canines infected with *E. granulosus* and *E. multilocularis* and environmental detection of *Echinococcus* eggs in soil samples (Abbasi *et al*. 2003; Deplazes *et al*. 2003; Eckert and Deplazes, 2004; Shaikenov *et al*. 2004; Stefanic *et al*. 2004; Reiterova *et al*. 2005; Naidich *et al*. 2006; Boufana *et al*. 2008). None of these tests, however, have been shown to have the sensitivity required for identification of a single egg, and this is clearly an area for future research.

Concluding comment

Since the 1970s, when I first began my research career, considerable advances have been made in the area of parasite biochemistry and physiology. Molecular biology provided a huge armamentarium of new and sophisticated approaches for the study of *Echinococcus* that firstly my group and then others have used to progress the field. For the future, one can predict complete genome sequencing of several *Echinococcus* taxa, the generation of a large *Echinococcus* transcriptome database and the application of post-genomic technologies, including DNA microarray profiling, advanced proteomics, glycomics, immunomics and RNA interference (RNAi), leading to a more complete understanding of the biochemistry, physiology and general biology of these important parasites, and to the development of effective interventions for hydatid disease, including new drugs and vaccines.

I would like to gratefully acknowledge my co-authors of the early papers that I published in *Parasitology* and which form the basis of this retrospective – Nicky Barrett, Arun Rishi and, of course, the late Des Smyth, who was such a guiding light and mentor to me in the years following my Ph.D. and beyond. I would also like to thank all my staff, students and numerous colleagues and collaborators with whom I have worked over the past 35 years and the support of the various funding bodies that have allowed me to undertake my research. Mimi Kersting's help with the figures is also much appreciated.

REFERENCES

Abbasi, I., Branzburg, A., Campos-Ponce, M., Abdel Hafez, S. K., Raoul, F., Craig, P. S. and Hamburger, J. (2003). Copro-diagnosis of *Echinococcus granulosus* infection in dogs by amplification of a newly identified repeated DNA sequence. *American Journal of Tropical Medicine and Hygiene* **69**, 324–330.

Agosin, M. (1968). Biochemistry and physiology of echinococcus. *Bulletin of the World Health Organization* **39**, 115–120.

Agosin, M. and Repetto, Y. (1965). Studies on the metabolism of *Echinococcus granulosus*. 8. The pathway to succinate in *E. granulosus* scolices. *Comparative Biochemistry and Physiology* **14**, 299–309.

Baz, A., Carol, H., Marco, M., Casabó, L., Jones, F., Dunne, D. and Nieto, A. (1998). Fc-binding molecules specific for human IgG1 and IgG3 are present in *Echinococcus granulosus* protoscoleces. *Parasite Immunology* **20**, 399–404.

Bonilla, M., Denicola, A., Novoselov, S. V., Turanov, A. A., Protasio, A., Izmendi, D., Gladyshev, V. N. and Salinas, G. (2008). Platyhelminth mitochondrial and cytosolic redox homeostasis is controlled by a single thioredoxin glutathione reductase and dependent on selenium and glutathione. *Journal of Biological Chemistry* **283**, 17898–17907.

Boufana, B. S., Campos-Ponce, M., Naidich, A., Buishi, I., Lahmar, S., Zeyhle, E., Jenkins, D. J., Combes, B., Wen, H., Xiao, N., Nakao, M., Ito, A., Qiu, J. and Craig, P. S. (2008). Evaluation of three PCR assays for the identification of the sheep strain (genotype 1) of *Echinococcus granulosus* in canid feces and parasite tissues. *American Journal of Tropical Medicine and Hygiene* **78**, 777–783.

Bowles, J., Blair, D. and McManus, D. P. (1992). Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. *Molecular and Biochemical Parasitology* **54**, 165–174.

Bowles, J. and McManus, D. P. (1993). NADH dehydrogenase 1 gene sequences compared for species and strains of the genus *Echinococcus*. *International Journal for Parasitology* **23**, 969–972.

Braschi, S., Curwen, R. S., Ashton, P. D., Verjovski-Almeida, S. and Wilson A. (2006). The tegument surface membranes of the human blood parasite *Schistosoma mansoni*: a proteomic analysis after differential extraction. *Proteomics* **6**, 1471–1482.

Braschi, S. and Wilson, R. A. (2006). Proteins exposed at the adult schistosome surface revealed by biotinylation. *Molecular and Cellular Proteomics* **5**, 347–356.

Bryant, C. (1975) Carbon dioxide utilisation, and the regulation of respiratory metabolic pathways in parasitic helminths. *Advances in Parasitology* **13**, 35–69.

Bryant, C. (1983). Intraspecies variation of energy metabolism in parasitic helminths. *International Journal for Parasitology* **13**, 327–332.

Camicia, F., Paredes, R., Chalar, C., Galanti, N., Kamenetzky, L., Gutierrez, A. and Rosenzvit, M. C. (2008). Sequencing, bioinformatic characterization and expression pattern of a putative amino acid transporter from the parasitic cestode *Echinococcus granulosus*. *Gene* **411**, 1–9.

Chemale, G., van Rossum, A. J., Jefferies, J. R., Barrett, J., Brophy, P. M., Ferreira, H. B. and Zaha, A. (2003). Proteomic analysis of the larval stage of the parasite *Echinococcus granulosus*: causative agent of cystic hydatid disease. *Proteomics* **3**, 1633–1636.

Craig, P. S., McManus, D. P., Lightowlers, M. W., Chabalogoity, J. A., Garcia, H. H., Gavidia, C. M., Gilman, R. H., Gonzalez, A. E., Lorca, M., Naquira, C., Nieto, A. and Schantz, P. M. (2007). Prevention and control of cystic echinococcosis. *Lancet Infectious Diseases* **7**, 385–394.

https://doi.org/10.1017/S0031182009005666 Published online by Cambridge University Press

Deplazes, P., Dinkel, A. and Mathis, A. (2003). Molecular tools for studies on the transmission biology of *Echinococcus multilocularis*. *Parasitology* **127**, S53–S61.

Eckert, J. and Deplazes, P. (2004). Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clinical Microbiology Reviews* **17**, 107–135.

Esteves, A., Dallagiovanna, B. and Ehrlich, R. (1993). A developmentally regulated gene of *Echinococcus granulosus* codes for a 15.5-kilodalton polypeptide related to fatty acid binding proteins. *Molecular and Biochemical Parasitology* **58**, 215–222.

González, G., Spinelli, P., Lorenzo, C., Hellman, U., Nieto, A., Willis, A. and Salinas, G. (2000). Molecular characterization of P-29, a metacestode-specific component of *Echinococcus granulosus* which is immunologically related to, but distinct from, antigen 5. *Molecular and Biochemical Parasitology* **105**, 177–184.

Hatch, C. (1975). Observations on the epidemiology of equine hydatidosis in Ireland. *Irish Veterinary Journal* **29**, 155–157.

Hou, Q. L., Wang, H., Zhang, Z. Z., Cao, W. Y., Zhang, F. C. and Zhang, W. B. (2007). [Immunolocalization of the antioxidant protein TPx of *Echinococcus granulosus*] [Article in Chinese] *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* **23**, 998–1000.

Jakobsson, E., Alvite, G., Bergfors, T., Esteves, A. and Kleywegt, G. J. (2003). The crystal structure of *Echinococcus granulosus* fatty-acid-binding protein 1. *Biochimica et Biophysica Acta* **1649**, 40–50.

Le, T. H., Pearson, M. S., Blair, D., Dai, N., Zhang, L. H. and McManus, D. P. (2002). Complete mitochondrial genomes confirm the distinctiveness of the horse-dog and sheep-dog strains of *Echinococcus granulosus*. *Parasitology* **124**, 97–112.

Macpherson, C. N. L. and McManus, D. P. (1982). A comparative study of *Echinococcus granulosus* from human and animal hosts in Kenya using isoelectric focusing and isoenzyme analysis. *International Journal for Parasitology* **12**, 515–521.

Matsumoto, J., Sakamoto, K., Shinjyo, N., Kido, Y., Yamamoto, N., Yagi, K., Miyoshi, H., Nonaka, N., Katakura, K., Kita, K. and Oku, Y. (2008). Anaerobic NADH-fumarate reductase system is predominant in the respiratory chain of *Echinococcus multilocularis*, providing a novel target for the chemotherapy of alveolar echinococcosis. *Antimicrobial Agents and Chemotherapy* **52**, 164–170.

McManus, D. P. (1981). A biochemical study of adult and cystic stages of *Echinococcus granulosus* of human and animal origin from Kenya. *Journal of Helminthology* **55**, 21–27.

McManus, D. P. (2002). The molecular epidemiology of *Echinococcus granulosus* and cystic hydatid disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **96** (Suppl. 1), S151–S157.

McManus, D. P. (2006). Molecular characterization of taeniid cestodes. *Parasitology International* **55** (Suppl.), S31–S37.

McManus, D. P. and Barrett, N. J. (1985). Isolation, fractionation and partial characterisation of the tegumental surface from protoscoleces of the hydatid organisms, *Echinococcus granulosus*. *Parasitology* **90**, 111–129.

McManus, D. P. and Bryant, C. A. (1995). Biochemistry, physiology and molecular biology of *Echinococcus*. In *Echinococcus and Hydatid Disease* (ed. Thompson, R. C. A. and Lymbery, A. J.), pp. 135–181. CAB International, Wallingford, UK.

McManus, D. P., Knight, M. and Simpson, A. J. G. (1985). Isolation and characterisation of nucleic acids from the hydatid organisms, *Echinococcus* spp. (Cestoda). *Molecular and Biochemical Parasitology* **16**, 251–266.

McManus, D. P. and Rishi, A. K. (1989). Genetic heterogeneity within *Echinococcus granulosus*: isolates from different hosts and geographical areas characterized with DNA probes. *Parasitology* **99**, 17–29.

McManus, D. P. and Simpson, A. J. G. (1985). Identification of the *Echinococcus* (hydatid disease) organisms using cloned DNA markers. *Molecular and Biochemical Parasitology* **17**, 171–178.

McManus, D. P. and Smyth, J. D. (1978). Differences in the chemical composition and carbohydrate metabolism of *Echinococcus granulosus* (horse and sheep strains) and *E. multilocularis*. *Parasitology* **77**, 103–109.

McManus, D. P. and Smyth, J. D. (1982). Intermediary carbohydrate metabolism in protoscoleces of *Echinococcus granulosus* (horse and sheep strains) and *E. multilocularis*. *Parasitology* **84**, 351–366.

McManus, D. P. and Thompson, R. C. A. (2003). Molecular epidemiology and taxononomy of cystic echinococcosis. *Parasitology* **127**, S37–S51.

McManus, D. P., Zhang, W., Li, J. and Bartley, P. B. (2003). Echinococcosis. *Lancet* **362**, 1295–1304.

Mühlschlegel, F., Sygulla, L., Frosch, P., Massetti, P. and Frosch, M. (1993). Paramyosin of *Echinococcus granulosus*: cDNA sequence and characterization of a tegumental antigen. *Parasitology Research* **79**, 660–666.

Naidich, A., McManus, D. P., Canova, S. G., Gutierrez, A. M., Zhang, W., Guarnera, E. A. and Rosenzvit, M. C. (2006). Patent and pre-patent detection of *Echinococcus granulosus* genotypes in the definitive host. *Molecular and Cellular Probes* **20**, 5–10.

Nakao, M., McManus, D. P., Schantz, P. M., Craig, P. S. and Ito, A. (2007). A molecular phylogeny of the genus *Echinococcus* inferred from complete mitochondrial genomes. *Parasitology* **134**, 713–722.

Oaks, J. A., Knowles, W. J. and Cain, G. D. (1977). A simple method of obtaining an enriched fraction of tegumental brush border from *Hymenolepis diminuta*. *Journal of Parasitology* **63**, 476–485.

Pappas, P. W. and Narcisi, E. M. (1982). A comparison of membrane-bound enzymes of the isolated brush border plasma membranes of the cestodes of *Hymenolepis diminuta* and *H. microstoma*. *Parasitology* **84**, 391–396.

Reiterova, K., Miterpakova, M., Turcekova, L., Antolova, D. and Dubinsky, P. (2005). Field evaluation of an intravital diagnostic test of *Echinococcus multilocularis* infection in red foxes. *Veterinary Parasitology* **128**, 65–71.

Rishi, A. K. and McManus, D. P. (1987). Genomic cloning of human *Echinococcus granulosus* DNA: isolation of recombinant plasmids and their use as

https://doi.org/10.1017/S0031182009005666 Published online by Cambridge University Press

genetic markers in strain characterization. *Parasitology* **94**, 369–383.

Rishi, A. K. and McManus, D. P. (1988). Molecular cloning of *Taenia solium* DNA and characterisation of taeniid cestodes by DNA analysis. *Parasitology* **97**, 161–176.

Rosenzvit, M. C., Camicia, F., Kamenetzky, L., Muzulin, P. M. and Gutierrez, A. M. (2006*a*). Identification and intra-specific variability analysis of secreted and membrane-bound proteins from *Echinococcus granulosus*. *Parasitology International* **55** (Suppl.), S63–S67.

Rosenzvit, M. C., Zhang, W., Motazedian, H., Smyth, D., Pearson, M., Loukas, A., Jones, M. K. and McManus D. P. (2006*b*). Identification of membrane-bound and secreted proteins from *Echinococcus granulosus* by signal sequence trap. *International Journal for Parasitology* **36**, 123–130.

Shaikenov, B. S., Rysmukhambetova, A. T., Massenov, B., Deplazes, P., Mathis, A. and Torgerson, P. R. (2004). Short report: the use of a polymerase chain reaction to detect *Echinococcus granulosus* (G1 strain) eggs in soil samples. *American Journal of Tropical Medicine and Hygiene* **71**, 441–443.

Smyth, D., McManus, D. P., Smout, M. J., Laha, T., Zhang, W. and Loukas, A. (2003). Isolation of cDNAs encoding secreted and transmembrane proteins from *Schistosoma mansoni* by a signal sequence trap method. *Infection and Immunity* **71**, 2548–2554.

Smyth, J. D. and Davies, Z. (1974). Occurrence of physiological strains of *Echinococcus granulosus* demonstrated by in vitro culture of protoscoleces from sheep and horse hydatid cysts. *International Journal for Parasitology* **4**, 443–445.

Smyth, J. D. and McManus, D. P. (1989). *The Physiology and Biochemistry of Cestodes*. Cambridge University Press, Cambridge, UK.

Smyth, J. D. and Smyth, M. M. (1964). Natural and experimental hosts of *Echinococcus granulosus* and *E. multilocularis*, with comments on the genetics of speciation in the genus Echinococcus. *Parasitology* **54**, 493–514.

Stefanic, S., Shaikenov, B. S., Deplazes, P., Dinkel, A., Torgerson, P. R. and Mathis, A. (2004). Polymerase chain reaction for detection of patent infections of *Echinococcus granulosus* (''sheep strain'') in naturally infected dogs. *Parasitology Research* **92**, 347–351.

Thompson, R. C. A. and McManus, D. P. (2002). Towards a taxonomic revision of *Echinococcus*. *Trends in Parasitology* **18**, 452–457.

Wachira, T. M., Bowles, J., Zehyle, E. and McManus, D. P. (1993). Molecular examination of the sympatric existence and distribution of sheep and camel strains of *Echinococcus granulosus* in Kenya. *American Journal of Tropical Medicine and Hygiene* **48**, 473–479.

Xiao, N., Qiu, J., Nakao, M., Li, T., Yang, W., Chen, X., Schantz, P. M., Craig, P. S. and Ito, A. (2005). *Echinococcus shiquicus* n. sp., a taeniid cestode from Tibetan fox and plateau pika in China. *International Journal for Parasitology* **35**, 693–701.

Xiao, S. H., Feng, J. J., Guo, H. F., Jiao, P. Y., Yao, M. Y. and Jiao, W. (1994). Effects of mebendazole, albendazole, and praziquantel on fumarate hydratase, pyruvate kinase, and phosphoenolpyruvate carboxykinase of *Echinococcus granulosus* cyst wall harbored in mice. *Zhongguo Yao Li Xue Bao* **15**, 69–72.

Xiao, S. H., Feng, J. and Yao, M. (1995). Effect of antihydatid drugs on carbohydrate metabolism of metacestode of *Echinococcus granulosus*. *Chinese Medical Journal* **108**, 682–688.

https://doi.org/10.1017/S0031182009005666 Published online by Cambridge University Press