Taenia crassiceps: *in vivo* and *in vitro* models

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SUMMARY

Taenia crassiceps is a cestode parasite of wild and domestic animals that rarely affects humans; it has been widely used as an experimental model. The asexual proliferation by budding is a useful attribute of *T. crassiceps* cysticerci, which allows the various strains to be maintained indefinitely in the peritoneal cavity of inbred mice. Over the last 50 years, experimental results using larval and adult stages of *T. crassiceps* have yielded much information on the morphology, infectivity, proliferation dynamics, host immune response, endrocrinological responses and vaccine research, all of which have contributed to our knowledge of cestode biology.

Key words: *Taenia crassiceps*, cestode, Cyclophyllidea.

INTRODUCTION

Taenia crassiceps (Zeder, 1800) is a tapeworm of arctic and red foxes, wolves and dogs (Loos-Frank, 2000; Bush *et al*. 2001). Natural infections with cysticerci have been reported in the field vole (*Microtus arvalis*) (Rietschel, 1981) and woodchuck (*Marmota monax*) (Bröjer *et al*. 2002), as well as in the field mouse (*Peromyscus* sp.) (Everhart *et al*. 2004). In natural infections, *T. crassiceps* cysticerci are found in the subcutaneous tissues of wild rodents (Freeman, 1962); other sites such as the peritoneal and pleural cavities have been described by Freeman *et al*. (1973), and others (Albert *et al*. 1972; Rietschel, 1981; Delvalle, 1989; Anderson and Kurland, 1998; Pétavy *et al*. 1996; Bröjer *et al*. 2002; Wunschmann *et al*. 2003; Everhart *et al*. 2004).

T. crassiceps is reported to have one of the highest reproductive capacities among invertebrate species. A number of strains have been found in the northern hemisphere in definitive hosts which include *Vulpes, Alopex, Canis, Mustela, Felis, Martes* and *Putorius* in natural environments as well as in experimentally infected dogs, gerbils and hamsters in the laboratory (Loos-Frank, 2000; Willms *et al*. 2004; Zurabian *et al*. 2008). In the intermediate host, multiplying larval stages have been found mainly in wild rodents, and a number of isolates have been extensively propagated in mice for the study of biological properties of both the larval and adult stages.

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Arme and Pappas (1983) and Smyth and McManus (1989) published books on the physiology and biochemistry of cestodes, so that we include in this review mostly advances published since 1989. The wild life cycle of this parasite is shown in Fig. 1. Foxes and dogs harbour the adult worm in the small intestine, from which infective eggs are released in faeces and when ingested by wild rodents, oncospheres traverse the intestinal epithelium, lodge in tissues and differentiate to the larval stage or cysticercus. These cysticerci can be harvested and injected into the peritoneal cavity of inbred mice and maintained indefinitely by serial sub-inoculation (Freeman, 1962; Esch and Smyth, 1976).

MORPHOLOGY

The ultrastructure of the larval stage and tapeworm of *T. crassiceps* does not differ significantly from that described for other taeniids or flatworms (Lumsden *et al*. 1982; Lumsden and Hildreth, 1983: Willms *et al*. 2003*a*, 2004, 2005).

Morphology of budding cysticerci

Experiments carried out in our laboratory with the ORF strain, showed that the subtegumentary cells incorporated ^{3}H-thymidine injected into the peritoneal cavity of BALB/c mice. Sections from these specimens were examined by autoradiography and exhibited a large number of labeled cells seen as an almost continuous array below the tegumentary cytons in buds (Fig. 2). By electron microscopy, the cell nuclei covered with silver granules appeared for the most to be undifferentiated cells, with abundant ribosomes and some mitochondria in cells located

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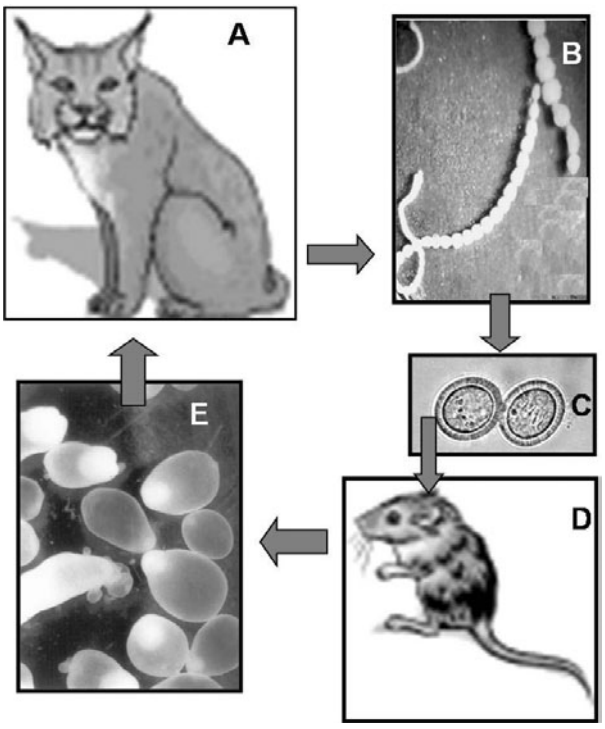

Fig. 1. Wild life-cycle of *T. crassiceps*. (A) Definitive host; (B) Adult worm in host intestine; (C) Infective eggs released in feces; (D) Ingestion of eggs by intermediate host; (E) Larvae grow in an intermediate host and are ingested by a definitive carnivore host where tapeworms grow in the duodenum.

irregularly below the layer of tegumentary cytons. The images suggest that the labeled cells may be tegumentary cell precursors which are eventually inserted into the tegument, contributing to the continuous growth of this tissue. A number of unlabeled cells with irregular odd-shaped nuclei were also found containing numerous microvesicles, cell types, which may correspond to differentiated tegumentary cytons before they are inserted into the tegument (Fig. 3).

PRODUCTION OF EXPERIMENTAL TAPEWORMS IN HAMSTER

The methods for obtaining experimental hamster tapeworms have been described in several publications (Sato and Kamiya, 1989; Kitaoka *et al*. 1990; Willms *et al*. 2003*a*, 2004; Zurabian *et al*. 2008).

Morphology of experimental tapeworms

The length of tapeworms grown in the hamster gut varies, but gravid proglottids with viable eggs are found in the 82nd proglottid at 27–34 d post-inoculation (Fig. 4). The scolex has four suckers and the rostellum carries thorn-shaped hooks. The microscopic structure of immature and maturing proglottids does not differ from tapeworms obtained from natural infections reported in the literature, except that the length and size of proglottids appear to be smaller than those obtained from natural definitive hosts (Fig. 5).

The ultrastructure of adult *T. crassiceps* WFU strain has revealed that the tegumentary surface is similar to that described for other flatworms (Willms *et al*. 2003*b*; Lumsden and Hildreth, 1983). The subtegument contains tegumentary cytons, flame cells, myocytes, calcareous corpuscles and glycogen storage cells. Adult tapeworms obtained from experimental hamsters after oral infection with WFU strain cysticerci also exhibit a continuous array of apoptotic cells in the sub-tegumentary tissues, similar to those found in *Taenia solium* (Fernández *et al*. 2005), suggesting that these tissues are being constantly replaced by new cells.

The immature proglottids, exhibit a myofilament lattice of connecting fibres and sarcoplamsic extensions of myocytons with cytoplasmic processes loosely attached to other cells, structures which be may be involved in the transport of cells and membrane-bound glycogen from the germinative tissue to the mature proglottid. Abundant membrane-bound glycogen is found between the tegumentary cytons of the neck tissue, and as single-stranded particles between the tegumentary cytons of mature proglottids. A large number of cell-to-cell adhesions, identified as gap junctions, are prominent in the immature proglottids and almost always connect to the membrane of cytoplasmic glycogen strands, similar to what has been described in the proglottids of *T. solium* (Willms *et al*. 2003*b*). The immature proglottid of *T. crassiceps* exhibits the formation of testes (Willms *et al*. 2004) along the inner wall of the longitudinal excretory ducts (Figs 6, 7). These structures can be identified by light and electron microscopy and are surrounded by a thin cytoplasmic wall. Ultrastructural analysis of mature proglottids showed that testes and vas deferens contain filiform spermatids, with a single axoneme and an elongated helicoidal nucleus inserted between the axoneme and the spiral cortical microtubules (Figs 8, 9). The morphology and characters of the spermatids in *T. crassiceps* conform to type III spermiogenesis described in other Taeniids (Justine, 1998; Ndiaye *et al*. 2003; Willms *et al*. 2004).

ASEXUAL PROLIFERATION OF THE LARVAL STAGE

Of particular interest for research in the biology of taeniids, is the capability of the larval stage to multiply asexually by budding in the intermediate host. Approximately 11 of 45 taeniid species exhibit asexual reproduction in the metacestode stage (Hoberg *et al*. 2000; Loos-Frank, 2000; Trouvé *et al*. 2003). This biological trait has been widely used since the first descriptions by Freeman (1962), who

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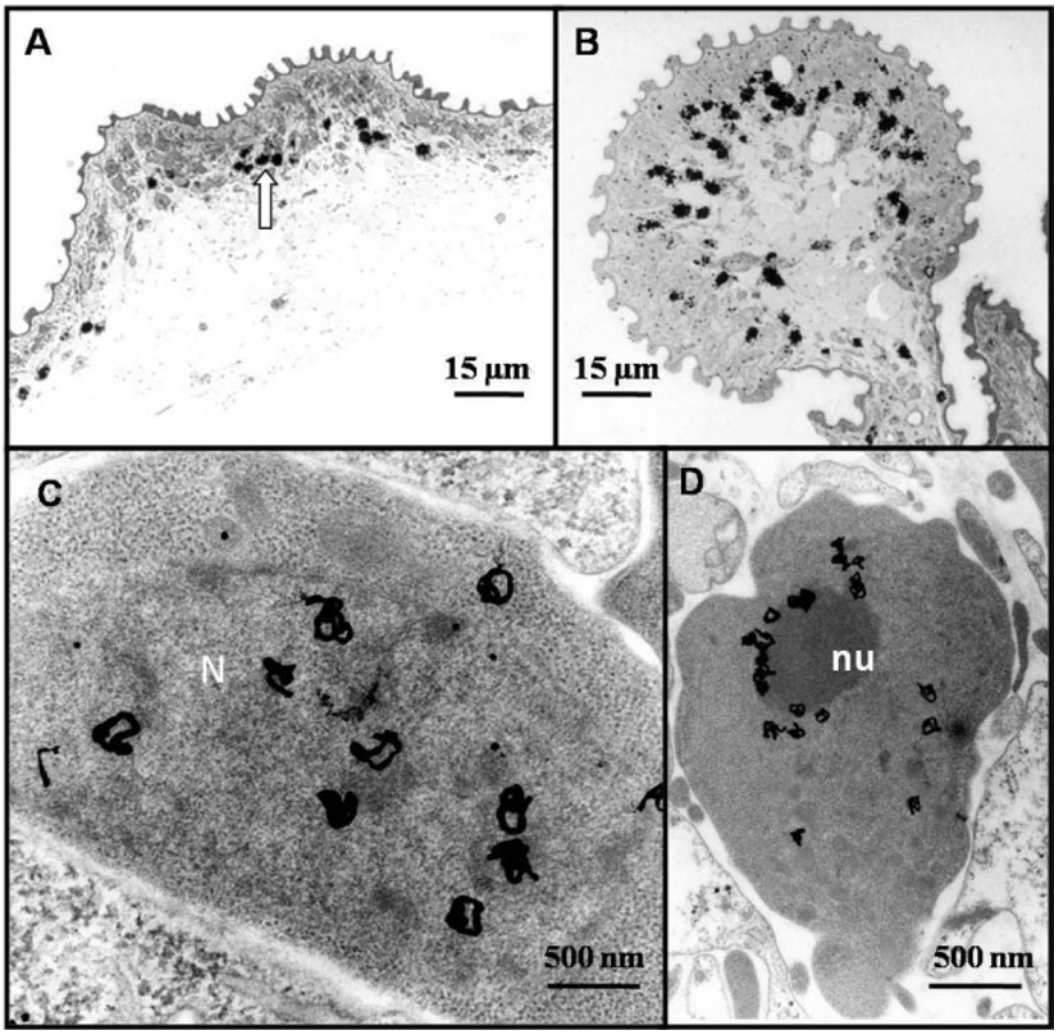

Fig. 2. Autoradiographs of *T. crassiceps* ORF strain tissues grown in BALB/c mouse peritoneum for 6 weeks and exposed for 3 h to 10 μCi of tritiated thymidine in the peritoneal cavity. (A) Arrow points to early budding area, with labeled cells on inner surface of bladder wall. (B) Bud illustrating labeled cells below the tegument. (C and D) Electron micrographs of labeled cells from area shown in (B).

showed that exogenous budding of the larval stages promotes the exponential growth of the parasite. In contrast to other widely studied tapeworms, such as *T. solium*, *Taenia saginata, Hymenolepis nana* and *Echinococcus granulosus*, *T. crassiceps* does not readily parasitize humans (Heldwein *et al.* 2006) and has therefore become a useful experimental model in the laboratory, resulting in at least 392 publications reported over the last 50 years (http://www.ncbi.nlm.nih.gov/).

Since asexual proliferation seems to have several independent and parallel appearances and losses through evolution (Hoberg *et al.* 2000; Trouvé *et al.* 2003), it has been suggested that these parasites possess remarkable genetic plasticity (Moore and Brooks, 1987). The development of asexual multiplication in taeniid species has been explained both by interspecific competition between species within hosts (Moore, 1981), and intraspecific competition between larvae in order to compensate for the loss of reproductive success in the definitive host (Trouvé *et al.* 2003). Asexual multiplication in cestodes may promote intraspecific genetic variation and the formation of new strains (Smyth and Smyth, 1964). A negative result of genetic variation is found in the *T. crassiceps* ORF strain, in which the loss of the scolex produces sterile larvae, incapable of developing adult tapeworms in a definitive host (Mount, 1968; Dorais and Esch, 1969) in addition to having lost two chromosomes (Smith *et al.* 1972). Anderson and Kurland (1998) have suggested ''genome decay with increased adaptation to the host'' occurring over long time periods, during which certain organisms lose genetic information after living in a restricted host environment. Lack of exposure to a definitive host for long periods may also be a contributing factor.

A number of *T. crassiceps* strains have been isolated and used for expansion in the laboratory, among them the HYG, KBS, Toi, the mutant ORF strain and, recently, the WFU strain obtained from a wild rodent infection in North America (Everhart

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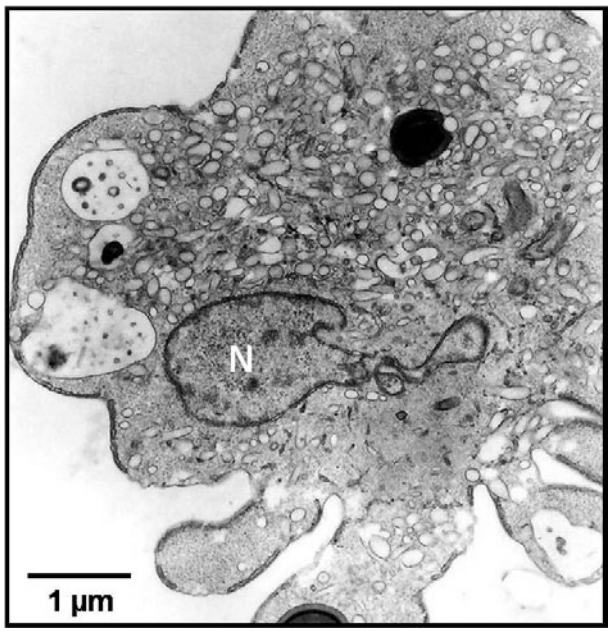

Fig. 3. Electron micrograph of a cell in the vesicular fluid of a *T. crassiceps* ORF bud, with an odd-shaped nucleus, numerous microvesicles in the cytoplasm and a number of pseudopods.

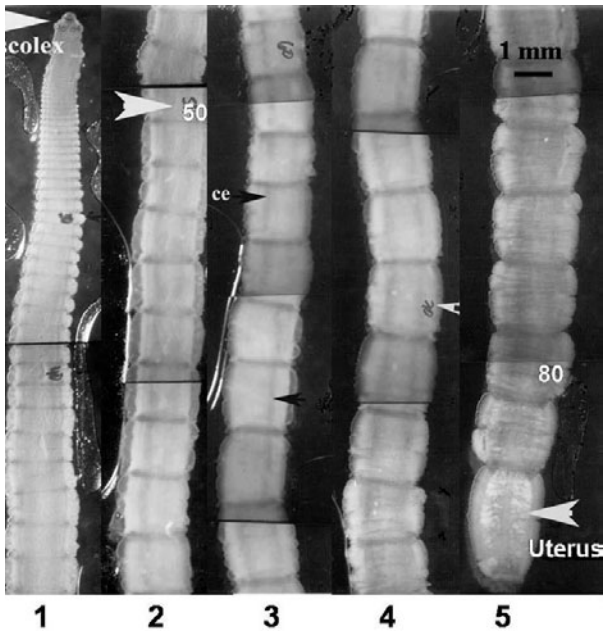

Fig. 4. Segments of a whole *T. crassiceps* worm with 82 proglottids recovered from hamster intestine, 34 days post infections. White numbers indicate approximate proglottid number.

et al. 2004). Additionally, a number of strains have been isolated in Germany, COLA and GIKS (Rietschel, 1981) and other strains in Japan (Sato and Kamiya, 1989; Miyaji *et al*. 1990). To our knowledge, with the exception of the ORF strain which is sterile, no specific differences have been identified between these strains.

The various strains of *T. crassiceps* have been used to explore a wide variety of cestode biology topics that are described below.

GENETIC SUSCEPTIBILITY OF MICE STRAINS

Susceptibility to *T. crassiceps* infection, as well as immune and hormonal responses have been described in several mouse strains and sub-strains. Mice with BALB/c background and H-2^d major histocompatibility genes develop the highest loads of ORF strain metacestodes (Sciutto *et al*. 1991), whereas C57BL mice were restrictive (Fragoso *et al*. 2008). Differences also exist between BALB/c mice sub-strains: significant susceptibility was observed in BALB/cAnN mice in contrast to the resistant Qa-2 protein expressed in BALB/cJ mice, which is also present in C57BL and BALB/cAnN × C57BL F1 Qa-2 transgenic males (Fragoso *et al*. 1996, 1998, 2008). Clear sexual dimorphism of the host was demonstrated by Fragoso *et al*. (2008), who showed that male mice of various strains are significantly more resistant than female mice infected with the ORF strain. Different mouse strains also exhibit diverse susceptibility to infection, as shown by differences in growth patterns in BALB/c and C57BL/6 mice, as well as differences in macrophage activity: macrophages from *T. crassiceps*-infected BALB/c mice showed stronger suppressive activity than those from C57BL/6 mice. These findings suggest that antigen presenting cell activation at both early and late time points during *T. crassiceps* infection is a possible mechanism that underlies the differential susceptibility to *T. crassiceps* infection displayed by these mouse strains (Reyes *et al*. in press).

In the case of the *T. crassiceps* WFU strain, BALB/cJ mice are extremely susceptible to larval development (Everhart *et al*. 2004). Experiments on growth dynamics reveal an initial delay in larval multiplication of the WFU strain when compared to the growth of the ORF larvae at 3 weeks post-inoculation (Everhart *et al*. 2004; Fragoso *et al*. 2008; Zurabian *et al*. 2008) and exponential growth and null mortality of the parasites were observed in susceptible BALB/c and BALB/cJ female mice.

METABOLIC PATHWAYS OF *T. CRASSICEPS*

Several metabolic pathways and a rather large number of enzyme complexes have been studied in this parasite, of which only a few will be described in this review. Their role in the survival of the parasite or of the host-parasite interaction have not been evaluated, because most of the studies, by necessity, have been carried out in parasite tissues removed from the host.

The main source of energy in the metacestode of this parasite appears to be glucose obtained from the host or from glycogen storage deposits (Willms *et al*.

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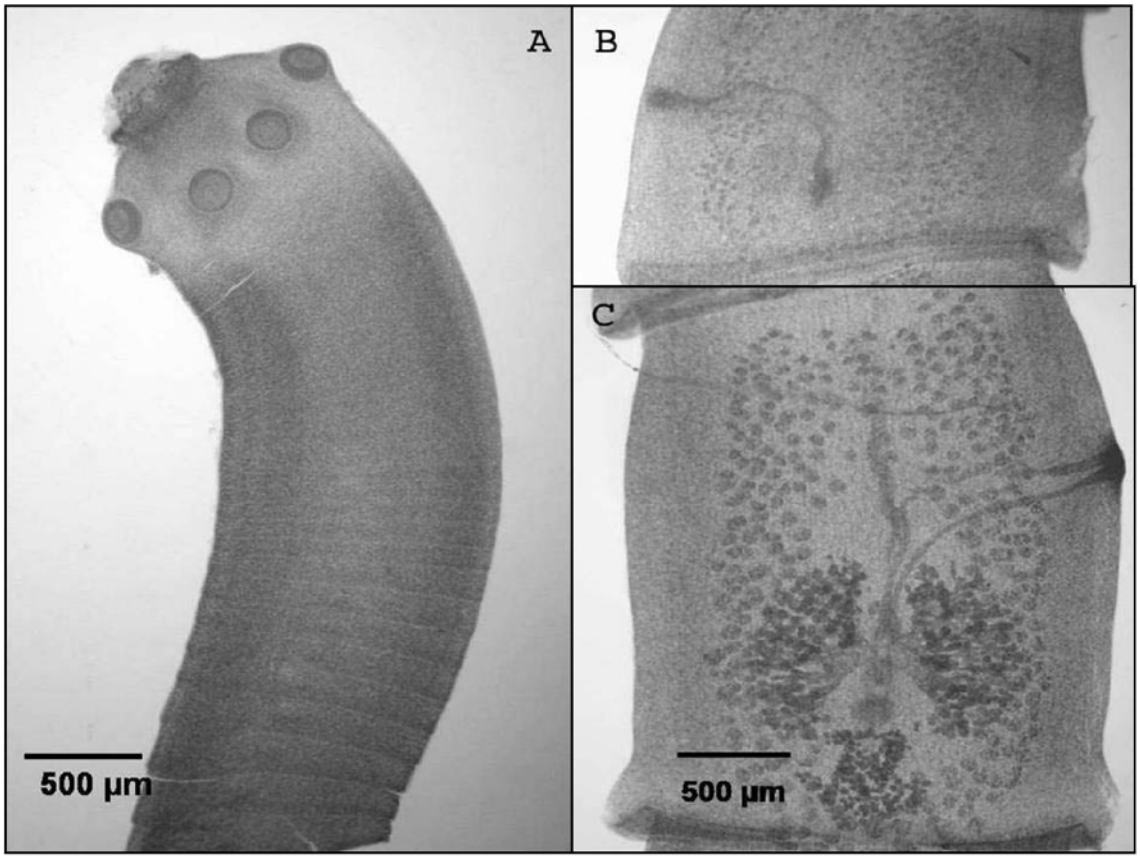

Fig. 5. *Taenia crassiceps* worm recovered from a 30 d hamster infection. (A) Scolex and initial formation of proglottids, (B) Immature proglottid, (C) Mature proglottid. Whole mounts stained with carmine red.

2005), which is then metabolized through the citric acid cycle (Tielens, 1994). However, Vinaud *et al*. (2009) have shown that *in vitro* sub-lethal doses of anthelminthic drugs which block glucose uptake cause cysticerci to use alternative energy pathways dependent on fatty acid oxidation with increased secretion of b-hydroxybutyrate. Cysticerci were also shown to secrete urea, an important metabolic pathway for the excretion of toxic substances. The existence of these pathways may increase the survival of the parasites in adverse host environments.

Transhydrogenases of NADPH/NAD in the mitochondria of cysticerci were described by Zenka and Prokopic (1988) and may be important for the survival of taeniid parasites. Mitochondrial transhydrogenase has been shown to protect *Caenorhabditis elegans* against reactive oxygen species (ROS) through the generation of reduced glutathione (GSH). Rydström (2006) has suggested that an important function of transhydrogenase is to maintain a high GSH/GSSG ratio and that this enzymatic complex may be relevant because mitochondrial ROS-mediated cell damage has been implicated in aging, apoptosis, necrosis and a number of pathological changes, including neurodegenerative diseases and cancer.

Cytoplasmic malate dehydrogenase activity has also been described in this parasite and is similar to the enzyme of other organisms (Zenka and Prokopic, 1989). Corbin *et al*. (1998) suggested that malic enzyme in cysticerci provides a direct passage of pyruvate from the cytosol to the mitochondrion.

In the absence of respiratory organs or a circulatory system and oxygen carrier proteins, supply of oxygen to tissues of cestodes depends on diffusion; in this regard, it has been suggested that an oxygen gradient is established and that, in consequence, aerobic metabolism is mainly limited to the outer layer (Arenal *et al*. 2005). Cyanide-resistant respiration in the mitochondrial fraction of *T. crassiceps* cysticerci, suggests the existence of an alternative respiratory pathway containing a cyanide-resistant terminal oxidase (Arenal *et al*. 2001). These results also indicate that mitochondria from cysticerci build up high levels of hydrogen peroxide during respiration and that respiratory complex I is the major site for H_2O_2 production. How or why these characteristics may benefit the metacestode stage of this parasite is not fully understood.

The pentose phosphate pathway (PPP) has also been found in *T. crassiceps* and it represents an alternative route for glucose oxidation in cells. The major products of its oxidative phase, NADPH and ribose 5-phosphate, are essential for cell survival in a variety of metabolic roles, e.g. lipid and nucleotide biosynthesis, as well as regulation of the redox state

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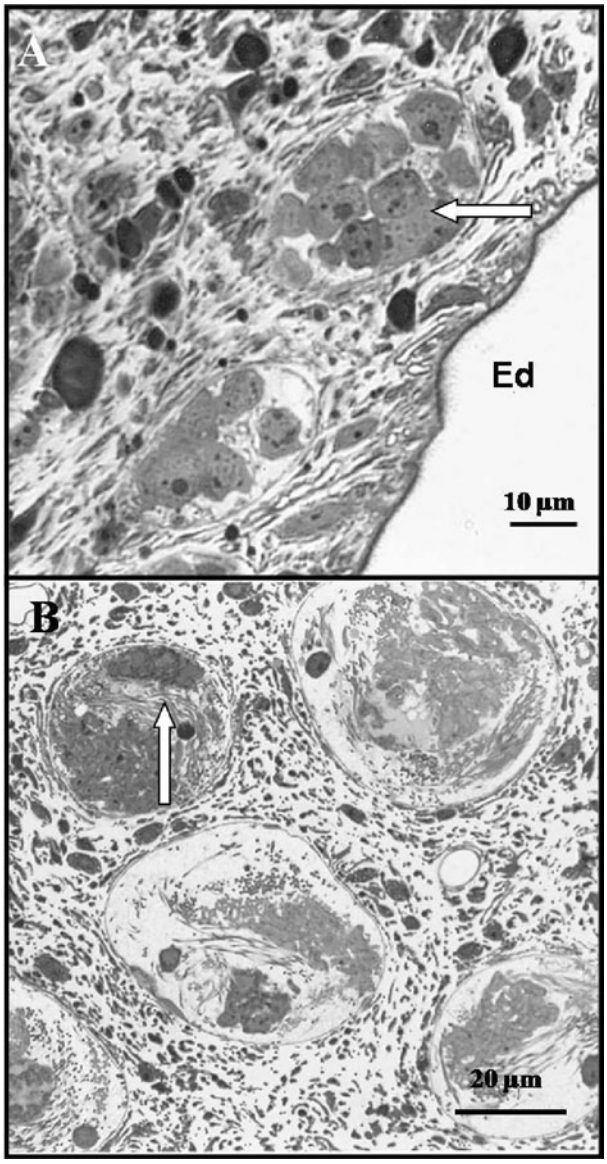

Fig. 6. (A) Light micrographs of section of an immature proglottid with testes next to excretory duct, arrow = spermatogonia. (B) Image of testes in mature proglottid. Arrow = spermatid bundles.

of the cell (Rendón *et al*. 2008). A multifunctional thioredoxin-glutathione reductase has also been described in *T. crassiceps* cysticerci. (Rendón *et al*. 2004).

STEROID METABOLISM IN HOST AND PARASITE

Early experimental results revealed that the ORF strain induces feminization in male mice of the BALB/cAnN strain – see Larralde *et al*. (1995), Morales *et al*. (1996) and Morales-Montor and Larralde (2005). They observed with ORF strain infections that oestrogens increase parasite loads and androgens decrease them, favouring or hindering parasite reproduction. This biological effect appears to be a peculiarity of the mutant ORF strain in BALB/cAnN males, since recent experimental evidence suggests that males of the BALB/cJ mouse

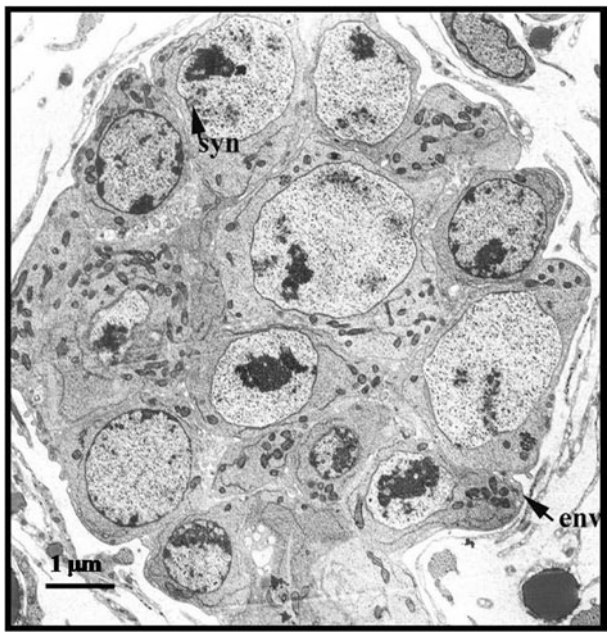

Fig. 7. Electron micrograph of *T. crassiceps* testes section from immature proglottid with developing spermatogonia. Syn = synaptolem; env = membrane surrounding testes.

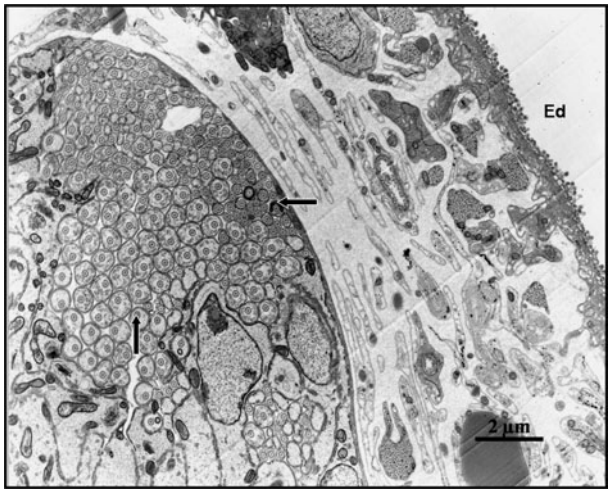

Fig. 8. Electron micrograph of *T. crassiceps* immature proglottid. Early formation of testes (arrow) on inner border of longitudinal excretory duct (Ed). Vertical arrow = maturing spermatids; horizontal arrow = testes epithelial wall.

strain do not feminize after infection with *T. crassiceps* ORF strain (Aldridge *et al*. 2007). The underlying causes for the feminization induced by the ORF strain are still poorly understood and are probably the result of complex interactions between the hormonal, immune and nervous systems (Fragoso *et al*. 2008).

In a further effort to understand the role of sex steroids in this parasite, Gómez *et al*. (2000), Romano *et al*. (2003) and Jiménez *et al*. (2006) have all investigated the capability of *T. crassiceps* ORF to produce sex steroids and found that the cysticerci

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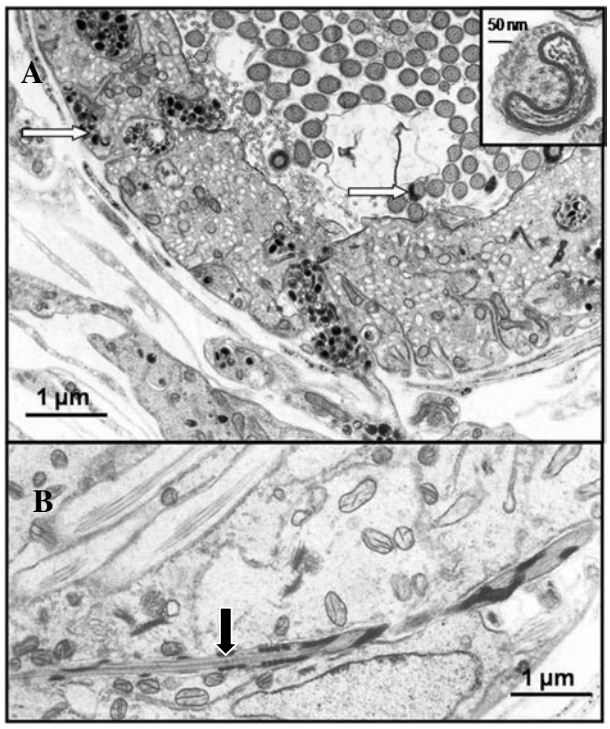

Fig. 9. *Taenia crassiceps*. Electron micrographs of (A) *Vas deferens* wall with spermatids in the lumen. (B) Longitudinal section of a whole spermatid. Insert, cross-section of a spermatid illustrating the helicoid nucleus surrounding the central axoneme.

produce testosterone from 3H-androstenedione. Recent results have shown that WFU strain tapeworms obtained in hamsters exhibited 3β-hydroxisteroid-dehydrogenase activity in the subtegumentary areas of the neck, immature proglottids and surrounding the testes in mature proglottids, following incubation with androstendiol (Fernández *et al*. 2008). The results strongly suggest that taeniid parasites have at least a part of the enzymatic chain required for androgen and oestrogen synthesis and that the enzymes are present in the larval and early strobilar stages and mature proglottids. How parasite steroids interact with steroid metabolism of the host is still not understood. Large numbers of cysticerci producing sex steroids in the peritoneal cavity may have significant effects on the steroid metabolism of the host, an aspect that remains to be explored.

EXPERIMENTAL PRODUCTION OF ADULT *T. CRASSICEPS*

In vitro *experiments*

In early studies of *T. crassiceps* infections, Esch and Smyth (1976), using the KBS and Toi strains, were able to produce segmentation of cysticerci *in vitro*, with testes and genital pores visible after 13 d in culture; however, in spite of varying culture conditions, no further development was obtained. These results suggest that the larval stage has the genetic information to differentiate but, for gonad development, requires host substances that can only be obtained by attaching to the intestinal wall of natural definitive hosts or by suppressing the immune response of experimental hosts (Sato and Kamiya, 1989; Willms *et al*. 2004).

In vivo *production of tapeworms*

The occurrence of *T. crassiceps* in naturally infected dogs that released eggs infective for field voles was reported by Rietschel (1981). Kroeze and Freeman (1982) found non-gravid tapeworms in the gut of mice fed HYG and KBS cysticerci.

Recent publications have described the growth of the adult *T. crassiceps* species in experimental definitive hosts. Sato and Kamiya (1989) and Kitaoka *et al*. (1990) were able to produce adult tapeworms in immune-suppressed hamsters after inoculation with an isolate of *T. crassiceps* cysticerci. The WFU strain has been used to infect experimental immune-suppressed hamsters and produced adult specimens (Figs 2, 3), which have been employed in ultrastructural analysis (Willms *et al*. 2004) and the identification of antigens and several enzymes shared by taeniid tapeworms (Kalinna *et al*. 1989; Larralde *et al*. 1990; Vaca-Paniagua *et al*. 2009, in press).

The experimental infection of mongrel dogs with the WFU strain larvae constitutes an important aid in the maintenance of laboratory strains. Because continuous passage of the WFU strain larvae in the peritoneal cavity of mice abrogates their ability to produce adult tapeworms in hamsters, it is necessary to inoculate dogs with mouse-derived larvae: two out of four dogs developed at least one gravid tapeworm at 30 days post infection, and the released proglottids were used to infect BALB/c mice, which developed larvae with renewed infectivity for hamsters (Zurabian *et al*. 2008). It was observed that the infectivity rate of cysticerci for hamsters was 33% during the first months after the strain was isolated, compared to a <1% infectivity found in hamsters fed cysticerci that had been re-inoculated for 10 generations in BALB/c mice. The progressive loss of infectivity in experimental models was first reported for *T. crassiceps* by Freeman (1962). The mechanisms for diminishing infectivity are not understood. The lack of reproducibility in recovering consistent numbers of strobila in the golden hamster has also been found in the production of adult experimental *T. solium* in hamsters (Willms *et al*. 2003*a*, *b*) and is probably due to a number of uncontrolled factors, such as the age of cysticerci, use of outbred hamsters, parasite strains and sex of the host, among others.

Long-term asexual proliferation may suppress or shut off the genes responsible for attaching to the intestinal wall of an experimental host such as the hamster, as long as the genes are not required for completion of the life cycle. Gene expression is possibly restored after cysticerci are released from

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the mouse peritoneum where the gene-silencing factor is functional or contact with a canid definitive host may somehow reactivate the genes responsible for attachment and differentiation. Gene silencing or shut-off (Kalinna and Brindley, 2007) is a recently developed experimental procedure in which double stranded RNA (dsRNA) molecules have been used experimentally to turn off specific parasite genes. The fact that these procedures are experimental does not exclude the possibility of their functioning *in vivo*. Gene shut-off may be an important energy saver during the continuous asexual multiplication of the larvae in the mouse peritoneal cavity, which involves the simultaneous duplication of a large number of subtegumentary cells in the budding process.

Infectivity of eggs obtained from experimental gravid worms

Sato and Kamiya (1989) reported that eggs obtained from experimental hamster tapeworms were infectious for voles and Mongolian gerbils. In the experience of the authors, the eggs obtained from gravid hamster worms infected with the WFU strain, did not infected BALB/c mice, possibly due to the extreme fragility of the egg shell. The oncosphere is easily released from the embryophore on contact with a glass slide (K. Willms, unpublished results). In contrast, gravid proglottids and eggs recovered from dog tapeworms of the WFU strain were consistently infective for BALB/c mice, possibly, because the parasite was able to reactivate the genes responsible for the production of infective eggs.

IMMUNE RESPONSE OF THE MURINE HOST

A large number of publications have dealt with the immunological response of the murine host to infections with *T. crassiceps*, many of which were designed to analyze the immune response in a laboratory model for the purpose of better understanding the immune reactions in human and swine cysticercosis. Early publications revealed that the mouse immune system destroys the peritoneal cavity larvae (Siebert *et al*. 1978). More recent evidence shows a depressed T cell proliferation in infected resistant and susceptible mice strains (Sciutto *et al*. 1995) and that during the early stages of infection, the response is of the non-permissive Th1 type, shifting to the Th2 type in the chronic stage with higher rates of parasite proliferation and the appearance of eosinophils and the cytokines IL-4, IL-6 and IL-10 produced by the host (Terrazas *et al*. 1998, 1999; Toenjes and Kuhn, 2003). This shift from Th1 to Th2 type responses has also been reported in *T. solium* neurocysticercosis (Chavarría *et al*. 2003). It is now fairly clear that shifts of the host immune response from a parasite-restrictive Th1 to a parasite-permissive Th2 response are the result of parasite modulation of the host immune response (Morales-Montor and Larralde, 2005). Of particular importance is the role of macrophages in the host response (Terrazas, 2008).

A number of studies found that, although *T. crassiceps*-infected BALB/c mice develop an initial but short-lived Th1 response, it is substituted by a strong Th2 response that is associated with an increase in the parasite load (Toenjes *et al*. 1999). It has also been demonstrated that the administration of anti-gamma interferon (IFN-γ) neutralizing antibodies to *T. crassiceps* infected mice during the early phase of infection renders them more susceptible to cysticercosis (Rodríguez-Sosa *et al*. 2004). STAT4 and STAT6 transcription factors are also involved in the modulation of the immune response to *T. crassiceps* in mice: infected STAT6$^{-/-}$ mice were shown to mount a strong Th1 response in the absence of Th2 development and were able to control the infection (Rodríguez-Sosa *et al*. 2002), and a STAT4-dependent Th1 response appears to be critical for the development of Th1 protective immunity (Rodríguez-Sosa *et al*. 2004). These observations suggest that, while Th1-type responses and IFN-γ are essential for the development of immunity against experimental cysticercosis, Th2-type responses may also play a role in the control of this parasite.

Landa *et al*. (2009) have recently shown that two peptides, Temporin A and Iseganan IB-367, produced by the innate immune response, are capable of damaging cysticerci *in vitro*, and have suggested that these findings may contribute to the design of new drugs in the prevention and treatment of these diseases.

VACCINE STUDIES

Taenia crassiceps antigens and recombinant antigens have been shown to induce high levels of protection against experimental murine *T. crassiceps* cysticercosis, an experimental model successfully used to test candidate antigens for use in vaccination against porcine *T. solium* cysticercosis. Cross-reaction of antigens between *T. solium and T. crassiceps* antigens has been clearly demonstrated in several laboratories (Larralde *et al*. 1990; Vaz *et al*. 1997; Espíndola *et al*. 2000). Vaccination with *T. solium* antigens protected mice against a challenge with *T. crassiceps*, showing that BALB/b male mice immunized with antigen extract from either *T. solium* or *T. crassiceps* were completely protected against challenge with cysticerci, whereas only partial protection was achieved in the susceptible BALB/c strain (Sciutto *et al*. 1990); swine cysticercosis has been diagnosed using indirect ELISA, by employing an heterologous antigen for *T. crassiceps* (Biondi *et al*. 1996) and various *T. crassiceps* antigen preparations have been used in the diagnosis of human neurocysticercosis (Larralde

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et al. 1990; García *et al*. 1995; García H. H. *et al*. 1997; Bueno *et al*. 2001; Espíndola *et al*. 2005).

More recently, Sciutto *et al*. (2002) and Toledo *et al*. (2001) have used recombinant antigens, the chemically synthesized KETc1 and KETc12 peptides obtained from the ORF strain, to immunize mice and found that immunization with KETc1 induced 66·7 to 100% protection against murine cysticercosis, and immunization with KETc12 induced 52·7 to 88·1% protection. The elicited immune response indicated that both peptides contain at least one B cell epitope (as demonstrated by their ability to induce specific antibodies) and one T cell epitope that strongly stimulated the proliferation of T cells primed with either the free peptide or total cysticercal *T. crassiceps* antigens. The high percentage of spleen cells expressing inflammatory cytokines points to the likelihood of a Th1 response being involved in protection. The protective capacity of the peptides and their presence in larval and adult stages of *T. solium* indicate that these two epitopes are strong candidates for use in a synthetic vaccine against *T. solium* pig cysticercosis.

CONCLUSIONS

Experimental models have demonstrated that the genetic background of the intermediate host is important for the establishment of the larval stage. Host specificity for the establishment of tapeworms in carnivores is also underlined by the necessity of suppressing the immune response to produce tapeworms in experimental definitive hosts such as the hamster. *T. crassiceps* larvae have also been useful in examining the role of enzymes involved in respiration, glucose oxidation, steroid metabolism, as well as the design of diagnostic tests for porcine and human cysticercosis, which take advantage of taeniids that share a number of antigenic epitopes. It can be concluded that both *in vitro* and *in vivo* models have been extremely useful in the characterization of the host-parasite relationship in *T. crassiceps* infections, and that experimental designs using these models can contribute much to further our understanding of diseases caused by taeniid parasites.

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