Research Article

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Evagination of metacestodes of the WFU strain of *Taenia crassiceps* and evaluation of the impact of immune suppression of hamsters during tapeworm development

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Abstract: Taeniosis-cysticercosis caused by *Taenia crassiceps* (Zeder, 1800) is a useful experimental model for biomedical research, in substitution of *Taenia solium* Linnaeus, 1758, studied during decades to develop effective vaccination, novel anti-helminthic drugs and diagnostic tools. Cysticercosis in mouse (*Mus musculus* Linnaeus) is achieved by the larval subculturing of the Wake Forest University (WFU) strain of *T. crassiceps*. Golden hamster, *Mesocricetus auratus* (Waterhouse), has been shown to be the most suitable host for adult forms of parasite in experimental taeniosis. Metacestodes of *T. crassiceps* WFU multiply by budding without restrictions once inoculated into the mouse, while the number of tapeworms developed from these larvae in hamsters remains highly variable. Three objectives have been proposed to improve the infection of *T. crassiceps* WFU in hamsters: (1) to re-evaluate the need of immune suppression; (2) to investigate the advantage of infecting hamsters with metacestodes with *in vitro* protruded scolices; and (3) to compare a number of tapeworms developed from metacestodes subcultured in hamsters against those proliferated in mice. Our results demonstrated that when the evagination of murine metacestodes was high, the number of *T. crassiceps* WFU adults obtained from hamsters was also high. Immunosuppressive treatment remains relevant for this experimental rodent model. The hamster-to-hamster cysticercosis-taeniosis by *T. crassiceps* overcame the mouse-to-hamster model in the yield of adult specimens. *In vitro* scolex evagination and metacestode asexual proliferation in hamsters place this rodent model by *T. crassiceps* WFU as the most affordable experimental models with taeniids.

Keywords: Parasite, Taeniidae, larvae, tapeworms, rodent cysticercosis-taeniosis

Taenia crassiceps (Zeder, 1800) is a taeniid cestode parasitising wildlife vertebrates, with three development stages: eggs, metacestodes and adults. Metacestodes or cysticerci are found in natural infections in rodents (Freeman 1962), but also in accidentally infected humans (Lescano and Zunt 2013). Two main laboratory strains of *T. crassiceps* are ORF metacestodes that have lost the scolices and the Wake Forrest University (WFU) strain, with an apparently normal larval phenotype (Everhart et al. 2004). Similar to the ORF, the WFU strain can multiply asexually in the peritoneum of mice (*Mus musculus* Linnaeus), but unlike the ORF strain it grows to the adult stage in hamsters as definitive hosts (Willms and Zurabian 2010).

Verster (1971) was the first to investigate immune suppression of unnatural definitive hosts, infected with taeniid parasites. In order to improve the yield of adult worms and

to obtain sexually mature specimens with infective eggs, Sato and Kamiya (1989) treated hamsters infected with *T. crassiceps* with corticosteroids. The average recovery of *T. crassiceps* from immunosuppressed hamsters varied between 11.7 and 76.7% (Sato et al. 1993, Zurabian et al. 2008). However, only Kitaoka et al. (1990) found eggs of *T. crassiceps* expelled in the faeces of hamsters, with and without steroid treatment.

Steroids were also used in the experimental taeniosis with *Taenia solium* Linnaeus, 1758 or *Taenia pisiformis* (Bloch, 1780) (Allan et al. 1991, Maravilla et al. 1998). The definitive hosts were treated with $\geq 5\text{mg/kg}$ of glucocorticoids, in a single or multiple applications, resulting in a highly variable number of recovered tapeworms with different degree of sexual maturity (Sato and Kamiya 1989, 1990, Kitaoka et al. 1990, Sato et al. 1994, Avila et

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Fig. 1. *Taenia crassiceps* (Zeder, 1800); metacystodes and adults of the WFU strain. **A** – heterogeneous population of metacystodes with different degree of development obtained from mouse peritoneum; **B** – larvae with elongated scolex after *in vitro* incubation; **C** – adult tapeworms localised in the small intestine of infected hamster and detached scolex (arrow).

al. 2006), and also inducing a cachexic state in the animals (Braun et al. 2014, Archer-Lahlou et al. 2018).

The present study was pursuing three objectives related to the experimental taeniosis: (1) to re-evaluate the importance of immune suppression in order to avoid cachexic state of the hosts; (2) to investigate the advantage of infecting hamsters with metacystodes with *in vitro* protruded scolices; and (3) to evaluate efficiency of model and infectivity of metacystodes proliferated either in mice or hamsters as intermediate hosts.

MATERIALS AND METHODS

Proliferation of metacystodes of *Taenia crassiceps* in rodent hosts

Metacystodes (Cs) of the WFU strain of *T. crassiceps* were isolated following Everhart et al. (2004). Parasites were maintained in mice by intraperitoneal (i.p.) passages as described previously (Zurabian et al. 2013). Female four weeks old BALB/c mice ($n = 9$) and female five months old golden hamsters ($n = 2$) each were inoculated with ten Cs. In total, nine consecutive in-frapopulations were obtained from mice (CsM) and two in-frapopulations from hamster's peritoneum (CsH).

Animals were supplied with commercial pellets and water *ad libitum* and kept under controlled conditions. Necropsies consisted of cervical dislocation of mice or injection of 400 mg/kg of sodium pentobarbital (Pisabental, Pisa Agropecuaria, Hidalgo, Mexico) in hamsters, as established by Mexican Official Regulation (NOM-062-ZOO-1999). The Ethical Committee of Faculty of Medicine (UNAM) had approved the experimental assays.

Criteria for selection of metacystodes and *in vitro* evagination

For *in vitro* culturing, 50–70 CsM or CsH with 3–4 mm large bladder and invaginated worms approximately 1 mm long were selected and evaluated for the contractile movements under the light microscopy. Cysticerci were washed in PBS and incubated in 10 mM glucose in RPMI 1640 medium (Sigma-Aldrich Co., St. Louis, Missouri, USA) during 1.5 h at 37°C and 5% CO₂. After incubation, evaginated Cs were counted, randomly collected and fed to the hamsters (Fig. 1B). Evagination of Cs was expressed in %. Only parasites with scolices irreversibly protruded out of the bladder were counted and grouped as 1–50% or 51–100%. Metacystodes with inverted scolex were selected from the same in-frapopulation upon necropsies and used as a control group (0% of evagination).

Adults of *Taenia crassiceps* in hamsters

Female 5–6 months old hamsters were treated with 20 mg of metronidazole (Degort's Chemical, Ciudad de México, México) per kg of weight given each day for three days. Albendazole (Zentel, Glaxo Smith Kline, Ciudad de México, México) was given in a dose of 30 mg per kg of hamster's weight per day during five days. Hamsters were divided into two groups for the infection assays: animals in the group 1 had immunosuppressive treatment (ISP) with 2 mg/kg of methyl prednisolone acetate (Upjohn, Ciudad de México, Mexico) at the same day of infection, and group 2 were animals without steroid treatment (nISP).

ISP ($n = 62$) and nISP ($n = 37$) hamsters were fed with CsM, and submitted to the evagination assay. All CsM used for assays were part of nine mouse in-frapopulations studied during five-year-peri-

Table 1. Overall distribution of the WFU strain of *Taenia crassiceps* (Zeder, 1800) developed from metacestodes proliferated in mice (CsM) according to the evagination rate. CsM fed to the hamsters with (ISP) and without (nISP) steroid treatment. KWT, Kruskal-Wallis test; FET, Fisher's Exact test.

% of evaginated CsM fed to the hamsters (n = 99)	Median of tapeworms (p25, p75)		KWT <i>p</i> value	Infection by at least 1 tapeworm				FET <i>p</i> value
	nISP n = 37	ISP n = 62		nISP n = 37		ISP n = 62		
				Negative = 33	Positive = 4	Negative = 18	Positive = 44	
0 (25)	0 (0, 0)	0.5 (0, 4)	0.0516	9 (27)	2 (50)	7 (39)	7 (16)	0.208
1–50 (24)	0 (0, 0) ^a	1 (0, 2)	0.0171	10 (30)	1 (25)	6 (33)	7 (16)	0.033
51–100 (50)	0 (0, 0) ^b	3 (1, 4)	0.0001	14 (42)	1 (25)	5 (28)	30 (68)	<0.001

Table 2. Overall distribution of the WFU strain of *Taenia crassiceps* (Zeder, 1800) developed from metacestodes proliferated in hamster (CsH) according to the evagination rate. CsH were fed to the non-treated with steroids hosts. KWT, Kruskal-Wallis test; FET, Fisher's Exact test.

% of evaginated CsH fed to the hamsters (n = 12)	Median of tapeworms (p25, p75)	KWT p value	Infection by at least 1 tapeworm		FET p value
			Negative n = 5	Positive n = 7	
0 (4)	3.5 (2.5, 4)		0 (0)	4 (57)	
1–50 (4)	2 (0.5, 4.5)	0.033	1 (20)	3 (43)	0.030
51–100 (4)	0 (0, 0)		4 (80)	0 (0)	

od. Twelve nISP hamsters were fed with evaginated CsH selected from two infrapopulations reared in the hamster's peritoneum. In infections with CsM, each experimental group of hamsters consisted of five to seven animals, and CsH were used to infect groups of four animals.

The infectivity was calculated using the next formula: (number of hosts infected with parasites $\times$ 100) / total number of hosts. The efficiency of infections was calculated using the following equation: (number of tapeworms $\times$ 100) / (number of hosts $\times$ 10 metacestodes).

Adults of *T. crassiceps* WFU specimens were obtained from the hamsters' gut after 1–2 weeks of growth, counted and measured. The largest tapeworm was fixed and stained following Paracarmin-Mayer method (Lamothe-Argumedo 1997) in order to evaluate the parasite's stage of development.

Statistical analysis

Two statistical tests were used to analyse the data related to the experimental taeniosis. The Kruskal-Wallis test (KWT) was used to evaluate the number of tapeworms obtained from each group of hamsters, and the Fisher Exact test (FET) was used to analyse the difference between the frequencies of hosts harbouring at least one tapeworm. The results obtained after infection of ISP or nISP hamsters with CsM or CsH with different evagination percentages (0, 1–50 or 51–100%) were compared using KWT or FET. Significant differences were considered when $p < 0.05$.

RESULTS

Evagination of metacestodes of the WFU strain of *Taenia crassiceps*

Each CsM or CsH infrapopulation remained in the rodent peritoneum for 5–6 months and comprised Cs with different degree of development (Fig. 1A). Selected for the *in vitro* assay CsM or CsH had apparently similar phenotypes and size, but displayed different evagination rates during incubation.

Adults of *Taenia crassiceps* WFU developed from CsM

Hamsters developed a significantly higher number of adults when infected with CsM from the 51–100% evagination group, compared to those with a lower evagination number or control ($p = 0.026$, KWT; data not shown in tables). Similarly, when greater evagination was achieved, the greater median was obtained for tapeworms from ISP hamsters (Table 1). More hamsters lodged at least one adult specimen when fed with CsM with 51–100% of evagination ($p = 0.024$, FET; data not in tables); the highest frequency of infection was also seen in ISP hosts (68%, $p < 0.001$, FET; Table 1).

Adults of *Taenia crassiceps* WFU developed from CsH

Control metacestodes (without evagination) produced a major number of adults per host ($p = 0.033$, KWT; Table 2). All hamsters fed with CsH without evagination harboured at least one adult specimen.

Taenia crassiceps WFU tapeworms in hamsters

Infectivity and efficiency was calculated for total populations of hamsters ($n = 111$) infected (during five-years period) either with CsM or CsH (Table 3). Tapeworms were found in 58% and 49% of hamsters infected with CsH or CsM, respectively. Infectivity of CsH (19%) was higher than CsM (15%) as shown in Table 3.

The number of tapeworms per host varied between one and nine, and 50% of the total number of hamsters used in the study had at least one intestinal tapeworm upon necropsy. The highest number of *T. crassiceps* WFU tapeworms was found attached to the intestinal wall of ISP hamsters. The largest tapeworms were also found in ISP hamsters, and measured about 15 cm in unrelaxed state (Fig. 1C). Staining with Mayer's paracarmin revealed pregravid proglottids and eggs in the process of formation.

Table 3. Efficiency of taeniosis model and infectivity of *Taenia crassiceps* WFU metacestodes. CsM, cysticerci proliferated in mice; CsH, cysticerci proliferated in hamsters. Efficiency = (number of hosts infected with parasites × 100) / total number of hosts. Infectivity = (number of tapeworms × 100) / (number of hosts × 10 metacestodes).

No. of the hamsters fed with metacestodes	Tapeworms (n)	Efficiency (%)	Infectivity (%)
CsM n = 99	149	49	15
CsH n = 12	23	58	19

DISCUSSION

Metacestodes of the WFU strain of *Taenia crassiceps* used in this study descended from i.p. passages of mice maintained during almost one decade; data for this study were collected from nine consecutive Cs infestations. As previously shown, this parasite loses the infectivity in experimental definitive hosts (hamsters) after long periods of asexual sub-culturing in mice, but it can be reactivated after parasite's natural life cycle (Zurabian et al. 2008).

Our results revealed that CsM with high evagination rates were able to continue with further development and, the number of tapeworms developed from these metacestodes, was significantly higher than the number of adults from its non-evaginated counterparts. We assume that because of the extra glucose administration, activated scolices might have an enhanced ability to anchor to the intestinal wall of the host. The cysticerci exposed to high glucose concentration might have an improved metabolism that, besides other developmental effects, induced a faster growth or differentiation of the hooks.

The presence of a sodium-dependent glucose transporter has been shown in larval and adult *Taenia solium* (see Cornford et al. 2001) and the parasite expresses enzymes of the pentose cycle (Rendón et al. 2008). It is well known that treatment of cysticerci with cysticide albendazole selectively inhibits the uptake of glucose, leading to glycogen storage depletion in the parasite (Vinaud et al. 2008).

We found that hamsters infected with evaginated WFU murine cysticerci (CsM) and a single dose of methyl prednisolone acetate developed a significantly higher number of worms, confirming that the immune suppression is important for the tapeworm development in the host. Therefore, our efforts to avoid immune suppression were not successful in mouse-to-hamster infections. Better results were obtained when asexually proliferated CsH were fed to hamsters non-treated with steroids, ending up in the taeniosis model with higher efficiency. Hence, the growth of

cysticerci in hamsters permits to exclude the host immunosuppression.

Similar to reports of experimental taeniosis caused by *Taenia pisiformis* or *T. solium* in immunosuppressed hamsters (Maravilla et al. 1998, Toral-Bastida et al. 2011, Domínguez-Roldán et al. 2016), we observed that almost half of the studied hamsters became infected with *T. crassiceps* WFU. It should be pointed out that cysticerci used in these studies were resected from their natural hosts (rabbit and pig), had a larger scolex and fixative structures when compared to the smaller ones in WFU larvae (Beveridge and Pickard 1976, Loos-Frank 2000). Efficiency of our model can be considered high enough, despite of the reduced infectivity over years of asexual proliferation (Zurabian et al. 2008), and the presence of morphological aberrations related to the hooks or suckers (Aguilar-Vega et al. 2016).

Regarding infectivity, compared to the above mentioned studies that gave 3–6 taeniid larvae to each definitive host, we administered ten WFU metacestodes, a strategy that prevented small worms from being swept away by peristalsis (Freeman et al. 2011, Pospekhova and Bondarenko 2014), and promoting a high load of helminths in the small intestine of the host. Upon necropsy, we did not search for detached complete tapeworms or gravid proglottids in the hamsters' faeces, and counted only those embedded in the intestinal mucosa.

For the first time, *T. crassiceps* WFU metacestodes were inoculated into the peritoneum of hamsters (CsH) and, after asexual multiplication, were evaginated and orally fed to another hamster. Only non-steroid treated hamsters were used for the assay, and the results suggest that better yields concerning the number of tapeworms were achieved when infection was done using non-evaginated metacestodes. As contradictory as it may seem, the greater the evagination of CsM, there was a greater infectivity.

In summary, a higher infectivity and efficiency were achieved in the new experimental model of taeniosis produced by *T. crassiceps* WFU cysticerci proliferated in hamsters compared to the mouse-based taeniosis model. The hamster-to-hamster model avoids the immune suppression and additional cysticerci manipulations before being administered to the definitive host. More studies should be done to set hamsters as the best hosts for larval and adult stages of *T. crassiceps* WFU.

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