

LABORATORY RODENT MODELS FOR THE TAPEWORM-STAGE OF *TAENIA SAGINATA* AND OTHER RELATED TAENIID SPECIES

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Abstract. Attempts were carried out to establish alternative definitive host systems for *Taenia saginata*, *T. crassiceps*, *T. hydatigena*, *Echinococcus multilocularis* and *E. granulosus*, in rodents. Following oral inoculation of cysticerci or protoscoleces, the tapeworm-stage development and sexual maturation of the 5 taeniid species in the intestine of a golden hamster (GH) and Mongolian gerbil (MG), both treated with prednisolone, were examined. Sexually mature *T. saginata* tapeworms were recovered from MG pretreated with prednisolone. The life cycles of *E. multilocularis* can be completed in MG and *T. crassiceps* in both MG and GH. *T. hydatigena* and *E. granulosus* developed to a certain extent at the tapeworm stage in MG pretreated with prednisolone. Potential use of rodents as an alternative definitive host for taeniid species is discussed.

INTRODUCTION

Taeniid cestodes cause parasitic diseases in livestock and humans, thus presenting public health as well as socio-economic problems (Schwabe, 1986; Lawson *et al.*, 1986). The life cycle of taeniid species generally depends upon the prey (intermediate host: herbivores)-predator (definitive host: carnivores) relationship. Most of the research on these taeniid species were done at the intermediate host level. Although it is generally agreed that more work needs to be done at the definitive host level, progress made was comparatively very slow due to the involvement of handling taeniid eggs, some of which are biohazardous.

Taeniid species show a high specificity for its definitive host. Besides humans and carnivores, which are the natural definitive hosts, attempts to experimentally create alternative definitive hosts had been reported, but none was able to obtain eggs from the tapeworms (Gnezdilov, 1957; Kovalenko, 1976; Verster, 1971, 1974). Investigations involving *Echinococcus* spp. (*E. granulosus* and *E. multilocularis*) infection in its natural definitive host, such as the dog, requires the use of expensive biohazard facilities and even that does not guar-

antee the safety of the researcher from accidental infection. In order to reduce the health hazard, it was noted that development of an *in vitro* culture system for *Echinococcus* adult tapeworm is needed (Smyth and Howkins, 1966). Although the *in vitro* system did provide much insight into the biology of *Echinococcus*, the tapeworm failed to become gravid and no eggs were obtained. Since no eggs were involved, the *in vitro* system is advantageous because there is no biohazard problem.

In order to obtain the eggs of *Taenia solium* and *T. saginata*, many attempts were made to establish an alternative experimental definitive host. Despite the observation that these tapeworms grew and developed to a certain extent, none grew to the gravid stage and, thus, no eggs were obtained.

Our research group also attempted to establish alternative definitive hosts for the taeniids species. We have experimented using *T. saginata*, *T. crassiceps*, *T. hydatigena*, *Echinococcus granulosus* and *E. multilocularis*. This article is an overview of the results obtained to date and also discusses the usefulness and limitations of the various alternative definitive host model systems. With the exception of *T. crassiceps* and *E. multilocularis*, the detailed

results for the other taeniid species mentioned above will be published elsewhere.

MATERIALS AND METHODS

Metacestodes of *T. hydatigena*, *T. saginata* and *E. granulosus* were collected from the omentum of sheep, tongue and lung of cattle, respectively, at Dagoretti abattoir, near Nairobi, Kenya. Those of *E. multilocularis* and *T. crassiceps* were obtained from Mongolian gerbils, which were used to passage the parasites in our laboratory, as described respectively by Kamiya and Sato (1990a; b) and Kitaoka *et al.* (1990). In experiments using *T. saginata*, *T. hydatigena* and *E. granulosus*, animal hosts and their treatments are shown in Table 1.

RESULTS

T. saginata: Three of group B and 2 of group C golden hamsters were each fed with 4 cysticerci, and only one tapeworm was recovered from a hamster of group B sacrificed on day 12 post-

inoculation(PI). Three Mongolian gerbils of group G were fed with 2 cysticerci, and sacrificed on days 2, 23 and 63 PI. From all of the gerbils, one tapeworm each was recovered from their intestine. Genital primordia were observed in the posterior segments of the 150 segments of the 23-day old worm. A sexually mature tapeworm having 348 proglottids was recovered on day 63 PI (Fig 1; Table 2).

T. hydatigena: Two and 3 golden hamsters of groups A and C, respectively, and 4 Mongolian gerbils of group E were each fed with 4 cysticerci, and 2 puppies with 6 cysticerci each. All of the hamsters were sacrificed on day 12 PI, and each gerbil on days 4, 12, 36 and 42 PI. No worms were recovered from the hamsters. In contrast, 2 worms each on days 4 and 12 PI, and 1 worm each on days 36 and 42 PI were recovered from the gerbils. The latter two worms had 70 and 98 proglottids, respectively, and also showed incipient genital development (Fig 2). In puppies, gravid segments began to appear in the feces on days 47 and 49 PI, respectively. Five gravid worms were recovered from each of the puppies sacrificed on day 90 PI (Table 3).

Table 1
Host groups and their treatment.

| Host group (age) | Day of PTBA-treatment (postinfection) | |
|--|---------------------------------------|---|
| Golden hamster (<i>Mesocricetus auratus</i>) | | |
| -2wkPGH (6wk) | -15, -13, -5, | 0, 3, 6, 9, 12, ... <every 4d>... |
| -1wkPGH (6wk) | | -5, -2, 0, 3, 6, 9, 12, ... <every 4d>... |
| PGH (6wk) | | 0, 3, 6, 9, 12, ... <every 4d>... |
| NGH (6wk) | not treated | |
| Mongolian gerbil (<i>Meriones unguiculatus</i>) | | |
| -3wkPMG (13wk) | -22, -18, -14, -10, -6, -2, | 2, 6, 9, 11, ... <every 4d>... |
| PMG (6wk) | | 0, 3, 6, 9, 12, ... <every 4d>... |
| PMG (3wk) | | 0, 3, 6, 9, 12, ... <every 4d>... |
| NMG (13wk) | not treated | |
| NMG (6wk) | not treated | |
| NMG (3wk) | not treated | |

PTBA: Prednisolone tertiary butylacetate (Codelcortone[®], TBA, Merck and Co, Inc)

PGH: Prednisolone treated golden hamsters; NGH: Normal golden hamsters

PMG: Prednisolone treated Mongolian gerbils; NMG: Normal Mongolian gerbils

Dose of PTBA / injection; Golden hamster: 5 mg

Mongolian gerbil: 4 mg (13 and 6wks), 3 mg (3wk)

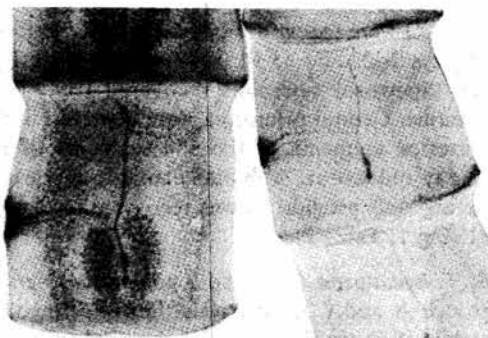


Fig 1—*T. saginata* from a prednisolone-treated Mongolian gerbil on day 63 PI.

E. granulosus: Seven or 8 animals each from groups A to E and 4 animals each from groups F and H (Table 1) were orally administered 5,000 protoscoleces of *E. granulosus*. On day 1 PI, 1 each of groups A to D were sacrificed, and a few worms was recovered from hamsters of groups A to C. Then some hamsters of groups B to D and G were reinfected with 20,000 protoscoleces on day 12 PI. The rest of the animals in groups A to D were sacrificed on day 18 and 21 PI. Worms were recovered only from 2 hamsters of group B sacrificed on day 21 PI (Table 4). None of them were segmented and, therefore, were determined to be worms from the secondary infection. Mongolian gerbils were killed on days 18, 28 and 36 PI, and some worms were recovered only from animals of groups E and G. The worms developed to stage II or III as designated by Smyth *et al* (1967) (Fig 3). Four puppies were orally administered 20,000

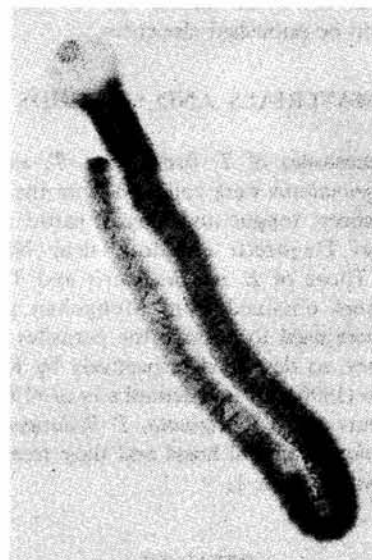


Fig 2—*T. hydatigena* from a prednisolone-treated Mongolian gerbil on day 36 PI.

protoscoleces. In a puppy killed on day 18 PI, 18.3% of the parasites had grown to adults, and in the other 3 puppies killed on day 36 PI, the recovery ratios were 6.5, 13.3 and 24.4%.

E. multilocularis and *T. crassiceps*: Results regarding these two parasites had been published (Kamiya and Sato, 1990a,b; Kitaoka *et al*, 1990; Sato and Kamiya, 1989a,b). Briefly, the life cycle of *E. multilocularis* can be completed in the Mongolian gerbil and *T. crassiceps* in both Mongolian gerbil and golden hamster.

Table 2

Worm recovery of *Taenia saginata* from each host group
[Number of positive host/number examined (number of worms recovered)].

| Host group (Age) | Number of metacestodes infected | Day of autopsy* | | | |
|---------------------|---------------------------------|-----------------|--------|--------|--------|
| | | 2d | 12d | 23d | 63d |
| Golden hamster | | | | | |
| -1wkPGH (6wk) | 4 each | - | 1/3(1) | - | - |
| PGH (6wk) | 4 each | - | 0/2 | - | - |
| Mongolian gerbil | | | | | |
| PMG (3wk) | 2 each | 1/1(1) | - | 1/1(1) | 1/1(1) |

*Days post-infection

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Table 3

Worm recovery of *Taenia hydatigena* from each host group
[Number of positive host/number examined (number of worms recovered)].

| Host group (Age) | Number of metacestodes infected | Day of autopsy* | | | | |
|---------------------|---------------------------------------|-----------------|--------|--------|--------|----------|
| | | 4d | 12d | 36d | 42d | 90d |
| -2wkPGH.(6wk) | 4 each | - | 0/2 | - | - | - |
| PGH (6wk) | 4 each | - | 0/3 | - | - | - |
| -3wkPMG (13wk) | 4 each | 1/1(2) | 1/1(2) | 1/1(1) | 1/1(1) | - |
| Dog (2mo) | 6 each | - | - | - | - | 2/2(5,5) |

* Days postinfection

Table 4

Worm recovery of *Echinococcus granulosus* from each host group
[Number of positive host/number examined (number of worms recovered)].

| Host group | Day of autopsy ¹⁾ | | | | | |
|------------------|------------------------------|-----------|------------------------|------------------------|---------|---|
| | 1d | 18d | 18d + 6d ²⁾ | 21d + 9d ²⁾ | 28d | 28d + 16d ²⁾ 36d + 24d ²⁾ |
| Golden hamster | | | | | | |
| -2wkPGH (6wk) | 1/1(2) | 0/2 | | 0/4 | | |
| -1wkPGH (6wk) | 1/1(4) | | 0/2 | 2/4(347, 5) | | |
| PGH (6wk) | 1/1(8) | | 0/2 | 0/5 | | |
| NGH (6wk) | 0/1 | | 0/2 | 0/5 | | |
| Mongolian gerbil | | | | | | |
| -3wkPMG (13wks) | | 2/2(4, 3) | | | 1/3(62) | 0/3 |
| PMG (6wks) | | 0/4 | | | | |
| PMG (3wks) | | | 2/2(296, 3) | | 1/2(8) | 1/3(56) |
| NMG (13wks) | | 0/4 | | | | |
| NMG (6wks) | | 0/1 | | | | |
| NMG (3wks) | | | 0/2 | | | |

1) Days post-infection

2) [Days after first infection] + [days after second infection]

Recovery rate of worms from dogs administered orally with 20,000 protoscoleces : 18d - 18.3%; 36d - 6.5%, 13.3%, 24.4%

DISCUSSION

Strobilar development, to a certain extent, of taeniid species occurs not only in the intestine of natural definitive hosts, such as canids and humans, but also in laboratory rodents. This was first shown by Gnezdilov (1957) when he observed

strobilation and development of genital primordia in the posterior proglottids of *T. solium* recovered from the intestine of a golden hamster following the oral inoculation of cysticerci. Verster (1971; 1974) found that the susceptibility of the golden hamster to *T. solium* can be increased by treating them with antilymphocytic serum or chemical



Fig 3—*E. granulosis* from a prednisolone-treated Mongolian gerbil on day 18 PI.

immunosuppressants, but could not reproduce the same effect for *T. saginata*. She succeeded in obtaining sexually mature but not gravid *T. solium* tapeworms by this method. In addition to the two taeniid species, incipient strobilar development in the intestine of rodents has been described for the following species; *E. granulosis* and *E. multilocularis* in ground squirrels, *Citellus citellus* (Movsesijan *et al*, 1967; Zukovic *et al*, 1975), mice, cotton rats, Dzungarian hamsters and golden hamsters (Kovalenko, 1976); *T. crassiceps* in mice (Kroeze and Freeman, 1982; 1983) and *T. hydatigena* in golden hamsters (Verma and Ahluwalia, 1987).

With reference to our present study, *E. multilocularis* and *T. crassiceps* developed to sexual maturity in the intestine of the golden hamster, and also in the prednisolone-treated golden hamster, as well as the Mongolian gerbil. We have shown that these two parasites can complete their life-cycles in laboratory rodents alone. We also found that the Mongolian gerbils show remarkable resistance to opportunistic microbial infection even when treated with high doses of prednisolone.

Considering our results with oral administration of taeniid metacestodes, the present rodent models can rejuvenate research into the tapeworm

stages and stimulate various lines of investigation, such as immunoexpulsion against adult worms, drug screening, strain differentiation and even preclude the use of natural definitive hosts, including dogs, cats and possibly human volunteers in future experiments.

REFERENCES

- Gnezdilov VG. The golden hamster (*Mesocricetus auratus* Waterhouse) as a potential definitive host of the tapeworm *Taenia solium*. *Zool Zh* 1957; 36 : 1770-3 (in Rus).
- Kamiya M, Sato H. Survival, strobilation and sexual maturation of *Echinococcus multilocularis* in the small intestine of golden hamsters. *Parasitology* 1990a; 100 : 125-30.
- Kamiya M, Sato H. Complete life cycle of the canid tapeworm, *Echinococcus multilocularis*, in laboratory rodents. *FASEB J* 1990b (In press).
- Kitaoka M, Oku Y, Okamoto M, Kamiya M. Development and sexual maturation of *Taenia crassiceps* (Cestoda) in the golden hamster. *J Parasitol* 1990; 76 : 399-402.
- Kroeze WK, Freeman RS. *Taenia crassiceps*: fate of cysticerci following ingestion by mouse. *Exp Parasitol* 1982; 54 : 425-31.
- Kroeze WK, Freeman RS. Growth and development of *Taenia crassiceps* (Cestoda) in the small intestine and peritoneal cavity of mice following oral infection. *Can J Zool* 1983; 61 : 1598-604.
- Kovalenko FP. Survival of *Echinococcus multilocularis* and *E. granulosis* protoscoleces in the intestine of laboratory rodents. *Med Parazitol Parazit Bolezni* 1976; 45 : 350-2 (in Rus).
- Lawson JR, Roberts MG, Gemmell MA, Best SJ. Benefit/cost analysis of options for the control and eradication of hydatosis and cysticercosis. MAF Wallaceville, 1986; 162pp.
- Movsesijan M, Sokolic A, Mladenovic Z. Preservation and irradiation of *Echinococcus granulosis* forms for immunological studies. *Veterinarski Arhiv Zagreb* 1967; 37 : 384-91.
- Sato H, Kamiya M. Viable egg production of *Taenia crassiceps* developed in the intestine of prednisolone-treated golden hamster. *Jpn J Parasitol* 1989a; 38 : 46-53.
- Sato H, Kamiya M. Deleterious effect of prednisolone on the attachment of *Taenia crassiceps* cysticerci to the intestine of gerbils. *Jpn J Vet Sci* 1989b; 51 : 1099-101.

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- Schwabe CW. Current status of hydatid disease; a zoonosis of increasing importance. In: Thompson RCA, ed. *The Biology of Echinococcus and hydatid disease*. George Allen and Unwin, 1986: 81-113.
- Smyth JD, Howkins AB. An *in vitro* technique for the production of eggs of *Echinococcus granulosus* by maturation of partly developed strobila. *Parasitology* 1966; 56 : 763-6.
- Smyth JD, Miller HJ, Howkins AB. Further analysis of factors controlling strobilization, differentiation, and maturation of *Echinococcus granulosus in vitro*. *Exp Parasitol* 1967; 21 : 31-41.
- Verma TK, Ahluwalia SS. Some observations on the development of *Taenia hydatigena* in pups and laboratory animals. *Indian J Anim Sci* 1987; 57 : 804-11.
- Verster A. Preliminary report on the golden hamster as a definitive host of *Taenia solium* Linnaeus, 1758 and *Taenia saginata* Goeze, 1782. *Onderstepoort J Vet Res* 1971; 38 : 63-4.
- Verster A. The golden hamster as a definitive host of *Taenia solium* and *Taenia saginata*. *Onderstepoort J Vet Res* 1974; 41 : 23-8.
- Zukovic M, Wikerhauser T, Dzakula N, Yelewera G. On the development of *Echinococcus granulosus* in the ground squirrel (*Citellus citellus*). *Acta Parasitol Jugosl* 1975; 6 : 67-71.
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