



Production of *Hymenolepis diminuta* in the Laboratory: An Old Research Tool with New Clinical Applications

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Abstract

Hymenolepis diminuta, the rat tapeworm, was first described in 1819 by Rudolphi and was studied extensively in several laboratories during the mid to latter part of the twentieth century. More recently, the primary use of the organism had been for educational purposes. The organisms require an intermediate insect host to complete their life cycle, making them non-transmissible to other rats or to humans under typical laboratory or educational environments. The organisms effectively colonize rats, but not humans or mice, and are easily maintained in laboratory. They are, with exceedingly rare exceptions, benign (e.g., nonparasitic) in humans, mice, and laboratory rats. Although the benign character of the helminth makes it ideal for educational purposes, the fact that no pathology is associated with colonization has led to decreased interest in the *H. diminuta* as a model for modern research where efforts are largely motivated by interests in medicine and health. However, more recently work with the “biota alteration” model of inflammatory disease has established that reintroduction of helminths into Western society, a practice often referred to as “helminthic therapy,” is potentially a way of lowering inflammation without compromising immune function. For this effort, the lack of pathology and benign nature of the organism makes *H. diminuta* an ideal subject for study. In this chapter, we describe production of *H. diminuta* using laboratory rats and introduction of the organisms into laboratory mice as a model for their effects in humans.

Key words Helminthic therapy, Helminth, Biological therapeutic, Inflammation, Anti-inflammatory

1 Introduction

Animal models for the study of inflammatory disease are extremely helpful to biomedical research efforts. This is increasingly true as the prevalence of a wide range of inflammatory-related diseases continues to rise in Western society [1–3]. Inflammation-related diseases of Westernization include a broad range of allergic disorders, autoimmune conditions, digestive diseases, and neuropsychiatric disorders. An intuitive view is that models aimed at dealing with the root causes of these inflammatory disease will be the most beneficial to medical progress [4]. These root causes include inflammatory diets, sedentary lifestyles, chronic psychological stress, and vitamin D deficiency. At the same time,

changes in the human biota, the life associated with the ecosystem of the human body, are being recognized as important contributors to the ever-increasing prevalence of inflammation-related diseases in Western society [1, 2]. Among the most impactful changes to the biota is the virtual annihilation of helminths from humans [4]. It is now becoming apparent that helminths, ubiquitous symbionts until very recently in human history, are important for immune function and stabilization. With that in mind, animal models to study the use of helminths as therapeutic agents in clinically relevant scenarios are of considerable interest.

Hymenolepis diminuta, the rat tapeworm, is now one of the most widely used helminths for therapeutic purposes [5, 6]. However, *H. diminuta* is not currently approved by any regulatory agency for therapeutic use, and the study of the effects of this helminth on humans and even on laboratory animals is in its infancy. Since helminthic therapy effectively alleviates many of the effects of “biota alteration,” one of the primary causes of disease in Western society, it is expected that the study of a wide range of helminths, including *H. diminuta*, will increase in the foreseeable future.

Similar to many rodent models of helminth colonization, *H. diminuta* exposure leads to increased type 2 cytokines in the intestine of both mice and rats. Although IL-4 appears to be the dominant cytokine at low worm burdens in the tolerant rat model, administration of 50 worms leads to a significant increase in IL-13 mRNA and protein production [7]. This increase in cytokine correlates with enhanced mucus production, goblet cell hyperplasia, and worm expulsion. Mice are less tolerant to prolonged colonization when exposed to low-dose *H. diminuta*. Mice colonized with five cysticercoids mount a rapid and robust type 2 immune response which leads to clearance of adult worms in both Balb/c and C57BL6 backgrounds [8, 9]. Expulsion in each of these cases is dependent on T cells and is dominated by the production of IL-4 and IL-13. Importantly, signaling induced by IL-4 and IL-13 is required for worm expulsion, mucus production, and goblet cell hyperplasia as STAT6-deficiency, a key factor in IL-4 receptor signaling, substantially prolongs adult worm engraftment [10].

H. diminuta is easily maintained in the laboratory with no specialized equipment (*see Note 1*). The organism is so easily maintained that it is now used in middle school and high school biology classes for educational purposes. Adding further to its utility as a laboratory animal model, the safety profile of the organism is excellent, posing no hazards to humans working with the organisms or to the laboratory rats that serve as their primary hosts (*see Notes 2 and 3*).

Unlike most roundworms and some other flatworms, *H. diminuta* lives exclusively in the lumen of the gut. That is, it does

not breach the epithelium of the gut, but rather remains in the fecal stream. The organisms have no mechanism by which breaching the epithelium is possible and essentially “swim” in the intestine [11]. Further, they do not form lesions at the site of attachment in their natural hosts [11].

In humans, helminthic therapy with *H. diminuta* is accomplished by repeated exposure to the cysticercoid life stage of the organisms at 1–6-week intervals [5, 6]. Unlike laboratory rats, neither humans nor mice can host mature, reproducing *H. diminuta*. For this reason, a mouse model may be the most clinically relevant for studying the use of *H. diminuta* for therapy in humans. In this review, methods are described for (a) maintenance in *H. diminuta* in the laboratory rat and (b) use of mice as a model for the effects of helminthic therapy with *H. diminuta* in humans (see **Notes 4–6**).

2 Materials

2.1 Maintaining the Beetles (*Tenebrio molitor*)

1. Quaker brand, 100% natural whole grain oats: follow the directions on the container to store the oats, and discard if mold is visible.
2. Freshly washed organic celery: the celery must be certifiably organic and stored in a refrigerator. Remove thin ends or leafy parts before storing.
3. Nutritional yeast (Bragg Live Food Products, Santa Barbara, CA) at a ratio of approximately 0.06 g nutritional yeast per gram of oats: add only to the nursery (see point 5 below for definition of the nursery).
4. Small plastic containers: reusable food-grade plastic containers are used as housing adult beetles. A typical setup is shown in Fig. 1. These containers are modified by cutting a hole in the lid and gluing a screen mesh onto the lid. In this figure, six batches of beetles are shown (two in the front, one open). See Methods for the definition of a “batch.”
5. Nursery: a container typically larger than that used to contain adult beetles, used to contain mealworms and pupae. It is modified by cutting a hole in the lid and gluing a screen mesh onto the lid.
6. Plastic dome enrichment: these are made from a section of a BPA-free (polypropylene) plastic drinking cup for the beetles in each batch to hide under. The surface of the cup is scored with sandpaper so that the beetles can climb on the plastic.
7. Dehumidifier: is recommended for beetle housing. Our laboratory uses an Eva-Dry EDV-100 petite dehumidifier in each “isolator.”



Fig. 1 Beetle housing conditions in the laboratory. In this setup, a front opening box is used as an “isolator,” and individual “batches” of beetles loaded with HDCs are kept in food-quality containers modified with wire screens to allow air to circulate in each batch. A dehumidifier is visible in the back, right of the container. The four batch containers and dehumidifier are resting on a mealworm nursery container (bottom of isolator), where mealworms are allowed to pupate and mature to adults

8. A chemical fume hood to store the isolators.
9. Unfitted disposable dust mask is adequate for most purposes; however, fitted respiratory protective equipment may be needed for individuals who are sensitive to dust or mold or who spend considerable time maintaining beetle colonies.

2.2 Maintaining the Rats

1. Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN).
2. A disposable fine tip transfer pipette (Samco Scientific Corp).
3. Maintained in AAALAC-approved barrier facilities at Duke University Medical Center in accordance with institutional guidelines. All animal care and procedures were approved by the institutional animal care and use committee at Duke University (*see Note 2*).

2.3 Isolating *H. diminuta* Cysticercoïds (HDCs)

1. 0.6% saline solution: distilled water, solid NaCl.
2. A disposable fine tip transfer pipette (Samco Scientific Corp).
3. Sterile petri dishes.
4. Dissecting microscope with 40× magnification.

3 Methods

3.1 Maintenance of Grain Beetles (*Tenebrio molitor*) in the Laboratory

1. Build the nursery out of a large plastic container and add the worms and pupae. They are provided with fresh Quaker oats, organic celery, and nutritional yeast seasoning. The oats to yeast ratio is 10:1 in the nursery, with celery as needed. Feeding the beetles a richer diet than oats and celery has reportedly increased the production of beetles but has decreased the therapeutic effect of the HDCs (*see Note 7*).
2. To prevent the celery from becoming buried in the oat/yeast mix, toothpicks are inserted into the celery. This will also prevent mold from developing (*see Notes 8 and 9*).
3. Newly hatched beetles are collected over a 2-week period and placed into a new “batch” box, which is labeled. We define one “batch” of HDCs as all HDCs contained in a group of beetles that is “loaded” with HDCs at the same time in the same container. Each batch of beetles consists of between 15 and 70 beetles and is loaded as described below in Subheading 3.2. This batch box will contain roughly 60 grams of fresh Quaker oats. Add the plastic dome enrichment and organic celery. The celery should be at a minimum of 0.23 g/cm², where the cm² reflects the area (length by width) of the container.
4. Each “batch” is kept in a separate container, or isolator, as depicted in Fig. 1. The isolator (the large green container in Fig. 1) is a container meant to house multiple batches of beetles and a nursery. Each isolator is outfitted with a dehumidifier and a large front opening for easy access to the batch boxes.
5. The celery in each batch box is changed twice weekly. In addition, the boxes are monitored for pupae or mold during this time.

3.2 Loading Grain Beetles with HDCs

Although the process is straightforward, substantial variation in the number of eggs eaten by individual beetles (and thus the eventual numbers of HDCs per beetle) is observed. It is possible to feed more than 100 eggs to an individual beetle, but the procedure can be adjusted so that an average of between 15 and 70 eggs per beetle are ingested (*see Note 10*). Since the eggs are produced by the adults living in rats and are present in the rat feces, the procedure involves feeding of rat feces to beetles.

1. To create a “batch” of HDC-loaded beetles, newly hatched (within 5–6 weeks) beetles are selected (*see Note 11*). Beetles are moved to a new container, or “starvation chamber,” without access to food or a water source (celery) for 2 days. Water and food deprivation ensures that the beetles will eat rat fecal pellets containing *H. diminuta* eggs.

2. Harvest fecal pellets from rats colonized with *Hymenolepis diminuta*. After collection from cages, feces containing *Hymenolepis diminuta* eggs are stored for no more than 2 days at room temperature prior to use. Do not store feces at 4 °C.
3. Prior to placing the droppings into the starvation chamber, add water dropwise if the droppings appear dry. Drying rat fecal pellets will kill the eggs. One or more drops of water per pellet are typically added immediately before feeding to the beetles. One pellet will feed a few dozen beetles, and five pellets will feed several hundred beetles. Keep the beetles in the “starvation chambers” with the rat fecal pellets for another 2 days.
4. After feeding for 2 days, the beetles should be removed from any remaining feces and placed in a fresh container with oats and organic celery.

3.3 Maintenance in the Rat

The rats must be maintained with HDCs isolated from grain beetles to ensure production of *H. diminuta* eggs (*see Note 12*). For maintenance in a mouse model, *see Notes 4–6*.

1. The HDCs are isolated from the grain beetles (*see Subheading 3.4*) and are suspended in 0.6% saline. They are then placed in a disposable “fine tip transfer pipette” (Samco Scientific Corp) as described below.
2. The pipette tip is placed in the rat’s mouth, on the tongue, while gently holding the rat, and the liquid is expelled. The rat is watched carefully during this time to ensure that it swallows. Four to five HDCs are administered per rat. There is generally no need for oral gavage, as the rodents will readily accept the HDCs when fed by an experienced technician.
3. After at least 21 days following the ingestion of HDCs and then monthly thereafter, the feces should be checked for HDC eggs using a standard fecal flotation test. A positive result of a fecal flotation test is shown in Fig. 2, and most veterinarians working in animal research facilities will be able to help with positive identification of tapeworm colonization.

3.4 Isolation of HDCs from Grain Beetles

1. The beetle is placed in a sterile petri dish. The beetle can be placed in the upside-down or upright position for this step of the procedure.
2. The head (or the thorax plus the head, it does not matter) is removed with a swift motion using a clean scalpel or knife.
3. The legs are removed using forceps and dissecting scissors.
4. The wings are removed using forceps and dissecting scissors.

eggs at 40x magnification

eggs at 100x magnification

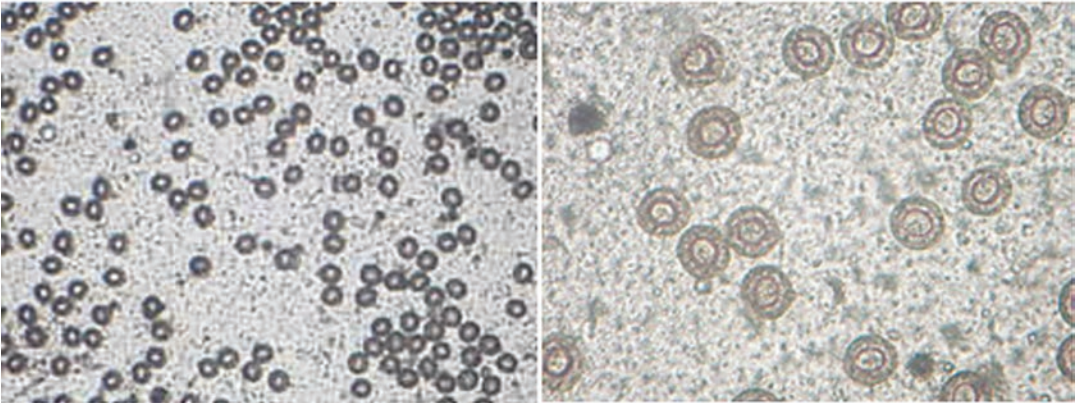


Fig. 2 Observation of HD eggs by fecal flotation. Rats colonized with *H. diminuta* have copious amounts of eggs in their fecal material that are readily observed after fecal flotation

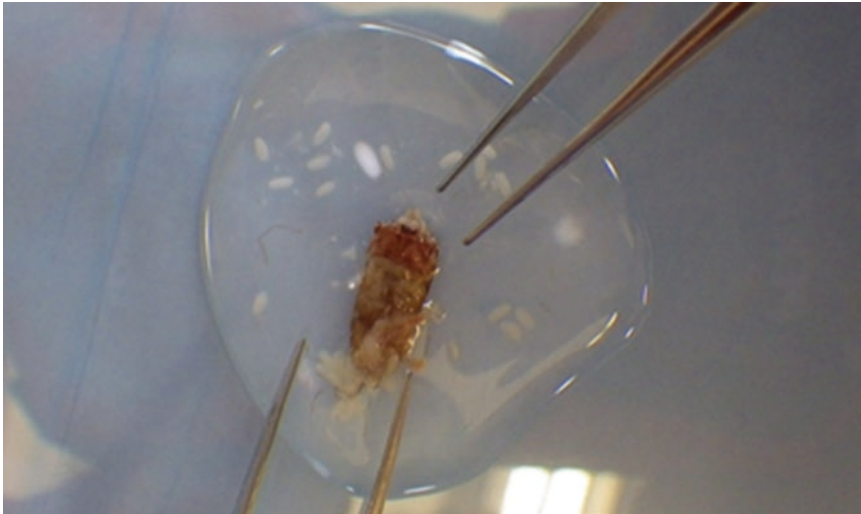


Fig. 3 Dissection of HDCs. Although eggs are clearly visible in this photo, HDCs are not discernable without the use of a microscope. The abdomen of a single grain beetle, bottom side up (with legs and wings removed), is shown. In this specimen, the contents of the abdomen have been partially removed and dispersed in the 0.6% saline solution, as described in the Methods

5. The abdomen of the beetle is placed topside (wing side) up in a new sterile petri dish, and about 3 mL of 0.6% saline solution is added (Fig. 3).
6. Using two pairs of tweezers and, if desired, a small knife, the interior of the beetle abdomen is gently scraped out into the saline solution (Fig. 3). This frees the HDCs from the beetle. The HDCs are visible to the naked eye, but will not be

discernable from other components of the beetle's abdomen without a microscope.

7. Once the contents of the beetle's abdominal cavity are suspended in solution on a petri dish, the dish is placed under a dissecting microscope, and the HDCs are harvested. A 20- to 40-fold total magnification (ocular + objective lens) is desirable to obtain a balance between identification of individual HDCs and observation of a broad field.
8. A disposable "fine tip transfer pipette" (Samco Scientific Corp) is used in collection of HDCs.

4 Notes

1. The life cycle of *H. diminuta* in the laboratory is shown in Fig. 4. Adult, egg-laying helminths are maintained in the laboratory using rats as the primary hosts. The eggs, present in the feces of the rats, are consumed by grain beetles, *Tenebrio molitor*, which serve as the intermediate host in the laboratory. *H. diminuta* achieve a distinctive, cysticeroid stage in the extraintestinal space of the abdomen of the beetle, which is readily extracted for inoculation of additional laboratory rats. This stage is often referred to as an "HDC" (*Hymenolepis diminuta* cysticeroids), although the acronym HDC is sometimes used as a general name for *H. diminuta* by individuals using helminthic therapy. In this manuscript, HDC (or HDCs, plural) will refer strictly to the cysticeroids stage of *H. diminuta*. Mature HDCs have been used for therapeutic purposes in humans and can be used in laboratory mice as a model for its therapeutic effect in humans (Fig. 4).
2. Older beetles can also be loaded, but they will not live as long after they are colonized by the HDCs, so they will be of less utility in further studies. It will take 5–7 weeks for the HDCs to mature once the beetles have ingested the eggs.
3. Results of loading will vary depending on the number of eggs in the pellets and other factors such as the relative humidity, and the procedure will be adjusted to maintain an average colonization rate between 15 and 70 HDCs per beetle (average) by increasing or decreasing the number of pellets used per beetle. If the procedure yields more than 70 HDCs per beetle on average, then the number of beetles fed by a single pellet will be increased. If the procedure yields less than 15 HDCs per beetle on average, then the number of beetles per pellet can be decreased.
4. Rats need to be exposed to *Hymenolepis diminuta* once every few months at most, and sometimes colonization will last for the lifetime of the rat. (*Hymenolepis diminuta* will live for 4–8

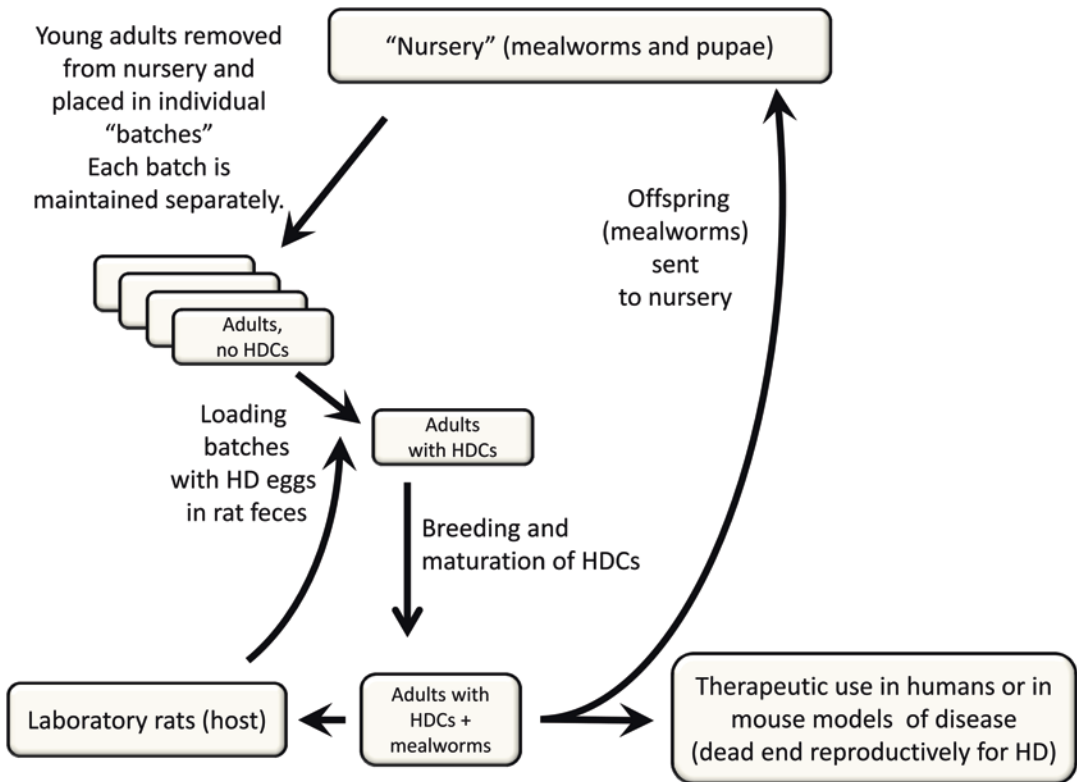


Fig. 4 Overall schematic of laboratory use of *H. diminuta* (HD), including maintenance of adults in rats and therapeutic use in humans or in mouse models

months in some rats and longer in others.) This depends in large part upon the breed of the rat, but it can vary for unknown reasons. Thus, colonization should be evaluated periodically, and rats should be recolonized as needed.

5. Rat-to-rat transmission of HDCs is not possible under standard laboratory conditions, and thus no special housing of the rats is required. The safety and training procedures needed to work with laboratory rodents are well documented and part of routine practice in any laboratory.
6. The risk of *H. diminuta* transmission to humans following exposure is negligible and requires no special safety precautions. The effects of ingestion of the larval stage of the organisms appear to be generally beneficial rather than harmful in humans based on sociomedical studies [5, 6], indicating that no particular safety precautions are warranted for work with *H. diminuta*. Thus, precautions that need to be taken are primarily dictated by precautions that need to be taken when working with its vertebrate and invertebrate primary and secondary hosts, respectively.

7. The primary concern when working with grain beetles is to minimize exposure to particulate antigens. The conditions of cultivation of grain beetles are conducive to growth of yeast/mold. Precautions, such as inserting toothpicks into celery, taken so that the source of food for the beetles (oats) does not come into extensive contact with the source of moisture (celery) for the beetles. A dehumidifier prevents excess growth of yeast or mold which might increase microbial-derived airborne antigens.
8. In addition, it is recommended that dust be kept to a low level by weekly or twice-weekly cleaning of housing. This is done by dumping the entire nursery into a sieve, allowing the droppings to pass through. After the droppings have been removed, the worms and pupae retained in the sieve are returned to the nursery and provided with fresh organic oats, celery, and nutritional yeast seasoning (10:1 ratio of oats to yeast in the nursery, with celery as needed).
9. The methods described are based essentially on methods acquired from individuals producing HDCs for therapeutic purposes in humans. The acquisition of these methods was conducted during the course of IRB-approved sociomedical studies, as described previously [5, 6]. These methods are slightly modified from methods described by Carolina Biological Supply (Greensboro, NC), which sells *H. diminuta* strictly for educational purposes in both the cysticercoid and egg life stages. Modification of the methodology to enhance the production of HDCs or beetles may decrease the therapeutic effect of the HDCs. In particular, feeding the grain beetles a richer source of nutrition repeatedly yielded improved production of grain beetles but also HDCs with decreased therapeutic impact. Using HDCs that were between 5 weeks and 5 months of age, but not older or younger, was also reported to have the most therapeutic benefit.
10. Unlike laboratory rats, laboratory mice will not readily ingest HDCs administered using the tip of a pipette placed in their mouth. Rather, the mice will do their best to avoid the pipette, and will often bite through the pipette, rendering the pipette ineffective for delivering the HDCs. Oral gavage needles are readily available, but HDCs tend to get hung up in the junctions in the needles, making delivery through a standard gavage needle unreliable. For reliable delivery of HDCs to mice, a disposable “fine tip transfer pipette” (Samco Scientific Corp) can be used for delivery, but it must be shielded to prevent the mice from biting through the pipette. For this purpose, our laboratory uses a modified 14-gauge IV catheter (Angiocath: Becton, Dickinson and Company, Franklin Lakes, NJ) as shown in Fig. 5 to shield the pipette. To feed the mice, the animals are held by the scruff of the neck, and the shielded pipette is inserted behind the tongue of the mouse with the

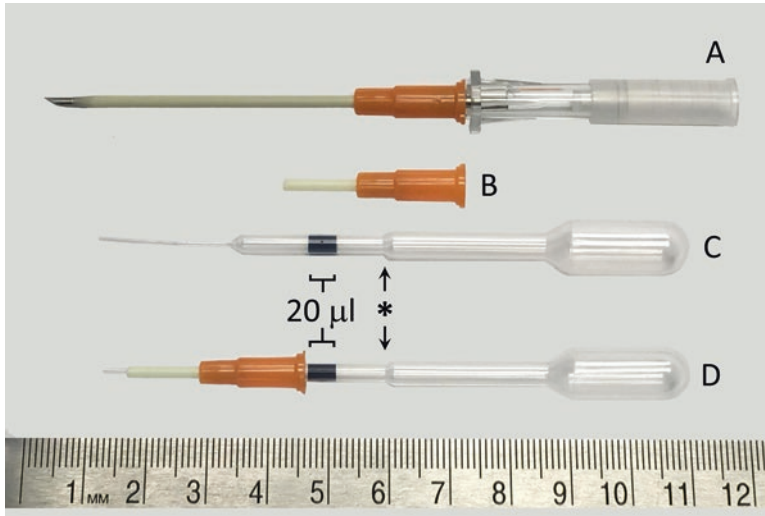


Fig. 5 Device for feeding HDCs to mice. Modification of a 14-gauge IV catheter to shield a pipette is shown. (a) Catheter with needle, (b) catheter modified to make a shield for the pipette, (c) pipette loaded with 20 μL of opaque liquid, (d) pipette loaded with 20 μL of opaque liquid and shielded with a modified catheter, ready for use in feeding HDCs to mice. The asterisk in the diagram indicates the furthest point to which HDCs should be drawn in the pipette. Beyond that point, the HDCs have an increased tendency to stick in the pipette. Of note is the fact that the unshielded pipette, as loaded in diagram c, is appropriate for feeding of rats. The numbers on the scale indicate centimeters

head in the vertical position. After insertion of the shielded pipette, the liquid is immediately dispensed, and the animal is watched closely to ensure that it swallows.

11. Controls in the mouse model should be fed beetle abdomen extracts from beetles that lack HDCs. These controls compensate for the fact that beetle extracts contain both nutritional material and microbial content. Although the microbial content of the extracts (as will all insect-associated bacteria) is benign, it will alter the flora in the mice. Although HDCs can be cleaned to avoid the nutritional and microbial contamination, HDCs in pure saline are excessively “sticky” and difficult to pipette. Thus, either the unpurified (in beetle abdomen extract) HDCs should be used or a carrier protein should be incorporated into the purification medium.
12. *H. diminuta* initially begin to grow in mice with good efficiency, with most of the HDCs maturing [12]. However, maturation is short lived. Hopkins provides an extensive list of mouse strains that reject *H. diminuta*, concluding that “all strains” reject the helminths [13]. However, Hopkins also concludes that side-by-side studies have not been conducted, so it is difficult to know if there are strain-dependent differences in rejection. Andreassen and colleagues concluded that *H. diminuta* were expelled from *nu/nu* mice between days 10 and 20 and were expelled from *+/nu* mice sooner, at less than 10 days [14].

References

1. Parker W, Perkins SE, Harker M, Muehlenbein MP (2012) A prescription for clinical immunology: the pills are available and ready for testing. *Curr Med Res Opin* 28:1193–1202. <https://doi.org/10.1185/03007995.2012.695731>
2. Parker W, Ollerton J (2013) Evolutionary biology and anthropology suggest biome reconstitution as a necessary approach toward dealing with immune disorders. *Evol Med Public Health* 2013:89–103. <https://doi.org/10.1093/emph/eot008>
3. Bickler SW, DeMaio A (2008) Western diseases: current concepts and implications for pediatric surgery research and practice. *Pediatr Surg Int* 24(3):251–255
4. Bilbo SD, Wray GA, Perkins SE, Parker W (2011) Reconstitution of the human biome as the most reasonable solution for epidemics of allergic and autoimmune diseases. *Med Hypotheses* 77(4):494–504. <https://doi.org/10.1016/j.mehy.2011.06.019>
5. Cheng AM, Jaint D, Thomas S, Wilson J, Parker W (2015) Overcoming evolutionary mismatch by self-treatment with helminths: current practices and experience. *J Evol Med* 3:235910
6. Liu J, Morey RA, Wilson JK, Parker W (2016) Practices and outcomes of self-treatment with helminths based on physicians' observations. *J Helminthol FirstView* 91:1–11
7. Webb RA, Hoque T, Dimas S (2007) Expulsion of the gastrointestinal cestode, *Hymenolepis diminuta* by tolerant rats: evidence for mediation by a Th2 type immune enhanced goblet cell hyperplasia, increased mucin production and secretion. *Parasite Immunol* 29(1):11–21. <https://doi.org/10.1111/j.1365-3024.2006.00908.x>
8. McKay DM, Halton DW, McCaigue MD, Johnston CF, Fairweather I, Shaw C (1990) *Hymenolepis diminuta*: intestinal goblet cell response to infection in male C57 mice. *Exp Parasitol* 71(1):9–20
9. Palmas C, Bortoletti G, Gabriele F, Wakelin D, Conchedda M (1997) Cytokine production during infection with *Hymenolepis diminuta* in BALB/c mice. *Int J Parasitol* 27(7):855–859
10. McKay DM, Khan WI (2003) STAT-6 is an absolute requirement for murine rejection of *Hymenolepis diminuta*. *J Parasitol* 89(1):188–189. [https://doi.org/10.1645/0022-3395\(2003\)089\[0188,SIAARF\]2.0.CO;2](https://doi.org/10.1645/0022-3395(2003)089[0188,SIAARF]2.0.CO;2)
11. Arai HP (1980) *Biology of the tapeworm Hymenolepis diminuta*. Academic Press, New York
12. Hopkins CA, Subramanian G, Stallard H (1972) The development of *Hymenolepis diminuta* in primary and secondary infections in mice. *Parasitology* 64(3):401–412
13. Hopkins CA (1980) Immunity and *Hymenolepis diminuta*. In: Arai HP (ed) *Biology of the tapeworm Hymenolepis diminuta*. Academic Press, New York, pp 551–614
14. Andreassen J, Hindsbo O, Ruitenberg EJ (1978) *Hymenolepis diminuta* infections in congenitally athymic (nude) mice: worm kinetics and intestinal histopathology. *Immunology* 34(1):105–113