Published in final edited form as:

J Parasitol. 2007 October ; 93(5): 1084–1089. doi:10.1645/GE-1234R.1.

THE SUGAR GLIDER (*PETAURUS BREVICEPS*): A LABORATORY HOST FOR THE NEMATODE *PARASTRONGYLOIDES TRICHOSURI*

Thomas J. Nolan, Xiaodong Zhu, Andrea Ketschek, Joan Cole*, Warwick Grant†, James B. Lok, and Gerhard A. Schad

University of Pennsylvania, 3800 Spruce Street, Philadelphia, Pennsylvania 19104

Thomas J. Nolan: parasit@vet.upenn.edu

Abstract

Parastrongyloides trichosuri is a nematode parasite of the Australian brush-tailed possums that can be propagated through many generations in vitro. This makes *P. trichosuri* uniquely suited for genetic investigations, including those involving transgenesis. However, an obstacle to its use as an experimental model has been the fact that its host is limited to Australia and New Zealand and that it cannot be exported because of its status as a protected species or agricultural pest, respectively. In previous studies, conventional laboratory animals such as rats, mice, rabbits, ferrets, and chickens have failed to support infections. In the present study, gerbils and short-tailed opossums proved similarly refractory to infection. In contrast, the sugar glider (*Petaurus breviceps*, family Petauridae) proved to be a good host for *P. trichosuri*. Patent infections resulted using as few as 6 infective larvae (L3i) and as many as 2,000 L3i. Large numbers of L3i (1,000–2,000) produced patent infections of much shorter duration than those seen when 100 L3i were initially given to the sugar glider. In one case, an infection initiated with 100 L3i was patent for over 1 yr. *Parastrongyloides trichosuri* is easily cryopreserved using a method developed for *Strongyloides stercoralis*. Thus, we have identified an experimental host for *P. trichosuri* that will make it possible to conduct research on this parasite in laboratories outside the endemic sites.

Parastrongyloides trichosuri, first described by Mackerras (1959) from the Australian brush-tailed possum, *Trichosurus vulpecula*, has also been reported from *T. caninus* (Spratt et al., 1991), and the recently described *T. cunninghami* (Viggers and Spratt, 1995; Lindenmayer et al., 2002). Parasitic males and females are found in the small intestine of the possum, and eggs pass out with the feces. Outside the host, eggs hatch and the larvae develop into free-living males and females. This nematode may undergo many free-living generations in the soil, but under conditions of starvation or crowding, some larvae develop into a morphologically distinct infective third-stage larvae (L3i; Grant et al., 2006). These L3i penetrate the host's skin and eventually reach the small intestine where they develop to the parasitic adult stage.

That *P. trichosuri* can undergo a virtually unlimited number of free-living generations makes this parasite especially useful for genetic investigations of parasitic nematodes (Crook et al., 2005). However, an obstacle to its use in laboratory investigations is that its natural host, the brush-tailed possum, is an agricultural pest (Cowan et al., 2006) and a reservoir of bovine tuberculosis in the Australasian region, and it is banned from import into

most countries. Attempted infections of rats, mice, rabbits, ferrets, and chickens failed (Ralston et al., 2002). Here, we report our successful infection of the sugar glider, *Petaurus breviceps*, an Australian marsupial and our unsuccessful attempts to infect gerbils, and South American short-tailed opossums.

MATERIALS AND METHODS

Parastrongyloides trichosuri

The organism was originally shipped to the United States as a culture of free-living stages from an infected *T. vulpecula* in New Zealand. This culture was passaged twice a week onto NGM plates with *Escherichia coli* strain HB101 as described previously (Grant, 2006), with the exception that an autoclaved gerbil fecal pellet was substituted for the possum fecal extract. The cultures were kept in a moist chamber at 20 C. Subsequent cultures were started from nematodes obtained from charcoal coprocultures made from the feces of infected *P. breviceps*. Larvae were recovered from these cultures after 24 hr of incubation at 20 C with a Baermann apparatus and transferred onto the NGM plates. L3i were obtained from cultures that had been allowed to grow for 2 or more weeks and thus depleted of bacteria. To develop the capacity to store many different isolates and transgenic lines of this nematode, we undertook the following cryopreservation study. The free-living stages were washed and incubated in the cryoprotectant (10% dimethyl sulfoxide [DMSO] and 10% dextran; Nolan et al., 1988) for 0, 30, or 75 min at room temperature. Once incubated in the cryoprotectant, they were placed in either a -75 C freezer or the vapor phase of liquid nitrogen for 3–8 days. The worms were thawed quickly under hot running water and washed twice in Dulbecco's modified Eagle's medium. Once washed, they were transferred to 5 ml of an invertebrate saline solution (BU; Hawdon and Schad, 1991), and stored at 26 C for 24 hr. The percentage of larvae that survived was recorded at 2 different time points, approximately 10 min and 24 hr thawing. The Fisher exact test was used to compare survival rates.

Animals

Water and commercially formulated pelleted diets (Purina Mills, St. Louis, Missouri) were available to all animals ad libitum. In addition to pelleted food (Animals Exotique, Pearland, Texas), the sugar gliders were given fresh fruit sprinkled with a calcium supplement and hard boiled eggs daily.

Outbred male Mongolian gerbils (*Meriones unguiculatus*) were obtained from Charles River Laboratories, Inc. (Wilmington, Massachusetts) and used between 2 and 4 mo of age. Two gerbils were inoculated subcutaneously (s.c.) with 200 L3i and necropsied 3 wk later. One gerbil was inoculated with 600 L3i SC and necropsied 8 wk later. Four gerbils were inoculated with 2,000 L3i SC and necropsied 4 wk later. All infected gerbils were given an s.c. injection of 2 mg of methylprednisolone acetate (MPA; Depo-Medrol, Pharmacia, Kalamazoo, Michigan) at the time of infection and weekly thereafter. Necropsy and examination of the small and large intestine for worms were done as described by Nolan et al. (1993) for gerbils infected with *Strongyloides stercoralis*. Feces were collected twice a week by placing the gerbils for 6 to 12 hr in a cage with a wire grid suspended above wet cardboard covering the cage bottom. Feces were mixed with bone-charcoal and placed in a petri dish containing a filter paper disk saturated with water. These charcoal coprocultures were incubated at 20 C in a humid environmental chamber for 3 to 5 days before being examined for free-living *P. trichosuri* using a Baermann apparatus and a stereomicroscope at $\times 20$ magnification.

Male short-tailed opossums (*Monodelphis domestica*) were obtained from the Southwest Foundation for Biomedical Research (San Antonio, Texas) and used between 3 and 4 mo of age. One opossum was injected s.c. with 70 L3i and 1 with 430 L3i. The feces from these 2 animals were collected and cultured twice a week as described above for gerbils. The first opossum was necropsied at 32 days postinfection (PI), whereas the other opossum was necropsied at 33 days PI.

Adult male sugar gliders were obtained from the unsalable stock of breeders in the United States who were raising the animals for the domestic pet trade. Available gliders were retired breeders and others that could not be sold to the pet trade. Thus, we do not know the actual age of these animals. Fecal examinations were done using the zinc sulfate centrifugational technique when the gliders first arrived in our facility. Only one glider was found to be infected with a parasite, *Hymenolepis nana*, a common tapeworm of rodents bought in pet stores (Duclos and Richardson, 2000). It was successfully treated with praziquantel (11 mg s.c.). The sugar gliders were kept in a large wire cage equipped with stocking-like sleeping pouches and a fecal collection pan beneath the wire cage bottom (Animals Exotique, Pearland, Texas). These highly social marsupials were housed 3 to 5 in a cage at all times. They were inoculated with between 10 and 2,000 L3i of *P. trichosuri* (the number depended on the experiment) by s.c. injection in the right rear flank. In Investigation 1, which was designed to assess the effects of parasite dose on the outcome of infection (Fig. 1A), 4 sugar gliders were each given a different number of L3i: 100, 500, 1,000, or 2,000. After the infection was no longer patent in 2 of these gliders, they were given weekly subcutaneous injections of 2 mg of methylprednisolone acetate beginning on day 76 PI and continuing until the end of the study on day 158 PI in an attempt to reactivate the infection. These animals were reinfected on day 97 PI after 3 injections of the steroid; the glider that had originally been given 1,000 L3i was injected with 97 L3i, and the glider that had received 2,000 L3i was injected with 974 L3i. In Investigation 2, which was designed to identify the minimum number of L3i needed to establish an infection, each of 5 gliders was given a nominal 10 L3i (counts of larvae left in the syringe after injection showed that the animals received between 6 and 10 L3i). The animals in these 2 investigations were necropsied as described above, with the time of necropsy varying with the study. In Investigation 3, 3 gliders were inoculated with 100 L3i; 2 of the animals received larvae recovered from a culture that had been made originally from the feces of an infected sugar glider (from Investigation 1, designated UPPU004) and passed continuously for 28 to 40 generations. The third glider was given larvae from a culture made with worms from the original New Zealand strain (10th passage) that had been cryopreserved for 186 days. The infections in gliders in Investigation 3 were followed by fecal culture to determine the maximum duration of the infection.

The final experiment was designed to determine the course of a secondary *P. trichosuri* infection in sugar gliders that had cleared a primary infection with the parasite. Three gliders were reinfected with *P. trichosuri* after egg passage ended. Two of these animals (from Investigation 1) were given 2 mg of MPA, s.c. on a weekly basis before injecting them with L3i (97 or 974 larvae), whereas the third animal (from Investigation 3) was not immunosuppressed with the steroid before inoculating it with 99 L3i. Later, this glider was given a third inoculation with 69 L3i after weekly injections of MPA had begun.

Feces were collected from individual sugar gliders by removing the animals from their sleeping pouch, placing them on the wire floor of the cage, and allowing them to defecate into a clean pan beneath the cage. Using this method, 0.20 ± 0.13 g (range 0.01–0.59 g) of feces was collected from individual gliders in about 10 min. Gliders were marked with an ear punch for individual identification.

Enumeration of eggs in fecal samples

Two methods were used to determine the egg output from infected sugar gliders. Fresh fecal pellets were collected from individual gliders as described above, and the total weight of the collected feces was recorded. In the first method, the feces were examined using the zinc sulfate centrifugational technique (Faust et al., 1938). The numbers of eggs seen on the slides were counted, and the eggs per gram of feces (EPG) was calculated by dividing the number of eggs found by the weight of feces examined. In the second method, the feces were weighed, mixed with 2 g of bone-charcoal, and transferred to a moist paper disk in a petri dish (60 × 15 mm). This mixture was incubated at 20 C for 24 hr and then placed into a Baermann apparatus for 6 hr to recover the larvae for counting. The EPG was calculated by dividing the number of larvae recovered by the weight of the feces initially cultured. A comparison of these techniques was made by thoroughly mixing individual fecal samples and using an aliquot from the mixture for each method. This comparison was repeated on a total of 8 fecal samples. In both techniques, the limit of detection was dependent on the amount of feces collected and theoretically varied from 2 to 100 EPG.

RESULTS

Infections in gerbils and South American short-tailed opossums

Nine gerbils were injected with L3i, but none was found infected on necropsy (3–8 wk after injection). No worms were seen in fecal cultures, nor were eggs seen in ZnSO₄ flotations made at various times between infection and necropsy. Cultures made between days 9 and 30 PI from the feces of the 2 injected *M. domestica* opossums were negative for *P. trichosuri*, and no worms were found in the intestine or body of these animals at necropsy.

Infection of *P. breviceps*—Investigation 1: Effect of dose

All tested doses (100–2,000) of *P. trichosuri* L3i led to patent infections in the injected sugar gliders. A composite fecal specimen was positive at 10 days PI (the first day examined) and all 4 gliders were patent by 20 days (the first time point at which individuals were examined). The maximum EPG, as determined by the flotation method, occurred early in the infection (between days 20 and 27 PI in gliders receiving 100, 1,000, and 2,000 L3i, and on day 48 PI for the animal receiving 500 L3i). The maximum EPGs were directly related to infecting dose, and they ranged from 1,852 to 12,800 EPG. The gliders infected with 100 and 500 L3i were killed on days 105 and 85 PI, respectively, due to complications arising from ocular bacterial infections. Both of these gliders had 22 adult worms in the small intestine at the time of death. These worm burdens represent 22 and 4.4% of the larvae initially inoculated (100 and 500, respectively). The gliders given 1,000 or 2,000 L3i were last patent on days 45 and 40 PI, respectively. They received steroids for 3 wk beginning on day 76 PI, but no eggs were seen in the feces over that time; in fact, no eggs were observed in the feces of either glider from day 45 to day 124 PI. They were reinfected on day 112 PI. The glider injected with 97 L3i (originally infected with 1,000 L3i) became patent 12 days later, and it remained so for at least 22 days. At necropsy, 46 days after reinfection, this glider had 2 female adult worms in the small intestine (2.1% of the reinfecting dose). The glider reinfected with 974 L3i (having originally received 2,000 L3i) never became patent, and no worms were seen in the intestine at necropsy 46 days after reinfection.

Investigation 2: Determining a minimum dose

In this investigation, we attempted to infect sugar gliders with only 10 L3i, because it was assumed that the number of larvae available after genetic transformation might be severely limited. Because some larvae were left in the syringe after injection, the actual numbers of worms injected were 6, 6, 8, 9, and 10. The 3 sugar gliders that received the higher doses

were patent on day 15 PI; one sugar glider that received 6 L3i was patent on day 16 PI. The second animal receiving 6 L3i was never patent by the flotation method, but it was patent by culture on day 25 PI (the only time point at which it was cultured). The maximum EPG ranged from 8 to 50 for the individual gliders found patent by the flotation method, but there was no correlation between infecting dose and the maximum EPG. Feces from 3 of the sugar gliders were cultured on days 22 and 25 PI, and cultures from the other 2 were made on one of these days. The sugar glider that received 9 larvae was not patent by culture on the 2 days its feces were cultured (22 and 25 days PI); all other animals were positive each time their feces were cultured. Thus, all 5 gliders were found to be patent by either the flotation or the culture method by 25 days PI. These gliders were necropsied on day 28 PI, and all had adult worms in their small intestines (range 1–5 worms, accounting for 16.7–55.6% [$\bar{x}$ = 36.6%] of the inoculated worms). No worms were found in the large intestine or in the body tissues of any necropsied sugar glider, either in this study or in Investigation 1. The sugar glider that received 9 L3i, and was never positive by culture, had only female worms at necropsy.

Investigation 3: How long do infections persist?

When the EPGs of 8 fecal samples were compared by the flotation and culture methods, the culture method always produced a larger value, and this was statistically significant when tested with the nondirectional Wilcoxon test ($W = -36$, $P = 0.01$). The EPG values determined by the culture method were 9.3 times greater, on average, than those determined using our flotation method; therefore, the EPG values for this final investigation were obtained using the culture method.

The 2 gliders infected with L3i from continuously cultured worms were patent by day 19 PI. The third glider was kept with the 2 infected gliders for 44 days before it was injected with cryopreserved L3i; no eggs were observed until day 11 PI (36 days after the first 2 gliders had become patent), suggesting that animal-to-animal transmission was not taking place under the housing conditions in this study. For the rest of this analysis, these 3 animals were considered as having been infected with identical doses. The prepatent period, as determined from the 8 gliders in Investigations 2 and 3, was 16.8 ± 4.2 days.

One glider infected with L3i from the sugar glider-derived isolate UPPU004 remained patent for at least 453 days, whereas the other animal receiving L3i from this isolate was last patent on day 89 PI (70 days of patency; see Fig. 2A, C, respectively). The glider receiving the cryopreserved New Zealand strain L3i (see Fig. 2B) was last patent on day 279 PI (269 days of patency).

The sugar glider that was last patent on day 89 PI was given a second inoculation of 99 L3i on day 156 PI, but it did not become patent any time over the next 177 days (out to day 333 postinital inoculation). On day 303 after the first inoculation, this animal was started on weekly injections of MPA, and on day 317 (161 days after the second inoculation), it was given a third inoculation (69 L3i) and became patent 20 days later. Weekly MPA injections were discontinued 35 days after the third inoculation, and the sugar glider remained patent for an additional 68 days (a total of 83 days of patency). Larvae collected from charcoal coprocultures made on day 36 of this last inoculation were plated onto the standard agar culture plates and passaged 10 times before being cryopreserved.

Cryopreservation

Incubation in the cryoprotectant before freezing was found to be deleterious to the worms during cryopreservation in liquid N₂ vapor. With no incubation, there was a 39% survival at 10 min postthawing versus 12% survival with 75-min incubation ($P < 0.0001$). Survival was higher when the worms were frozen (with no incubation in the cryoprotectant) at -75 C than

when freezing was done in the liquid N₂ vapor phase (51 and 39% survival, respectively, when measured at 10 min postthawing; $P = 0.0285$). There was no difference in survival when the worms were examined at 24 hr postthawing compared with 10 min postthawing ($P = 0.2$ for freezing at -75°C and $P = 0.7$ for freezing in the liquid N₂ vapor phase). In the experiments using no incubation and freezing at -75°C , more larvae (59.3%) survived thawing than adults (38.1%; $P < 0.0001$), and by 24 hr postthawing all adults had died, but the larvae had continued to develop to adulthood, and the thawed worms could be cultured for many generations. Third-stage larvae recovered from cultures made from worms thawed after cryopreservation at -75°C (with no incubation before freezing) were able to produce an infection in a sugar glider. This infection became patent at 11 days PI and persisted for at least 279 days, values similar to infections started by L3i that were derived from continuously cultured worm populations (see results of Investigation 3).

DISCUSSION

Parastrongyloides trichosuri is a parasitic nematode uniquely suited for the investigation of the genetic control of the infective process (Grant et al., 2006; Newton-Howes et al., 2006). The unlimited generations in its free-living cycles will allow for the isolation of transgenic lines, which could then be forced into the infective L3 for infection of the host. The major obstacle to general use of this nematode as a laboratory model has been the lack of a host that was widely available outside the endemic zone. The natural definitive host, the brush-tailed possum, can be used only in countries where it exists naturally (Australia and New Zealand), because its importation to other countries is banned due to its status as a carrier of bovine tuberculosis and an agricultural pest. Additionally, its size (3–4 kg) and its aggressive disposition are problematic in adapting it as a laboratory host. Furthermore, wild-caught possums from areas where *P. trichosuri* is present have presumably been previously infected. Common laboratory animals such as rats, mice, rabbits, ferrets, and chickens have proven refractory to *P. trichosuri* (Ralston et al., 2002). In this study, we attempted to infect a limited number of gerbils, and short-tailed opossums, but no patent infections resulted in any of these animals.

An unidentified species of *Parastrongyloides* has been reported from sugar gliders in Australia (Spratt et al., 1991), where *T. vulpecula* and *P. breviceps* are native species. In the studies reported above, we found that the sugar glider could easily be infected with *P. trichosuri* and that the infections could remain patent for more than 1 yr. In our studies, sugar gliders infected with *P. trichosuri* passed eggs as early as 10 days PI, with an average observed prepatent period of 16.9 ± 4.2 days. The prepatent period in *T. vulpecula* (the natural host) inoculated with 1,000 L3i, has been reported as 14 days, with patent infections lasting between 29 and 274 days (Grant et al., 2006). In sugar gliders inoculated with 100 L3i (Investigation 3), the animals remained patent for 70 to more than 436 days. In contrast, the 2 sugar gliders infected with 1,000 or 2,000 L3i (Investigation 1) remained patent for only 45 days or less.

Although sugar gliders are bred in captivity and available from commercial suppliers, they are not to our knowledge currently used as laboratory animals, so their availability is limited and they are expensive. Thus, we assessed the possibility of reinfecting sugar gliders that had self-cured. In 2 of the 3 animals given MPA before reinoculation, patent infections were produced that lasted for 21 and 83 days. Both of these infections resulted in sufficient fecal egg output to initiate cultures of free-living stages (Fig. 2C). The self-cured sugar glider that did not become patent after reinoculation and treatment with steroids was initially infected with 2,000 L3i and reinoculated with 973 L3i. It is possible that the immunity developing after the initial infection was, in this case, strong enough to prevent reinfection despite steroid treatment. The self-cured animal that was exposed to 99 L3i without being given

steroids did not develop a patent infection. Thus, if a sugar glider that has self-cured (or otherwise outlived its infection) is treated with steroids, it can be reinfected and enough eggs produced to initiate new cultures. The ability to re infect the sugar glider, coupled with the ability to cryopreserve *P. trichosuri*, provides a safeguard against loss of genetic lines due to unforeseen circumstances during passage in vitro. Regarding cryopreservation of larvae, the addition of dextran to the cryoprotectant was probably unnecessary, because we found a similar percentage survival among thawed parasites (59%) as seen by Grant et al. (2006) when 10% DMSO alone was used as the cryoprotectant (75%).

Because the number of L3i in the initial stages of establishing a transgenic line is likely to be small, we infected sugar gliders with between 6 and 10 worms, numbers that we thought would be available in the initial development of a transgenic line. We were able to produce patent infections in all 5 of the animals that we infected with these low doses. Thus, the sugar glider seems to be a good laboratory host for working with low numbers of *P. trichosuri*, and it is therefore useful in transgenic studies.

Acknowledgments

We thank A. Freeman, M. Samuels, and M. Brenes for help in maintaining the free-living life cycle of *P. trichosuri*. This work was supported by grants from the National Institutes of Health (R01 AI 22662 and R01 AI 50668) and The Ellison Medical Foundation (ID-IA-0037-02).

LITERATURE CITED

- Cowan PE, Ralston MJ, Heath DD, Grant WN. Infection of naive, free-living brush tail possums (*Trichosurus vulpecula*) with the nematode parasite *Parastrongyloides trichosuri* and its subsequent spread. *International Journal for Parasitology*. 2006; 36:287–293. [PubMed: 16442541]
- Crook M, Thompson FJ, Grant WN, Viney ME. *daf-7* and the development of *Strongyloides ratti* and *Parastrongyloides trichosuri*. *Molecular and Biochemical Parasitology*. 2005; 139:213–223. [PubMed: 15664656]
- Duclos LM, Richardson DJ. *Hymenolepis nana* in pet store rodents. *Comparative Parasitology*. 2000; 67:197–201.
- Faust EC, D'Antoni J, Odom V. A critical study of clinical laboratory technics for the diagnosis of protozoan cysts and helminth eggs in feces. I. Preliminary communication. *American Journal of Tropical Medicine*. 1938; 18:169–183.
- Grant WN, Stasiuk S, Newton-Howes J, Ralston M, Bisset SA, Heath DD, Shoemaker CB. *Parastrongyloides trichosuri*, a nematode parasite of mammals that is uniquely suited to genetic analysis. *International Journal for Parasitology*. 2006; 36:453–466. [PubMed: 16500655]
- Hawdon JM, Schad GA. Long term storage of hook-worm infective larvae in buffered saline solution maintains larval responsiveness to host signals. *Journal of the Helminthological Society of Washington*. 1991; 58:140–142.
- Lindenmayer DB, Dubach J, Viggers KL. Geographic dimorphism in the mountain brushtail possum (*Trichosurus caninus*): The case for a new species. *Australian Journal of Zoology*. 2002; 50:369–393.
- Mackerras MJ. *Strongyloides* and *Parastrongyloides* (Nematoda: Rhabdiasoidea) in Australian marsupials. *Australian Journal of Zoology*. 1959; 7:87–104.
- Newton-Howes J, Heath DD, Shoemaker CB, Grant WN. Characterization and expression of an Hsp70 gene from *Parastrongyloides trichosuri*. *International Journal for Parasitology*. 2006; 36:467–474. [PubMed: 16469320]
- Nolan TJ, Aikens LM, Schad GA. Cryopreservation of first stage and infective third stage larvae of *Strongyloides stercoralis*. *Journal of Parasitology*. 1988; 74:387–391. [PubMed: 2454310]
- Nolan, Megyeri Z, Bhopale VM, Schad GA. *Strongyloides stercoralis*: The first rodent model for uncomplicated and hyperinfective strongyloidiasis, the Mongolian gerbil (*Meriones unguiculatus*). *Journal of Infectious Diseases*. 1993; 168:1479–1484. [PubMed: 8245532]

- Ralston, M.; Cowan, P.; Heath, D. Biological management of possums. Ministry of Agriculture and Forestry; Wellington, New Zealand: 2002. Measuring the spread of the candidate possum biocontrol vector *Parastrongyloides trichosuri*; p. 56-61.
- Spratt, DM.; Beveridge, I.; Walter, EL. Records of the South Australian Museum, Monograph Series. Vol. 1. Adelaide; Australia: 1991. A catalogue of Australian monotremes and marsupials and their recorded helminth parasites; p. 1-105.
- Viggers KL, Spratt DM. The parasites recorded from *Trichosurus* species (Marsupialia: Phalangteridae). Wildlife Research. 1995; 22:311–332.

Figure 1.

Diagrammatic representations of experimental protocols for experimental infections of sugar gliders (*Petaurus breviceps*) with *Parastrongyloides trichosuri* (time line in days). **(A)** Investigation 1, designed to assess the effect of parasite number on the onset and duration of patent infection. **(B)** Investigation 2, designed to identify the minimum infection dose of *P. trichosuri* L3i in *P. breviceps*. **(C)** Investigation 3, designed to ascertain the infectivity of cryopreserved L3i, as well as the maximum duration of patency.

Figure 2.

Fecal egg output from sugar gliders inoculated with 100 L3i (Investigation3). Points represent fecal egg counts obtained by the culture method and expressed as EPG. Points plotted as 0 EPG denote that no worms were seen in cultured feces, i.e., below the limit of detection. **(A)** Sugar glider infected with UPPU004 strain larvae. **(B)** Sugar glider infected with New Zealand strain larvae that had been cryopreserved. **(C)** Sugar glider infected with the UPPt1004 strain larvae. This animal was reinoculated twice (arrows), at 156 days post-PI with 99 L3i and at day 317 PI with 69 L3i. Weekly steroid injections were given between days 303 and 352 PI.