

# Host response of flour beetles, *Tribolium confusum* to reinfection with larval stage of *Hymenolepis nana*

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## Abstract

Adult beetles, *Tribolium confusum*, were exposed to gravid proglottides of worms, *Hymenolepis nana* obtained from an already infected mice. Fourteen days after initial infection the beetles were infected as before. Four days later they were dissected before the cysticercoids of the second infection were fully developed. The results revealed that the beetles can be infected more than once, and cysticercoids can live through the life of the beetles, there is no effective host resistance associated with a number of infections, nor is there effective interference with cysticercoids development.

**Key words:** Host response, Beetles, *Hymenolepis nana*, Cysticercoids and Reinfection.

## Introduction

The tapeworm, *Hymenolepis nana* commonly called the dwarf tapeworm, is cosmopolitan species that is one of the most common cestodes of humans in the world, especially among children (Karnaukov and Laskovenko, 1984). Beside humans, domestic mice and rats also serve as suitable hosts for *H. nana* (Ferreti *et al.*, 1981). The most common intermediate hosts capable of transmitting the larval stages of *H. nana* are arthropods such as the flour beetles *Tribolium confusum*. (Lloyd, 1998). When eggs are ingested by an intermediate host they hatch release an oncosphere (hexacanth), which penetrates the gut wall within approximately 90 minutes and develops in the body cavity into a proceroid (Anderson and Lethbridge, 1975). Some further development of the proceroid into a cysticeroid (Gibson, 1998). Cysticercoides develop to an infective stage in beetles within approximately 5.5 days (Freeman, 1983), although others have suggested a longer time period of 14 days is usual (Nakamura and Okmoto, 1993). Cysticercoids are not transmissible between beetles (Yan and Norman, 1995) and are only infective to mammalian hosts. When the infected intermediate host is ingested by a definitive host the resting cysticeroid excysts in the host's anterior small intestine and migrates to lower ileum (Henderson and Hanna, 1987) where it attaches to the wall of the intestine and mature into an adult worm. The response of insects to single or repeated infections have been little studied, and is of increasing interest. We have therefore studied here, first, the response of adult flour beetles, *Tribolium confusum*, to reinfection, and the structure and number of cysticercoids derived from both primary and secondary infection. Secondly we studied the tolerance of beetles to primary cysticeroid infection, i. e. how long cysticercoids can remain in the intermediate host.

## Materials and Methods

### 1. Parasite strain

A laboratory reference of *H. nana* was provided by Dr. Abdul-Muhsein Hameed Jasim, College of Veterinary Medicine, University of Baghdad, in two forms – eggs in phosphate buffered saline (PBS) and approximately 30 flour beetles, *Tribolium confusum*, infected with cysticeroid stage of *H. nana*. The isolate has been passaged through laboratory mice for more

than 20 years. Approximately 2000 *H. nana* eggs were inoculated into 4 – 5 week old, male Balb/ c mice.

## 2. Maintenance of the intermediate host

Adult and larval *Tribolium confusum* were kept in stock culture in an oven at a temperature of 30 °C , in jars filled with flour containing 5% brewer's yeast. Infected beetles were kept in a separate incubator under the same condition.

## 3. Infection of beetles with *H. nana* eggs

Adult beetles were starved for two days at 30 °C and then exposed to gravid proglottides of worms obtained from an already infected mouse. Beetles were allowed to feed 4 – 6 hours and were then transferred to a clean jar filled with flour. The beetles were maintained at 30 °C. Fourteen days after initial infection the beetles were starved for two days and infected as before. Four days later they were dissected before the cysticercoids of the second infection were fully developed. Control beetles were infected once in the same way and dissected four days after infection. The number of primary mature and secondary immature cysticercoids were recorded from experimental and control beetles. To determine the longevity of the cysticercoids in the beetles, three groups of beetles were taken and infected with a primary infection only, and dissected at two month intervals. The number of cysticercoids from each group was counted. The age of the beetles used in these experiments was unknown: they were at the same age, however.

## Results

### 1. Secondary infection

Cysticercoids of *H. nana* from a secondary infection were successfully grown in beetles. They were structurally normal in comparison with those from a single infection in control beetles, as shown in fig. 1 and 2. Fig. 1 shows cysticercoids of primary and secondary infection in the same beetles. It also shows the presence of two suckers and the ring of the hooks in secondary (four days old) cysticercoids, which indicate its normal structure and the normal speed of growth in comparison with the four days old control cysticercoid in fig. 2. Tab. 1 and 2 summarises counts of cysticercoids obtained from each of two successive infections. There were 54 beetles out of 82 infected with both primary and secondary infection.

### 2. Longevity of cysticercoids

Cysticercoids in primary and secondary infections can establish in beetles throughout their life. The cysticercoids can live long as six months without loss, as shown in tab. 3.

**Table 1:** Number of beetles infected with *H. nana* cysticeroids in primary and secondary infection out of 82 beetles.

Number of beetles infected in		
Primary infection	secondary infection	Primary + secondary infection
62	65	26 + 28 : 54

**Table 2:** Number of cysticeroids and range per infected beetles from primary and secondary infection of beetles out of 82 beetles.

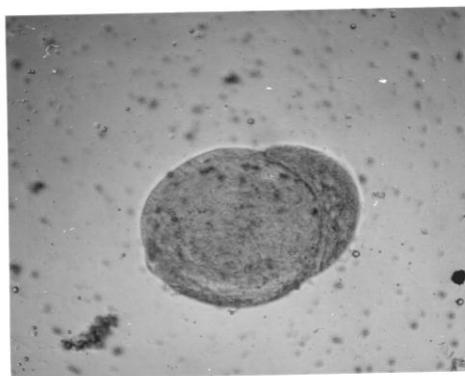
Number of beetles infected in			
	Primary infection	secondary infection	Control
Number:	955	920	889
Range:	1 – 65	1 – 52	1 - 66

**Table 3:** Number of cysticeroids after two months intervals of dissection.

	Days after infection		
	60	120	180
No. of cysticeroids in 50 beetles:	869	992	950
Range per infected beetles:	1 – 110	1 – 85	1 – 96
Percentage of infected beetles:	81%	76%	78%

**Figure 1:** Cysticeroids of a primary (14 days old) and secondary (4 days old) infection in the same beetle.





**Figure 2 :** Four days old control cysticercoid.

## Discussion

The observation on the longevity of cysticercoids and reinfection of the beetles indicate that in *T. confusum* there is no effective host resistant to a single or repeated infection. The structure, speed of development and the number developing in a superimposed infection was not reduced as a result of primary infection, at any rate within the range of cysticercoid number investigated. The cellular and humoral defence reaction of vector species also act as determinants of infection, but much has yet to be learned in the exciting new research field, including the methods adopted by the parasite to evade the vector's immune defence (Roitt, 2001). No information is available on cellular response of beetles from this study, but Cavier and Leger (1965) found that there is no cell coating of *H. nana* developing in *T. confusum*, this enables *H. nana* to persist in the environment even when conditions become unfavorable for the egg stage (Kennedy, 1983). Heyneman and Vog (1971) succeeded in reinfesting beetles three times with *H. diminuta*, *H. citteli* and *H. microstoma*. They showed that the cellular response of beetles to developing *H. microstoma* and *H. diminuta* was limited to a small number of cells which collected around the cysticercoids, although *H. citteli* was found to be covered one to three cells thick, perhaps because *T. confusum* is not a normal intermediate host for this species.

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### المستخلص

عُرِضَتْ خنَافِس الدَّقِيقِ المُحَيَّرَةِ إِلَى القِطْعِ الحَبْلِيِّ لِلدُّودَةِ المَحْرَشَفَةِ القَزْمَةِ الَّتِي تَم الحِصُولُ عَلَيْهَا عَادَةً مِنْ فَنْرَانِ مَخْمَجَةٍ. خَمَجَتِ الخَنَافِسُ بَعْدَ أَرْبَعَةِ عَشْرَ يَوْمًا مِنْ جَرَعَةِ الخَمَجِ الأَوَّلِ وَبِنَفْسِ الطَّرِيقَةِ. شَرَحَتِ الخَنَافِسُ بَعْدَ أَرْبَعَةِ أَيَّامٍ قَبْلَ وَصُولِ كَيْسَانِيَّاتِ الخَمَجِ الثَّانِي لِمَرِحْلَةِ التَّنَامِي الكَامِلِ. أَظْهَرَتِ النَتَائِجُ بِأَنَّ الخَنَافِسَ مُمْكِنٌ أَنْ تَخْمَجَ لِأَكْثَرِ مِنْ مَرَّةٍ وَأَنَّ الكَيْسَانِيَّاتِ المَذْنَبَةَ بِإِمكَانِهَا أَنْ تَبْقَى مَعَ أُسْتَمْرَارِ حَيَاةِ الخَنَافِسِ وَلَمْ يَكُنْ هُنَاكَ أَيُّ تَأْتِيرٍ لِمَقَاوِمَةِ العَائِلِ عَلَى عِدَدِ الإخْمَاجِ وَلا يَوجَدُ أَيُّ تَأْتِيرٍ فَعَالٍ عَلَى تَّنَامِي الكَيْسَانِيَّاتِ المَذْنَبَةَ فِي عَائِلِهَا.