# The Infectivity of Frozen *Gnathostoma spinigerum* Encysted Larvae in Mice

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## Abstract

The infectivity of encysted larvae of *Gnathostoma spinigerum* in mice, after being frozen in the freezer of a household refrigerator (-2 to -4 °C), was evaluated. The larvae used in the experiment, which were obtained from laboratory-infected rats and mice, were 6 months old. Those recovered from naturally infected eels were of unknown age. Each group of larvae was placed in a blockglass containing physiological saline, kept in the freezer for 1, 6, 12, 18, 24, 30, 36 or 48 hours, and fed to clean mice, 5 larvae per mouse. Both the 6-month-old and the unknown-age encysted larvae lost their infectivity completely after being frozen for 48 hours. When the larvae were rolled up inside the mice flesh and frozen at the same temperature for 48 hours, they could still infect clean mice, with an infectivity of 20.0%. It was concluded that although *G. spinigerum* encysted larvae could survive several days in the freezer of a household refrigerator they lost their infectivity only after 1-2 days. However, the mimetic infected meat containing encysted larvae resisted this freezing temperature for at least 48 hours.

**Keywords:** *G. spinigerum*, infectivity, frozen, encysted larvae, mice, eels

*Gnathostoma spinigerum*, a nematode parasite of carnivores, can cause infection in humans as an accidental host following ingestion of fish or meat which harbor advanced third-stage (infective-stage) larvae [1-2]. In Thailand, the larvae were found to naturally infect 48 species of animals that serve as their second intermediate hosts or paratenic hosts [1, 3-4]. The infectivity rates, of *G. spinigerum* unencysted larvae (3 weeks old) and encysted larvae (8 weeks, 6 months and 1 year old) when fed to mice, which had been obtained from laboratory mice infected with cyclops harboring early third-stage larvae, were 60.0%, 61.6%, 66.0% and 69.3%, respectively. The infectivity rates for mice with encysted larvae (unknown age) that had been obtained from naturally infected eels was 62.0% [5]. The larvae were found to be very resistant not only to various kinds of chemicals and drugs [1, 6-7] but also irradiation and environmental conditions [8-10]. Encysted larvae could survive 12 days in physiological saline at room temperature (27 °C), 22 days in the refrigerator (4 °C) and 12 days in the freezer (-4 °C) [9]. Although the encysted larvae could survive several days in the household refrigerator and freezer, no data were gathered concerning their infectivity after being kept in these conditions.

*G. spinigerum* encysted larvae used in the experiment were obtained from 2 sources: laboratory-infected rats and mice, and naturally infected eels. The larvae that were maintained in rats and mice were 6 months old, while those from eels were of unknown age. The infected rats and mice were killed, their livers and muscles were cut into small pieces and pressed between two thick glass plates (a compression technique), and the larvae were collected and placed in a blockglass containing physiological saline. They were divided into groups of 50 larvae (25 larvae in some groups) and then kept in the freezer of

a household refrigerator (-2 to -4 °C) for 1, 6, 12, 18, 24, 30 or 48 hours. Each group of larvae were fed to mice, 5 larvae per mouse, using a stomach tube connected to a syringe. As for the control group, the 6-month-old larvae were fed to mice without prior freezing. These infected mice were killed 3 weeks thereafter and the compression technique was performed to recover all the existing larvae. The infectivity percentage was calculated. In the case of the naturally infected eels, only the liver was used for the recovery of encysted larvae. The recovered larvae were frozen and fed to mice as described for the larvae that were obtained from the infected rats and mice. Some other 6-month-old larvae were divided into groups of 50 or 25 larvae. They were rolled up inside the flesh of mice, so as to imitate the infected meat, into a flat ball (3.5 cm thick, 6 cm in diameter) and put in a container. The meat with the larvae inside was kept in the freezer at about -4 °C for 6, 18, 24, 30, 36 or 48 hours. The frozen larvae were fed to clean mice, 5 larvae per mouse. Autopsy of these infected mice for larval recovery was also done at 3 weeks post-infection.

The infectivity rates of *G. spinigerum* encysted larvae, which had been obtained from laboratory-infected rats and mice, and from naturally infected eels, after being frozen at about -4 °C are shown in Table 1. It was apparent that the longer the time the larvae (from both sources) were frozen, the lower the percentage of infectivity. However, the decrease in the total number of larvae recovered did not depend upon the increase in the time they had been frozen (Spearman Rank Correlation Test, $p > 0.05$). The total number of recovered larvae in the groups that were obtained from rats and mice, was significantly lower than the control when they had been frozen for at least 12 hours ($\chi^2$ = 10.5090, P-value = 0.001) and as short as 6 hours ($\chi^2$ = 10.6667, P-value = 0.001) in the groups that were obtained from eels. The encysted larvae from both sources lost their infectivity

**Table 1 Infectivity of *G. spinigerum* encysted larvae after being frozen in the freezer of a household refrigerator (-2 to -4 °C) and fed to mice.**

| Frozen time (hour) | Encysted larvae from rats and mice[a] | | | | Encysted larvae from eels[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of mice (5 larvae /mouse) | Total no. of larvae infected[a] | Total no. of larvae recovered[c] | Infectivity (%) | No. of mice (5 larvae /mouse) | Total no. of larvae infected[b] | Total no. of larvae recovered[c] | Infectivity (%) |
| 0 (control) | 10 | 50 | 37 | 74.0 | 10 | 50 | 38 | 76.0 |
| 1 | 10 | 50 | 37 | 74.0 | 10 | 50 | 33 | 66.0 |
| 6 | 10 | 50 | 28 | 56.0 | 10 | 50 | 22 | 44.0 |
| 12 | 10 | 50 | 21 | 42.0 | 10 | 50 | 9 | 18.0 |
| 18 | 10 | 50 | 10 | 20.0 | - | - | - | - |
| 24 | 10 | 50 | 0 | 0 | 10 | 50 | 0 | 0 |
| 30 | 5 | 25 | 1 | 4.0 | - | - | - | - |
| 48 | 5 | 25 | 0 | 0 | 5 | 25 | 0 | 0 |

[a] Encysted larvae of *G. spinigerum* at the age of 6 months maintained in laboratory rats and mice.
[b] Encysted larvae of *G. spinigerum* (unknown age) collected from naturally infected eels.
[c] Three weeks post-infection.

**Table 2 Infectivity of *G. spinigerum* encysted larvae.**

| Frozen time (hour) | No. of mice (5 larvae/mouse) | Total no. of larvae infected[a] | Total no. of larvae recovered[b] | Infectivity (%) |
|---|---|---|---|---|
| 0 (control) | 10 | 50 | 35 | 70.0 |
| 6 | 10 | 50 | 30 | 60.0 |
| 18 | 10 | 50 | 23 | 46.0 |
| 24 | 10 | 50 | 24 | 48.0 |
| 30 | 10 | 50 | 19 | 38.0 |
| 36 | 5 | 25 | 8 | 32.0 |
| 48 | 5 | 25 | 5 | 20.0 |

[a] Encysted larvae of *G. spinigerum* at the age of 6 months maintained in laboratory rats and mice. The larvae were wrapped inside the flesh of mice, frozen at -2 to -4 ˚C and then fed to clean mice.
[b] Three weeks post-infection.

completely after being frozen for 48 hours. However, at the same temperature and frozen time, the larvae could still infect clean mice (infectivity = 20.0%) if they had been previously rolled up inside the flesh of the mice (Table 2).

Rojekittikhun *et al* [5] reported that the infectivity (in mice) of advanced third-stage larvae of *G. spinigerum* at the age of 3 weeks to 12 months, obtained from laboratory-infected mice, was 60.0%-69.3%, while from naturally infected eels (unknown age) it was 62.0%. In the present study, slightly higher infectivities were obtained: 74.0% for the larvae (6 months old) from rats and mice and 76.0% for the larvae (unknown age) from eels. Setasuban *et al* [8] found that *G. spinigerum* encysted larvae could survive 12 days and 9 days at -4 ºC and -9 ºC, respectively; and over 8 days if they were mixed in minced meat salads and kept in the refrigerator (4 ºC). In the present study, all encysted larvae lost their infectivity completely after being frozen at -4 ºC only for 2 days. However, at the same temperature and frozen time, if they were previously rolled up inside the flesh of mice into a flat ball of about 3.5 cm in thickness and 6 cm in diameter, their infectivity was still 20.0%. Setasuban *et al* [8] also concluded that humans were still not safe from gnathostomosis if they ate improperly cooked fish/meat previously kept in a refrigerator for even 3 weeks. It would be safe if those foodstuffs were boiled (100 ºC) or steamed (110 ºC) for at least 5 or 9 minutes, respectively.

In conclusion, raw fish or meat may be edible and may not be the cause of gnathostomosis if kept in a deep freezer (-20 ºC) for at least 9 days, however, the safest way to avoid the infection is to cook them until well done.

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