[Jpn. J. Parasitol., Vol. 44, No. 4, 321–324, August, 1995]

Research Note

The Lethal Effect of Freezing on Spirurid Nematode Larvae in Firefly Squids, *Watasenia scintillans*

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(Accepted June 6, 1995)

Key words: Spirurina; firefly squids; freezing; creeping; larva migrans.

"Spiruroid type X nematode larvae" (Hasegawa, 1978), a causative agent of creeping eruption and ileus in humans, were recovered from the digestive organs of: sandfish, *Arctoscopus japonicus* (Otsuru *et al.*, 1974); Alaska pollack, *Theragra chalcogramma* (Hasegawa, 1978); sagittate calamari, *Ommastrephes soloanei pacificus* (Okazawa *et al.*, 1993); and firefly squids, *Watasenia scintillans* (Ando *et al.*, 1992). Of these hosts, firefly squids are often eaten raw by humans. Most cases in which humans are found to have ingested "type X" larvae are reported from March to July (Ando *et al.*, 1992). This period is consistent with the season for fishing firefly squids. In addition, some patients ate raw firefly squids just before the onset of creeping eruption. Therefore, some investigators have suggested that this type of larvae is chiefly responsible for creeping eruption and ileus (Fujihira *et al.*, 1992; Kagei *et al.*, 1992; Takahashi *et al.*, 1992; Hasegawa *et al.*, 1993; Shinozaki *et al.*, 1993).

Recently, the consumption of raw firefly squids has increased in Japan. Finding a way to kill the spirurid nematode larvae in firefly squids would prevent a related increase in the number of cases of creeping eruption and ileus. In a previous report, we revealed that larvae *in vitro* died in a 14% solution of sodium chloride within a day (Akao *et al.*, 1994). However, this procedure is not suitable for mass preparation. We therefore examined the effect of freezing on the "spiruroid type X" larvae.

In a preliminary study, 15 trays, each containing 21 firefly squids (7 rows × 3 columns), were placed side by side in a walk-in freezer (55.4 m^3) for a designated period. The surface temperature of the frozen firefly squids was monitored with a digital thermometer (SK-2000MC, Iuchi, Osaka, Japan). The surface temperature of firefly squids usually required 30 minutes to fall to –32°C; freezing times were measured and expressed from the point at which this temperature was reached. Only the squids' digestive organs (the esophagus, the stomach, and the intestine) were harvested. After digestion with artificial gastric juice (0.5% hydrochloric acid and 0.7% pepsin, pH 1.5), the larvae were recovered by the method of Okazawa *et al.* (1993). Table 1 shows the effects of freezing on the survival rate of the larvae. "Spiruroid type X" larvae were still alive when the firefly squids were kept at –32°C for 40 minutes. However, none of the larvae survived in squids kept frozen for 60 minutes or longer. These experiments were carried out on 23 and 30 June 1994.

On the basis of the preliminary study, we con-

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Table 1 Lethal effect of freezing on spiruroid type X larvae

	No. of firefly squids examined	Time after freezing*	Defrosted	No. of larvae recovered	No. of larvae alive
Exp. I	315	40	Spontaneously	12	2
Exp. II	315	60	Spontaneously	8	0
Exp. III	315	90	Spontaneously	5	0
Exp. IV	315	90	40°C, 10 sec in water bath	5	0

*Surface temperature of firefly squids was kept in –32°C.

ducted a further investigation on 27 February 1995, using a technique suitable for commercial preparation. Firefly squids captured in the Toyama Gulf were placed on a tray, as in the preliminary experiments, then kept in a carbon dioxide gas circulating freezer (C. P. F. Quick Freezer®, type: G-1, D. M. L., Co. Ltd., Kanazawa, Japan). We developed this apparatus specifically for this study. The surface and core temperatures of the firefly squids were measured simultaneously during the experiment. Figure 1 shows the relationship between the surface and core temperatures of the firefly squids. Carbon dioxide was supplied for 20 minutes and the surface temperature became –32°C within the first 8 minutes, but the core temperature was still –7.4°C. It took 12 minutes when the surface temperature was –40°C. The core temperature fell to –32°C after 18 minutes. The firefly squids were then kept for 30 minutes after the surface temperature became –40°C. This means that the core temperature was maintained below –32°C for at least 20 minutes. As a control in this experiment, we also tried to recover the larvae from 1234 unfrozen firefly squids captured on the same day and at the same place. From the raw control, 5 larvae of "spiruroid type X" were recovered, all living; from the 325 frozen squids, 11 larvae were recovered, all dead (Fig. 2).

These results suggest that keeping firefly squids frozen in a deep freezer (below –40°C) for 40 minutes could reduce the risk of infection from "spiruroid type X" larvae. During the freezing process, it is important to keep the core temperature of the squids below –32°C rather than simply maintaining their surface temperature below –32°C. Boiled firefly squids may be eaten safely. However, as long as the Japanese custom of eating raw fish remains unchanged, freezing squids is an alternative way to treat them to reduce the risk of infection.

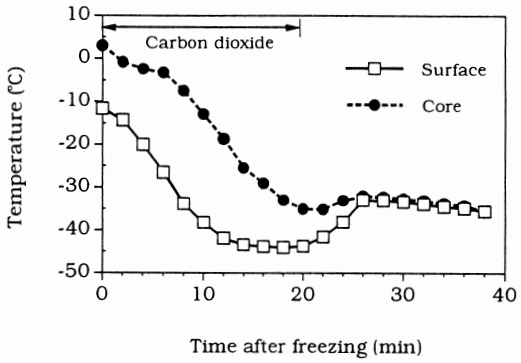

Fig. 1 The relationship between the surface and core temperatures of firefly squids frozen with carbon dioxide. Carbon dioxide was supplied for 20 minutes.

Acknowledgment

We wish to thank Dr. Satoshi Shinonaga, Department of Medical Zoology, Tokyo Medical & Dental University for his assistance in preparation of the photographs. We also thank Ms Michiyo Katayama and Ms Keiko Numazaki for their technical assistance.

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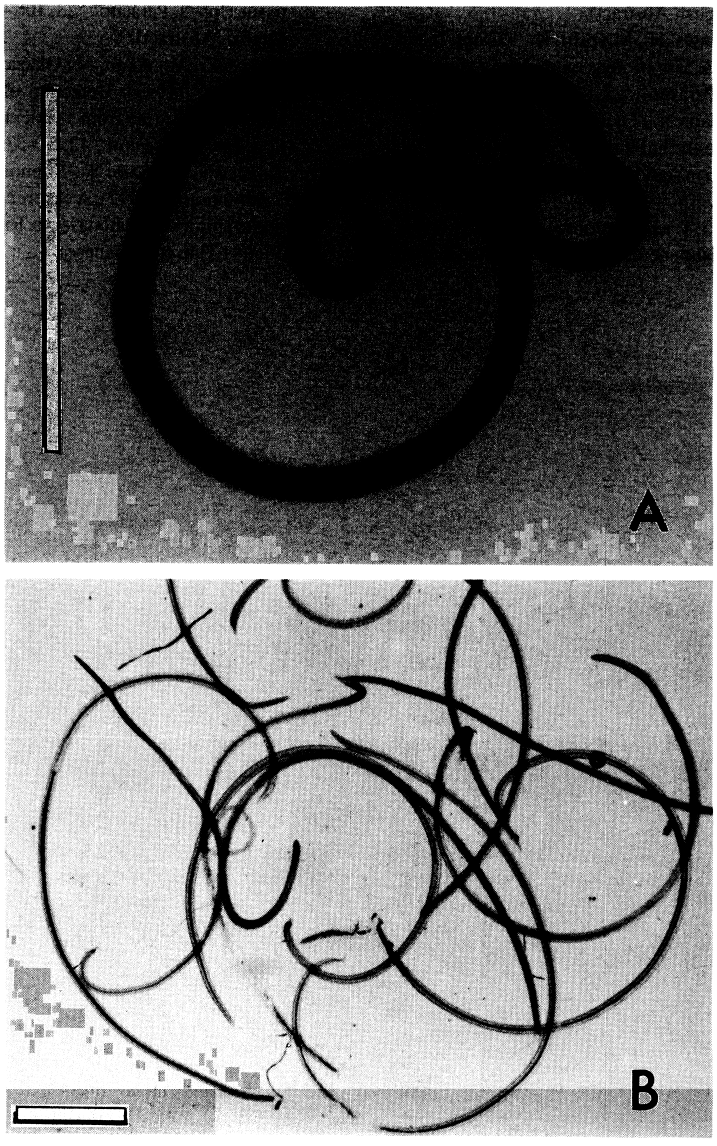

Fig. 2 Spiruroid type X larvae recovered from raw (A) or frozen (B) firefly squids. The bar indicates 1.5 mm.

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