PINEAPPLE JUICE FOR DIGESTION OF SWAMP EEL VISCERA FOR HARVESTING INFECTIVE-STAGE LARVA OF *GNATHOSTOMA* SPP

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Abstract. Third-stage larvae were used as antigen in the diagnosis of gnathostomiasis in Western blot analysis. Normally, the larvae were obtained from digestion of eel's liver (*Fluta alba*) by the enzyme pepsin. We used pineapple juice (*Ananus comosus)* instead of enzyme pepsin in harvesting *Gnathostoma spinigerum* third-stage larvae. The difference in recovered larvae numbers, between pineapple juice and pepsin, were not statistically significantly different ($p>0.05$). The larvae from pepsin and pineapple juice digestion were cultivated on BME for 7 days; the survival rates were not significantly different ($p>0.05$). Thus, pineapple juice is another enzyme of choice for recovering *Gnathostoma spinigerum* third-stage larvae.

INTRODUCTION

In Thailand, *Gnathostoma spinigerum* is the major causative agent of human gnathostomiasis (Daengsvang, 1980). The disease has also been reported in Japan, China, Malaysia, Indonesia, Philippines, Israel, and other areas where raw or pickled fish are consumed (Akahane *et al*, 1994; Ando *et al*, 1998; Rojekittikhun *et al*, 1998). Clinical manifestations of human gnathostomiasis are caused by the migration of immature larvae (L3s) as painful, pruritic swelling (cutaneous larva migrans). Migration in other tissues (visceral larva migrans) can result in severe manifestations. Diagnosis should be made as soon as possible in the latter case. At present, Western blot analysis is used as the gold standard, and antigen from third-stage larvae were used as specific antigen (Tapchaisri *et al*, 1991). However, acid pepsin is both expensive and must pass through demanding importation controls. An alternative substance that can be used instead of acid pepsin is required. Pineapples have been known to have enzymes with proteolytic effects (Kelly, 1996; Melendo *et al*, 1996). Recently, Prawang *et al* (2002) proposed pineapple juice as an alternative digestive agent for harvesting metacercaria of *Opisthorchis viverrini* from fishes. This study investigated the feasibility of using pineapple juice for digestion of swamp eel viscera for harvesting infective stage larva of *Gnathostoma* spp.

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MATERIALS AND METHODS

The pineapples, *Ananus comosus*, were purchased from a local market at Klong Toey in Bangkok. The crude pineapple juice extract was prepared using a juice extractor (National Model MJ 68 M). All eel livers were separated from other viscera and washed with tap water, then equally divided into two parts. One part (10 grams) was digested with pepsin (1.5% pepsin in water, adjusted to pH 2.0 with 1M HCl) 80 ml. Digestion was performed in a water bath (37ºC, 4 hours) with frequent agitation. The digested liver was washed repeatedly by sedimentation with 0.85% NaCl solution. Gnathostoma L3s were identified according to the characterization described by Daengsvang (1981) and counted by stereomicroscopy. The second part of the eel's liver was digested by pineapple juice in the same fashion as for pepsin. Digestion was performed in a water bath as mentioned earlier, Gnathostoma L3s were identified and

counted by stereomicroscopy. Some larvae were chosen for cultivation on BME culture medium (Gibco, Grand Island, NY, USA). Preparation of this medium was done by using one pack of BME powder (9.2 g) dissolved in 1 liter of distilled water and adjusted to pH 7.2-7.4 by $NaHCO_3$. Sterilization was done by 0.45 μm millipore membrane filtration.

RESULTS

The lavae recovered from the eel's liver were divided into encysted and excysted forms. Some excysted larvae were alive and some were dead. With the equivalent weight of eel's liver used, the acid pepsin digestion yielded much higher numbers of larvae than pineapple, at 182 and 158, respectively. The percent recovered is shown in Table 1. Comparison of each group by one-way ANOVA found that there was no significant difference in both solutions ($p > 0.05$). After 7 days on BME medium, almost all larvae survived (93 and 95%, respectively). There were no significant differences between the two survival rates ($p > 0.05$).

Table 1
Number of larvae obtained from pineapple juice and pepsin digestion.

No. of test	1.5% Acid pepsin			Pineapple juice		
	Encysted	Excysted		Encysted	Excysted	
		Active	Dead		Active	Dead
1	0	21	0	5	8	0
2	1	24	4	2	1	0
3	0	17	7	0	9	1
4	1	7	0	3	12	1
5	22	12	0	29	24	3
6	7	16	11	0	16	10
7	1	14	0	0	13	2
8	0	13	2	0	16	1
Total (%)	32 (17)	124 (69)	24 (14)	39 (25)	99 (63)	18 (12)

$p > 0.05$ by one-way ANOVA

Table 2
Survival of 3rd stage larvae on BME medium.

No. of tests	Number of larvae on 1st day		Number of larvae on 7th day	
	1.5% Acid pepsin	Pineapple juice	1.5% Acid pepsin	Pineapple juice
1	20	11	15	10
2	20	10	18	9
3	8	14	8	13
4	33	47	30	45
5	5	5	5	5
6	12	12	12	12
7	9	9	9	9
8	10	10	9	9
9	15	15	15	15
10	20	20	20	19
Total (%)	152	153	141 (93)	146 (95)

$p > 0.05$ by one way ANOVA

DISCUSSION

The freshly prepared pineapple juice (crude) and 1.5% acid pepsin solution yielded similar numbers of healthy third-stage larvae, even when kept for 7 days. The seven-day period may be useful for those wanting to cultivate the parasite for excretory antigen. Pineapple juice has proteolytic properties equal to those of acid pepsin solution. The proteolytic action of pineapple juice arises from its cysteine proteinase enzymes. Rowan *et al* (1990) reported at least four distinct cysteine proteinases, *ie* bromelain (fruit and stem), anamain and comasain. In pineapple juice, fruit bromelain FA2 is the main proteinase component (Yamada *et al*, 1976). This study found that crude pineapple juice is an alternative to acid pepsin, not only for the recovery of third-stage larvae of *Gnathostoma spinigerum* but also for harvesting metacercariae (Prawang *et al*, 2002). Dried pineapple juice would be the best way to preserve all contents. Prawang *et al* (2002) proved that juice frozen for 30 days yielded the same results as freshly-prepared juice. Dried pineapple juice would be meaningful for commercial products, not only for use in digestion, and can be used as a soft drink. In conclusion, pineapple juice confers several advantages, such as year-round availability, easy and inexpensive preparation, and non-toxicity.

REFERENCES

Ando K, Tanaka H, Taniguchi Y, *et al*. Two human cases of gnathostomiasis and discovery of a second intermediate host of *Gnathostoma nipponicum* in Japan. *J Parasitol* 1998; 74: 623-7.

Akahane H, Lamothe-Argumedo R, Martinez-Cruz JM, *et al*. Amorphological observation of the advanced third - stage larvae of *Gnathostoma. Jpn J Parasitol* 1994; 43: 18-22.

Daengsvang S. A monograph on the Genus *Gnathostoma* and Gnathostomiasis in Thailand. Tokyo: Southeast Asian Medical Information Center, International Medical Foundation of Japan, 1980: 1-85.

Daengsvang S. Gnathostomiasis in Southeast Asia. *Southeast Asian J Trop Med Public Health* 1981; 12: 319-32.

Kelly GS. Bromelain: a literature review and discussion of its therapeutic applications. *Alt Med Rev* 1996; 1: 243-57.

Melendo JA, Beltran J, Jaime I, Sancho R, Roncales P. Limited proteolysis of myofibrillar proteins by bromelain decreases toughness of coarse dry sausage. *Food Chem* 1996; 57: 429-33.

Prawang T, Sukontason K, Sukontason KL, Choochote W, Piangjai S. Pineapple juice as an agent for the digestion of fish prior to the harvesting of metacercariae. *Southeast Asian J Trop Med Public Health* 2002; 33 (suppl 3): 99-102.

Rojekittikhun W, Pubampen S, Hiranyachattada P, *et al*. A suvey on the infective larvae of *Gnathostoma spinigerum* in fresh water fish sold in the local markets of Bangkok. *J Trop Med Parasitol* 1998; 12: 7-12.

Rowan AD, Buttle DJ, Barrett AJ. The cysteine proteinases of the pineapple plant. *Biochem J* 1990; 266: 869-75,

Tapchaisri P, Nopparatana C, Chaicumpa W, Setasuban P. Specific antigen of *Gnathostoma spinigerum* for immunodiagnosis of human gnathostomiasis. *Int J Parasitol* 1991; 21: 315-9.

Yamada F, Takahashi N, Murachi T. Purification and characterization of a proteinase from pineapple fruit, fruit bromelain FA2. *J Biochem (Tokyo)* 1976; 79: 1223-34.