THE EXSHEATHMENT OF *NECATOR AMERICANUS* INFECTIVE LARVAE

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Abstract. Infective 3rd-stage larvae of *Necator americanus* were treated with human sweat under various conditions, and compared with human serum, 1.5% saline solution, and distilled water. The infective larvae were observed under inverted microscopy. The highest percentage (14.0%) of the exsheathed larvae was found in human sweat after 2 hours' incubation at 37ºC. The proportion of exsheathed larvae in human sweat was significantly different from human serum ($p<0.001$), 1.5% saline solution ($p<0.001$), and distilled water ($p<0.001$). This may reflect the effect of human sweat on the process of skin penetration by *Necator americanus* larvae.

INTRODUCTION

Necator americanus is a human pathogen that causes hookworm disease by active penetration of host skin by infective larvae. Infective 3rd-stage larvae (L_3) of this genus generally exhibit 3 patterns of migratory behavior (Hotez *et al*, 2004). They enter the epidermis and migrate laterally to produce cutaneous larva migrans. From the skin, they move to the viscera and display visceral larva migrans, or develop into adults in the intestine to reveal human hookworm infection.

During the invasion phases, the infective 3rd-stage larvae may be exposed to human sweat when they penetrate human skin or contact human serum after reaching the viscera. Although the behaviors of larvae crawling on surfaces and penetrating the host have already been described (Haas *et al*, 2005), the relevance of human serum and human sweat to larval exsheathment has not been determined. The aim of this study was to analyze the exsheathment of *Necator americanus* infective larvae in human sweat, human serum, 1.5% saline solution, and distilled water under various conditions. An understanding of the host factors affecting larval exsheathment is important for preventing and controlling hookworm transmission.

MATERIALS AND METHODS

Parasites

Fecal samples with hookworm eggs were concentrated by gravity sedimentation. The fecal pellets were cultured for 3rd-stage larvae by Harada-Mori filter-paper-strip method, as described by Garcia and Ash (1979). The cultured larvae were identified microscopically to species and kept in distilled water at room temperature before use. Individual larvae were used within one week post-harvest.

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Human sweat and human serum

Human sweat was collected from volunteers after heavy exercise and used immediately. Human serum was obtained via routine venipuncture at the Center of Medical Laboratory Service, Faculty of Medical Technology, Mahidol University.

Exsheathment of *Necator americanus* infective larvae

The larvae were harvested and placed in 15 ml tube containing 12 ml of distilled water. The solution was centrifuged at 200g for 5 minutes and the supernatant removed, leaving a volume of 2 ml in the bottom of the tube. The larvae in a 20 µl aliquot were counted under a microscope and the volume adjusted for a concentration of approximately 20 larvae/100 µl.

Approximately 10 live and active larvae (in 50 µl of water) were added to each well of a 96-well microtiter plate, and kept at room temperature for 30 minutes. All larvae moved to the bottom of the microtiter plate and then 30 µl of the supernatant was removed. In this study, human serum, human sweat, 1.5% saline solution, and distilled water were used to assess hookworm-larva exsheathment. Each medium, at 180 µl volume, was added to 2 rows of a microtiter plate that contained approximately 10 live and active larvae per well. Assays were performed in 2 microtiter plates. One plate was held at room temperature, while the other was incubated at 37ºC. Larval exsheathment in individual wells was observed under an inverted microscope after 1- and 2-hours' incubation.

Statistical analysis

For all assays, the numbers of exsheathed larvae was expressed as a percentage of the total number of larvae per well. Data were analyzed by chi-square test.

p-values were calculated using Epi-Info version 6.0; statistical significance was set at $p < 0.001$.

RESULTS

Identification of hookworm

The Harada-Mori filter-paper-strip method was used to collect and enable species identification of hookworm larvae using a light microscope. All harvested larvae showed marked striations on their sheaths, especially at the posterior end. By morphology, the hookworm larvae were identified as *Necator americanus*.

Exsheathment of *Necator amercanus* infective larvae

To determine the percentage exsheathment of hookworm infective larvae in 4 types of media, *in vitro* assays were conducted; the results are shown in Table 1. Larval exsheathment (Fig 1) occurred quite often after extended incubation of 1-2 hours, especially in human sweat. *Necator americanus* larvae exsheathed increasingly at 37ºC in human sweat and human serum. The highest percentage (13.9%) of exsheathed larvae was found in human sweat when incubated at 37ºC for 2 hours (Fig 2). Chi-square analysis showed the proportion of exsheathed larvae was significantly higher in human sweat than human serum ($p<0.001$), in 1.5% saline solution ($p<0.001$), and distilled water ($p<0.001$).

DISCUSSION

Previous studies have suggested that exsheathment of hookworm larvae occurs upon skin penetration; however; various studies noted some controversy, *ie*, that sheathed larvae may also successfully penetrate (Goodey, 1925; Mathews, 1972, 1975). Although our results did not resolve whether exsheathment occurs at the point of entry, this study showed that human

Table 1
Numbers of *Necator americanus* exsheathed larvae in 4 media after incubation at 25ºC and 37ºC for 1 hour and 2 hours.

Media	Temperature (ºC)	Incubation time (hour)	Numbers of exsheathed larvae (Total numbers of larvae)
Distilled water	25	1	6
		2	12
			(n=463)
	37	1	3
		2	6
			(n=478)
1.5% saline solution	25	1	7
		2	15
			(n=439)
	37	1	6
		2	12
			(n=417)
Human sweat	25	1	18
		2	24
			(n=489)
	37	1	32
		2	61
			(n=436)
Human serum	25	1	3
		2	7
			(n=435)
	37	1	9
		2	15
			(n=454)

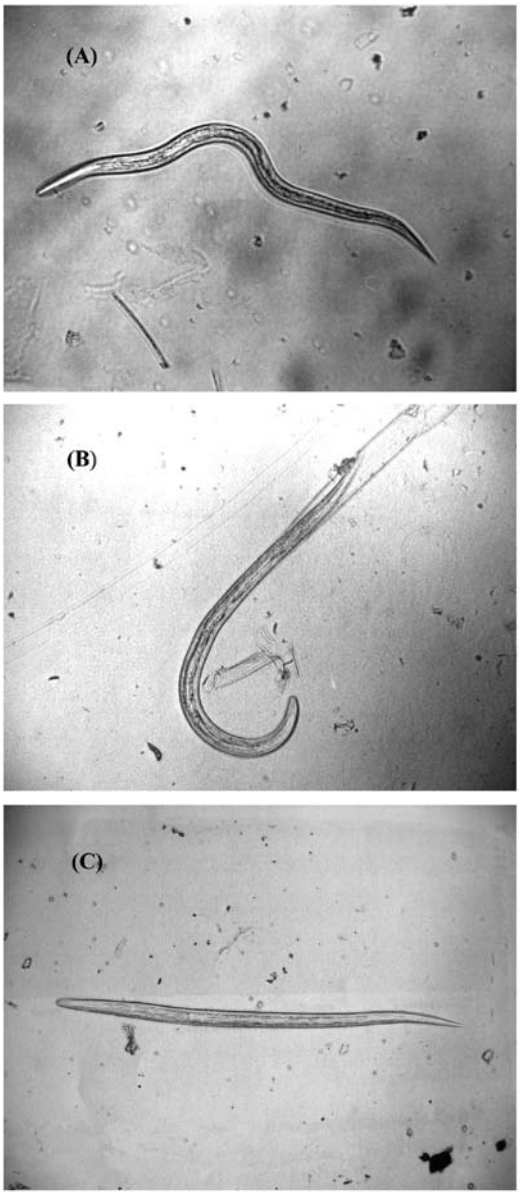

Fig 1- Exsheathment of *Necator americanus* infective larva (A) before exsheathment; (B) during exsheatment; (C) after exsheathment.

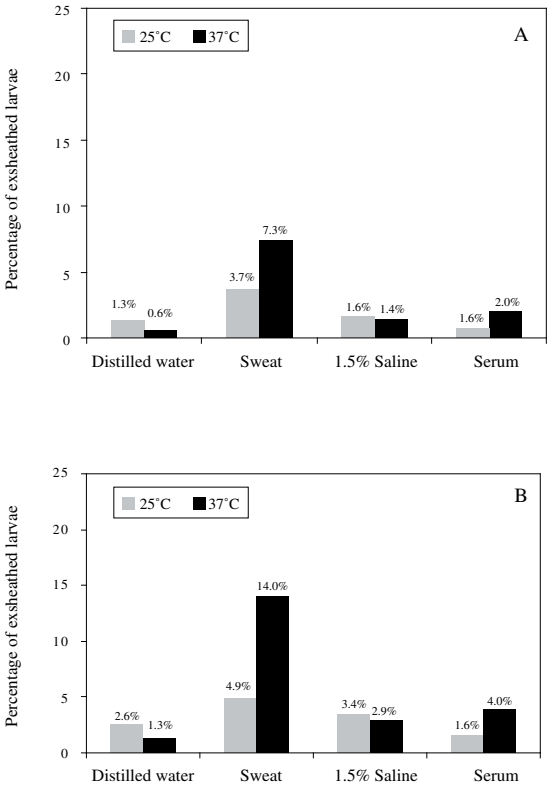

Fig 2- Percentage of *Necator americanus* exsheathed larvae after incubation in distilled water, human sweat, 1.5% saline solution, and human serum at 25ºC and 37ºC for (A) 1 hour, (B) 2 hours.

sweat is likely to be an important contributory factor for larval exsheathment of *Necator americanus*.

Since human sweat contains water and a few minerals (*ie*, sodium, calcium), we compared larval exsheathment in human sweat and 1.5% saline solution. The results showed that exsheathment mainly occurred in human sweat rather than 1.5% saline solution, human serum, or distilled water. These findings suggest that sodium is unlikely to be crucial for larval exsheathment. It is possible that the pH of human sweat, which is usually acidic (between pH 6.0- pH 6.3), compared with human serum and distilled water, may mediate larval exsheathment. Further study is required to demonstrate which components of human sweat result in larval exsheathment, and whether other species of hookworm could exsheath in human sweat.

In conclusion, the results indicate the importance of human sweat in achieving larval exsheathment by *Necator americanus* infective larvae, which may be required for either penetration of the host skin or development of hookworms.

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