FACTORS AFFECTING THE HATCHING OF HUMAN PINWORM OVA

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Abstract. Parasite life-history traits reflect past environmental and host selective pressure that act to produce strategies that maximize successful transmission. Pooled human pinworm eggs were pretreated with 0.9% NaCl, acid digestive enzyme, and alkaline solutions (pH 9.0) and then incubated in 0.9% NaCl at room temperature and 37˚C both with and without 5% CO_2. Eggs pretreated with both acid and base had the same hatching pattern, which was markedly different to that of the untreated eggs. At room temperature (RT), hatching of the pretreated eggs occurred on the first day and reached its peak rate (>90%) on day 3; at 37˚C hatching occurred on the second day and was more than 80% by day 5. Hatching of the untreated eggs was evident on day 2 at RT and between days 3-5 at 37˚C although in smaller numbers (<20%). The CO_2 did not affect the hatching of larvae. The larvae could survive after hatching in 0.9% NaCl for 2 and 4 days at 37˚C and 25˚C, respectively. The present investigation gives a different information that human pinworm ova can hatch into larvae with or without exposure to acid digestive enzyme or alkaline solutions.

INTRODUCTION

Human enterobiasis has been known since around 3000 BCE; the prevalence of the infection has changed little since the 1930s and, with no means limited to the tropics (Cook, 1994; Russell, 1991; Aroujo *et al,* 1985). Prevention remains difficult, and reinfection is common. Parasite life-history including the resistance and viability of eggs of human pinworms to desiccation, media, chemicals and at different temperatures were investigated by previous authors many years ago (Sondak, 1935; Jones and Jacobs, 1940; Hulinska, 1974; Beaver *et al,* 1984). It was found that the gastric digestive enzymes stimulated the release of larvae and hatching in the duodenum(Cook, 1994; Jones, 1988). We studied the factors influencing the hatching of pinworm eggs in a laboratory experiment that resemble natural conditions of the egg exposure by pH and temperature.

MATERIALS AND METHODS

Parasite egg collection

Eggs were obtained by swabbing the anal folds of elementary schoolchildren in Bangkok where the children with massive enterobiosis were treated. The swabs were placed in screw-cap test tubes with normal saline at room temperature and transported to the laboratory within 2 hours. Eggs for experimental purpose were pooled from the positive samples and washed in physiological saline.

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Experimental design

The pooled pinworm eggs were divided into three groups, each of 90 eggs.

Group I: Untreated eggs in NSS and was used as the control.

GroupII: Eggs were treated with an acid digestive solution (0.1% pepsin in 0.7% conc HCl) at 37˚C for 30 minutes.

Group III: Eggs were treated with 0.1M PBS pH 9.0 solution at 37˚C for 30 minutes.

After treatment, the eggs were washed three times with physiological saline and incubated with NSS plus antibiotics (streptomycin and penicillin) in a 24-well tissue culture plate at room temperature (RT); at 37˚C with and without 5% CO_2. Thirty eggs were used for each condition and experiments were conducted in triplicate. Hatching was observed and counted daily by inverted microscopy for 10 days.

Student's paired *t*-test was used for statistical analysis.

RESULTS

Affect on egg hatching

Of the untreated eggs, 6.8% lavae hatched on day 4 at RT and 2.4-17.5% hatched between days 5-10 (Fig 1). At 37˚C, 13.8-17.2% hatched between days 3-5 (Figs 2-3).

Eggs pretreated with both acid and base had the same hatching pattern (Figs 1-3) which was not statistically significant different and CO_2 showed no effect. At RT, hatching occurred on the first day and reached the peak rate of 91.8-96% between days 3-10 (Fig 1). At 37˚C hatching was evident on day 2 and

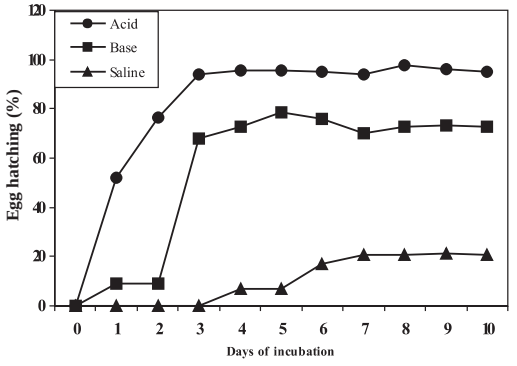

Fig 1- Effect of pretreatment with acid pepsin solution (●), basic solution (■), and untreated in NSS (▲) on the hatching of *Enterobius vermicularis* larvae which were incubated at room temperature. The difference in the hatching curve between the groups that had been pretreated with acid and base after day 5 was not significant.

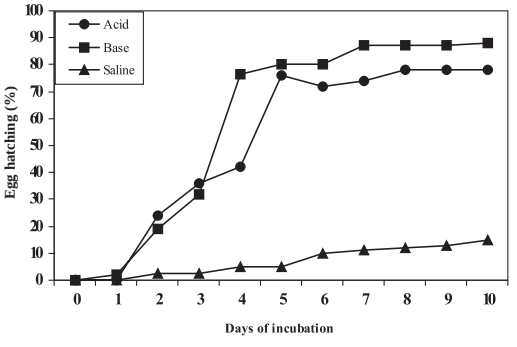

Fig 3- Effect of pretreatment with acid pepsin solution (●), basic solution (■), and untreatment in NSS (▲) on the hatching of *Enterobius vermicularis* eggs which were incubated at 37˚C. The difference in the hatching curve between the groups that had been pretreated with acid and base was not significant.

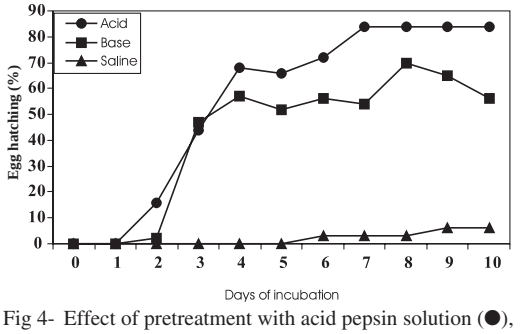

Fig 4- Effect of pretreatment with acid pepsin solution (●), basic solution (■), and untreated in NSS (▲) on the hatching of *Enterobius vermicularis* eggs which were incubated at 37˚C with 5%CO_2. The difference in the hatching curve between the groups that had been pretreated with acid and base was not significant.

reached its peak of 80-84% between days 4-10 (Figs 2-3).

Viability of the hatching larvae

The hatched larvae were able to survive in 0.9% NaCl solution for 1-4 days at RT and for 1-2 days at 37˚C; the presence or absence of 5% CO_2 made no difference to these survival times.

DISCUSSION

In our experiment, the egg hatching could occur in saline even in low number; this may due to the operculum-like of the egg shell that makes them able to hatch without chemical stimulation as occured around the perianal area and caused re-infection (Hulinska and Hulinsky, 1973). The acid and base can stimulate egg hatching in a shorter time and in higher numbers which may due to the outer proteinaceous and chitin elements of the egg shell. These elements caused them to hatch when exposed to gastric and pancreatic juices when they reached the stomach and duodenum (Cook, 1994; Russell, 1991; Leng and Liu, 1982).

Since the eggs recovered by anal swabs are of various stages of development, we found that larvae hatched at different times or simply failed to hatch (Hulinska, 1974).

At room temperature, hatching occurred earlier and in higher numbers than those at 37˚C; this was true for both treated and untreated eggs, as claimed in a previous study; the mechanism for this has not been elucidated (Hulinska, 1974).

The larvae could survive after hatching for 2 and 4 days which may be long enough for them to find a suitable place for further development and molting.

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