HOST-FINDING BEHAVIOR OF *STRONGYLOIDES STERCORALIS* INFECTIVE LARVAE TO SODIUM CATION, HUMAN SERUM, AND SWEAT

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Abstract. The host-finding behavior of *Strongyloides stercoralis* infective larvae was examined by *in vitro* agarose assay method. As human body fluid contains 0.85% (*ca* 0.15 molar) NaCl, various concentrations of sodium chloride, from 0.5M to 0.01M (7 steps), were examined. Many larvae were attracted at concentrations between 0.5 and 0.05M of sodium chloride. The concentration of 0.05M attracted the most larvae. The concentration of 0.02M of sodium chloride showed greatly reduced larval attraction compared with 0.05M. Therefore, the threshold concentration was determined as 0.05M. Then, 0.05M of chemicals were examined in a further experiment. Chloride compounds (NaCl, KCl, $CaCl_2$, $MgCl_2$) were investigated. These chemicals are components of human body fluids. Distilled water was used as the control in all experiments. Only sodium chloride attracted the larvae. Next, alkaline compounds were examined [NaOH, KOH, $Ca(OH)_2$, and $Mg(OH)_2$]. Larvae accumulated only at the NaOH site. The results suggested that the Na cation is important for larval attraction. A high pH value did not influence attraction at all. Next, human serum was tested. The human serum used was from normal serum to 1:32 diluted sera by distilled water (7 steps). Hierarchical attraction was seen according to serum concentration. Next, human sweat was collected from a limited zone of chest skin where only eccrine glands were distributed. Non-diluted sweat attracted the most larvae. Sweat might act as one of the most probable factors for infection by this skin-penetrating nematode.

INTRODUCTION

Strongyloides stercoralis has a peculiar life cycle. The adult females are only parasitic in the small intestine of humans. The adults produce eggs in the crypts of the lamina propria, and the eggs hatch in the small intestine and grow into rhabditoid larvae. Most rhabditoid larvae are shed together with feces. A few grow into infective larvae in the large bowel and reinfection occurs through the large bowel walls (autoinfection). Most soil-dwelling larvae may develop into free-living adult males and females, but some grow directly into filariform larvae (infective larvae) which infect humans through the skin (direct infection). The free-living females lay eggs on the ground. Then the eggs grow into infective larvae and start to find human skin to continue their reproduction.

Regarding chemotactic studies of nematodes, *Caenorhabditis elegans* was first examined and found to have chemotactic behavior to sodium chloride (Ward, 1973). This nematode is not parasitic but is a soil-dwelling nematode in all of its stages. Then, plant parasitic nematodes and entomopathogenic nematodes were examined (Riddle and Bird, 1985; Zuckerman and Jansson, 1984). For mammalian host parasitic nematodes, Zietse *et al* (1981) examined the chemotaxis *in vitro* using *Ancylostoma caninum* infective larvae. Animal-infecting parasites have to find their host for infection and migrate inside the host body to reach appropriate organs or tissues to become adults; the females of which produce eggs there. Unless they can find a host, they eventually die and their generation ceases. Amphids are widely accepted as chemorecepters in nematodes (Poinar *et al*, 1968). The amphids of nematodes that infect mammals must be different from free-living nematodes. Ashton and Schad (1996, 1999) described the precise structure of *S. stercoralis* amphids. In *Strongyloides* species chemotaxis, Tada *et al* (1997) examined the chemoattraction behavior of *S.ratti*. Next, Koga and Tada (2000) reported the chemotaxis of *S. ratti* larvae to serum proteins. The chemokinetics of *S. ratti* (Tobata-Kudo *et al*, 2000a,b) were examined on agarose gel plates of NaCl gradients. As for *S. stercoralis*, Sciacca *et al* (2002) investigated the response to carbon dioxide and concluded that the larvae resumed crawling movement after exposure to 5% CO_2; this related to larval active host-finding behavior. Forbes *et al* (2003) examined the infective larval behavior on sodium chloride gradient agarose gel. In the present study, we show that the Na cation is essential for host finding by *S. stercoralis*, using

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some chemicals, as well as NaCl, human serum, and sweat *in vitro*.

MATERIALS AND METHODS

The third-stage larvae (L_3) of *Strongyloides stercoralis* used in this experiment were originally obtained from patient feces in Thailand. The seven-day copro-cultured L_3 of *S. stercoralis* were harvested by the filter-paper culture method. The larvae were washed three times with distilled water and then filtered through a nylon mesh (15 μm pore size, Nytrel-TI, UGB, France) to remove copro-debris. Small particle debris was removed by sucking up the supernatant after all the larvae sank to the bottom of the beaker (10 ml). In the beaker, a larvae-rich solution remained; from this, only the larvae were sucked up using a small tipped pipette and were then transferred to a small Petri dish (2 ml). Finally, the larval number was adjusted to 100 L_3/5 μl. Five ml of melted 0.9% agarose (GP-42, Nakarai Tesque) in distilled water was poured into a polystyrene Petri dish (Nissui-P Dish Co, 82 mm x 15 mm at the bottom). After cooling, at a position 2.0 cm apart from a central dot, 4-5 dots were radially allocated equidistant from each other. Next, all points were marked at the outside of the bottom of each dish, and each peripheral point was numbered. The center dot was prepared to place infective larvae, and the peripheral dots were used to place a 0.5 cm round piece of filter paper (Advantec No 3, Toyo Roshi quantitative low ash).

Inorganic compounds [NaCl, KCl, $MgCl_2$, $CaCl_2$, NaOH, KOH, $Ca(OH)_2$ and $Mg(OH)_2$] were used as attractants. All reagents were of analytical quality. In addition, human serum and human sweat were tested. Normal human serum was a mixture of an equal volume from two individuals. Human sweat was collected, using a plastic spoon, and limited to the sternum zone of the chest skin, immediately after emergence of droplets of sweat (not heavy sweating) where the eccrine sweat glands are distributed. Attractant solution-impregnated pieces of filter paper (5 mm in diameter) were placed on the agarose plates at the marked sites. After placing the papers, a round margin was marked along each circle on the outside of the bottom. The papers were then removed after 45 minutes by forceps. As a control, distilled water (DW) was always used.

A 5.0 μl larval suspension containing approximately 100 viable larvae was placed at the center of the agarose plate. The water droplets were then absorbed by piling on about 2 mg of agarose powder. Next, the assay plates were covered with a lid and maintained at room temperature in darkness for 45 minutes. The number of larvae that had accumulated in the circular area (0.5 cm) was counted using a dissecting microscope (Nikon, Japan) under a dark field illuminator. Ten agarose plates with the same experimental design were simultaneously run to assay larval movement.

The results were expressed as the average number of larvae ± standard deviation. Each larval number represents the mean of ten experiments. The Student's *t*-test was used for the statistical analysis.

RESULTS

First, to examine whether sodium chloride had a larvae-attracting characteristic for the infective larvae of *S. stercoralis*, five larvae were placed at the central point of an agarose plate. Human serum contains about 0.85% NaCl, which is most of the inorganic substances in human body fluids. A 0.15 molar concentration of NaCl is a similar to that of physiological saline solution. Concentrations of 0.15 M, 0.1 M, 0.05 M, and 0 M attractants had been prepared and placed on the peripheral sites, and larval movement was detected. Larval tracks were exposed one hour after the larvae were released onto the agarose plate. *S. stercoralis* infective larvae moved to NaCl attractant sites but not to the control site (Fig 1). Then, we sought to determine a threshold concentration of NaCl for further studies. Sodium chloride concentrations, ranging from 0.5 M to 0 M solutions (7 steps), were prepared and tested on the agarose assay. The larvae were attracted to concentrations from 0.5 M to 0.05 M (Fig 2). A 0.05 M solution attracted larvae the most. There were no significant differences between 0.5 M and 0.2 M, 0.2 M and 0.1 M, or 0.1 M and 0.05 M ($p>0.05$). There were significant differences between 0.5 M and 0.05 M, and 0.05 M and 0.02 M ($p< 0.01$). We determined that the threshold concentration favorable for these larvae was 0.05 M. In subsequent experiments, using the inorganic substances, the concentrations were adjusted to 0.05M. Distilled water (DW) was used as a control in all experiments.

Next, chloride compounds were examined (NaCl, KCl, $CaCl_2$, $MgCl_2$, and DW). NaCl attracted the most larvae (Fig 3). Next, alkaline compounds (NaOH, KOH, $Ca(OH)_2$, $Mg(OH)_2$, and DW) were examined, although these chemicals are not components of human body fluids. NaOH attracted the larvae the most (Fig 4). Thus, the Na+ cation was an attracting component for these larvae. Next, human serum was examined. We examined sera ranging from normal to 1:32 dilution (7 steps). Larval attraction numbers increased

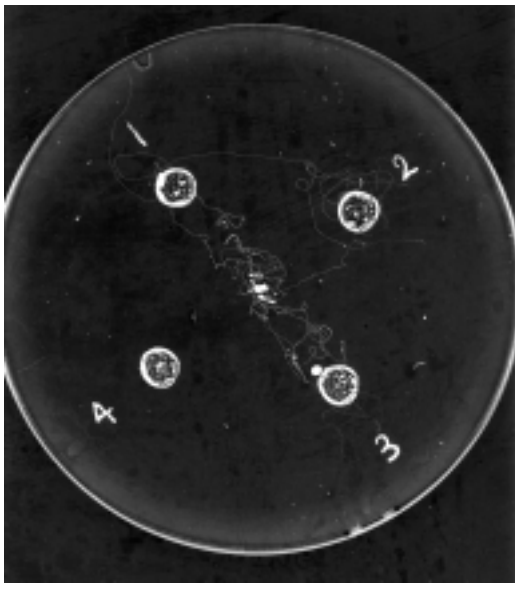

Fig 1- Tracks of 5 larvae placed at the center of an agarose plate. Upside-down photo. Attractants of 0.15M(1), 0.1M(2), 0.05M(3) and 0M(4) sodium chloride solution placed in the peripheral circles. Larvae were attached only to NaCl sites. Exposure was done about 1 hour after larval release.

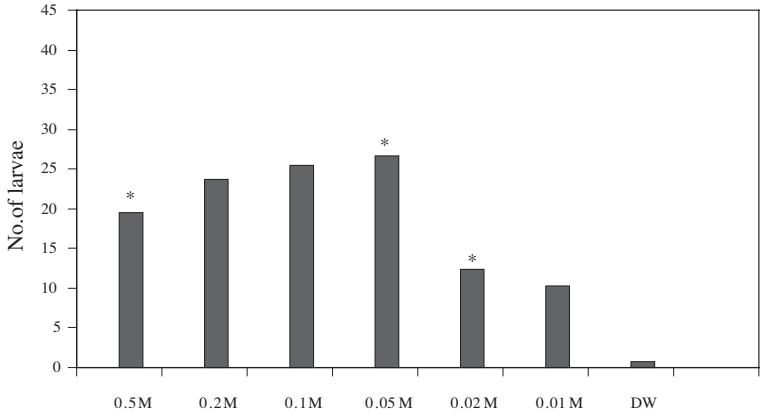

Fig 2- Chemoattraction of *S. stercoralis* larvae to various concentrations of NaCl. Asterisks show the significant differences.

according to the concentration of the serum (Fig 5). Significant differences were found between larval numbers at each serum concentration. However, 1:8 diluted serum (0.018 M of NaCl) could attract these larvae because 20.6 (average) larvae were attracted in another experiment at this concentration (data not shown). Finally, human sweat was examined. Whole sweat attracted the larvae most (Fig 6). There were significant differences between whole sweat and 1:2 dilution, and between 1:2 dilution and 1:4 dilution, respectively.

DISCUSSION

Forbes *et al* (2003) examined the larval movement of *S. stercoralis* infective larvae on an agarose plate of a sodium chloride gradient. The larvae showed repeated ascending movements until a 1.1 M concentration, when 15-20 larvae released at 0.01 M, and then they returned to a comfort concentration of 0.03-0.07 M NaCl. Tobata-Kudo *et al* (2000a) described using almost the same sodium chloride gradient as the above experiment. *S. ratti* infective

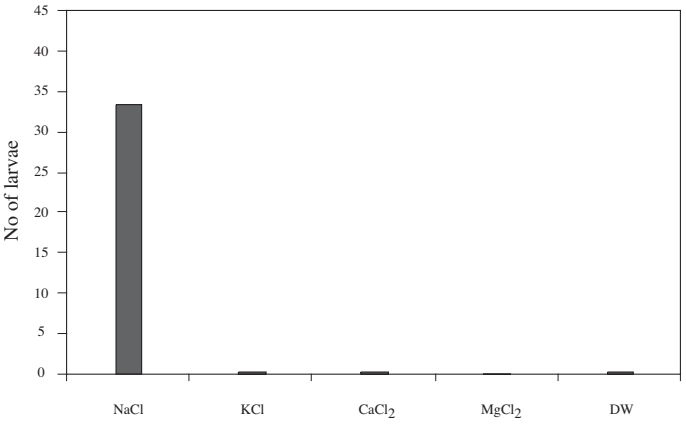

Fig 3- Chemoattraction of various chloride compounds by *S. stercoralis* larvae to various chloride compounds.

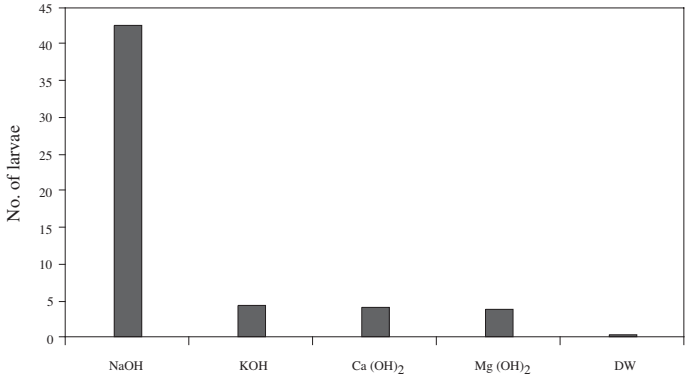

Fig 4- Chemoattraction of *S. stercoralis* larvae to various alkaline compounds.

larvae showed frequent ascending movements toward a 0.1 M concentration when one larva was released at the 0 M point; then the larva turned back again to the 0 M area. It seems somewhat surprising that the larvae ascended to 1.1 molarity of sodium chloride in the above description. In the present study, 0.05 M of NaCl was the most preferred concentration. We examined up to 0.5 M of NaCl concentration, however, even 0.5 M seemed too concentrated for these larvae. Our results seem to support the comfort (optimal) zone of *S. stercoralis* as found by Forbes *et al* (2003). *S. stercoralis* infective larvae prefer a range of 0.05-0.1 M NaCl concentration. We did not examine a 0.3 M concentration. However, 0.2 M sodium chloride could not attract the larvae. A study by Tada *et al* (1997) described an accumulating response of *S. ratti* larvae toward sodium ions (Na^+), instead of potassium (K^+), calcium (Ca_2^+) and magnesium (Mg_2^+) ions, indicating that chemoattraction of this larva to NaCl could be due to the presence of Na^+ ions. In our study, we examined 0.05 M of chloride compounds (NaCl, KCl, $CaCl_2$, $MgCl_2$, and DW), and found only NaCl strongly attracted *S.stercoralis* larvae. When OH (alkaline) compounds were examined, only sodium chloride attracted the larvae. Alkaline pH values did not influence larval attraction. These results correspond to those of Tada *et al* (1997).

As regards serum examination, several researchers (Zietse *et al*, 1981; Wauters *et al*, 1982; Vetter *et al*, 1985; Granzer and Haas, 1991) investigated *Ancylostoma caninum* infective larvae-attraction to dog serum and dog serum components. They fractionated dog serum by its molecular weight, and three former authors found that the larvae were attracted to serum components with molecular weights < 500 Dalton. However, they concluded that intact serum was more effective than every fractionated component of serum. Conversely, Granzer and Haas (1991) reported that a

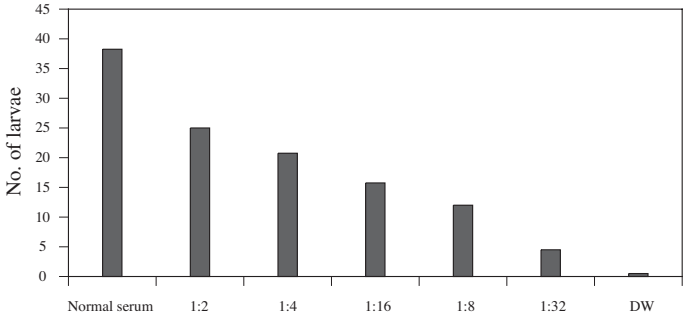

Fig 5- Chemoattraction of *S. stercoralis* lavae to human serum at various dilutions.

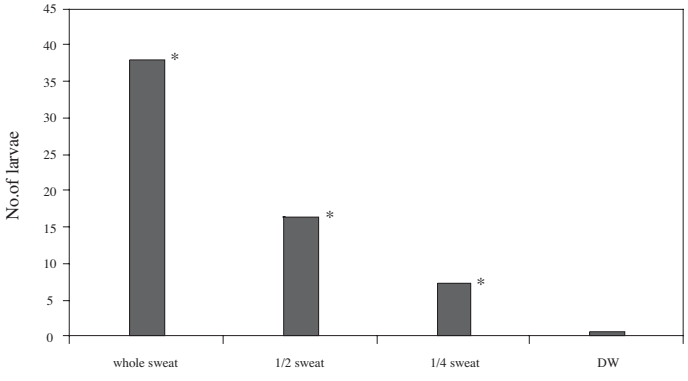

Fig 6- Chemoattraction of *S. stercoralis* larvae to whole and diluted human sweat. Asterisks show the significant differences ($p<0.01$).

molecular weight of dog serum >10 kDa was effective in the larval attraction of *A. caninum*. Koga and Tada (2000) demonstrated that *S. ratti* infective larvae were attracted to rat serum proteins (> 10 kDa) and bovine serum albumin, ovalbumin and peptides, as well as sodium chloride.

In the present study, we examined normal human serum and diluted serum. A 1:4 dilution of serum was the most similar concentration to 0.05 M NaCl, but there were significant differences between normal serum and 1:4 diluted serum. Hierarchical attraction was seen according to serum concentration. Human serum showed no stronger attraction than other 0.05 molar Na cation solutions. Further study is needed to determine whether human proteins have the ability to attract *S.stercoralis* larvae. However, we would like to elucidate the host-finding cues on human skin. Human proteins and albumin are not components of human body fluids. We used the serum for our examination instead of human body fluid which is very similar in composition to plasma (Pocock and Richards, 1999).

For host-finding, human skin surface substances are important. Mashima (1978) described in his textbook of human physiology that human sweat from the eccrine gland that is the predominant sweat gland in human skin that contains 0.65% NaCl, 0.08% urea, and 0.03% lactic acid. At the beginning of secretion, the precursor of human sweat from the eccrine glands contains 0.9% NaCl, but sodium chloride is reabsorbed by these glands as the sweat flows along the duct to maintain human body fluid electrolyte as close as possible as normal. In heavy sweating, the sodium chloride concentration of sweat rises up to 0.9% because there is less time for reabsorption (Mashima and Muramatsu, 1987). We collected fresh eccrine gland sweat so as not to be contaminated by apocrine gland sweat, which is distributed in the auricle, armpit and crotch areas. A little skin lipid from the holocrine gland might have contaminated our sweat. Patterson

et al (2000) investigated the regional composition of sweat in normal human bodies. They collected human sweat from the forehead, chest, scapula, abdomen, lower back, forearm, hand, thigh, calf, and foot. The chest sweat contained 0.0476 M of sodium cation, which is not heavy exercise sweating. This result corresponds with our threshold of 0.05 M. Sweat was first investigated in a nematode behavioral experiment in this report. Human sweat may sometimes wash sodium chloride from the skin surface during heavy sweating, and sometimes repeated mild sweating will concentrate sodium chloride by drying on the skin. This may become an attractant factor for host finding for this nematode.

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