# Literature Reviews

## 1. Etiology

Biologically, *S. stercoralis* is unique worm, which is capable of reproducing within the human host. Strongyloidiasis is caused by infection of humans with this small (2.2 mm by 40 μm) adult female worms or parasitic female. It is a small, colorless, semitransparent filariform nematode with a finely striated cuticle. It has a short buccal cavity and long, slender, cylindrical esophagus. The body of the worm contains one-third of esophagus at the anterior portion, while the posterior part contains ovaries, oviducts, and uteri. Only parasitic adult female worms are found in the small intestine of the host, mostly the duodenum and upper jejunum. In the absence of parasitic adult male worms, they reproduce by parthenogenesis. Adult females release eggs, sized 54 by 32 μm; covered by a thin, transparent shell containing mature larvae in the intestinal mucosa. Eggs hatch in the mucosa releasing first stage larvae (L1), which burrow through the epithelium to the intestinal lumen where they are passed in the feces to the environment and can be seen during fecal examination (Brown and Neva, 1983).

The worm has three types of life cycle (Brown and Neva, 1983; Grove, 1989; Bogish and Cheng, 1998).

1) Indirect cycle: free-living males and females in the soil are about 1 mm and 2.0-2.5 mm long, respectively. After fertilization, the free-living female produces eggs that develop into L1 and require only a few hours for complete development. After a

short feeding period of 2 to 3 days in the soil, L1 molt into L2, L3, L4 and free-living adults. The eggs hatch in the soil, where the larvae feed actively on organic debris.

2) Direct cycle: The direct cycle appears to be associated with the optimal environmental conditions. It is believed that it depends on environmental conditions such as excessively high or low pH, temperature and level of nutrition, the L2 larvae will change to L3. When L3 penetrate the skin, the lymphatic vessels or the small cutaneous veins carry them to the postcaval vein, where they enter the right side of the heart and are carried to the lungs via the pulmonary artery. In the lungs, the larvae undergo a third molt, rupture from the pulmonary capillaries, and enter the alveoli. From the alveoli, they move up the respiratory tree to the epiglottis. Coughing and swallowing cause the migrating of larvae over the epiglottis to the esophagus and down to the small intestine. In the small intestine, the larvae reach a final molt and become parasitic worms. The worms burrow into the mucosa of the small intestine and produce eggs within 25-30 days postinfection. The autoinfection explains persistent strongyloidiasis in patient who live in non-endemic areas. The larvae may develop rapidly into the L3 stage in the intestine and, by penetrating the intestinal mucosa or the perianal skin, establish a developmental cycle within the host.

**2. Clinical syndromes**

Up to 50% of *S. stercoralis* infections are asymptomatic. When symptoms develop, gastrointestinal complaints are common (Grove *et al.,* 1975; Bannon *et al.,* 1995). Patients may present with a multitude of symptoms including nausea, vomiting, fever, malaise, abdominal pain, distension, and flatulence. This may be associated with watery diarrhea. Pruritic anus is common. Weight loss, steatorrhoea,

occult blood loss, and malabsorption of sugar, fat, and vitamins have been described. Intestinal bleeding has been reported (Carp *et al.*, 1987). Larvae currens is strong clinical evidence of *Strongyloides* infection. Pulmonary symptoms include wheezing, chest pain, dyspnea, and cough. X-ray findings of the abdomen are nonspecific, but may include dilated thickened loops of small bowel that may mimic a small bowel obstruction. Migration of larvae through the lungs can cause the Löffler's syndrome (Bannon *et al.*, 1995).

Mahmoud (1996) and Bogish and Cheng (1998) divided the symptoms of human strongyloidiasis into three phases: cutaneous, pulmonary, and intestinal. Cutaneous phase is characterized by slight hemorrhaging, swelling, and intense itching (ground itch) at sites that have been invaded. Primary infection with *S. stercoralis* is initiated through skin penetration by infective L3. Acute infection in the human host is usually asymptomatic. Larva currens is a cutaneous manifestation that occurs more frequently in association with autoinfection. It is an allergic reaction to L3 that migrate in the skin at a relatively fast rate and leave itchy urticarial tracks. The condition is usually detected over the lower abdominal wall, buttocks, or thighs. Larva currens may disappear in a few days but usually recurs over prolonged periods. Pulmonary phase is caused by larval migration through the lung that may cause lung damage. When this happens, the patient develops burning sensations in the chest, cough, and other symptoms of bronchial pneumonia. However, symptomatic pulmonary strongyloidiasis due to migrating larvae is seen rarely. Clinically, it resembles Löffler's syndrome with cough, shortness of breath, wheezing, and transient pulmonary infiltrates. Peripheral blood eosinophilia is usually detected in individuals with this type of strongyloidiasis. Intestinal phase begins when the adult

female worms reach maturity in the gastrointestinal tract. The symptoms appear when female worms become embedded in the mucosa. Moderate to heavy infections produce pain and intense burning in the abdominal region, accompanied by nausea, vomiting, and intermittent diarrhea. Long-standing infections result in chronic dysentery and weight loss. The very heavy infections may be fatal causing by massive invasion of tissues by L3 following by the bacterial infection from ulceration of intestinal mucosa.

Since cell-mediated immunity is thought to participate in the regulation of *S. stercoralis* autoinfection, frequent and severe infections with *S. stercoralis* have been expected to emerge in patient with AIDS. However, this has not happened, even in areas of the world where both *S. stercoralis* and AIDS are endemic, and, in general, there does not appear to be a higher incidence of chronic strongyloidiasis with AIDS (Zumla and Croft, 1992).

**3. Prevalence**

The prevalence of *S. stercoralis* is lower than that of hookworm in the temperate zones. It is especially prevalent in tropical and subtropical areas, where warmth, moisture, and lack of sanitation favor its free-living cycle (Brown and Neva, 1983). Humans usually contact the infective larvae in the soil; less frequently, larvae are ingested in contaminated water. It has been estimated that human cases of strongyloidiasis currently number 100-200 million worldwide. This is a general assessment and the prevalence of this infection varies considerably in different areas of the world (Bogish and Cheng, 1998).

From 1995 to 2000, the prevalence rates varied from less than 1% to 13% in Brazil (Kobayashi *et al.,* 1995; 1996; de Razende *et al.,* 1997; Machado and Costa-Cruz, 1998; Miranda *et al.,* 1998), 3.5 to 33% in Nigeria (Udonsi *et al.,* 1996; Agi, 1995; 1997), 4% in eastern Kentucky and rural Tennessee of the United states (Mahmoud, 1996), less than 1 to 3.3% in Indonesia (Bangs *et al.,* 1996; Widjana and Sutisna, 2000) to 2.2% in Laos (Chai and Hongvanthong, 1998). In Thailand, the prevalence in 1989-2000 was found varying from 1.8 to 15.5% in northern and southern regions (Kasuya *et al.,* 1989; Koga *et al.,* 1990; Anantaphruti *et al.,* 2000). However, all of the prevalence rates mentioned above may not be comparable since some studies used the less sensitive methods for stool examinations.

**4. Diagnosis**

One of the problems related to strongyloidiasis is the difficulty in the detection of *S. sterceralis* larvae in feces specimens in chronic and low-level infections. There have been many reports addressing this problem either by coprologic or serologic method. The parasitologic diagnosis based on coprologic examination is probably one of the most difficult problems in clinical parasitology. Direct fecal smear, formalin-ether concentration, and filter paper culture (Harada-Mori method) are conventional methods for detecting larvae in fecal specimens. However, these methods are not considered sensitive enough for diagnosis of chronic strongyloidiasis. In such cases, the larvae are present only in very small numbers or are frequently absent from individual fecal specimens. To determine the efficacy of fecal examination for the detection of *Strongyloides* infection, fecal samples collected in Japan, Brazil, and Thailand were examined by four different methods (direct fecal

smear, formalin-ether concentration, Harada-Mori, filter paper culture, and agar plate culture). The agar plate culture method was highly effective; more than 96% of the positive cases were diagnosed by the agar plate method. In contrast, only 25.68%, 46.78% and 27.8% were detected by direct smear, formalin concentration and filter paper methods, respectively (Sato *et al.*, 1995). Previously, Koga *et al.* (1990) examined fecal specimens from school children in northern Thailand and found that the agar plate method was more efficient (78% sensitivity) than traditional methods (56.5%, 47.8%, and 17.4% sensitivity for direct smear, filter paper and formalin-ether concentration methods, respectively). The sensitivities of the agar plate method for the detection of *S. stercoralis* infection from various studies varied from 78-100% (Arakaki *et al.*, 1990; Koga *et al.*, 1990; Sukhavat *et al.*, 1994). Later a number of studies have shown that the detection rates could be improved by repeated examinations (Sato *et al.*, 1995; Dreyer *et al.*, 1996; Moustafa, 1997). Agar plate culture was considered more sensitive than traditional method especially in endemic areas. In one study, follow-up examinations by the agar plate method of the initially negative subjects could detect *S. stercoralis* larvae in 0-5.9% of the subjects over the four-week period (Uparanukraw *et al.*, 1999). This method is also necessary to discriminate *Strongyloides* microscopically from other similar nematode larvae, especially in an area, such as northern Thailand, with a high prevalence of hookworms. Arakaki *et al.* (1990) has shown that culturing feces for more than 2 days can differentiate between *S. stercoralis* and *Necator americanus* larvae, which was also confirmed by Jongwutiwes *et al.* (1999). However, the filter paper and agar plate culture methods are labor-intensive and are not routinely used by diagnostic laboratories in endemic areas (Lindo *et al.*, 1994). Despite its high sensitivity, the

agar plate culture method suffers from its high cost. The cost for the material alone is the cheapest for the direct smear; the cost increases 4 times for the modified Baermann method and 15 times for the agar plate culture method. The last method also requires a better equipped laboratory and demands more time for preparation and examination and the well-trained personnel (de Kaminsky, 1993).

For the past several years, there have been reports on serologic diagnosis of human strongyloidiasis using antigens prepared from L3 of either the human parasite, *S. stercoralis*, or the rat parasite, *S. ratti*. Most of these studies have relied on the ELISA, IFA or IHA to detect immunoglobulin G (IgG) antibodies reactive with larval somatic antigens (Carroll *et al.*, 1981; Neva *et al.*, 1981; Gam *et al.*, 1987; Bailey *et al.*, 1989; Sato *et al.*, 1990). The ELISA was shown to be 83.8% sensitive as compared to 77.9% of the IHA (Gam *et al.*, 1987). The ELISA was found to be more practical IHA or IFA or radioimmunoassay. Although the report of Grove and Blair (1981) showed a high correlation between IFA and ELISA, ELISA did not seem to be as sensitive as the IFA. However, in practice IFA is not easy to interprete since it depends on subjective visual assessments of fluorescence and the results are usually expressed as the serial dilutions of the serum that gives the least fluorescence. Regarding the radioimmunoassay, although it is highly sensitive, the isotope presents medical hazards and has a short half-life. Although ELISA is not usually quite as accurate as radioimmunoassay, its reagents present no health hazards, are stable, and have long shelf-life. Moreover, the estimation of results can either be made by visualization or by measuring optical density with a rather simple spectrophotometer of the type found in most laboratories. The range of application of ELISA is potentially as wide as that of radioimmunoassay and it may also reinforce or replace

other serologic tests, such as complement fixation, haemagglutination, and immunofluorescence (Voller *et al.,* 1976). Although ELISA is considered to replace IFA, IHA or radioimmunoassay but the problem of cross-reaction between antigens of *S. stercoralis* and filarial worms was encountered. However, it has been shown that preabsorption of sera with the extract of adult filarial worms could remove cross-reactive IgG induced by hookworm and filarial infections, and increase the specificity of the ELISA from 25% to 65% (Conway *et al.,* 1993a). Sato *et al.* (1990) using the Western blot method have detected epitopes in proteins of 41 and 26 kDa in *S. stercoralis*, which were absent in *S. ratti* and *S. venezuelensis*. Similarly, immunodominant 41-, 31-, and 28-kDa proteins of *S. stercoralis* L3 appear to be specific to *S. stercoralis* and were not present in extracts of *S. cebus* or *S. ratti* (Conway *et al.,* 1993b; 1994). Serum IgG recognition of these bands as a criterion for immunodiagnosis gave a sensitivity ranging from 85% for recognition of all three bands to 93% for recognition of any of the three bands. When the three bands were considered separately, the 41-kDa protein was the most sensitive being recognized by IgG in 91% and 100% of confirmed and suspected strongyloidiasis sera, respectively. Serum IgG was reactive with the 41-kDa band in 9% of subjects with other nematode infections and with all three bands in 7%. ELISA and Western blot for strongyloidiasis did not cross-react with sera from individuals with *N. americanus* hookworm, mixed *Ascaris lumbricoides* and *Trichuris trichiura*, or no infection. However, 35% of lymphatic filariasis sera and 95% of onchocerciasis sera were positive by ELISA whereas the minority of these sera were reactive with any of the 41-, 31-, and 28-kDa bands on Western blots. Douce *et al.* (1987) found no cross-reactivity of antibodies to *N. americanus* and *Opisthorchis viverrini*, hookworm,

*Schistosoma mansoni*, and *Taenia* with L3 of *S. stercoralis* but some patients infected with *Wuchereria bancrofti, Loa loa*, and *Onchocerca volvulus* exhibited weak cross-reactivity. To increase the specificity and sensitivity of ELISA, Lindo *et al.* (1994) preincubated the sera with *Onchocerca gutturosa* antigens to remove cross-reactivity with other helminths. The sensitivity of the ELISA increased from 80% to 85% following preincubation. Similarly, there was an increase in the specificity from 94% to 97%. The IgG recognition of 41-, 31-, and 28-kDa L3 larval components showed sensitivities of 100%, 85%, and 65%, respectively. The specificities of recognition of these proteins were 94%, 89%, and 75%, respectively. Both the ELISA following incubation of sera with *O. gutturosa* extract and serum IgG reactivity to the 41-kDa larval component using immunoblotting are sensitive and specific techniques for diagnosis of endemic strongyloidiasis.

Although the development of immunologic methods has provided highly specific and sensitive diagnosis of gastrointestinal tract infections, it, however, cannot differentiate between an ongoing and a past infection and are not helpful in the diagnosis of disseminated infection. This problem could be overcome by the demonstration of parasite antigen in infected humans as this would be indicative of current infection. In recent years, diagnosis of parasite in feces by coproantigen detection has been developed using either monoclonal or polyclonal antibody to detect heat-, cold-, or formalin-resistant antigens. These helminth coproantigens can be detected by ELISA, and such coproantigen ELISA has certain advantages over conventional serum antibody assays. The coproantigen detection avoids handling of blood or serum products, and should indicate the presence of a current infection only. In addition coproantigens rapidly disappear from feces after treatment (Allan *et al.*,

1992; Deplazes *et al.,* 1992). Coproantigen ELISA has recently become an important tool, especially in community screening and surveillance of control programs for taeniid cestodes due to its specificity, sensitivity, robustness, and ability to handle large numbers of samples (Allan *et al.,* 1992; Deplazes *et al.,* 1992; Craig *et al.,* 1995; Malgor *et al.,* 1997). The coproantigen ELISA has been successfully applied to detect *Echinococcus granulosus* antigen in dog feces, *Faciola hepatica* antigen in human feces and *Entamoeba histolytica* antigen in human feces with 89-100% sensitivity and 96-100% specificity (Deplazes *et al.*, 1992; Espino and Finley, 1994; Malgor *et al.*, 1997; Singh *et al.*, 1999). Allan *et al.* (1992) described three coproantigen ELISA that used hyperimmune rabbit serum raised against adult cestode somatic antigens for the detection of *Taenia*- and *Echinococcus*-specific antigens in host feces. The first assay, using an antiserum against *Taenia pisiformis* antigen extract, was shown to be considerably more sensitive than microscopic detection of eggs in feces. Antigen was present in feces before patency and antigen levels were independent of *T. pisiformis* egg output. The second assay, involving a human taeniasis test based on antibodies against *T. solium* was highly specific and capable of diagnosing individuals who would not have been detected by coproscopy or treatment to recover the tapeworm. The third assay was designed for *E. granulosus* and demonstrated 87.5% sensitivity and 96.5% specificity with samples from dogs naturally and experimentally infected with *Echinococcus* or *Taenia*. The detection of circulating antigens was far less sensitive than coproantigen detection in *F. hepatica* infection (Espino *et al.*, 1998). It gave only 8.5% sensitivity as compared to 89.8% sensitivity of coproantigen detection.

Lastly, the coproantigen ELISA was applied to rats experimentally infected with *S. ratti* (Nageswaran *et al.*, 1994). It was shown that during the period of parasite excretion, coproantigen detection was more sensitive than parasitological diagnosis. The first detection of coproantigen coincided with patency and the coproantigen level remained elevated even when the parasite count was low.

**5. Therapy**

The goal of therapy in strongyloidiasis is eradication of the infection. Thiabendazole is the most effective agent currently available that can be used for both intestinal and tissue forms (Zygmunt, 1990). The efficacy of thiabendazole is considerable; rates of parasite eradication are in the 90% range. However, this therapy may not be sufficient for infection due to worms capable of maintaining low levels in the host, which may result in the hyperinfection syndrome if the host's immune status changes. Therefore, close follow-up of treated individuals is required to assure complete elimination of the worms. The other considerable problem with thiabendazole therapy is its frequent side effects (*e.g.*, nausea, headache, hypotension, and hypersensitivity reactions). These side effects may occur in about 30% of treated individuals. Rare and severe neuropsychiatric reactions as well as rare mortalities have been reported (Mahmoud, 1996). Extended and/or repeated pulse therapy may be needed. Thiabendazole is generally dosed 25 mg/kg twice a day for three days. In the hyperinfection syndrome, this therapy may need to be given for two to three weeks and still may be unsuccessful (Grove, 1982). In the report of Zaha *et al.* (2000), they found that thiabendazole had a problem in safety, since a high incidence of severe adverse effects and liver dysfunction occured. The search for alternative

chemotherapeutic agents has resulted in experimenting with albendazole and ivermectin. Human trials with both compounds have demonstrated rates of parasite eradication ranging from 60 to 90% (Mahmoud, 1996). A comparative study showed that ivermectin appears to be equally effective as thiabendazole for treating uncomplicated chronic strongyloidiasis and is associated with far fewer side effects (Gann *et al.,* 1994). Both albendazole and ivermectin are not yet approved for use in cases of strongyloidiasis (Mahmoud, 1996). Recently, Zaha *et al.* (2000) also showed that ivermectin had a strong anthelmintic effect with the least toxicity whereas the eradication effect of albendazole was not sufficient.and that a high incidence of liver dysfunction was observed with mebendazole.

**6. Objectives of this study**

- To produce and characterize the monoclonal antibody (mAb) to the 41-kDa protein of *S. sterocoralis* L3.

- To evaluate the mAb-based sandwich ELISA for coprodiagnosis of human strongyloidiasis