Chapter 10
Strongyloides stercoralis and Strongyloidosis

Masataka Korenaga and Fabrizio Bruschi

Abstract Strongyloidosis is a chronic, soil-transmitted, intestinal parasitic disease. *Strongyloides stercoralis* is a roundworm and the main causative agent of this disease. *S. stercoralis* has a unique life cycle, which consists of direct (homogonic) development and indirect (heterogonic) development. Parasitic adult females produce both sexes of the next generation parthenogenetically. Female larvae can choose the direct or indirect development depending on various environmental conditions. Autoinfection is one of the characteristic features of this parasite, which causes hyperinfection and disseminated infection. Strongyloidosis occurs mostly in humid tropics and subtropics of more than 70 countries, affecting people between 30 million and 100 million or higher. However, the precise number is not known up to the present, because of difficulties in diagnosis. Even in highly developed countries, like the USA, serious problems have been caused by transmission of *S. stercoralis* through organ transplantation. We describe the current status of strongyloidosis with special reference to biology, epidemiology, immunology, and vaccine development.

10.1 Introduction

Strongyloidosis is one of the chronic, soil-transmitted, intestinal helminth infections which affect the health of over one-third of the world population. 30–100 million people are estimated to be infected with *Strongyloides* spp. (CDC homepage). *Strongyloides stercoralis* is widespread, mainly in the tropics and subtropics and of species naturally infecting humans. Besides this species,

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S. fuelleborni infection in humans has been reported but restricted in Africa and the Southeast Asian country of Papua New Guinea. The burden of strongyloidosis to humans has been underestimated in an aspect of global health. Strongyloidosis is one of the neglected tropical diseases and perhaps the most neglected (Olsen et al. 2009).

In this chapter, we focused mainly on human strongyloidosis together with recent advances of experimental models relating to human strongyloidosis. The comprehensive review articles regarding strongyloidosis and *Strongyloides* spp. have been published elsewhere (Grove 1989a, b; Sato 2003; Montes et al. 2010; Krolewiecki et al. 2013).

10.2 The Agent

10.2.1 Life Cycle and Morphology

The life cycle of *S. stercoralis* is unique. Infective third-stage larvae (L3i) penetrate the intact skin of hosts and migrate into the lungs via the bloodstream. The larvae pass the capillary walls and move to the alveoli, bronchus, and trachea and then go down the esophagus via the pharynx. Finally the larvae molt twice and mature to parasitic females. Adult worms parasitize in the mucosa of the small intestine. The sizes of the adult worms are 2.1–2.7 (2.4 in average) mm in length and 30.0–40.0 (37.0) μm in width, whereas those of *S. fuelleborni* are 2.9–4.2 (3.5) mm in length and 43.0–55.0 (51.0) μm in width. The ovaries of *S. fuelleborni* spiral around the intestine (Little 1966). Parasitic females lay eggs parthenogenetically. The early stages of *S. stercoralis* larvae pass through the gut of the host with feces and develop in the external environment (Little 1966). Female and male first-stage larvae may develop to free-living adults, mate, and reproduce offspring (which become L3i eventually). This type of development is known as heterogonic (indirect). Under certain conditions (temperature, nutrients, pH, etc.), female larvae can take either of two different life cycles: a heterogonic development as above or a homogonic (direct) development. In homogonic development, first-stage rhabditiform larvae molt twice to grow to L3i. L3i are threadlike in shape (filariform), 490–630 (563) μm in length, and 15–16 (15.8) μm in width in *S. stercoralis* and 560–680 (616) μm in length and 14–17 (15.8) μm in width in *S. fuelleborni*. Filariform larvae are characterized in the notched tip of the tail. Four molts occur in the development of both the parasitic and free-living adults (Little 1966). When second-stage larvae transform within the intestine into L3i, they can penetrate the perianal skin or bowel mucosa to complete their life cycle, which is called an autoinfection. The life cycle of *S. stercoralis* is shown in Fig. 10.1.

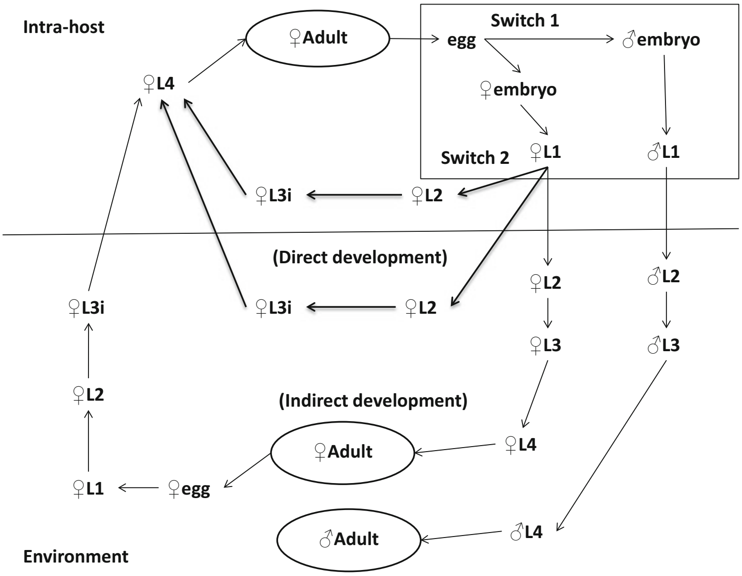

Fig. 10.1 The life cycle of Strongyloides stercoralis. The life cycle consists of direct (homogonic) and indirect (heterogonic) developments. Two developmental switches, a sex determination and a female-only developmental choice, have been demonstrated to control the development in *S. ratti* which is close phylogenetically to *S. stercoralis* (Nemetschke et al. 2010; Viney 2006). Such switches might be hypothesized in *S. stercoralis*

10.2.2 Mechanisms of Development

The temperature-sensitive developmental switch was demonstrated clearly to be controlled by the neuron pair ALD (amphidial neuron: lamellar dendrite, cell body "D") (Nolan et al. 2004). Sensing the environment is the function of the amphidial neurons, serving as thermoreceptors similar to neuron pair AFD in *Caenorhabditis elegans* (Mori and Ohshima 1995). Nolan et al. (2004) showed that first-stage larvae enter the homogonic development at temperature of 34 °C and above, whereas larvae enter the heterogonic pathway and develop to free-living adult worms at temperature below 34 °C. These results coincide with former observation that some larvae developed to the infective third-stage larvae when passage along the gut was delayed in an experimental canine model (Nishigori 1928). Thus internal development to infectivity makes autoinfection possible (Schad 1989). Autoinfection continues throughout the lifetime of the hosts. Persistent infections lasting for 40 years have been recorded, for example, as "war strongyloidosis" from various countries (Pelletier et al. 1988; Suzuki et al. 1989; Robson et al. 2009).

In the heterogonic development, eggs reproduced develop only into L3i (Yamada et al. 1991). It has been suggested that *S. stercoralis* free-living females reproduce by automictic thelytoky and pseudogamy (Hammond and Robinson 1994).

Molecular biology and genomics of *Strongyloides* spp. are reviewed elsewhere (Charlesworth 2010; Nemetschke et al. 2010; Streit 2008; Viney 2006).

S. stercoralis L3i was shown to be strongly attracted to an extract of the mammalian skin. The active component in the skin extract was urocanic acid, which is abundant in the mammalian skin and skin secretions. The attractant activity of urocanic acid was inhibited by divalent metal ions. This suggests the possibility to develop an inexpensive, practical, topical preventive for use on exposed body surfaces in people at risk of infection with *S. stercoralis* (Safer et al. 2007).

Metalloproteinases play roles widely in parasitism, ranging from tissue penetration, digestion of host tissue for nutrition, and evasion of host immune responses to developmental molts of larvae (Tort et al. 1999). With several *Strongyloides* spp., a proteinase activity was implicated in skin penetration by the larvae (Lewert and Lee 1954). Cysteine and metalloproteinases were active during the skin penetration process (Dresden et al. 1985; Rege and Dresden 1987). With *S. stercoralis*, the larvae rapidly penetrated the dermal extracellular matrix with the aid of a secreted, neural metalloproteinase (McKerrow et al. 1990). An astacin-like metalloproteinase transcript was reported from the infective larvae of *S. stercoralis* (Gallego et al. 2005). The *S. stercoralis* metalloproteinase has been designated as strongylastacin depending on the results of phylogenetic and structural analysis (Gallego et al. 2005).

10.3 Epidemiology of Infection

Strongyloidosis occurs mostly in humid tropics and subtropics of more than 70 countries (Olsen et al. 2009). The number of people infected with *S. stercoralis* is estimated to be between 30 million and 100 million or higher (Siddiqui and Berk 2001). The precise number is not known up to the present, because the prevalence obtained in each research depends on sensitivity and specificity of the methodology applied. Several reports, however, give us current epidemiological status showing the worldwide spread of strongyloidosis (Table 10.1). These figures imply that the number of population suffering strongyloidosis is more than we imagined. Most of them live in conditions of poor hygiene. Wang et al. (2013) reviewed that most of the patients with strongyloidosis in China were peasants or field-workers and that evident clustering in families in rural areas (e.g., Guangdong and Guangxi Provinces, etc. in southern China) was seen when they examined cumulative cases and distribution of strongyloidosis during 1973–2011.

Strongyloidosis poses a serious problem even in highly developed countries. Transmission of *S. stercoralis* has occurred through organ transplantation in the USA. The donor was from a Caribbean endemic area. The kidneys, pancreas, liver, and heart were transplanted. This fact emphasizes the importance of considering the possible occurrence of donor-derived infection with *S. stercoralis*, although the most relevant problem in organ transplant recipients is represented by reactivation of chronic infection after initiation of immunosuppressive treatment (Hasan

Table 10.1 Prevalences of strongyloidosis in various regions and/or countries in the world

Regions and/or countries	No. of subjects surveyed	No. of positives	%	CI (95 %)[a]	Year of survey	Methods of survey	Reference
Oran, Argentina	228 patients	67	29.4		2007	Agar plate, Baermann, sedimentation conc. Harada-Mori	Krolewiecki et al. (2010)
	262 patients	214	81.7		2007	NIE-LIPS	
Rome, Italy	4,695 Italian	2	0.04		2006–2008	Agar plate	Masucci et al. (2011)
A large teaching hospital	656 non-Italian	2	0.3				
Rural area, Brazil	ND[b]	ND	4.8		1999–2009	Parasitological methods	Paula and Costa-Cruz (2011)
Urban area, Brazil	ND	ND	5				
Eastern Uganda	113 mothers	9	8	3.7–14.7	2009	Baermann	Sousa-Figueiredo et al. (2011)
	213 preschool children	8	3.8	1.6–7.3			
	120 mothers	88	73.3	64.5–81.0		ELISA	
	225 preschool children	61	27.1	21.4–33.4			
Northeast Poland	120, 5 months–18 years old	7	5.83		2008–2009	Decantation	Żukiewicz et al. (2011)
Northern Laos	14 households × 6 villages	ND	8.9	7.4–10.4	2009	Formalin-ether concentration	Conlan et al. (2012)
	Household members >6 years old						
	Randomly selected						
41 GeoSentinel clinics in 19 countries	854 children (<18 years old)	40	4.7		1997–2009	ND	McCarthy et al. (2013)
	6,751 adult (>19 years old)	344	5.1				
	International migrants[c]						

(continued)

Table 10.1 (continued)

Regions and/or countries	No. of subjects surveyed	No. of positives	%	CI (95 %)[a]	Year of survey	Methods of survey	Reference
Flores Island, Indonesia, semi-urban area	675, 18–80 years old	5	0.7		2009	qPCR	Wiria et al. (2013)

[a] CI: confidence intervals

[b] ND: not described

[c] Diagnoses with strongyloidosis by region of migrant origin were of 7 % in Southeast Asia ($n = 1{,}200$), 3 % in South Asia ($n = 844$), 6 % in North Africa ($n = 503$), 4 % in East Africa ($n = 1{,}253$), 5 % in West Africa, and 5 % in South Africa ($n = 698$)

et al. 2013). Two cases with strongyloidosis were recorded on 1,046 kidney and 708 liver transplant recipients registered in four medical centers in Brazil from 2001 to 2006 (Batista et al. 2011). Expanded infectious disease screening program was done in the USA for Hispanic transplant candidates (recipients) between 2006 and 2008, minimizing the risk of posttransplant infectious complications. On 83 patients screened, most were from Mexico (74.7 %), and the others from Ecuador, Puerto Rico, and Peru. The seropositive rate was 6.7 % for *S. stercoralis* (Fitzpatrick et al. 2010).

Roxby et al. (2009) have warned that physicians in the USA often miss opportunities to identify patients with chronic strongyloidosis and stressed an importance of screening and treatment before transplantation. Repetto et al. (2010) also suggested the need to include strongyloidosis as a presumptive diagnosis in patients with past risk of infection and especially if they develop eosinophilia although not originating from endemic areas. Based on mortality data during 1991–2006 in the USA, a population-based case-control study showed that strongyloidosis caused 347 deaths (0.79 per 10 million deaths, 14–29 deaths per year) and that strongyloidosis deaths were related with chronic obstructive pulmonary disease (COPD) and infection with human immunodeficiency virus (HIV). However, in the second half of the study period (1999–2006), strongyloidosis deaths were associated only with HIV infection (Croker et al. 2010).

10.4 The Host Response to the Parasite and Immunopathological Processes

Pathophysiological aspects in human strongyloidosis were reviewed extensively by Genta and Caymmi Gomes (1989). Patients with chronic strongyloidosis had parasite-specific IgE antibodies (Genta et al. 1983). Total IgE levels were above 200 IU/mL in 10 of the 15 patients examined (66.7 %), and eosinophilia in peripheral blood was seen in 73.3 % of the patients (Genta et al. 1983). Recently, eotaxin and IL-5 serum levels were found significantly increased in patients with strongyloidosis (Mir et al. 2006). The antigen-specific Th2 responses are protective against helminth infections including *Strongyloides* spp. In relation to this, the role of basophils was reported: basophils derived from mice infected with *Strongyloides venezuelensis* produce spontaneously in vitro IL-4, IL-6, and IL-13, along with IL-3. They express MHC class II and induce the development of naïve $CD4^+$ cells into Th2 cells (Yoshimoto et al. 2009).

Larvae of *S. stercoralis* possess collagenase-like and other proteolytic activities (Rege and Dresden 1987; Mckerrow et al. 1990; Brinley et al. 1995). Penetration by *Strongyloides* larvae caused alteration of the extracellular glycoprotein-containing materials of the skin, especially in the basement membrane. The larvae were able to pass through the basement membrane easily and to reach within the dermis

3 minutes after they were placed on the skin in an experimental rodent model using *Strongyloides ratti* (Lewert and Lee 1954).

Immune responses caused by larval penetration/migration are an important study subject. Recently, tissue factors (TFs) have been considered important for initiating innate and adaptive responses. Thymic stromal lymphopoietin (TSLP) is one of the TFs, an interleukin 7 (IL-7)-like cytokine. TSLP is expressed mainly by epithelial cells at barrier surfaces (the skin, gut, and lungs) (Ziegler and Artis 2010). Myeloid dendritic cells (DCs) express TSLP receptor and IL-7 receptor-α (Reche et al. 2001). Since parasitic infections cause epithelial damage, it might be suggested that TSLP expression is induced through the protease-activated receptor pathway (Demehri et al. 2009). TSLP can drive a Th2 response, potentially through effects on DCs, granulocytes, natural killer (NK) cells, and CD4+ T cells (Ziegler and Artis 2010). TSLP was shown to promote protective immunity to *Trichuris muris, Nippostrongylus brasiliensis, Heligmosomoides polygyrus,* and *Schistosoma mansoni* in mice, but the role in protective immunity to *S. stercoralis* still remains uncertain (Ziegler and Artis 2010).

Trefoil factor 2 (TFF2) produced by epithelial cells has a critical role in their wound healing during larval migration through the lungs in mice infected with *N. brasiliensis*, a rodent nematode which is very similar to hookworm (Wills-Karp et al. 2012). This factor regulates interleukin-33 (IL-33) production by epithelial cells. This cytokine stimulates IL-5 production resulting in eosinophilia, contributing to protective immunity against *S. venezuelensis* in mice (Yasuda et al. 2012). IL-5 and/or eosinophils induced by IL-5 were shown to be involved in reducing susceptibility and/or fecundity in a primary infection with *S. ratti* (Ovington et al. 1998; Watanabe et al. 2003) and *S. venezuelensis* (Korenaga et al. 1994) in mice, while duration of the infection is similar in normal and IL-5-deficient mice. IL-5 was shown to be critical for the protective immunity to migrating larvae in a secondary infection with *S. ratti* (Watanabe et al. 2003) and *S. venezuelensis* (Korenaga et al. 1991) in mice, but not for adult worm expulsion from the gut.

Granulocytes are also crucial for the host's early defense against larval *S. stercoralis* (Galioto et al. 2006) and migrating larvae of *S. ratti* (Nawa et al. 1988; Watanabe et al. 2000). A histopathological study indicated that migrating larvae of *S. ratti* at the inoculation site are surrounded by neutrophils and eosinophils at 12–24 h after infection (Dawkins et al. 1981). Motile *S. ratti* larvae were shown to stimulate neutrophils' release of eosinophil chemotactic factor (ECF). Neutrophils were considered to be an important source of ECF, responsible for eosinophil accumulation around the larvae (Owhashi et al. 1986). Furthermore, eosinophil chemoattractants are produced by larval *S. stercoralis.* The chemoattractants are both protein and chitin that are major components of nematode cuticle, stimulating multiple receptors on the eosinophil surface (Stein et al. 2009).

Classical NK cells, retinoid-related orphan receptor γ^+ (RORγ^+) lymphoid tissue inducer-related cells, and Th2-type innate lymphocytes have distinct roles in innate immune responses, producing Th1, Th17, and Th2 cytokines, respectively (Koyasu and Moro 2012). Th2-type innate lymphocytes include natural helper cell (NH cell) (Moro et al. 2010), nuocyte (Neill et al. 2010), innate helper 2 cell (Ih2) (Price et al. 2010), and multipotent progenitor type 2 cell population (MPP$^{\text{type2}}$) (Saenz et al. 2010). Recent evidences indicate an involvement of Th2-type innate lymphocytes in the early phase of following Th2-type responses in murine helminthiasis models (Maizels et al. 2012). To date, however, a relation between Th2-type innate lymphocytes and immune responses to *S. stercoralis* remains obscure.

Toll-like receptors (TLRs) on dendritic cells and other various cells recognize invading pathogens through pathogen-associated molecular patterns (PAMPs) during both the innate and the adaptive responses (Akira et al. 2001). Among them, TLR4 is critical for protective adaptive immunity to migrating larvae of *S. stercoralis* in murine model. TLR4 is expressed on the surface of neutrophils. TLR4 has been shown to be required for activating neutrophils in mediating larval killing but not for T- and B-cell function (Kerepesi et al. 2007). Since the first report of Abraham et al. (1995), his group has published excellent papers on protective immunological mechanisms against *S. stercoralis* using an innovative method consisting in a diffusion chamber containing L3i implanted subcutaneously in mice, to assess in vivo survival rates of larvae (Abraham's implantation method). This allowed to identify the different factors involved in protective immunity against *S. stercoralis* (Table 10.2). Refer to an excellent review of Bonne-Année et al. (2011).

A macrophage migration inhibitory factor (MIF) is one of the cytokines identified originally as an inhibitor of the random migration of macrophage. It regulates both innate and adaptive immune responses as well as inflammation (Nishihira 2012). L3i of *S. ratti* secretes MIF (*Sra*-MIF) which binds monocyte/macrophage lineage to induce IL-10 but not TNF-α production. Sequence analysis of the full-length cDNA of the parasite-derived cytokine indicated the highest homology to *S. stercoralis* (Younis et al. 2012). There is a possibility that MIF derived from *S. stercoralis* might regulate host immune responses.

It is hard to analyze immunological and inflammatory responses to the adult stage of *S. stercoralis*, for lack of adequate experimental systems except an immunosuppressed canine model (Schad et al. 1984). Although rodents are not definitive hosts for *S. stercoralis*, a Mongolian gerbil (jird) infection model in which the parasite can develop to the adult has been used to analyze hyperinfection of *S. stercoralis* (Nolan et al. 1993, 1995). Autoinfection occurs only when the intestinal population of the first-stage larvae was very large in the jird model (Nolan et al. 2002). We expect that a good model will be developed to clarify the interaction between adult worms of *S. stercoralis* and host immune mechanisms. More information regarding protective intestinal immunity to *Strongyloides* spp. is available in the papers written by Nawa (2003) and Iriemenam et al. (2010).

Finally, in general, regions of developing countries with high parasitic infection rates have a reduced incidence of autoimmune diseases relating to Th1 immune responses and/or CD4+ regulatory T-cell function. Chronic liver diseases such as primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH) are thought to have an autoimmune basis to their pathogenesis (Aoyama et al. 2007). A particular situation to study is represented by Okinawa prefecture in Japan which is endemic for strongyloidosis. Aoyama et al. (2007) examined the relationship between autoimmune liver diseases and *S. stercoralis* infection. They found that the

Table 10.2 Factors of protective immunity against larval *S. stercoralis*

Innate immunity	Adaptive immunity	References
	Granulocytes (neutrophils, eosinophils) Compliment (C3) IgM	Brigandi et al. (1996)
	Granulocytes, eosinophils	Rotman et al. (1996)
	Eosinophils IgM	Brigandi et al. (1997)
	CD4, IL-4, IL-5 rIL-12 (suppress immunity)	Rotman et al. (1997)
	Eosinophils	Brigandi et al. (1998)
IL-5, eosinophils	IgM (induced by IL-5)	Herbert et al. (2000)
B cells (-)	B-1 cells (IgM)	Herbert et al. (2002a)
	IgM, IgG, complement (C3) Granulocytes (neutrophils) IL-5 (-)	Ligas et al. (2003)
	Human IgG, complement (C3) Granulocytes IgA + IgE + IgM (-) IL-5 + eosinophils (-) Ab-dependent cytotoxicity (-)	Kerepesi et al. (2004)
Eosinophils (CCR3) Neutrophils (CXCR2)	Neutrophils (CXCR2)	Galioto et al. (2006)
Eosinophils (Ag presenting)	Eosinophils (Ag presenting)	Padigel et al. (2006)
C3 C5 (-)	C3, C3a C5 (-)	Kerepesi et al. (2006)
TLR4 (-)	TLR4 PEC (neutrophils?)	Kerepesi et al. (2007)
Eosinophils (Ag presenting)	Eosinophils (Ag presenting)	Padigel et al. (2007a)
	Gαi2 protein signaling (neutrophil recruitment) Immune serum	Padigel et al. (2007b)
MPO (neutrophils) MBP (eosinophils)	MPO (neutrophils)	O'Connell et al. (2011a)
IL17A (-), IL17F (-) CXCR2 (neutrophil recruitment)	IL17A (-), IL17F (-) CXCR2 (neutrophil recruitment)	O'Connell et al. (2011b)

(-): not essential

frequency of *S. stercoralis* infection in the autoimmune liver disease group (1 %) was significantly lower than that in the control group (7 %). It might be postulated that the pathogenesis of autoimmune liver diseases is modulated by *S. stercoralis* infection through Th1–Th2 cross-inhibitory process and/or induction of CD4+ regulatory T cell which produce IL-10 and transforming growth factor-β (Aoyama et al. 2007).

10.5 Clinical Manifestations and Prognosis in Immunocompetent and Immunocompromised Patients

Morbidity caused by *S. stercoralis* infection ranges from asymptomatic light infections to severe and often fatal clinical manifestations. Symptoms are abdominal pain, anorexia, nausea with or without vomiting, diarrhea, constipation, pruritus ani, urticaria, larva currens, chest pain, dyspnea, weight loss, malaise, and nervousness (Grove 1989a). Severe infections produce various manifestations depending on the intensity of infection, the organs involved, and the presence or absence of secondary bacterial infection (Grove 1989a). Disseminated infection is related to the migrating larvae to the organs beyond the range of the normal migratory route and is often complicated by Gram-negative sepsis (Kishimoto et al. 2008).

Chronic strongyloidosis is sustained by a relatively low and stable number of adult worms by means of well-regulated autoinfection. When the stable interaction between the parasite and host is impaired, an increasing number of autoinfective larvae complete the life cycle, and the population of adult worms increase. This status is called hyperinfection (Siddiqui et al. 2006). Since Purtilo et al. (1974) described 32 cases hyperinfected by *S. stercoralis*, its association with host immunosuppression has become recognized (Grove 1989a). Those patients showed depression of cell-mediated immunity, protein-calories malnutrition, malignant conditions (carcinoma, lymphoma, leukemia, etc.), and chronic illnesses (tuberculosis, syphilis and lepromatous leprosy, etc.). Hyperinfection has been described in various reports in patients receiving renal transplantation or affected by systemic lupus erythematosus, nephritic syndrome (Grove 1989a), rheumatoid and bronchial asthma (Altintop et al. 2010), hypogammaglobulinemia (Sheet et al. 2005), and malignant lymphoma (Suzuki et al. 1989; Abdelrahman et al. 2012). These diseases/clinical conditions are treated with corticosteroids and other immunosuppressants or can cause immunosuppression by themselves (Grove 1989a). It has been hypothesized, but not proven, that hyperinfection might be mediated through steroid hormone receptors in *S. stercoralis* larvae (Siddiqui et al. 2000b).

IgG subclasses in the humoral response to *S. stercoralis* were examined in 20 patients with uncomplicated strongyloidosis and 21 immunocompromised patients with extraintestinal disease (hyperinfection). Specific IgG2 and IgG4 levels were significantly higher in immunocompetent than in immunocompromised patients. Especially IgG4 response was prominent. By immunoblotting, there was no difference in parasite antigens which were recognized by antibodies of sera from either immunocompetent or immunocompromised patients with strongyloidosis (Genta and Lillibridge 1989).

The first report indicating an association between *S. stercoralis* infection and human T-lymphotropic virus-1 (HTLV-1) infection was done by Nakada et al. (1984). HTLV-1 infection in certain individuals coinfected with *S. stercoralis* might cause an immunological unbalance which favors the parasite (Newton et al. 1992; Satoh et al. 2002a). In fact, the coinfection with HTLV-1 decreases IL-5 levels, peripheral eosinophil counts, and IgE responses consistent with a relative switch from Th2 to Th1 response (Hirata et al. 2006; Porto et al. 2001) while expanding the regulatory T-cell subset (Montes et al. 2009). Furthermore, *S. stercoralis* infection induces polyclonal expansion of HTLV-1-infected cells through activating the IL-2/IL-2R system (Satoh et al. 2002b). Thus host's immune systems seem to be modulated by coinfection with *S. stercoralis* and HTLV-1. It has been suggested that regulatory T cells play an important role in susceptibility to *S. stercoralis* hyperinfection (Montes et al. 2009).

Coinfection with HIV and *S. stercoralis* is common in endemic areas. However, HIV infection is not always a cause for disseminated strongyloidosis and hyperinfection syndrome (Lucas 1990). HIV-associated immune reconstitution disease (IRD) is the clinical presentation or deterioration of ongoing opportunistic infections that results from enhancement of pathogen-specific immune responses among patients responding to antiretroviral treatment (ART) (Lawn and Wilkinson 2006). The number of reports of IRD associated with parasitic diseases (leishmaniasis, toxoplasmosis, schistosomiasis, and strongyloidosis) has been increasing (Kim and Lupatkin 2004; Lanzafame et al. 2005; Lawn and Wilkinson 2006). IRD develops when immune responses suppressed markedly by HIV are rapidly restored during ART. In cases of disseminated strongyloidosis and hyperinfection syndrome in HIV patients, a relation between $CD4^+$ T cell and the parasite's developmental pathway seems to be most important. Interestingly, significant negative correlations were shown between $CD4^+$ cell counts and the proportions of free-living male and female worms. Homogonic development of *S. stercoralis* seems to be favored in individuals with preserved immune function (Viney et al. 2004).

In contrast to these, no cases of hyperinfection syndrome have occurred in an urban US AIDS cohort studied by Nabha et al. (2012), with the exception of a few patients with signs and symptoms referable to *Strongyloides*-associated IRD following ART. However, HIV-infected patients remain at risk of hyperinfection with *S. stercoralis*, when receiving corticosteroids to treat *Pneumocystis jirovecii* pneumonia, extrapulmonary tuberculosis, and so on. HIV-positive immigrants from endemic areas should be screened systemically for strongyloidosis (González et al. 2010; Llenas-García et al. 2012; Mascarello et al. 2011).

10.6 Diagnosis (Inclusive Histopathology)

10.6.1 Microscopic Examination and Histopathology

Detection of *S. stercoralis* larvae can be done by microscopic examination of feces, duodenal aspirates, or bronchoalveolar lavage. A filter paper method is useful to recover filariform larvae for identification of the parasites. Using an agar plate (Fig. 10.2), fecal cultures can increase the sensitivity even if larvae are low in number in feces examined (Arakaki et al. 1990; Ines et al. 2011; Kaminsky 1993;

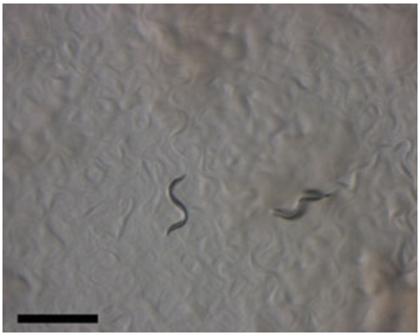

Fig. 10.2 Motile larvae of *Strongyloides venezuelensis* and furrows seen on agar plate (Bar indicating 0.5 mm)

Machicado et al. 2012; Salazar et al. 1995). When compared to the efficacy of four different methods (direct fecal smear, formalin-ether concentration, Harada-Mori filter paper culture, and agar plate culture), the agar plate culture (using 3 g of feces) was highly effective (Sato et al. 1995). Results of a single stool examination by use of conventional technique fail to detect larvae in up to 70 % of cases (Siddiqui and Berk 2001). Even when the examinations were repeated daily for three days, the reconfirmation rate was 51.5 % by the direct smear and 45.5 % by the concentration method (Sato et al. 1995). These results indicate that it is difficult to detect *S. stercoralis* larvae in stool specimens because the majority of cases involve chronic low-level infection (Sato et al. 1995).

Khieu et al. (2013) conducted a cross-sectional study in 458 children from four primary schools of semirural villages in Cambodia, using agar plate culture (for a hazelnut-sized stool sample) and Baermann techniques (for a walnut-sized stool sample) on three stool samples. The sensitivity of agar plate culture and Baermann was 88.4 % and 75.0 %, respectively. The negative predictive values were 96.4 % and 92.5 %, respectively. The estimated prevalence according to a model of Marti and Koella (1993) was 24.8 % of the study population. The cumulative prevalence increased from 18.6 % with a single test to 24.4 % after analyzing three stool samples. This figure was close to the Marti and Koella model's true prevalence. Khieu et al. (2013) suggested that the examination of multiple stool samples with different diagnostic methods is required to reach a reliable estimate of the prevalence in absence of a gold standard.

Histological examination of duodenal or jejunal biopsy specimens might reveal adults and/or larvae embedded in the mucosa. Kishimoto et al. (2008) clearly showed that observation and biopsy from a total of 25 cases by an esophagogastroduodenoscopy (EGD) were effective tools for diagnosing strongyloidosis, besides gastroduodenal drainage and stool analyses. Abnormal endoscopic findings in the duodenum were edema (69.5 %), white villi (56.5 %), erythema (39.1 %), erosion (26.0 %), stenosis (17.3 %), fine granule (17.3 %), hemorrhage (13.0 %), dilatation (13.0 %), and ulcer (8.6 %) (Fig. 10.3, after Kishimoto et al. 2008). The histopathological changes in fatal cases were classified into three categories (De Paola et al. 1962). First, catarrhal enteritis is a minor form characterized by mild mucosal congestion with larvae restricted to the mucosal membrane. Second,

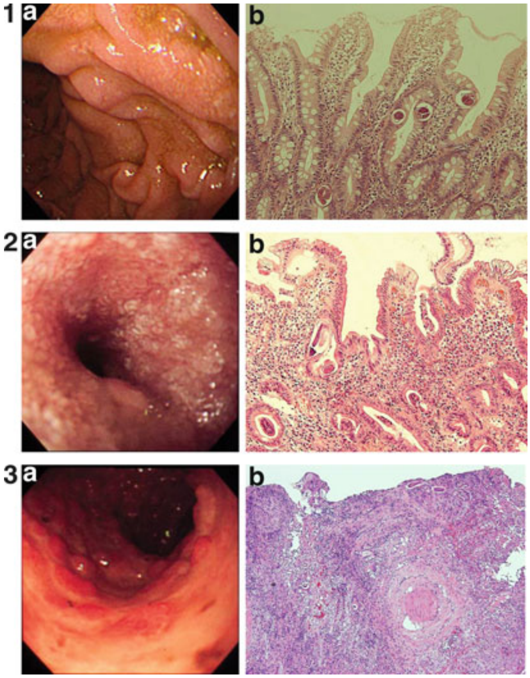

Fig. 10.3 Endscopic and histopathological observations on the duodenum of *Strongyloids stercoralis* hyperinfection. **1.** (**a**) Endoscopic image showing white villi and edematous mucosa in the second part of duodenum. (**b**) Biopsy specimen from the mucosa showing numerous larvae with villous atrophy and mild inflammatory cell infiltration (HE staining, ×200). **2.** (**a**) Endoscopic image showing white villi and stenosis in the second part of duodenum. (**b**) Biopsy specimen from the mucosa showing numerous larvae with severe villous atrophy and moderate inflammatory cell infiltration (HE staining, ×200). **3.** (**a**) Endoscopic image showing large ulcers and pseudopolyps in the second part of duodenum. (**b**) Biopsy specimen from the margin of the ulcer showing formation of granulation tissue and complete destruction of the villi. Numerous larvae are observed within the granulation and lymph vessels (HE staining, ×100). Reference: Kishimoto K, Hokama A, Hirata T et al. (2008) World Journal of Gastroenterology 14(11): 1768–1773. The publisher and Hokama (correspondent author) gave us permission

edematous enteritis is a moderately serious form characterized by edematous thickening of the wall, swelling folds, and villous atrophy with larvae invading lymph vessels. Third, ulcerative enteritis is a serious form characterized by ulcers and fibrosis. Larvae were found in the entire wall.

S. stercoralis infection disturbs the mucosal integrity and compromises the intestinal barrier. Infection is associated with high apoptosis rates concomitant with low cell proliferation in duodenal and jejunal biopsies. The proliferative index is significantly reduced in patients compared to controls in both duodenal and jejunal biopsies, using an immunostaining method with Ki-67 which identifies cells in different cell-cycle phases (Werneck-Silva et al. 2006).

10.6.2 Serological Diagnosis

Serological tests have been developed to detect antibodies against *S. stercoralis* crude (CrAg), purified or recombinant antigens.

Indirect immunofluorescence using larval *S. stercoralis* antigen showed a 92 % positivity for IgG antibodies with no cross-reactivity to *Schistosoma mansoni*, *Loa loa*, or hookworm or in patients with idiopathic hypereosinophilia. A weak positivity was found in Bancroftian filariasis patients (Genta and Weil 1982). Relatively low molecular weight proteins (41, 26, and 22 kDa or 41, 31, and 28 kDa) from larval *S. stercoralis* were shown to be reactive to IgG and to be applicable for immunodiagnostic tools such as enzyme-linked immunosorbent assay (ELISA) and immunoblotting (Sato et al. 1990; Conway et al. 1993). Highly immunodominant 41 kDa antigen (P5) exhibited immunoreactivity with 83 % of patients with strongyloidosis. Sequential analysis showed that P5 antigen is γ-subunit of isocitrate dehydrogenase (NAD$^+$) (Siddiqui et al. 2000a).

Although ELISA using larval antigens is thought to be useful for immunodiagnosis, there is a problem with supplying antigenic materials sufficiently. Therefore, a recombinant 31 kDA antigen (NIE) derived from L3i of *S. stercoralis* was developed, which resulted in the specificity of 87.5 % with 48 sera from the patients with strongyloidosis. The NIE antigen was reactive with both parasite-specific IgE and IgG from the pooled patients' sera. There was no cross-reactivity to *Onchocerca volvulus*, *L. loa*, and *Mansonella perstans,* but in tropical pulmonary eosinophilia presumably caused by *Wuchereria bancrofti*, false-positive results were obtained (Ravi et al. 2002).

Furthermore, luciferase immunoprecipitation systems (LIPS) were applied to detect parasite-specific IgG using recombinant antigens, NIE and SsIR. LIPS assays using either NIE or SsIR as antigen exhibited the same or higher performance in sensitivity or specificity compared to ELISA using the same antigens. When the assay was applied to combine NIE with SsIR as antigens, LIPS was 100 % sensitive, and specific, with an optimal negative (NPV) and positive predictive values (PPV) (Ramanathan et al. 2008). An excellent community-wide study on strongyloidosis was reported using stool examination (agar plate, Baermann, sedimentation concentration, and Harada-Mori) and serodiagnosis (CrAg-ELISA, NIE-ELISA, NIE-LIPS, and NIE-SsIR-LIPS). The prevalence of *S. stercoralis* infection was 29.4 % by stool examination using agar plate, Baermann, sedimentation concentration, or Harada-Mori methods. The optimal cutoff point for each immunoassay was determined by plotting the sensitivity and specificity for cutoff point values by means of the receiver operating characteristic (ROC) curves. NIE-LIPS revealed the highest sensitivity (97.8 %) and specificity (100 %) for detecting specific IgG (Krolewiecki et al. 2010).

While serodiagnosis using CrAg and NIE is slightly cross-reactive to Bancroftian filariasis as mentioned above, recombinant strongylastacin, a 40 kDa metalloproteinase, does not cross-react with IgE antibodies from either patients with *W. bancrofti* or patients with tropical pulmonary eosinophilia and increased level of IgE antibodies (Varatharajalu et al. 2011). Interestingly, the immunoblots and ELISA revealed the presence of IgG antibodies to strongylastacin in all individuals, irrespective of *S. stercoralis* infection status. IgG antibodies to strongylastacin are ubiquitous, because they are thought to result from zinc metalloproteinases, including astacin-like enzymes in food and/or in the gut's normal biota (Varatharajalu et al. 2011).

10.6.3 PCR-Based Examination

Since the paper by Putland et al. (1993), 18S rDNA and mitochondrial DNA of *S. stercoralis* have been utilized for phylogenetic analysis and diagnostic purposes (Dorris et al. 2002; Hu et al. 2003). Hasegawa et al. (2009) critically showed that hypervariable regions in 18S rDNA are suitable for markers with species-specific diagnosis in strongyloidosis. Some isolates of *Strongyloides* spp. were analyzed with 18S rDNA, showing that the genetic relationship among parasite populations is not related to the host species (human, chimpanzee, and canine) but to geographical distribution (Pakdee et al. 2012).

A *S. stercoralis* real-time PCR has been developed and achieved higher specificity and sensitivity comparing to Baermann sedimentation and coproculture (Verweij et al. 2009). The primer and probe set from the 18S rRNA gene sequence was 10-fold to 100-fold more sensitive than the PCR designed from the cytochrome c oxidase subunit I gene or the *S. stercoralis*-specific repeated sequence. However, the real-time PCR applied in asymptomatic cases in Cambodia showed a lower sensitivity compared to studies undertaken with symptomatic patients (Schär et al. 2013). Fluorescence resonance energy transfer (FRET) real-time PCR techniques have been applied to detect 18S rRNA (Janwan et al. 2011) or 28S rRNA gene sequences (Kramme et al. 2011) in fecal samples. Kramme et al. (2011) suggested that FRET real-time PCR reduced nonspecific binding in comparison with TaqMan minor groove binder probe for amplicon detection used by Verweij et al. (2009).

A nested PCR targeting the internal transcribed spacer I (ITS1) region of the ribosomal DNA gene has been used to amplify *S. stercoralis* DNA (Nilforoushan et al. 2007) and to apply to fecal samples for field survey (Ahmad et al. 2013).

10.7 Treatment

According to Centers for Disease Control and Prevention (USA) (www.cdc.gov/parasites/strongyloides/health_professionals/index.html) and Segarra-Newnham (2007), a treatment for strongyloidosis is recommended as follows:

10.7.1 First-Line Therapy

Ivermectin (Merck Sharp & Dohme Research Laboratories, NJ, USA)

200 μg/kg/day, 1 dose; repeat same dose after 2 weeks.

In case of immunosuppressive patients or disseminated patients, repeat totally 4 doses or more every 1–2 weeks. Follow-up stool examination should be done to verify eradication of worms.

Contraindications are as follows: there is no safety data for pregnant or lactating women and child patients weighing <15 kg. Confirmed or suspected concomitant *Loa loa* infection may cause serious side effects.

Most of the patients treated with ivermectin had no side effects in Japan. But some complained of nausea, anorexia, dizziness or vertigo, blurred vision, and malaise after the first treatment and itching and borborygmus after the second treatment (Shikiya et al. 1992).

Refer to WHO recommendations:

http://whqlibdoc.who.int/publications/2006/9241547103_eng.pdf.

10.7.2 Alternative

Albendazole, 400 mg orally twice a day for 7 days.

Some patients complained of diarrhea and abdominal pain (Segarra-Newnham 2007).

Contraindications are as follows: patients with hypersensitivity to benzimidazole. Its use should be avoided in the first trimester of pregnancy.

Refer to WHO recommendation: http://whqlibdoc.who.int/publications/2006/9241547103_eng.pdf.

Basic pharmacology of various drugs for strongyloidosis was reviewed by Grove (1989b).

10.8 Prevention and Control

Personal hygiene is important to prevent strongyloidosis, wearing shoes and using lavatory not to contaminate soil of living places and working fields. For public health, unfortunately, no vaccine for *Strongyloides* has been put into practical use so far. Recent advances in molecular biology give us some clues to potential chemotherapeutic and/or vaccine targets for strongyloidosis.

DNA microarrays are powerful tools to advance the development of vaccine discovery and chemotherapeutics. The microarray-based analysis of differential gene expression between L3i and L1 revealed differences in the expression of genes encoding putatively as well as between *S. stercoralis* L3i and *C. elegans* dauer stage larvae (Ramanathan et al. 2011). Furthermore, transcriptome analysis of L3i has provided us targets for potential chemotherapeutics using 454 sequencing coupled with semi-automated bioinformatic analyses. More than 50 % of *S. stercoralis* putative proteins examined have no homologues present in humans. Among them, several putative proteins have been searched for homologues to *C. elegans* proteins with lethal RNAi phenotype, which cause death of *C. elegans* when knocked down via RNA interference (Marcilla et al. 2012).

Deoxycholate (DOC)-soluble proteins extracted from *S. stercoralis* L3i were shown to induce protective immunity, using Abraham's implantation method. Then, larval antigens were purified by an IgG affinity chromatography. Eluted antigens, in combination with alum, generated significant protective immunity in mice (Herbert et al. 2002b). DNA vaccine induced protective immunity against *S. stercoralis* L3i in mice. Three proteins recognized by the patients' serum IgG were candidates for vaccine. Successful immunization was done with plasmid containing DNA encoding Na^+-K^+ ATPase and plasmid containing DNA encoding granulomacrophage-colony stimulating factor (GM-CSF) (Kerepesi et al. 2005). Furthermore, a recombinant antigen SsIR that is highly immunogenic in humans generated protective immunity through an antibody-dependent manner, so that SsIR plus alum may have the potential to be used for a prophylactic vaccine in humans (Abraham et al. 2011).

10.9 Concluding Remarks

The most important measure to prevent tropical infectious diseases such as strongyloidosis is the development of society and promotion of healthcare system in developing countries. According to the repot of Khieu et al. (2013), almost two-thirds of the soil-transmitted helminth infections could be avoided by proper sanitation in Cambodia.

Educational program on strongyloidosis for medical students and residents has been suggested to let them recognize the risk of strongyloidosis as well as to improve basic parasitological knowledge (Bjorklund et al. 2011). Strongyloidosis is a silent disease in most cases so that physician and health professionals may misdiagnose and/or tend to underestimate its morbidity. Precise knowledge on strongyloidosis for people concerned is needed as well as the development of effective vaccine and diagnostic tools that have specificity, sensitivity, and simplicity.

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