Table I. Distribution of *Bartonella henselae* seroprevalences according to different demographic information in 295 veterinary-associated individuals[a].

Variable	No. of people[a]	No. of seropositive cases (%)	*P*-value[b]
Gender			0.655
Male	168	2 (1.2)	
Female	127	3 (2.4)	
Age (y)			0.715
< 20	10	0 (0.0)	
21–30	94	3 (3.2)	
31–40	49	1 (2.0)	
41–50	28	0 (0.0)	
> 50	14	1 (7.1)	
Occupation			0.890
Clinician	108	3 (2.8)	
Veterinary technician	25	0 (0.0)	
Public health veterinarian	24	0 (0.0)	
Veterinary student	128	2 (1.6)	
Staff	10	0 (0.0)	
Residential area in Taiwan			0.022
Northern	158	2 (1.3)	
Middle	115	1 (0.9)	
Southern	19	1 (5.3)	
Eastern	3	1 (33.3)	
Clinic work experience (year)			0.081
0–3	124	2 (1.6)	
4–10	37	2 (5.4)	
11–20	25	0 (0.0)	
21–30	4	1 (25.0)	
> 30	4	0 (0.0)	
Scratch or bite incidents in last 6 months			0.162
Yes	125	5 (4.0)	
No	70	0 (0.0)	
Stray animal exposure			1.000
Yes	88	3 (3.4)	
No	80	2 (2.5)	
Cat/dog at home			
Yes	161	5 (3.1)	0.589
No	34	0 (0.0)	

[a] No. of people in each variable may not fulfill a total of 295 because of incomplete responses.
[b] Fisher's exact test for homogeneity.

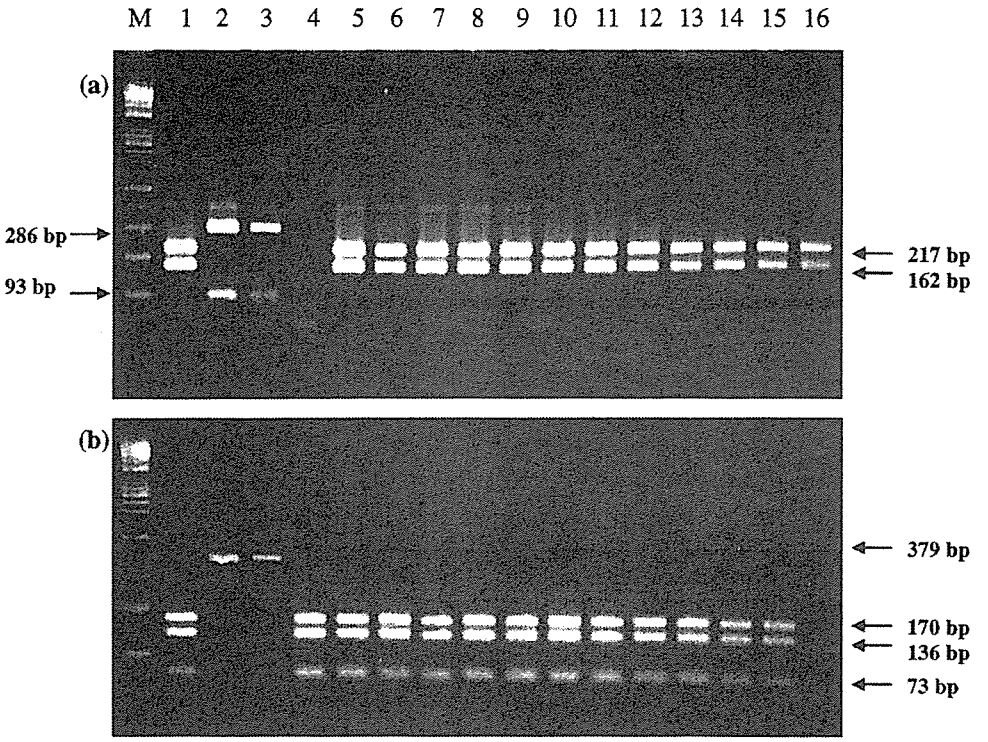

Figure 1. PCR/RFLP of the *glt*A gene for the cat isolates: (a) with *Hha* I digestion; (b) with *Taq* I digestion. M: standard 100-bp molecular ladder; lane 1: *B. henselae* ATCC 49882; lane 2: *B. clarridgeiae* ATCC 51734; Negative control: lane 4 of (a) and lane 16 of (b); the other lanes were isolates from cats tested (lane 3 is the cat positive for *B. clarridgeiae* and the other 12 lanes are *B. henselae* positive cats).

Table II. Comparison of *Bartonella* bacteremic status and seropositivity using *B. henselae* Houston-1 as the antigen.

Bacteremic status	No. of cats	No. of seropositive cats (%)
B. henselae	24	12 (50.0)
Type I only	7	6 (85.7)
Type II only	3	0 (0.0)
Co-infection of type I and type II	12	4 (33.3)
Type unidentifiable (fungal contamination)	2	2 (100)
B. clarridgeiae	1[a]	0 (0.0)
Total	25	12 (48.0)

[a] The cat was co-infected with *B. henselae* type II.

results showed that at least 29.2% (7/24) and 12.5% (3/24) of *B. henselae* bacteremic cats were only infected with *B. henselae* genotypes I or II, respectively. Interestingly, at least 50% (12/24) of *B. henselae*-infected cats were found to be co-infected

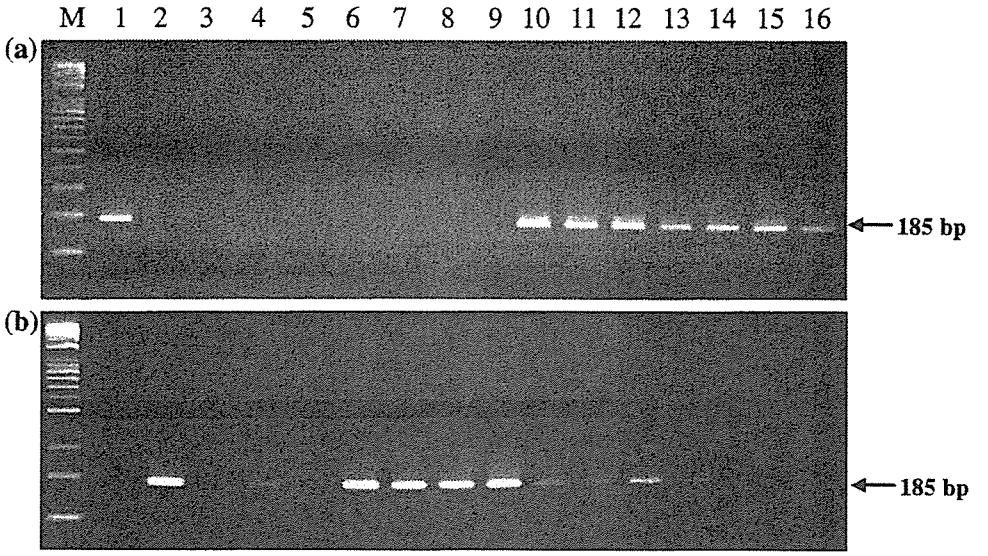

Figure 2. Using 16S rRNA gene for genotyping of *B. henselae*: (a) cats infected with genotype I; (b) cats infected with genotype II; M: standard 100-bp molecular ladder; lane 1: *B. henselae* ATCC 49882; lane 2: *B. henselae* U-4 strain; lane 3: negative control; the other lanes were isolates from cats tested.

with genotypes I and II (Fig. 2). It was identified that one cat was dually-infected by *B. clarridgeiae* and *B. henselae* type II (Tab. II).

The bacteremia level varied from 30 to 115 000 CFU/mL. Fifteen of the 25 bacteremic cats were found to have high level of bacteremia (> 1000 CFU/mL). Most of the bacteremic cats were male (60.0%), adult (88.0%), with flea infestation (88.0%) and impounded cats (80.0%) (Tab. III). According to observation by investigators, flea infestation rates varied in cats from origins, ranging from 79.9% in impounded cats, 46.7% in pet cats, and 0% in cats from the cat farm.

Thirty-one (23.7%) cats were seropositive for *B. henselae*. The geometric mean titer of the cats tested was 1:128. By univariate analysis (Tab. III), cats with flea infestation were more likely to be bacteremic and seropositive for *Bartonella* ($P < 0.05$). Cat origin was significantly associated with seropositivity and bacteremia status. Impounded cats had the highest percentages of seropositivity and bacteremia, followed by owners' cats and then cats from cat farm. None of the cats from the cat farm were seropositive nor bacteremic for *Bartonella*. Although gender was not a statistically significant factor associated with bacteremia status, male cats were 2.34 times more likely to be bacteremic than female cats. Although there was no significant association (correlation coefficient = –0.021, $P > 0.05$) between *B. henselae* antibody titer and bacteremia level, it was found that seropositive cats were more likely to be *Bartonella* bacteremic than seronegative cats (38.7% vs. 13.0%, $P < 0.05$). Furthermore, it was found that more than half (13/25) of bacteremic cats did not raise antibodies against to *B. henselae* Houston-1, mainly in cats infected with only *B. henselae* type II (3/3) and with *B. clarridgeiae* infection (1/1) and with *B. henselae* types I and II co-infection (8/12) (Tab. II). When these 12 bacteremic but seronegative cats were re-tested by IFA slides coated with type II antigen (*B. henselae* U-4, University of California, Davis, USA), 9 cats were identified to be seropositive, with ranges of antibody titers from 1:128 to 1:1024.

To evaluate the validity of direct PCR detection for determination of bacteremic status, 44 cats were blindly chosen for

Table III. Univariate analysis of the characteristics of cats associated with *Bartonella* bacteremia and seropositivity.

Variable	No. of cats	No. of seropositive cats (%)		No. of cats with bacteremia (%)	
Gender					
Male	49	11	(22.4)	15	(30.6)
Castrated male	10	2	(20)	0	(0)
Female	61	17	(27.9)	8	(13.1)
Unknown	11	1	(9.1)	2	(18.1)
Age					
Adult	114	27	(23.7)	22	(19.3)
Juvenile	17	4	(23.5)	3	(17.6)
Flea infestation					
Yes	65	23	(35.4)[a]	22	(33.8)[a]
No	66	8	(12.1)	3	(9.1)
Holding condition					
Owner's pet	30	8	(26.7)	5	(16.7)
Cat farm	37	0	(0)	0	(0)
Impounded cats	64	23	(40)	20	(31.3)
Total	131	31	(23.7)	25	(19.1)

[a] $P < 0.05$ by Fisher's exact test for homogeneity.

comparison. The sensitivity and specificity of direct PCR test of the 16S-23S rRNA intergenic region were 60% (3/5) and 67% (26/39), respectively. The PCR test was with low positive predictive value of 19% (3/16) and high negative predictive value of 93% (26/28). Further using the combination of IFA test and direct PCR test to determine bacteremic status, it was found that 96% (25/26) of the cats with negative results by both IFA and direct PCR test were non-bacteremic (Tab. IV).

4. DISCUSSION

This is the first epidemiologic study of *Bartonella* infection in veterinary professionals and cats in Taiwan, since the first human CSD case reported in 1998 [22]. The seroprevalence of *B. henselae* was 1.7% in veterinary-associated individuals. This prevalence was lower than in previous reports from Japan, which were 15% in veterinary professionals [20] and 10.9% in veterinary school students [17]. It was also lower than the 7.1% in the veterinary population that attended the veterinary Conference in Ohio, USA [30]. Because of the low seroprevalence of *B. henselae* in humans in our study, no significant risk factors were identified to be associated with the infection. Nevertheless, through clinical interviews, all of the five seropositive individuals had a history of animal bite or scratch incidents during the previous 6 months before this survey. Therefore, knowing how to handle animals properly seems to be an important way to reduce the risk of getting CSD infection in Taiwan.

Table IV. Evaluation of serodiagnosis and PCR method to determine cats with bacteremia.

	No. of cats with bacteremia	No. of cats without bacteremia
Sero-negative and PCR-negative	1	25
Sero-negative but PCR-positive	2	12
Sero-positive but PCR-negative	1	1
Sero-positive and PCR-positive	1	1

The overall prevalences of seropositivity and bacteremia in cats in Taiwan were 23.7% and 19.1%, respectively. Our data further indicated that *Bartonella* seropositivity ranged from 0% to 16.7% in pet cats and 31.3% in impounded cats. Comparing to the results in other Asian countries, the prevalence of *Bartonella* bacteremia in cats in Taiwan was between that in Japan (7.2–9.1%) [25, 26], and in the Philippines (61%) [8]. The study subjects in Japan were mainly pet cats; however, only stray cats were investigated in Philippines [8, 25, 26]. Prevalence of *Bartonella* infection in cats has been shown to be associated with climatic factors in the USA [15] and Japan [28]. That is, high seroprevalence of the infection in cats correlates with warm and humid climates. As *Bartonella* infections are mainly transmitted by arthropods, it was hypothesized that climatic factors may affect the distribution of arthropod vectors, including fleas. Our data further suggested that the prevalence of *Bartonella* infection in Asian cats was associated with countries with different latitudes, from the lowest prevalence in the temperate country (e.g. Japan), the moderate in the sub-tropical country (e.g. Taiwan) and the highest in the tropical country (e.g. Philippines).

Similar to the previous reports from other countries [3, 6, 8], cats with *Bartonella* bacteremia was strongly associated with flea infestation in our study. In Japan, the prevalence of *B. henselae* in flea-infested cats was significantly higher than that of flea-free individuals [28]. Owing to the humid and warm climate in Taiwan, the flea infestation rates were 79.9% and 46.7% in the impounded cats and in the owners' cats in our study, respectively. Among cats from a breeding farm with strict ectoparasite control, none of them were seropositive or bacteremic for *Bartonella*. The results highlight the importance of flea control in cats to prevent the disease transmission in Taiwan.

Among the 25 bacteremic cats, ten cats were identified to be only infected with *B. henselae* type I (7 cats) or type II (3 cats). However, 12 cats were co-infected with *B. henselae* type I and type II. This is the highest prevalence (9.2%, 12/131) of co-infection with *B. henselae* type I and type II in cat population that has ever been reported worldwide [4, 13, 27]. In most Asian countries, *B. henselae* isolates from cats belong predominantly to type I, even if the number of cats tested is rather small [5, 26, 27]. Similarly, we identified a higher proportion of *Bartonella*-bacteremic cats with *B. henselae* type I infection than type II infection.

Yamamoto et al. [35], reported that cats primarily infected with *B. henselae* type I and challenged with *B. henselae* type II showed cross-protection from bacteremia, whereas no cross-protection was previously shown for cats primarily infected with *B. henselae* type II and challenged with *B. henselae* type I [34]. Therefore, it would be reasonable to hypothesize that antibodies against the antigens of *B. henselae* type II might not reacted with the antigens of *B. henselae* type I. Previous reports [11, 21] have also shown that cats with *B. henselae* type II or *B. clarridgeiae* bacteremia could

be seronegative by IFA when using *B. henselae* Houston-1 as the antigen. The same results were also identified in our study. Therefore, using *B. henselae* Houston-1 only as the antigen for sero-diagnosis would underestimate the number of seropositive cats with *B. henselae* type II infection.

A valid test for CSD diagnosis in cats is important for prevention of the disease transmission. Our results indicated that significant association between seropositivity and bacteremic status, while IFA test was with low positive predictive value (42%) and moderate negative predictive value (86%). Nowadays, PCR machines are available almost in every diagnostic laboratory. Molecular identification of *Bartonella* DNA using whole blood samples may offer quick reference data. We found direct PCR test of 16S-23S intergenic region offers 93% negative predictive value for determining *Bartonella* non-bacteremic cats. If the cat was both PCR and IFA negative, the probability of being a non-bacteremic cat could be even higher (with a negative predictive value of 96%). Clinically, it seems to be a feasible way to determine *Bartonella*-free cats from unknown origins.

In conclusion, the study implied the importance of stray cat control in Taiwan for CSD prevention, on the basis of high prevalence of *Bartonella* bacteremia in this population. Although the seroprevalence of CSD was not high in the veterinary populations that we investigated, people still need to be aware of acquiring the infection through accidental transmission from stray cats living close to human environments.

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Evaluation of the anthelmintic effects of artesunate against experimental *Schistosoma mansoni* infection in mice using different treatment protocols

Lu Shaohong [a,b], Takashi Kumagai [b], Ai Qinghua [b], Yan Xiaolan [a,b], Hiroshi Ohmae [c], Yoshisada Yabu [b], Li Siwen [a], Wen Liyong [a], Haruhiko Maruyama [b], Nobuo Ohta [b,*]

[a] *Institute of Parasitic Diseases, Zhejiang Academy of Medical Sciences, Hangzhou 310013, China*
[b] *Department of Molecular Parasitology, Nagoya City University Graduate School of Medical Sciences, Nagoya 467-8601, Japan*
[c] *Institute of Basic Medical Sciences, University of Tsukuba, Ibaraki 305-8575, Japan*

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Abstract

The therapeutic effects of artesunate against experimental *Schistosoma mansoni* infection in mice were analyzed. Previous studies showed that artesunate is highly effective against *S. japonicum* infection, but the action of this drug against *S. mansoni* remained uncovered. The present study examines the optical conditions for artesunate against *S. mansoni* and evaluates the effects of inhibiting the sexual maturation of adult worms. Mice infected with *S. mansoni* were orally administered with artesunate according to different schedules. Four consecutive administrations of 300 mg/kg of artesunate at 2-week intervals conferred almost total protection without the development of pathological lesions in the liver. The significant reduction in the number of eggs produced by surviving worms and the status of egg maturation suggested that artesunate inhibits sexual maturation. Electron microscopy revealed that artesunate caused morphological damage, especially on the worm tegument. Artesunate was also very effective in iron-deficient mice. Furthermore, the efficacy of artesunate was equal to or better than that of artemether against *S. japonicum* infection. Considering that artemether is more toxic, artesunate is currently one of the most efficient drugs against immature *S. mansoni*.

Keywords: *Schistosoma mansoni*; Artesunate; Prophylaxis; Iron-deficiency; Fecundity

1. Introduction

Schistosomiasis remains an important parasitic disease in terms of large endemic area and the number of infected individuals [1]. Among the five major species of human schistosomes, *Schistosoma mansoni* is the most prevalent, being endemic in 55 countries mainly of sub-Saharan Africa as well as in some parts of South America [2].

Metrifonate, oxamniquine and praziquantel have been used to treat schistosomiasis over the past few decades [3]. Among these, praziquantel is presently the only drug that is highly effective against the adult stage of all human schistosome species with no or minimal side effects [4–6]. However, a series of recent laboratory studies and clinical trials has indicated that schistosomes are developing resistance to praziquantel [7–10]. Thus, the present widespread use of praziquantel might eventually negate the benefits of this drug. Considering that the current state of vaccine development is still far from practical application [11], effective drugs for the prophylaxis and therapy of schistosomiasis are urgently required.

Artesunate (dihydroartemisinin-10-α-succinate) is a derivative of artemisinin that has improved solubility and chemical stability, as well as enhanced anti-malarial activity [2,12,13]. It was originally synthesized and used as an anti-malarial drug in China in 1987 [14]. It has low toxicity and no mutagenicity [15]. Li et al. discovered that artesunate could kill schistosomula and that it had prophylactic properties against *S. japonicum* [16]. Malic dehydrogenase, 6-phosphate mannosidase and acid phosphatase are inhibited in *S. japonicum* and tegument damage arises in worms after exposure to artesunate

Abbreviations: CMC-Na, sodium carbonyl methylcellulose; FWRR, female worm reduction rate; PBS, phosphate buffered saline; PI, post infection; SD, Standard deviation; WRR, Worm reduction rate.

* Corresponding author. Tel.: +81 52 853 8184; fax: +81 52 842 0149.
E-mail address: nohta@med.nagoya-cu.ac.jp (N. Ohta).

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[17–20]. The worm reduction rates (WRR) were 77.5–99.5% when *S. japonicum*-infected mice (300 mg/kg), rabbits (20–40 mg/kg) and dogs (30 mg/kg) were orally administrated with artesunate once each week for 4 consecutive weeks [21]. Patients infected with *S. japonicum* in endemic areas of China were treated with praziquantel, and then divided into one group that was inoculated with 6 mg/kg of artesunate and another that received a placebo [22]. The protective rates of artesunate were 83.9–100% and 68.2% in areas of light to moderate, and heavy endemic infection, respectively. Recently, artesunate has also been reported to be effective against *S. mekongi* infection [23].

The efficacy of artesunate against infection with *S. mansoni* as opposed to *S. japonicum* remains controversial. An early study by Araujo et al. showed that artesunate obviously modified the reproductive organs of *S. mansoni* [24]. The results of our preliminary study showed that artesunate was therapeutically effective against *S. mansoni* infection in mice. We investigated details of the prophylactic and therapeutic aspects of artesunate against *S. mansoni* infection with a view of expanding the use of this drug. Since recent report has suggested that artesunate is less effective against *S. mansoni* than artemether, we attempted to reproduce these findings [25]. Chronic *S. mansoni* infection is associated with a latent iron-deficiency and iron is thought to be important for the activities of artemisinin-derivatives [26–28]. We therefore studied the effects of artesunate against *S. mansoni* infection in iron-deficient and in normal mice.

2. Materials and methods

2.1. Parasites and infection of mice

Cercariae of Puerto Rican strain of *S. mansoni* were released from *Biomphalaria glabrata* snails after exposure to artificial light for 3–5 h. BALB/c female mice weighting about 20 g (SRL, Hamamatsu, Japan) were infected with 200 cercariae by the tail dipping method. Developmental stages of the parasites were divided into the following: pre-lung stage at <7 days after infection, lung stage at 7–8 days after infection, post-lung stage at 2–3 weeks after infection, young adult stage at 4–5 weeks after infection, and adult stage at 6 or more weeks after infection. The Ethical Committee for Animal Experiments, Nagoya City University Graduate School of Medical Sciences, approved our experimental protocol.

2.2. Treatment protocol

Artesunate provided by the Guilin Pharmaceutical Corp (lot #021205) (Guilin, China) was suspended in 1% sodium carbonyl methylcellulose (CMC-Na) for treatment of *S. mansoni*-infected mice. Mice were administrated orally with the aid of stainless stomach tube attached to a syringe. In each experiment, there was a control group(s) being administrated with 1% CMC-Na alone. In all experiments described below, one group contained 6 or 7 mice.

The first experiment was to determine which developmental stage of the parasite was most susceptible to artesunate. Five groups, each of which contained 7 mice, were orally administrated with dose of 300 mg/kg for 2 consecutive days on the day 7–8, 14–15, 21–22, 28–29, or 35–36 post infection (PI). This covered different developmental stages (7–36 days) of the parasite. Adult worms were collected by perfusion method 56 days PI. The dosage of 300 mg/kg was referenced from our previous results from testing *S. japonicum* and *S. mansoni* [19,24].

In the second experiment, 6 groups, each of which again contained 6 or 7 mice, were treated orally in different time schedule at the dose of 300 mg/kg. Comparisons were made for three points: start of treatment (14 or 21 days PI), frequency of treatment (3 or 4 times), and interval of treatment (every 2 weeks or 3 weeks). Adult worms were collected by perfusion method 70 days PI.

For electron microscopic observation and for in vitro oviposition study, we prepared mice with sub-optimal artesunate treatment, because full-dose treatment of artesunate eliminated almost all worms. For this purpose, mice were treated orally with 100 mg/kg of artesunate on 14 days PI, followed by two consecutive treatments on day 28 and 42. Adult worms were collected by perfusion method 56 days PI.

2.3. Iron-deficient mice

To test the efficacy of artesunate in the iron-deficient host animals, 2 groups had been fed with normal or iron-deficient feeding for 7 weeks, and we tested the iron concentration in serum every week by Fe-IC diagnostic kit (Wako, Osaka, Japan). After Fe level in the serum decreased significantly, groups of mice were infected with *S. mansoni*, and then given the drug on day 14 PI at dose of 300 mg/kg once a week for 4 consecutive weeks. WRR were compared between the two groups as described below.

2.4. Assessment of the therapeutic effects

Mice were sacrificed and worms were recovered by portal perfusion with phosphate-buffered saline (PBS). The reduction rates of total and female worms were calculated by comparing the mean worm number with that of control group. The whole small intestine and almost all of the liver, from which a small part was removed for histological examination, were digested in 4% KOH overnight at 37 °C in separate tubes. The number of eggs were counted after centrifugation and washing. The fecundity of female worm was assessed by the parameter of eggs per female worm calculated as follows: total number of eggs recovered from mice was divided by the number of female worms. Paraffin-embedded sections of the liver were examined histologically to assess the hepatic lesions due to *S. mansoni* infection. Wet weight of the liver from each mouse was measured.

2.5. Scanning electron microscopic analysis

Adult worms obtained from the group treated with artesunate and from the corresponding control group were

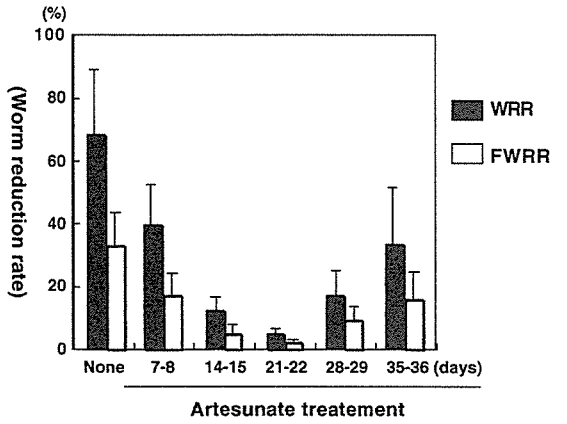

Fig. 1. Optimal time for artesunate prophylaxis against *S. mansoni* infection. Artesunate was orally administrated at the dose of 300 mg/kg to infected mice at different times from day 7/8 to day 35/36 after infection. Parasites were collected by perfusion 8 weeks after infection. WRR was highest at 21 days after infection.

tested for electron microscopic observation. The worms were collected by perfusion method 56 days PI, and processed for scanning electron microscopic analysis according to standard methods [18]. Briefly, samples were fixed with 1–2% glutaraldehyde, washed, and post fixed in 0.1 M osmium tetroxide, dehydrated using ethanol, coated with gold-palladium and then examined with a scanning electron microscope (S-4800, Hitachi, Japan).

2.6. In vitro evaluation of artesunate on oviposition by adult worms

Adult *S. mansoni* pairs of 8 weeks PI were obtained from control or artesunate-treated mice, and were cultured in vitro to compare their egg production in vitro. Two worm pairs were dispensed to each well of 24 well plates (Nunc, Roskilde, Denmark) in RPMI 1640 (Sigma, St Louis, USA) supplemented with 10% fetal bovine serum (FBS; GIBCO BRL, Rockville, USA) and 100 μg/ml streptomycin, 100 U/ml penicillin and 20 mM L-glutamine (GIBCO). After incubation for 3 days with 50 μg/ml of hemin in CO_2 incubator at 37 °C, produced eggs were collected and compared microscopically between the worms from artesunate-treated and non-treated mice. Results were shown as mean egg number per one worm pair.

Table 2
Effect of artesunate (300 mg/kg) with different regimens on oviposition and hepatomegaly in *S. mansoni* infection

Group	*N*	Administration (days)	Eggs/female[a] ± SD	Mean liver weight (g) ± SD
1	6	–	4449±641	2.00±0.40
2	5	14–28–42	448±91*	1.14±0.02*
3	4	14–35–56	155±26*	1.15±0.01*
4	4	14–28–42–56	270±33*	1.28±0.11*
5	5	21–35–49	702±226*	1.17±0.04*
6	6	21–42–63	1339±320*	1.28±0.13*
7	6	21–35–49–63	684±150*	1.23±0.16*
Cont[b]	4	–	–	1.10±0.01*

[a] Number of eggs deposited in the intestine and the liver divided by number of female worms recovered.
[b] Cont: Age-matched no infection control.
* $p<0.01$ (vs Group 1).

2.7. Statistical analysis

Student's *t*-test was employed to assess the statistical difference between treatment group and control group. Differences were considered when p values were <0.05.

3. Results

3.1. Susceptible stages of S. mansoni

Fig. 1 shows the susceptibility of *S. mansoni* at various developmental stages to artesunate in mice. The anthelmintic effect was significant at a WRR of 41% in the group administered with artesunate at the lung stage (days 7–8 PI), and reached a maximum of 93% WRR in the group medicated at the post-lung stage (days 14–15 and 21–22 PI). The efficacy decreased when mice were treated after day 21 PI, but the effect against the young adult stage remained significant (days 35–36 PI) at a WRR of 46% ($p<0.01$).

3.2. Optimal protocol for artesunate treatment

To identify a suitable treatment schedule, groups of mice were initially given artesunate on either of day 14 or 21 PI, and

Table 1
Effect of artesunate (300 mg/kg) with different regimens against *S. mansoni* infection

Group	*N*	Administration (days)	Total worms ± SD	WRR (%)	Female worm ± SD	FWRR (%)
1	7	–	25.0±11.2		6.43±2.63	
2	6	14–28–42	3.17±3.58*,#	87	1.67±1.77*	73
3	6	14–35–56	4.16±2.61*,#	83	2.33±1.89*	63
4	6	14–28–42–56	0.67±0.90*	97	0.33±0.51*	95
5	6	21–35–49	4.83±2.90*,$	81	2.17±1.48*	66
6	6	21–42–63	8.50±4.17*,$	66	3.16±1.91*	50
7	6	21–35–49–63	1.00±1.63*	96	0.50±0.80*	92

All mice were sacrificed 70 days PI.
*$p<0.01$ (vs Group 1), #$p<0.05$ (vs Group 4), and $$p<0.05$ (vs Group 7).
WRR: worm reduction rate, percent reduction in worm burden compared with Group 1.
FWRR: female worm reduction rate, percent reduction in female worm burden compared with Group 1.

repeatedly medicated at different frequencies and intervals. The WRR and FWRR were highest in mice given 4 doses of artesunate at 2-week intervals (Groups 4 and 7), regardless of whether drug administration was started 14 or 21 days PI (Table 1). The WRR values for the groups given 4 consecutive doses were significantly elevated compared with given 3 consecutive doses ($p<0.05$). Artesunate was more effective when administered at 2-week than at 3-week intervals, although the difference was not statistically significant. The reduction in oviposition of all groups given artesunate was statistically significant compared with the control group (Table 2) ($p<0.01$). The diminished egg production indicated a pathological improvement, because morbidity is mainly due to eggs being deposited in the liver and other organs. Mice that did not receive artesunate developed apparent hepatomegaly, whereas no changes were evident in infected mice given artesunate compared with uninfected mice (Table 2). Histological observations revealed that liver sections from mice treated with artesunate contained neither eggs nor circumoval granuloma (data not shown).

3.3. Efficacy of artesunate in iron-deficient mice

After 50 days of feeding with an iron-deficient diet, the mean serum iron concentration in mice decreased to 63 ± 12 μg/dl, compared with 272 ± 19.9 μg/dl in mice fed with a normal diet. Table 3 shows that worms were not recovered from either normal or iron-deficient groups treated with artesunate, while the worm burden was high in non-treated mice. The worm burdens in mice fed with iron-deficient and normal control diets did not differ. These results indicated that a serum iron-deficiency at the level tested here did not influence the efficacy of artesunate against *S. mansoni* infection.

3.4. Electron microscopic observation

Scanning electron microscopy revealed that artesunate induced damage mainly on the worm tegument. Fig. 2a and b shows normal tubercles with small sharp spines on the tegument of control 8-week-old adult worms and alterations on the tubercles of 8-week-old adult worms treated with artesunate, respectively. The tubercles on the surface of male worms were retracted and small, and the sharp spines were shortened or absent, although the sensory structures were unaffected. The suckers of both male and female worms were damaged and collapsed (Fig. 2c) and the tegument ridges were focally swollen and fused (Fig. 2d). Host leukocytes adhered to the damaged tegument, although cellular characterization of those leukocytes was not clarified (Fig. 2e).

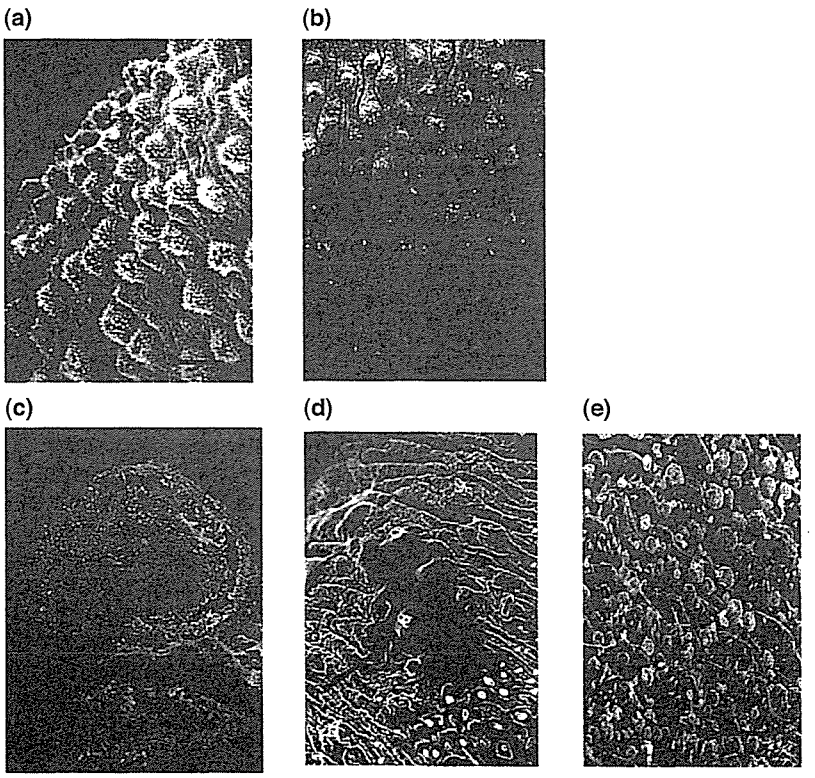

Fig. 2. Morphological damage observed by scanning electron microscopy. Worms were collected 8 weeks after infection from mice treated with artesunate at sub-optimal dose (100 mg/kg) on 14 days PI. (a) Normal tubercles on tegument of male worms from control group. (b) Alterations on tubercles of male worms from group given artesunate. (c) Damage to the oral sucker of treated group. (d) Focal swelling and fusion of tegumental ridges of treatment group (arrow). (e) Host leukocytes adhered to surface of parasites treated with artesunate, although cells were not characterized. Bars: 1.00 μm.

Table 3
Effect of artesunate on *S. mansoni* infection in iron-deficient or normal mice

Group	*N*	Fe-deficient	Artesunate[a]	Total worms recovered	WRR (%)	No. of female worms	FWRR (%)
F-1	6	+	–	24.8±3.9		13.3±2.3	
F-2	6	+	+	0*	100	0	100
N-1	6	–	–	28.8±9.1		14.0±4.0	
N-2	6	–	+	0*	100	0	100

All mice were sacrificed 70 days PI.
[a] Artesunate was treated at the dose of 300 mg/kg on day 14–21–28–35 PI.
* $p<0.01$ vs artesunate non-treated groups.

3.5. Inhibitory effects of artesunate on egg production in vitro

The number of eggs and degree of egg maturation were compared between adult pairs of *S. mansoni* recovered from mice with or without artesunate treatment. Worm pairs from control mice produced significantly more eggs in vitro than those from mice given a sub-optimal dose of artesunate (45.0±3.6 vs 12.3±1.8 eggs/pair, $p<0.01$). Over 80% of eggs produced by worms from control mice were mature and/or normally developed, whereas worms from mice treated with artesunate did not produce any mature eggs in vitro.

4. Discussion

The present study found that artesunate prominently affected the immature stages of both *S. mansoni* and *S. japonicum* but at different levels of efficacy, since *S. mansoni* was the most susceptible to artesunate 1 or 2 weeks later than *S. japonicum* in a previous study in mice [21]. The lung stage schistosomula of *S. japonicum* were the most susceptible to artesunate [21], whereas immature adult *S. mansoni* worms at the post-lung stage were most affected by the drug. This coincides with their growth profiles: *S. mansoni* takes approximately one week longer than *S. japonicum* to develop from the schistosomula to the adult stage. A common metabolic profile, which is sensitive to artesunate, might be expressed in the highest level at a particular developmental stage of schistosome parasites, but there might be a time lag of one week between the two parasite species. Our results regarding the effectiveness of artesunate are equal to the efficacy of artemether reported by Xiao et al. [29]. The difference in developmental time course could explain the different time schedule for effective drug prescription between *S. mansoni* and *S. japonicum*. To combat *S. japonicum* infection, the first medication should be given 7 days after infection and 3 consecutive administrations once a week are recommended [21], although early diagnosis method should be developed when we implement the protocol. For *S. mansoni*, drug administration starting 14 or 21 days after infection followed by 3 repeated doses at 2-week intervals provided optimal protection. Considering the lengthy exposure period in heavy endemic areas, a treatment regimen with long intervals might be practical for field application. Other morphological observations have shown that damage caused by a single administration of artemether recovers within one week [24], indicating a need for repeated administration.

In addition to immature worms, artesunate also seems to kill mature adults, because the WRR was enhanced when additional doses were administered at 8 or 9 weeks after infection (Group 4 vs Group 2, or Group 7 vs Group 5: Table 1). The mechanism of the effects of artesunate has not been defined. Electron microscopy showed that artesunate caused morphological changes in *S. mansoni*, especially tegument damage similar to that caused by artemether [18,20,30]. The adhesion of host leukocytes to the surface of the parasites suggests that parasite antigen is released and these cells could cause immune-mediated damage, although no direct evidence is available.

Artesunate not only diminished the number of eggs produced, but also retarded their maturation. Although the reduced worm burden and inhibition of sexual maturation might be responsible for preventing the development of hepatic lesion, one study has shown that artesunate protects against liver injury induced by acetaminophen and carbon tetrachloride by stimulating hepatic-metabolizing enzymes [31]. Artesunate also inhibits the growth of hepatocellular carcinoma by inducing cancer cells to undergo apoptosis and by increasing topoisomerase activities [32,33]. We speculate that the schistosomicidal effects suppressed hepatic lesions, but an additional mechanism of hepatic cell protection conferred by artesunate could not be ruled out.

Iron-dependent free radicals generated by interaction between artemisinin and a high concentration of heme generated by extensive hemoglobin digestion might be toxic for the parasite [26]. Iron is an important factor for artesunate action. Mansour et al. suggested that chronic *S. mansoni* infection is associated with a high incidence of latent iron-deficiency [34]. Artesunate exerted striking schistosomicidal effects against *S. mansoni* in iron-deficient mice at levels comparable to those in control mice with normal iron levels. We thus conclude that even a severe iron-deficiency would not influence the efficacy of the artesunate.

Among several artemisinin derivatives, artesunate and artemether are usually used as anti-malarial drugs. Artesunate is less toxic than artemether [35], but a recent study has reported that artemether is more effective than artesunate against *S. mansoni* [25], although the experimental design in these studies and artesunate lots in the present study differed. Further studies are required to conclude which should be recommended for use as an anti-schistosome drug in humans.

In conclusion, artesunate is a promising prophylactic and therapeutic agent with which to combat *S. mansoni* infection,

and human trials should be implemented in the near future. In view of the short half-life of artesunate, parasite will probably not develop tolerance or resistance. Nevertheless, artesunate should be extensively applied in areas where malaria and schistosomiasis are co-endemic to minimize any latent risk of resistance.

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Parasitology in Japan

Disease burden and epidemiology of soil-transmitted helminthiases and schistosomiasis in Asia: the Japanese perspective

Nobuo Ohta[1] and Jitra Waikagul[2]

[1] Section of Environmental Parasitology, Graduate School, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan
[2] Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Bangkok 10400, Thailand

The disease burden due to soil-transmitted helminthiases (STH) and schistosomiasis is not well documented in Asia. Both STH and schistosomiasis are chronic diseases but case detection is not easy because of the absence of clinical symptoms. STH and schistosomiasis are, however, endemic in Asia and their burden is significant. At the preparatory meeting for the Hashimoto Initiative in Japan in 1997, STH and schistosomiasis were categorized as Group 2 diseases. Parasitic infections in this category were well understood at the time but sophisticated control strategies were lacking. Japan has promoted comprehensive collaborative projects to reduce the burden of STH and schistosomiasis throughout Asia, creating an international network to collect epidemiological information and to implement and improve disease control, thus extending the school-based control method that had proved so successful in Japan.

Evaluation of disease burden

Helminth infections are important health problems in many parts of Asia but an exact evaluation of disease burden due to soil-transmitted helminthiases (STH) and schistosomiasis is not available because they are so-called 'neglected diseases'. There is no registration system for STH and schistosomiasis in Japan or other Asian countries. The current status of STH and schistosomiasis varies across the region. A recent increase in trade and human migration within Asia has highlighted the need to evaluate the epidemiological status of STH and schistosomiasis.

Japanese researchers have been proactive at building partnerships with Asian parasitologists to find applicable and effective strategies for parasite control. In the Hashimoto Initiative for global parasite control (HI) of 1997, STH and schistosomiasis were designated as Group 2 diseases [1]. The HI working group categorized parasitic diseases into three groups: Group 1 diseases require investment from the basic research stage to develop new treatments. Group 2 (which includes the filariases, in addition to STH and schistosomiasis) is a group of diseases for which control drugs already exist, and the priority is to establish a mechanism to deliver them to endemic areas. Group 3 falls between Group 1 and Group 2. This means that applied or operational research is needed, rather than basic research, for implementing disease control for STH and schistosomiasis. To discuss the Japanese perspective on STH and schistosomiasis in Asia, it is important to understand the current status of these parasitic infections in Japan and in East or Southeast Asian countries.

STH and schistosomiasis in Japan: then and now

Heavy disease burden due to STH and schistosomiasis in the first half of the 20th century was a strong driving force for parasitology research in Japan. In 1949, the incidence of STH peaked at 73% of the population [2] but the picture regarding schistosomiasis remains unclear. In 1950, only 1.6% of residents in the Kofu basin, which is in the central Yamanashi part of Japan, tested positive for *Schistosoma japonicum* in stool examinations using the direct smear method [3]. This relatively low incidence was probably a gross underestimation because a positive rate of *S. japonicum* infection of 44.2% was reported in the same area when some, but not all, health centers tested stools of schoolchildren using the centrifugal concentration (AMS III) method. Furthermore, single testing results in underestimates because repeated testing of fecal samples from residents of the Kofu basin raised the positive rate to more than twice that detected by the merthiolate-iodine-formaldehyde concentration (MIFC) method [4].

The most common STH in Japan in the first half of the 20th century was *Ascaris lumbricoides* infection, followed by *Necator americanus* infection. Night soil was widely used as a fertilizer for cultivation, resulting in contaminated vegetables, which were the main source of

Corresponding author: Ohta, N. (matata.vip@tmd.ac.jp).
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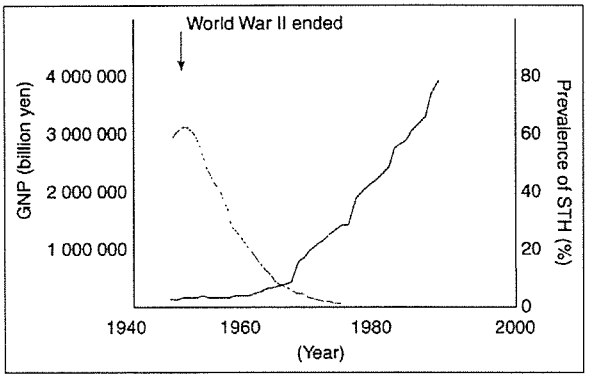

Figure 1. Control of parasitic diseases and economic development in Japan. The GNP (blue line) of Japan started to rise in the mid-1960s, whereas the prevalence of STH (green line) decreased rapidly before the economic growth in Japan. Reproduced, with permission, from M. Shimada, Nagasaki University.

STH. With the rapid improvement in quality of life in Japan (including the cessation of night soil use), the prevalence of STH fell rapidly and reached a negligible level in the late 1970s (Figure 1). Despite rapid economic growth in Japan in the 1960s, economic development was not the main factor behind successful STH control. Japan created a unique and effective scheme for parasite control after World War II, which was led by the private sector, the Japan Association of Parasite Control (JAPC) and supported by local governments [5]. JAPC implemented a school-based approach, in which teachers had the main role in health education and deworming, that was backed by the Japanese Government via proclamation of the School Health Law in 1958. The Japanese Organization for International Cooperation in Family Planning (JOICFP: http://www.joicfp.or.jp/) was also important and was established based on the unique idea of integrating STH control with family planning and nutritional improvement [6]. The parasite control activities of JAPC and the integrated programs contributed substantially to health promotion, not only in schools but also in communities because parasite control has proven to be a good starting point for encouraging community participation. Members of academia also had important roles in parasite control activities, not only by providing scientific guidance but also by evaluating the efficacy of the control interventions.

Currently, a small number of sporadic cases of STH infection still occurs in Japan. Several factors contribute to this: (i) there is a movement to use non-chemical fertilizer for vegetation, such as night soil, the use of which places more people at risk of *A. lumbricoides* infection; (ii) a specific group at risk comprises those who have lived for prolonged periods in other endemic countries, with >10% of people returning from Africa bringing back STH [7]; (iii) recently, food-borne STH from fresh food imported from endemic countries has also been identified [8]; and (iv) strongyloidiasis is still endemic in Okinawa and other southern Japanese tropical islands. A recent survey reported that the incidence of *Strongyloides stercolaris* infection is 5–10% in Okinawa, although infections were observed mainly in older age groups [9]. A strong association between strongyloidiasis and adult T-cell leukemia virus has been reported [10,11], although the biological mechanisms of the association remain to be elucidated. In addition, there is a small number of cases of opportunistic infection with *S. stercolaris* in immunocompromized people in Okinawa [12].

Schistosomiasis japonica was endemic in Japan, with two Japanese pathologists, Katsurada and Fujinami, discovering the causative parasite, *S. japonicum*, in 1904 [13]. Nine years later, Miyairi discovered *Oncomelania nosophora*, which is the intermediate host snail for *S. japonicum* [14]. These discoveries enabled the implementation of schistosomiasis control in the early 20th century [15]. Schistosomiasis is prevalent in several foci where intermediate snail host colonies exist. Although the intermediate host snails in Japan are of a single species, *S. japonicum* in each endemic focus in Japan has adapted only to *O. nosophora* of the same geographical origin [16,17]. This indicates that imported strains of *S. japonicum* are not readily introduced into Japan.

Since 1977, no newly infected cases of schistosomiasis have been reported in Japan and, in 1996, the local government in Yamanashi (Japan) declared that transmission had ceased. Since then, only imported cases have been reported, most of which were schistosomiasis from Africa. The disease control strategy for schistosomiais in Japan comprised three main approaches: (i) control of the snail intermediate host; (ii) treatment of all infected people; and (iii) concreting over the wetland habitat of the snail host [18]. Because schistosomiasis japonica is zoonotic, health checks of human residents and sampling of wild mice were the methods used for case detection. At a health check, intradermal skin tests were used to screen for schistosomal infection [19]. Although the cause is unclear, the hepatitis C virus appeared earlier in schistosomiasis-endemic areas than in schistosomiasis-free areas [20]. A similar association was reported in Egypt [21], where schistosomiasis is also endemic. *S. japonicum* infection might be carcinogenic [22], with epidemiological studies showing significant association between rectal and hepatic cancers and *S. japonicum* infection in Japan [23,24]. Cercarial dermatitis has also been reported, which is caused by schistosomes of birds (e.g. *Gigantobilharzia sturniae*). Rice farmers, in particular, are at risk because there are snails in paddy fields, and birds that feed on the snails perpetuate the life cycle of the parasite.

STH in Southeast Asia and China

STH and schistosomiasis are the most common helminth infections worldwide, especially in poor communities in Southeast Asia. STH are widely distributed throughout the region (Figure 2). It is estimated that, in 2003, 33.9 million people in Vietnam and 74.7 million people in the Greater Mekong Subregion (GMS) countries were infected with *A. lumbricoides*, and 17.6 million people in Vietnam and 32.9 million people in the GMS were infected with *Trichuris trichiura* [25,26].

There is only a small amount of recent published data about the disease burden and epidemiology of STH, and data are not available from many countries. Brooker *et al.*

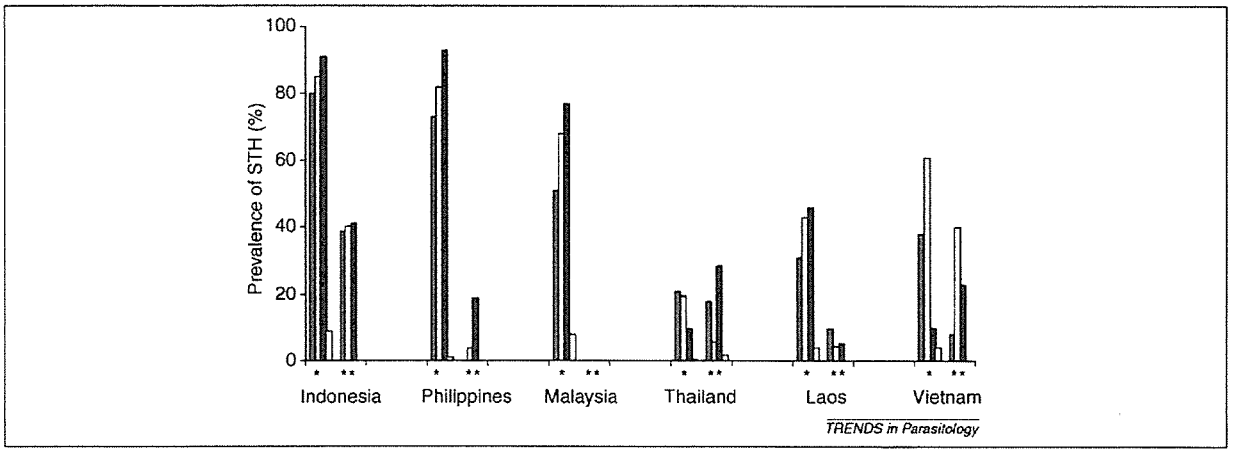

Figure 2. Prevalence of STH in Southeast Asia. The prevalence of STH is high in the south-central part (*) compared with the northern part (**) of the countries shown. Key: *Ascaris lumbricoides*, orange bars; hookworm, green bars; *Strongyloides stercoralis*, unfilled bars; *Trichuris trichiura*, blue bars.

[26] used a geographical information system (GIS) to collate and map STH distribution in Southeast Asia and found a distinct geographical variation throughout the region. In Vietnam, for instance, the prevalence of STH infection declined from the north to the south of the country [25], whereas higher prevalence occurred in the south of Thailand than in other parts of the country [27]. A report from Bali showed that wet highland had significantly higher STH prevalence than did wet lowland, dry highland or dry lowland [28]. In Malaysia, high levels of STH infection were reported in Orang Asli, an aboriginal tribe resettlement village [29]. This variation in STH occurs because transmission is strongly related to environmental and host behavioral factors (Table 1).

A national survey was carried out in China in 2003 to analyze the epidemiological status of helminthic infections [30]. Results showed that the infection rate of STH was 19.6%, of which ascariasis accounted for 12.7% (86 million people), followed by hookworm infections with 6.12% (39 million people) and whipworm infections with 4.63% (29 million people). One-third of infected individuals carried two or more parasites, with some infected by six species of parasite at the same time. The highest infection rate, with >10 000 hookworm and/or whipworm eggs per gram and >50 000 *A. lumbricoides* eggs per gram, was noted in Hainan Province (54.7%), with other southern areas such as Guizhou, Sichuan, Guangxi and Hunan showing an infection rate of at least 30%. STH in northern areas were less frequent, with the lowest infection rate reported in Xinjiang Province (0.72%). STH are most common in primary-school children and illiterate people in China, indicating that the STH infection rate decreases with higher education level. The helminthic infection rate in China seems to be declining, with heavy infections of *A. lumbricoides* and hookworm found in fewer than 2% of infected people in 2003.

Table 1. Factors influencing the transmission of STH and schistosomiasis

STH	Schistosomiasis
Environmental	
Tropical climate	Tropical climate
High humidity	Water (e.g. river)
Unhygienic sanitation	Unhygienic sanitation
Land surface temperature	Snail intermediate host
Night soil fertilizer	Reservoir hosts
Behavioral	
Toilet usage	Toilet usage
Personal cleanliness	Water contact
Occupation (e.g. farmer)	Occupation (e.g. farmer, fisherman)
Wearing shoes	

Schistosomiasis in Southeast Asia and China

Schistosoma blood flukes in Southeast Asia belong to the *S. japonicum* complex, which comprises three different species: *S. japonicum*, *Schistosoma mekongi* and *Schistosoma malaysiensis* [31]. They differ in morphology, geographical distribution, snail intermediate host and enzyme polymorphisms. Approximately 60 000 Laotians and 80 000 Cambodians are estimated to be at risk from schistosomiasis mekongi [32], and ~6.7 million Filipinos are at risk from schistosomiasis japonicum [33]. The best-known species is *S. japonicum*, which is found in China, the Philippines [34] and certain areas of Sulawesi, Indonesia [35]. In China, endemic foci occurred in seven provinces, and 843 000 infected individuals were reported in 2003. Two distinct types of schistosomiasis are endemic in China: marshland type and hill type. Approximately 11 million people are at risk from the marshland type and 5 million people are at risk from the hill type. Marshland schistosomiasis occurs in the mid- or lower reaches of the Yangtze and is under the direct influence of the environmental conditions of both the river and the surrounding lakes. Hill-type schistosomiasis occurs in western China. Although the endemic foci are not big, disease control is difficult because the foci are located in remote areas.

S. mekongi occurs in the Khong District of Laos and along the Mekong in Cambodia [32]. Up to 150 000 inhabitants were at risk of *S. mekongi* infection in the 1980s. However, the recent situation in the endemic area is much improved because of a mass treatment program led by the Cambodian Government, the World Health Organization (WHO: http://www.who.int) and Medicins sans Frontieres (http://www.msf.org). The program started in 1995, with a Japanese non-governmental organization (NGO), the

Sasakawa Memorial Health Foundation (http://www.sasakawa-igaku.or.jp), joining in 1997 [36].

S. malayensis infects various indigenous tribes in the upper Rejang river basin, Sarawak, Malaysia [37]. Among animal schistosome species, *Schistosoma spindale* is a common cause of cercarial dermatitis in humans in Indonesia, Malaysia, Thailand and Vietnam. This dermatitis is strongly associated with farmers and fishermen working in rice paddies [38].

More than ten species of mammal, including water buffalo, wild pigs, deer, horses, dogs, cats and rodents, are reservoir hosts of *S. japonicum*. Water buffalo and cattle are the most important hosts of this parasite in China, whereas dogs, but not water buffalo, are important hosts in the Philippines [32]. In the case of *S. mekongi*, 12.2% of pigs in Laos and 0.3–3.6% of dogs in Cambodia are hosts [39–41]. *Oncomelania hupensis hupensis* and *Oncomelania hupensis quadrasi* are the snail hosts for *S. japonicum* in China and the Philippines, respectively; however, apparent strain differences are noted within *O. h. hupensis* [42]. *Neotrichura aperta* is the only species of snail known to be a host of *S. mekongi* [43], and *S. malayensis* uses *Robertsiella kaporensis* as its intermediate host [37]. The restricted distribution of these snails limits the endemic areas of schistosomiasis. A study of rats in proximity to snail colonies showed that 95.5% of rats caught within a snail colony were positive for schistosomiasis, 56.5% of rats caught within 100 m of a snail colony were positive and no rat caught $\geq$1 km from a snail colony was positive for schistosomiasis. Existing control programs in endemic areas aimed at improving sanitation and reducing both the number and the size of snail habitats have led to decreased infection rates among rats and snails [44]. Common locations relating to snail breeding sites that increase the presence of the disease are irrigation networks and agricultural land [45] (Table 1).

Results of studies on the epidemiology and immunology of schistosomiasis in the Philippines indicate that the individuals who are most vulnerable to rapid reinfection are 5–14-year-old children. In China, however, high incidence is observed even in adults [46]. A drop in incidence at age 15–19 years and decreased intensity of infection at this age and in older Filippino people indicate the development of immunity [47]. Schistosomes, *T. trichiura* and hookworms cause anemia, and co-infections of these species increase the likelihood of anemia, particularly in 5–14-year-old children. Carcinogenesis associated with *S. japonicum* has also been found in China, and specific effects on mutagenicity have been suggested [48].

Schistosomiasis control in China has been implemented since 1955, when endemic situations were serious in areas along the Yangtze, including Shanghai, Wuhan and other big cities. Although the endemic situation has improved, a renewed effort to eliminate schistosomiasis was mounted as a collaborative project with the World Bank (http://www.worldbank.org) in 1992. Over eight years, a nationwide mass chemotherapy program was implemented and one endemic province, Zejiang, declared the disease to be eradicated in 1996. During the program, >200 research projects, both applied and operational, were promoted and new therapeutics, diagnostics, epidemiological techniques and cost-effective operational approaches were investigated [49]. China is considered to be in the final stage of disease eradication; however, there are several obstacles to overcome before reaching this goal [50]. Despite the intensive control program, a warning was recently issued by the Chinese Center for Disease Control and Prevention (http://www.chinacdc.net.cn) that there is a reemergence of schistosomiasis japonica in China. A nationwide survey was carried out in 2005, the results of which will be made public in the near future. Construction of the Three Gorges Dam will be completed in 2006 and the distribution of endemic foci is anticipated to change because of the changing water levels of the Yangtze. Because selective chemotherapy is undertaken during low disease prevalence in China, it is important to develop a sensitive, but cost-effective, case-detection system. Intensive surveys that use new tools and techniques are needed to create a new strategy for schistosomiasis control in China.

The viewpoint in Japan

Historically, the transmission of STH has been related to social infrastructure, including water supply, toilet facilities and sanitation, lifestyle, cultivation techniques and food distribution. Therefore, STH are considered to be a socioeconomic matter. However, in the case of Japan, the economy did not have an important role in the control of STH. Instead, the gross national product increased in Japan just after the successful control of infectious diseases such as STH (Figure 1). This means that improved public health conditions preceded economic growth. Attitude changes, based on improved knowledge and experiences, resulted in successful parasite control. Control is not expensive, yet the presence of STH and schistosomiasis is an inhibitory factor for socioeconomic development, and this is the most obvious consequence of disease burden due to helminth infection.

Japanese scientists have sought to build a close relationship with researchers in other Asian countries in both basic and applied reserach into parasitic diseases. Bilateral cooperative overseas aid orchestrated by the Japan International Cooperation Agency (JICA: http://www.jica.go.jp) has helped to strengthen the training aspects needed for parasite control, which are based on the lessons learned during previous success in Japan. One of the successful programs promoted by JICA is the HI [51]. In 2000, JICA established the Asian Center of International Parasite Control (ACIPAC: http://www.tm.mahidol.ac.th/en/seameo/thailand.htm) at Mahidol University, Bangkok, as the first center within the HI. Training courses for the school-based control of malaria and STH for program managers were organized by ACIPAC for health personnel and educators from central to provincial levels. Approximately 111 personnel, mostly from GMS countries, were trained between 2001 and 2005. Small-scale pilot projects (SSPPs) on school-based STH control, supported by JICA, have been conducted in Cambodia, Laos, Myanmar and Vietnam.

The contribution made by Japanese NGOs to parasite control should also be emphasized. The JAPC supported the Asian Parasite Control Organization (APCO), which was established in 1974. Between 1977 and 1999, an APCO

training course that used school-based STH control to gain entry into the community was conducted at Mahidol University and its partner institutions (the Faculty of Public Health and the Ministry of Public Health). More than 530 health personnel in Asia have been trained and evaluation of the training, conducted after the 20th course, showed that >50% of ex-participants continue to work in the field of parasite control (J. Waikagul, unpublished). Another bilateral cooperation scheme is the US–Japan Cooperative Medical Science Program, which was established in 1964 to focus research on diseases that are prevalent in Asia, with parasitology research being one of the main subjects. Through these cooperation schemes, the exchange of scientific information among Asian parasitologists is increasing and, by combining various bilateral cooperation programs, the Federation of Asian Parasitologists was officially established in 2001. More recently, the Japanese Government launched a new strategy for the research and control of infectious diseases by collecting biological and epidemiological information in Asia and Africa. In addition, three centers were established in Thailand, Vietnam and China as cooperative projects with Osaka University (http://ww.osaka-u.ac.jp), Nagasaki University (http://www.nagasaki-u.ac.jp) and the University of Tokyo (http://www.u-tokyo.ac.jp), respectively.

Future perspectives

Collaborative projects between Japan and other Asian countries have been ongoing in the field of basic research into parasitology and disease control, and will increase in number in the future. In particular, the development of vaccines and new therapeutics is an urgent research subject for *S. japonicum*. Several groups from Japan are, with Chinese colleagues, undertaking research projects on schistosomiasis vaccine development. Paramyosin and calpain of *S. japonicum* were tested as vaccine candidates [52–54], and partial but significant vaccine effects were observed in a field trial using domestic pigs [55,56]. Qinhaosu derivatives have been intensively investigated as new therapeutic and/or prophylactic drugs for Asian schistosomiasis [57,58]. Artemether was also shown to have prophylactic effects on various schistosome species [59]. Artesunate was also effective, not only against *S. japonicum* but also against *S. mansoni* infection; however, the optimal protocols for artesunate treatment were different between *S. japonicum* and *S. mansoni* infections [60]. Side effects were not observed and complete cure rate was confirmed.

The monitoring of intermediate host snails by remote sensing was investigated as a novel epidemiological tool for schistosomiasis. In the Philippines, a research group from Japan proposed a monitoring system that uses digital maps of Landsat images to which epidemiological information is added [61]. A similar system was developed to monitor the reemergence of schistosomiasis japonica in a former endemic focus of the disease in Japan [62].

Cooperative projects are being intensively promoted for STH and schistosomiasis control. Children of primary-school age are most affected by STH and schistosomiasis. Deworming is a preventive control measure but, to keep reinfection rates as low as possible, preventive education must be implemented widely and continuously throughout the region. A regional training program of effective education for trainers is necessary but the program remains in a low profile at present. ACIPAC activities are continuing as a collaborative project between Japan and Thailand, and SSPPs in the surrounding countries are encouraged to develop into country-level projects. In fact, as a result of this activity and the support of other international organizations, a National Intestinal Parasite Control Program has been started in Cambodia and Laos [63].

Although the prevalence of STH has decreased compared with the prevalence in the 1980s, these diseases remain a major public health problem in Southeast Asia. Extensive training programs are still needed in the region to support national programs for parasite control. ACIPAC, together with collaboration from Japan, is ready to be a partner of other international and local organizations and agencies to provide training based on the successful Japanese model of school-based control programs for parasites and other infectious diseases.

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