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STUDIES ON CURRENT TREND OF IMPORTED MALARIA IN JAPAN

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Abstract: Current epidemiologic and therapeutic aspects of imported malaria in Japan were examined from 1980 to 1995 by sending questionnaires every year to more than 1,500 major hospitals. Imported malaria gradually increased in number from 1980 and became more than 100 from 1990. The number of cases with falciparum malaria increased in number from around 1993 and became comparable to the number of vivax malaria. As the presumptive place of contraction, Africa has been increasingly important, especially for falciparum malaria. Recently the number of foreign cases, most often from India, has been becoming rather high, ranging 24 to 36% of the total cases. We found 7 fatal cases with falciparum malaria, all of whom seemed to be infected in Africa except for a Japanese female due to domestic transfusion malaria. Recrudescence and relapse were detected at significant rates, i.e., 2.2 to 9.5% and 5.4 to 17.2% of the cases with falciparum and vivax malaria analyzed, respectively. Concerning the antimalarial drugs available in Japan, mefloquine has been the drug of choice after the Research Group for Development of Chemotherapeutic Agents against Tropical Parasitic Diseases supported by the Japanese Ministry of Health and Welfare started its import for clinical trial. Further efforts are still needed to improve the diagnostic and therapeutic capacity against imported malaria in Japan.

Key words: Imported malaria, Mefloquine, *Plasmodium falciparum*, Recrudescence, Relapse

INTRODUCTION

Malaria has been increasingly a serious health hazard in most of the endemic areas, especially in Africa. At present it is estimated that more than 2 billion people are at the risk of infection. Approximately 300 to 500 million of clinical cases and 1.5 to 2.7 million deaths by malaria occur every year. Morbidity and mortality due to this infectious disease have not shown any clear trend of decline; rather even some social and civil unrests like large-scale population movements have produced new problems on malaria (Gilles, 1993). In addition, other factors, from the economic and agricultural to those concerning priority setting in the investment in public health have been apparently affecting the recent epidemiology of malaria (Suzuki, 1997). Thus, current situation of malaria is not ascribed to any single cause in the endemic area.

On the other hand, in developed countries, malaria has been of little concern, probably because new transmission of malaria parasites was terminated and the priority of medical research and education has been placed on such chronic diseases as cancer and vascular disease. However, as the number of travellers abroad has been increasing, some tropical parasitic diseases have been re-emerging as imported infections. Undoubtedly, malaria is one of the serious imported parasitic diseases in developed countries. Thus, measures for early diagnosis and effective treatment should be organized as quickly as possible.

As an effort for the chemotherapy of imported malaria in Japan, the activity of the Research Group for Development of Chemotherapeutic Agents against Tropical Parasitic Diseases has been playing an important role. It adopted mefloquine for the first time as one of the drugs supplied on the request of responsible physicians. Also on the basis of recent efforts by this Research Group, some epidemiologic issues of imported malaria in Japan have been made clear, a part of which is reported in this communication.

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Materials and Methods

The Research Group for Development of Chemotherapeutic Agents against Tropical Parasitic Diseases was organized in 1980 by Prof. Hiroshi Tanaka, Institute of Medical Sciences, University of Tokyo, on the basis of support from the Japanese Ministry of Health and Welfare. This Group has been responsible for the nationwide, comprehensive investigations on epidemiology of parasitic diseases including imported malaria in Japan in addition to a variety of other duties.

Every year from 1985, questionnaires concerning occurrence of malaria have been sent to 1,500-1,700 major hospitals in Japan. Responses to the questionnaires have been summarized by the authors. Identification of malaria parasites was essentially done by responsible physicians and collaborating laboratories, mostly parasitology departments in medical schools, and reported to us. When further identification seemed to be necessary, e.g., confirmation of the mixed infection, we requested stained specimens of patients' blood and identified species of malarial parasites. Informed consent, if needed, was obtained from the cases with imported malaria.

Results and Discussion

The annual number of malaria patients gradually increased and became more than 100 from 1990 (Table 1). Although there seemed to be a possibility that the number was underestimated, approximately 56% of the grand total cases were due to *Plasmodium vivax*, and 32% due to *Plasmodium falciparum*. The cases with *Plasmodium malariae* and *Plasmodium ovale* were much less and approximately the same in number; about 2% of the whole cases.

Table 1 The number of imported malaria cases in Japan from 1980 to 1995 summarized by the Research Group for Development of Chemotherapeutic Agents against Tropical Parasitic Diseases

Year	*P. vivax*	*P. falciparum*	*P. malariae*	*P. ovale*	Mixed	Unknown	Total
1980	58	29	1	1	2	3	94
1981	54	21	2	1	2	6	86
1982	51	26	5	1	1	3	87
1983	59	17	4	2	1	5	88
1984	54	27	4	0	0	5	90
1985	43	20	2	0	0	4	69
1986	49	25	0	3	2	4	83
1987	41	18	2	4	2	6	73
1988	47	28	1	2	0	0	78
1989	50	31	2	0	3	7	93
1990	62	40	0	3	5	6	116
1991	63	43	3	0	3	2	114
1992	70	26	0	3	4	9	112
1993	60	40	2	5	3	2	112
1994	39	46	3	4	5	7	104
1995	56	51	1	5	4	0	117
	856	488	32	34	37	69	1,516

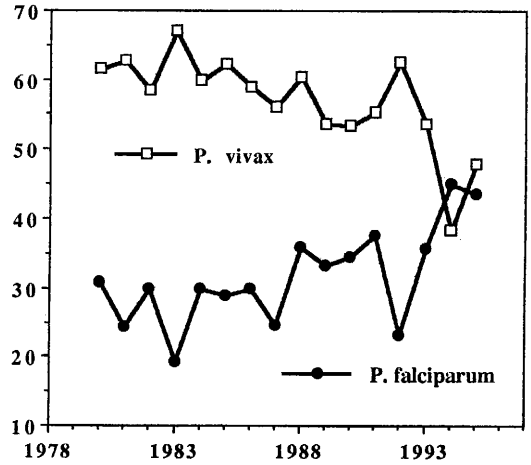

Figure 1 Annual number of imported falciparum and vivax malaria cases from 1980 to 1995. The vertical axis stands for the number of cases with either malaria each year.

Such a trend of falciparum and vivax malaria is more clearly illustrated in Figure. 1. From 1993, falciparum and vivax malaria became comparable in the number of patients. Of particular importance is that more than 70% of the Japanese cases were due to falciparum malaria and less than 25% due to vivax malaria during 1992 to 1995 (data not shown), which should be taken into consideration for establishing the countermeasures against drug resistant and/or complicated malaria.

Concerning the possible place of contraction, more than 70% of falciparum malaria seemed to be originated from Africa, while 60-70% of vivax malaria was from Asian regions (data not shown). This is generally in agreement with the data on imported malaria in other developed countries like Canada (Lynk and Gold, 1989).

Table 2 Ratio of Japanese and foreign imported malaria cases from 1990 to 1995

Year	Total	Japanese Cases	Foreign Cases
1990	116	88 (75.9%)	28 (24.1%)
1991	114	73 (64.0%)	41 (36.0%)
1992	112	74 (66.1%)	38 (33.9%)
1993	112	85 (75.9%)	27 (24.1%)
1994	104	76 (73.1%)	28 (26.9%)
1995	117	87 (74.4%)	30 (25.6%)

Table 3 Nationalities of foreign cases with imported malaria in Japan from 1990 to 1995

Countries	1990	1991	1992	1993	1994	1995	Total
1. Asian & Pacific Region							
Israel	2	2	2				6
India	11	6	7	5	6	6	41
Sri Lanka		3	1				4
Vietnam	2	2	1	3	1		9
Philippines		2	1		2	6	11
Thailand		1	2		1		4
Myanmar	1	3	1			1	6
Indonesia		3		3			6
Papua New Guinea		1		1	1		3
China	1		4	2		1	8
2. American Region							
Brazil		1	1	3	2		7
USA	1	2	3	3		2	11
3. European Region							
United Kingdom		1	2		1		4
4. African Region							
Nigeria	1	2	2			2	7
Tanzania	1	1	2		1		5
Sudan	1	1			1		3
Ghana	2	2	1		1	2	8
Ivory Coast		1			1	1	3
Cameroon	1		1	1	1		4

As one of the characteristics concerning recent trend of imported malaria in Japan, it is pointed out that rather many foreign cases with malaria have needed medical care. The rate of imported foreign malaria patients has been ranging from 24 to 36% of the whole cases since 1990 (Table 2).

Nationalities of the foreign cases with imported malaria were shown in Table 3. Among a variety of countries, India has been most prominent in number followed by Philippines, Brazil, United States, Vietnam and Ghana. The number of imported cases from India appears to be constant from 1990. Such a trend of recent imported malaria prompts medical professionals in Japan to face a novel situation and to employ another approach, e.g., understanding of different languages and customs.

Concerning the clinical aspects, 7 fatal cases due to falciparum malaria were recorded from 1990 to 1995 (Table 4). Among them, an attention must be paid to a 70 year old Japanese female, who had never been abroad and died of malaria after platelet transfusion conducted at her own resident city. However, the donor could not be identified (Kano and Suzuki, 1994). Virtually all of these cases died within 7 days after reentering into Japan, although some of them had administration of quinine, chloroquine, Fansidar and/or even artemisinin. Previous literatures also reported that the fatal cases with imported falciparum malaria had the average duration of illness from 1 to 8 days (Philpott and Keystone, 1987), which stresses the necessity of early diagnosis and prompt treatment. In accordance with the fact that falciparum malaria has recently been more serious in Africa, five of the six fatal cases other than the 70 year old Japanese female seemed to be infected in Africa. This also seems consistent with the general trend of falciparum malaria in Japan as noted above.

Rates of recrudescence and relapse of falciparum and vivax malaria, respectively, are summarized in Table 5. From 1990 to 95, the number of recrudescent falciparum malaria was 1 to 3 cases, corresponding to 2.2 to 9.5% of the cases analyzed each year. Though the

Table 4 Fatal cases with imported or domestic falciparum malaria in Japan from 1990 to 1995

Year	Case	Contraction†	Purpose‡	Period§	Treatment
1990	64, M	Ivory Coast	Industry, 14 days	4 days	Quinine
1991	70, F	Domestic	(Platelet transfusion)		
1991	28, M	Thailand	Commerce, 2 months	1 day	Quinine Chloroquine
1992	36, M*	Kenya	Sight-seeing, 7 days	7 days	Chloroquine Quinine Fansidar
1994	30, M	Mali	Investigation, 1 month	7 days	Fansidar Artemisinin
1994	30, M	West Africa	Sight-seeing, 10 days	3 days	
1995	50, F	Africa	Sight-seeing, 14 days	0 day	

*UK. All other cases were Japanese
† Presumptive place of contraction
‡ Purpose of trip with the period of stay
§ Period from re-entering into Japan to the onset of disease

Table 5 Recrudescence and relapse of imported falciparum and vivax malaria, respectively, in Japan from 1990 to 1995

	Falciparum malaria		Vivax malaria	
Year	No. Cases Analyzed	Recrudescence*	No. Cases Analyzed	Relapse*
1990	37	3	52	7
1991	36	3	44	4
1992	21	2	46	3
1993	36	3	48	6
1994	31	1	29	5
1995	45	1	37	2

* : Number of recrudescent and relapsing cases among the patients with falciparum and vivax malaria analyzed each year, respectively

rate appeared to decrease from 1994, it should not be expected that the rate will further decrease as judged from the widespread drug resistant falciparum malaria in the endemic areas. The number of relapsing vivax malaria was 3 to 7 cases, ranging from 5.4 to 17.2%. Primaquine has also been supplied by our Research Group by request; however, a rather high rate of relapse may be attributed to lack in primaquine administration because of the mixed infection with *P. falciparum* at least partially.

The drugs employed for recrudescent and relapsing malaria cases were summarized in Table 6. It is evident that recrudescent falciparum malaria were initially administered with a variety of drugs; however, 7 out of the 11 cases were given only chloroquine and/or Fansidar, which might represent recrudescence by chloroquine resistant *P. falciparum*, although the drug susceptibility was not examined. Quinine was administered to three of them. On the other hand, relapse in the cases with concomitant infection of *P. falciparum* with *P. vivax* seemed rather common. Analysis of the drugs given to these cases probably suggests that the coinfection with *P. vivax* may have been overlooked. It is also possible that the radical treatment with both schizontcides and primaquine was not attempted simply because of the lack in knowledge on the chemotherapy of relapsing malaria.

Table 6 Drugs employed for primary treatment of falciparum malaria and mixed infection with *Plasmodium falciparum* and *P. vivax* or *P. ovale* which resulted in recrudescence and relapse, respectively, in Japan

Falciparum malaria			Mixed Infection		
Contraction*	Drugs	Species	Contraction*	Drugs	Consequence†
Papua New Guinea	Halofantrine	Pf, Pv	Vietnam	Quinine Mefloquine Minomycin	Pv
Thailand	Quinine Minomycin	Pf, Pv	India	Chloroquine	Pv
Southeast Asia	Mefloquine Quinine	Pf, Pv	India	Mefloquine	Pv
Solomon Islands	Chloroquine Primaquine	Pf, Pv	Thailand	Fansidar Mefloquine Primaquine	Pv
India	Chloroquine	Pf, Pv	Papua New Guinea	Quinine Fansidar	Pv
Tanzania	Quinine Primaquine	Pf, Pv	Indonesia	Quinine, Fansimef Artemisinin	Pv
Myanmar	Fansidar	Pf, Pv	Indonesia	Mefloquine Artemisinin	Pv
Myanmar Thailand Indonesia Cameroon	Chloroquine Fansidar Chloroquine Fansidar Fansidar	Pf, Po	Africa	Chloroquine	Pv

* : Presumptive place of contraction
† : Species of malaria parasite detected after relapse
Pf : *P. falciparum*
Pv : *P. vivax*
Po : *P. ovale*

The activities of the Research Group for Development of Chemotherapeutic Agents against Tropical Parasitic Diseases has been significant in regard to establishing countermeasures against parasitic diseases in Japan. For these 15 years, this Research Group has been actively functioning in many aspects of education, consultation and surveillance on tropical parasitic diseases including imported malaria (Takeuchi, 1993). It has published a booklet for standard method of chemotherapy of parasitic diseases, which has been distributed free of charge. One of the most important activities of this Research Group is to import appropriate anti-parasitic drugs, which have not been officially approved in Japan, and to maintain them under appropriate conditions as well as supervisions. Based on the request, the Research Group has been supplying these drugs as their clinical trial under the responsibility of physicians with informed consent. Before the drugs were supplied, they were cleared for inspection of the standard for medicines by Japanese law. Current drugs which can be supplied by this Group includes those against amebiasis, giardiasis, trypanosomiasis, leishmaniasis and strongyloidiasis as well as malaria. Among these drugs, mefloquine has been requested most frequently. We have imported more than 1,500 tablets of mefloquine (Lariam tablet, Roche) since 1991.

Concerning the drugs chosen in Japan for chemotherapy of imported malaria before we officially started to import mefloquine, combination of Fansidar and chloroquine seemed to be employed most frequently (Table 7). Mefloquine was occupying only 5.6%, which was probably based on the personal import. However, after we started official import of mefloquine, it eventually occupied more than 60% in 1995, virtually all of which were supplied by the Research Group. Moreover, all of the records on outcome and laboratory data of mefloquine administration have been maintained by the Research Group. So far, we have found neither serious adverse effects nor resistant falciparum malaria concerning mefloquine.

Table 7 Comparison of drugs employed for chemotherapy of malaria between 1990 and 1995

1990 (n=36)		1995 (n=39)	
Fansidar+Chloroquine	22.2%	Mefloquine	61.5%
Fansidar	19.4%	Mefloquine+Fansidar	5.1%
Fansidar+Quinine	19.4%	Quinine+Fansidar	5.1%
Fansidar+Tetracycline	5.6%	Quinine+Tetracycline	5.1%
Chloroquine+Fansimef	5.6%	Mefloquine+Quinine	2.6%
Mefloquine	5.6%	Mefloquine+Quinine +Tetracycline	2.6%
MP Tablet*	2.8%	Mefloquine+Artemisinin	2.6%
Fansidar+Quinine +Chloroquine	2.8%	Quinine+Halofantrine	2.6%
Fansidar+Quinine +Tetracycline	2.8%	Quinine+Halofantrine +Tetracycline	2.6%
Chloroquine	2.8%	Quinine+Chloroquine	2.6%
Chloroquine+Quinine	2.8%	Quinine+Fansidar +Tetracycline	2.6%
Chloroquine+Fansimef +Tetracycline	2.8%	Fansidar+Tetracycline	2.6%
		Chloroquine	2.6%
Fansimef	2.8%		

*: Tablet containing both sulfamonomethoxine and pyrimethamine

There seems to be no doubt that malaria, mostly as an imported tropical disease, has been increasingly important in clinical medicine of Japan as well as other western countries (Miller *et al.*, 1989; Lynk and Gold, 1989). It is one of the infectious diseases which should be reported to the Ministry of Health and Welfare in Japan. However, this governmental surveillance system does not appear to cover all of the cases with imported malaria. In 1993, the Ministry reported occurrence of 58 cases of malaria, whereas the Research Group summarized 109 cases.

In Japan, currently available clinical measures against malaria do not seem entirely sufficient. First of all, at present, no antimalarial drugs are commercially available except for Fansidar. Moreover, the number of personnels as well as laboratories for accurate diagnosis of malaria is not large enough. Under such circumstances, it is quite important that antimalarial drugs like mefloquine have been available as orphan drugs through activities of such official organs as our Research Group.

As shown earlier in this communication, mefloquine, which has been supplied by our Research Group, is now the drug of choice for chemotherapy of imported malaria, especially of falciparum malaria. It is still of significant utility in such a developed country as in Japan despite the occurrence of mefloquine resistant *P. falciparum* in the endemic areas (Webster *et al.*, 1985). Moroever, there have been many requests for mefloquine for prophylaxis, though it is not acceptable for the Research Group. As far as we know, halofantorine, another antimalarial, as well as mefloquine is under clinical trial in Japan and is expected to appear in market soon. Artesunate, which has been employed for the chemotherapy of severe and complicated malaria with mefloquine (Hien *et al.*, 1996), has also been possessed by the Group.

Such Japanese situations of imported malaria and the system for its chemotherapy should be evaluated through comparison with those of other developed countries. At least it is evident that further official commitment to malaria by the government as well as medical

professionals must be sought in consideration of its seriousness as a re-emerging infectious disease.

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AN *ONCHOCERCA* SPECIES FROM CATTLE ON KYUSHU ISLAND IS *O. SUZUKII*, A TRANSFUGE PARASITE FROM THE JAPANESE ENDEMIC BOVID, *CAPRICORNIS CRISPUS*

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Abstract: In 1988-1989, microfilariae of an unidentified *Onchocerca* species were found in the skin of eight of the 16 cattle aged at least 5 years that were examined in Oita, Kyushu, Japan. Adult worms were not found in cattle, but the microfilariae taken from skin snips, which have coiled posterior parts, were grown to the infective stage in simuliid. In 1997, both adults and microfilariae of *O. suzukii* were recovered from Japanese serows (*Capricornis crispus*) in Yamagata, Honshu. Such microfilariae from serows, reported here for the first time, had coiled posterior parts and measurements {130-160 (mean, 140) μm long and 4-6 (5) μm wide} close to those from cattle. The findings showed that the *Onchocerca* sp. in cattle in Kyushu is *O. suzukii*, a transfuge parasite from the Japanese endemic bovid, *C. crispus*.

Key words: Filarioidea, Japanese serows, cattle, onchocerciasis

INTRODUCTION

After the detection in Oita of a zoonotic onchocercal nodule from a young girl in 1987, reported by Beaver *et al.* (1989) and Hashimoto *et al.* (1990), a survey of *Onchocerca* species in cattle and of their vectors was done in Kyushu (Takaoka *et al.*, 1989; Takaoka and Bain, 1990; Takaoka, 1990). In skin snips from cattle, the microfilariae of two ubiquitous species, *O. lienalis* (Stiles, 1892) and *O. gutturosa* Neumann, 1910, were identified and a third *Onchocerca* species, unknown elsewhere, was recovered only in microfilarial form. The unusual shape of these microfilariae (helicoidal posterior region and flattened body) suggested that they belonged to the genus *Cercopithifilaria* Eberhard, 1980 (Takaoka and Bain, 1990).

The microfilariae were injected into the thorax of simuliids, and infective *Onchocerca* larvae were obtained that corresponded to one of the three kinds of infective larvae that have been found in natural infections (Takaoka, 1990); the two others were already identified as *O. lienalis* and *O. gutturosa* (Takaoka and Bain, 1990). If the species from cattle is *Cercopithifilaria*, the vector may be ticks. As does the microfilaria, this *Onchocerca* sp. in the infective stage has primitive characteristics, including a long, thick body. These findings suggested that the parasite belonged to a primitive endemic species of *Onchocerca*.

The geographical isolation and morphological characteristics of the *Onchocerca* sp. from Oita suggested that its original host was the relict bovid, *Capricornis crispus*, Rupicaprinae, endemic in Japan, with herds in Mt. Sobo (1,758 m), Kyushu. Two unnamed *Onchocerca* species have been found in Japanese serows by Suzuki *et*

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al. (1982). In 1994, one of the *Onchocerca* species was reported to be *O. skrjabini* Rukhlyadev, 1964 (=*O. tarsicola* Bain and Schulz-Key, 1974), a common parasite of deer first found in the Caucasus mountains, and with a palearctic distribution (Yagi *et al.*, 1994). The other species, *O. suzukii* Yagi, Bain and Shoho, 1994, has been found only in *C. crispus*, and is among the *Onchocerca* species with primitive characteristics. Unfortunately, none of the female worms recovered had uterine microfilariae.

The purpose of this study is to describe microfilariae of *O. suzukii* from Japanese serows in the recent parasitological surveys done in Yamagata, reexamine our original records on the *Onchocerca* sp. from cattle in Oita, Kyushu, examined in 1988-1989, and compare the microfilariae of *O. suzukii* from serows with those from cattle in Oita.

Materials and Methods

Thirty-three Japanese serows were killed within a three-week period in February and March 1997 on Mt. Zao (1,841 m) in Yamagata Prefecture, Japan, in accordance with the conservation policies of the Agency of Cultural Affairs, Japan. Serows were immediately brought to Yamagata City and necropsied by the staff at the Animal Husbandry Research Center. Subcutaneous tissues were examined for filarial parasites, and tissues containing filarial parasites were stored in 2% formalin in saline; later, microfilariae were removed from the terminal portion of the uterus of *O. suzukii* females under a dissection microscope.

Separately, in 22 February 1998 seven Japanese serows were killed in Yamagata and examined for parasites at the same place. Skin snips were taken from these serows.

Results and Discussion

Adult worms of *O. suzukii* (10 intact male specimens and 16 intact female specimens) were found in subcutaneous tissues of trunks of 12 (36%) of the 33 serows examined in 1997. At this time, uterine microfilariae of *O. suzukii* were detected in only one of the 16 females found. The microfilariae (Figs. 1-4) were 130-160 (mean of 10 microfilariae, 140) μm long and 4-6 (5) μm wide. The posterior part was coiled and tapered conically. The other *Onchocerca* species, *O. skrjabini*, was found in the carpal and tarsal regions of 13 (39%) of the serows.

Two microfilariae were found in a skin snip of one of the seven serows examined. The microfilariae were 148 and 156 μm long and 5 μm wide, and the posterior part was coiled. Adult specimens of *O. suzukii* were found in this one serow that had dermal microfilariae.

The original records about microfilariae with coiled posterior parts found from cattle in our earlier studies in Oita (Takaoka, 1990) were inspected anew, because only illustrations were shown for these microfilariae from two kinds of specimens: skin snips and the stomach of simuliids. Measurements were not given in the text. Five microfilariae taken from skin snips were 156-181 (169) μm long and 4-5 (4) μm wide.

The morphological characteristics of *O. suzukii* microfilariae seemed to be identical to those of the microfilariae from cattle, showing that this *Onchocerca* sp. was *O. suzukii*. We therefore concluded that the *O. suzukii* discovered in cattle in Oita was a transfuge parasite from Japanese serows.

Adult worms of *O. suzukii* were not detected in cattle probably because only the carpal and tarsal regions of the cattle slaughtered in Oita were examined; it is now known that adult worms reside in the subcutaneous tissue of the flanks. The survey done at abattoirs in Oita showed that parasitism of cattle by *O. suzukii* was common: of the 16 animals aged 5 to 10 years, eight harbored dermal microfilariae of this species. None was found in 18 cattle that were 2 years old.

Another case of transfuge *Onchocerca* species has been described. The original host of *O. skrjabini* is red deer; the species has also been found in reindeer (Bain *et al.*, 1979). If *O. suzukii* has moved in the same way between serows and cattle, such infection may become more common in Japanese cattle in the future.

Is human onchocerciasis possible with this species? The causative species in the first case of human zoonotic onchocerciasis found in Japan resembled *O. gutturosa* in its annular cuticular ridges (Beaver *et al.*, 1989). Another case was reported in Oita in 1996 and the causative species was identified as *O. gutturosa* from features of the annular ridges (Takaoka *et al.*, 1996). In Oita, *O. suzukii* is transmitted by a blackfly species, *Simulium bidentatum*, which is anthropophilic (Takaoka and Bain, 1990). Another kind of human zoonotic onchocerciasis in which histological sections will not show annular cuticular ridges (Yagi *et al.*, 1994) may be found in areas inhabited by cattle and serows in Japan.

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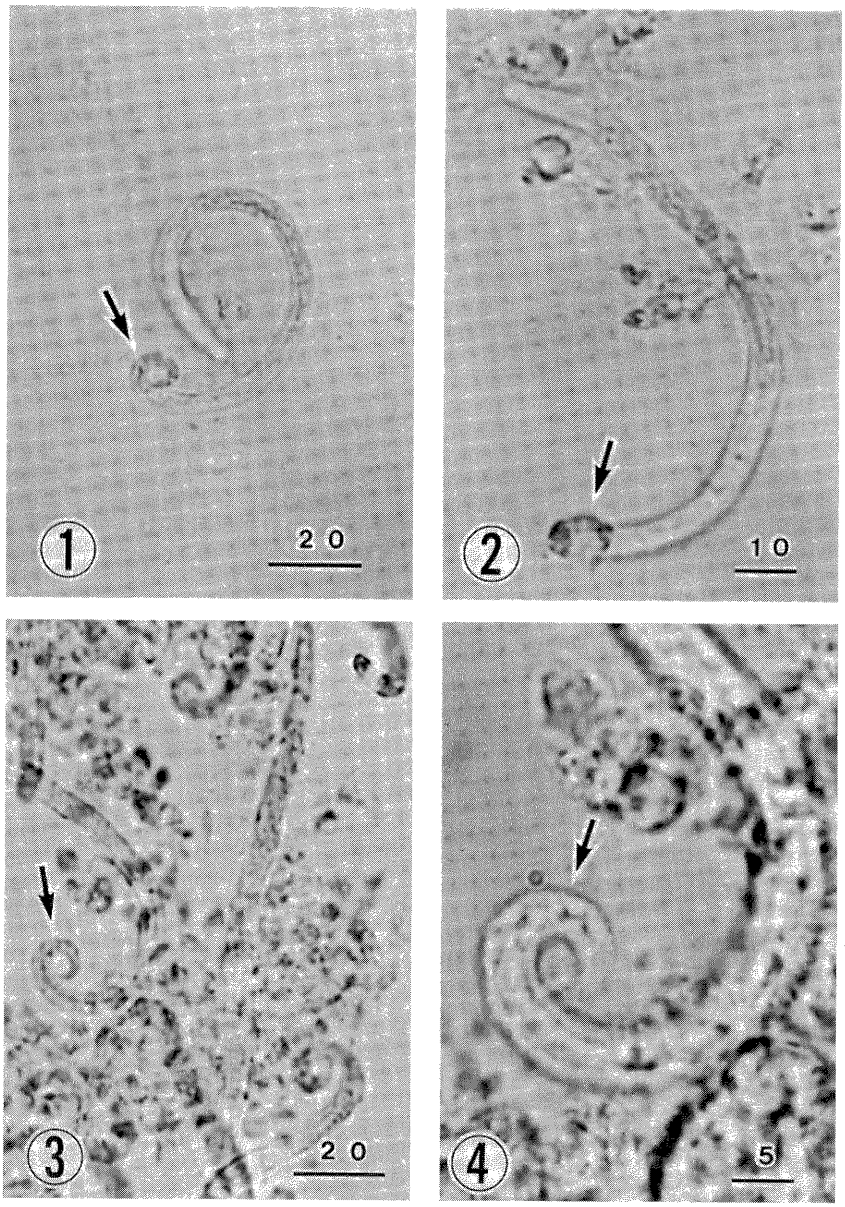

Figures 1-4 Microfilariae of *Onchocerca suzukii* from a Japanese serow. Arrows show the coiled posterior part of the microfilariae. 4, Enlarged area of Fig. 3. Units of the scale, micrometers.

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PREVALENCE OF CANINE HEART FILARIA, *DIROFILARIA IMMITIS*, IN DOGS IN OKINAWA PREFECTURE, JAPAN, 1991-1992

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Abstract: From 1991 to 1992, the prevalence of *Dirofilaria immitis* was clarified in stray dogs from Okinawa, Kume where the first survey was carried out, Miyako, and Ishigaki Isls. of Okinawa Prefecture, Japan. The number of dogs examined was 504 in Okinawa, 13 in Kume, 139 in Miyako and 184 in Ishigaki Isls. In the islands except Miyako Is., about 40% of adult dogs (one year or older) were determined to be infected with *D. immitis*; but in Miyako Is., 8.3% of adult dogs were positive. All the worms recovered were *D. immitis*. In Ishigaki Is., the incidence was higher than that reported by Asato *et al.* (1985). However, in Miyako Is. where young dogs were examined, the incidence was similar to that reported by Asato *et al.* (1985). In Kume Is., the incidence of *D. immitis* in house dogs was also examined by the presence of the microfilariae, being 25.5% in outdoor dogs reared outside and none in dogs retained indoor.

Key words: Canine filaria, *Dirofilaria immitis*, epidemiological survey, house dog, Japan, Okinawa, stray dog

INTRODUCTION

Surveys of dog filaria in Okinawa Prefecture were carried out by Pennington and Phelps (1969), Suenaga *et al.* (1976) and Asato *et al.* (1985). *Dirofilaria immitis* and *Dipetalonema roconditum* were found by Pennington and Phelps (1969) and Suenaga *et al.* (1976). The infection rate of stray dogs with *D. immitis* was 0.17% in the survey by Pennington and Phelps from 1966 to 1967 and 50.5% in the survey (1981 to 1984) by Asato *et al.* In the present paper, we report the recent situation of stray dogs infected with *D. immitis* in Okinawa, Miyako and Ishigaki Isls., and stray and house dogs in Kume Is. of Okinawa Prefecture, Japan from 1991 to 1992.

MATERIALS AND METHODS

From April 1991 to June 1992, examination was made on infection of dog filaria among captured dogs caged and brought to the Animal Protection Center of Okinawa Prefecture for mercy killing by carbon dioxide. The sex and age of the dogs were recorded. Blood (20 μl) were collected from the heart of the dogs. The microfilariae abbreviated as mf hereafter were collected from the blood using the method of Suenaga *et al.* (1972) and counted, and the outline of the body was drawn under a microscope with 100× magnification. The length of some mf was measured by a digital curvimeter (Uchida). The number of adult worms, male and female, in the internal organs, such as the heart and the pulmonary arteries of the dogs, was also counted at the same time. The infection rate for different ages of the dogs was determined for 4 islands, Okinawa (1,199 km^2), Kume (59 km^2), Miyako (158 km^2) and Ishigaki Isls. (222 km^2); the last three islands are located approximately 100 km west, 300 km and 400 km southwest of Okinawa Is., respectively. The number and age of dogs registered in Miyako and Ishigaki Isls. in 1992 were also examined by the records kept in the Animal Protection Center of Okinawa Prefecture (Table 1). In Kume Is., the infection rate of house dogs with *D. immitis* was also examined in May 1991 during the rabies vaccination of house dogs in Okinawa Prefecture. A drop of blood from the earlobe of the dogs was taken and the presence of mf was determined under a microscope (×100). In

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Table 1 Number of resistered dogs in 1992 in Ishigaki and Miyako Isls.

Age (year)	No. (%) of dogs registered	
	Ishigaki	Miyako
<2	317(53.2)	272(51.6)
<1	112(18.8)	83(15.8)
1	105(17.6)	99(18.8)
2	100(16.8)	90(17.1)
≧3	279(46.8)	255(48.4)
3	80(13.4)	64(12.1)
4	54(9.1)	65(12.3)
≧5	145(24.3)	126(23.9)
Total	596(100)	527(100)

Kume Is., 90% of the 70 dogs registered were examined in 1991. The age of dogs was estimated by the degree of teeth wearing. Statistical analysis for the difference between percentages and number of worms were made by chi-square and Fisher tests, respectively.

RESULTS

1. Incidence of stray dogs with *D. immitis*

As shown in Table 2, the average incidence of puppies (less than one year old) and adults of one year and older infected with *D. immitis* in Okinawa Is. was 3.6% (2/55) and 40.1% (180/449), respectively. The incidence tended to be higher in older dogs. *D. immitis* worms were detected in 36.4% of the 11 dogs in Kume Is. In Miyako Is., 7.2% of 139 dogs and 37.5% of 184 dogs in Ishigaki Is. were positive. The difference of incidence was highly significant between puppies and adult dogs under three years old ($X^2=23.3$, $p=0.001$), and the incidence in dogs under three-years old was significantly lower than that in three and more than three years old ($X^2=64.2$, $p<0.001$). In total, 31.6% of 838 stray dogs examined, being 35.9% of the males and 27.7% of the females, were infected.

In Ishigaki Is., the percentage of dogs under three years old was almost the same in both the registered and examined dogs, being 53.2% and 61.4%, respectively (Tables 1, 2). On the other hand, in Miyako Is., 51.6% of the registered dogs and 84.2% of the examined dogs were under three-years old, and were significantly different ($X^2=48.0$, $p<0.001$).

2. Incidence of *D. immitis* adult worms from the heart and mf from the blood of stray dogs

As shown in Table 3, the incidence of stray dogs infected with both stages of *D. immitis*, adult worms and mf was 54.9% in Okinawa, 50.0% in Kume, 90.0% in Miyako and 47.8% in Ishigaki Isls., with a total of 54.3%. The percentage of dogs with *D. immitis* adult worms of both sexes, but without the mf was 15.4% in Okinawa, 25% in Kume, 0% in Miyako and 18.8% in Ishigaki Isls., with a total of 15.8%. The incidence of dogs with mf only was 1.6% in Okinawa and 1.4% in Ishigaki Isls.

3. Body length of mf from the blood of stray dogs with and without adult worms of *D. immitis*

There was no significant difference in the length of mf recovered from stray dogs with *D. immitis* adult worms and those without adult worms (Table 4). The length of the mf from the dogs in three different islands,

Table 2 Incidence of *D. immitis* among stray dogs in 4 islands of the Ryukyu Archipelago (April 1991-June 1992)

Dog age (year)	Number of dogs examined and infection rate (%)						
	Okinawa Is.	Kume Is.	Miyako Is.	Ishigaki Is.	Male	Female	Total
<2	340(26.2)	5(0.0)	117(6.0)	113(23.0)	240(22.1)	235(29.4)	575(21.2)
Puppy							
<1	55(3.6)	1(0.0)	30(3.3)	12(0.0)	34(5.9)	64(1.6)	98(3.1)
Adult							
1	164(25.4)	2(0.0)	47(2.1)	45(11.1)	98(19.4)	160(17.5)	258(18.2)
2	121(38.0)	2(0.0)	40(12.5)	56(37.5)	108(29.6)	111(36.0)	219(32.9)
≧3	164(56.7)	6(66.7)	22(13.6)	71(60.6)	158(57.0)	105(50.5)	263(54.4)
Adult							
3	95(45.3)	2(50.0)	12(8.3)	35(68.6)	82(51.2)	62(43.5)	144(47.9)
4	46(71.7)	2(100)	8(12.5)	22(45.5)	47(61.7)	31(54.8)	78(59.0)
≧5	23(73.9)	2(50.0)	2(50.0)	14(64.3)	29(65.5)	12(75.0)	41(68.3)
Total(1-≧5)	449(40.1)	10(40.0)	109(8.3)	172(40.1)	364(38.7)	376(32.2)	740(35.4)
Grand total	504(36.1)	11(36.4)	139(7.2)	184(37.5)	398(35.9)	440(27.7)	838(31.6)

Table 3 Incidence of *D. immitis* adult worms in the heart and microfilariae in the heart blood of stray dogs in 4 islands of Okinawa Prefecture (April, 1991-June, 1992)

Islands	Okinawa	Kume	Miyako	Ishigaki	Total
No. of dogs examined	504	11	139	184	838
No. of dogs infected with *D. immitis* (A)	182	4	10	69	265
with adults of ♂, ♀, mf (B)	100(54.9)*	2(50.0)	9(90.0)	33(47.8)	144(54.3)
with both sexes of adults, without mf (C)	28(15.4)†	1(25.0)	0(—)	13(18.8)	42(15.8)
with ♂, without ♀, without mf (D)	25(13.7)‡	1(25.0)	0(—)	8(11.6)	34(12.8)
without ♂, with ♀, without mf (E)	26(14.3)§	0(—)	1(10)	14(20.3)	41(15.5)
without adult, with mf (F)	3(1.6)¶	0(—)	0(—)	1(1.4)	4(1.5)

( )*—¶ show the incidence of B, C, D, E and F in infected dogs (A), respectively.

Table 4 Body length of microfilaria from the heart blood of dogs with and without adult worms of *D. immitis*

Locality (Is.)	Dogs with		No. mf examined	Av. length (μm) of mf (min.-max.)
	Adult worm	mf		
Okinawa	+	+	42	304.8(255.8-355.0)
Kume	+	+	10	287.8(271.3-300.7)
Okinawa	−	+	23	291.8(263.5-320.9)
Ishigaki	−	+	10	303.5(285.2-325.5)

mf: microfilaria

Kume, Okinawa and Ishigaki Isls., was almost the same (Table 4).

4. Incidence of indoor and outdoor house dogs with *D. immitis* in Kume Is.

In Kume Is., 20.6% of the house dogs were positive mf of *D. immitis* (Table 5). None of the puppies examined were infected. Likewise, all the adult dogs reared indoor were not infected. Only adult dogs older than 2 years and reared outdoor were infected with *D. immitis* (Table 5) ($X^2=3.85$, $p<0.05$).

Table 5 Incidence of house dogs with *D. immitis* in Kume Is.

Dog age (year)	No. of dogs examined and incidence (%) of *D. immitis*		
	Rearing place		Total
	Outdoor	Indoor	
Puppy <1	7(0.0)	0(—)	7(0.0)
Adult			
1	15(0.0)	0(—)	15(0.0)
2	8(25.0)	0(—)	8(25.0)
3	12(41.7)	3(0.0)	15(33.3)
4	5(60.0)	5(0.0)	10(30.0)
≧5	4(75.0)	4(0.0)	8(37.5)
Total(1-≧5)	44(29.5)	12(0.0)	56(23.2)
Grand total	51(25.5)	12(0.0)	63(20.6)

5. Relationship between the age of stray dogs and the number of adult worms of *D. immitis*

The mean age of 261 stray dogs with adult worms was 2.9±1.6 years. In general, the number of adult worms increased as the age of the dog increased (Table 6). The average number of worms detected in puppies, adult dogs under three-years old, and three and more than three-years was significantly different ($F=9.9$, $p<0.001$), being 2.3±0.6, 4.3±4.0 and 7.7±7.8, respectively. The number of the dogs with a single adult worm of *D. immitis* was most frequent, 31.8% with one male and 28.4% with one female worms (Fig. 1). The average number of worms recovered from 261 infected dogs was 6.1±6.6; 2.9 male and 3.2 female worms (Table 6). The maximum number of adult worms in a dog was 39, 21 male and 18 female worms (Fig. 1).

Table 6 Age of stray dogs and number of adult worms of *D. immitis*

Dog age (year)	No. of examined dogs with *D. immitis*	Average no. of adult worms of *D. immitis*			
		Male	Female	Total	min-max
<1	3	1.0	1.3	2.3±0.6	2-3
1-2	117	2.0	2.3	4.3±4.0	1-24
1	47	2.3	2.7	4.9±4.1	1-14
2	70	1.8	2.0	3.8±3.9	1-24
≧3	141	3.6	4.1	7.7±7.8	1-39
3	68	2.6	2.9	5.5±5.8	1-30
4	45	4.1	4.8	8.9±8.5	1-39
≧5	28	5.4	5.9	11.2±9.6	1-32
Total	261	2.9	3.2	6.1±6.6	1-39

6. Relationship between the number of female worms in a dog with both sexes of adult worms and microfilarial density

The results are shown in Fig. 2. The number of female worms in dogs harboring both sexes of *D. immitis* showed good correlation with the number of mf per 20 μl blood. The line was indicated by $Y=38.707X^2-1.054X+527.7$ ($r=0.52$, $p<0.001$). The number of the mf in the dogs infected with both sexes of *D. immitis* worm was increared with increasing of the number of female *D. immitis* worms in dogs.

DISCUSSION

After the World War II, Pennington and Phelps (1969) reported the first record of *D. reconditum* in Okinawa Is., Japan. The incidence of *D. reconditum* was high, being 23% of 557 stray dogs examined in Okinawa, and microfilariae of both *D. reconditum* and *D. immitis* were also detected in a dog from compouned Kadena airbase of Okinawa Is. from 1966 to 1967. On the other hand, Keegan *et al.* (1967) reported high incidence of *D. immitis*; 44.5% in dogs from the wooded foothills outside Kochi City and 12.5% in urban Kochi of Sikoku, Japan. Also in Nagasaki of western Japan, 29.1% of 690 dogs had microfilariae of *D. immitis* (Suenaga *et al.*, 1971). Subsequently, in Okinawa Is. from 1972 to 1974, Suenaga *et al.* (1976) conducted a dog filarial survey and reported that the incidence of *D. reconditum* in stray dogs was 12.1% and in house dogs 2.9%; the incidence of *D. immitis* was 0.2% in stray dogs and 1.2% in house dogs. *D. reconditum* was predominant in the dogs in Okinawa at that time. The high incidence of *D. reconditum* from 1962 to 1974 in Okinawa Is. might be due closely to the introduction of U.S. army dogs, when Okinawa Prefecture was under American rule after the World War II. Ten years after the survey by Suenaga *et al.* (1976), the situation of dog filaria in Okinawa Prefecture changed drastically. An extensive survey carried out from 1981 to 1984 by Asato *et al.* (1985) showed that 67.2%, 9.1% and 6.5% of stray adult dogs in Okinawa, Miyako, and Ishigaki Isls. respectively were infected with only *D. immitis* and that no *D. reconditum* was detected in 225 dogs examined. After 1972 when Okinawa Prefecture returned to Japan, many dogs with *D. immitis* were brought from mainland Japan to Okinawa Prefecture. The presence of the vector mosquitoes (Toma *et al.*, 1978) of *D. immitis* and reduction of the vector of *D. reconditum* might be closely related to the present situation of high infection rate of *D. immitis* in Okinawa, which is similar to those in other prefectures of Japan

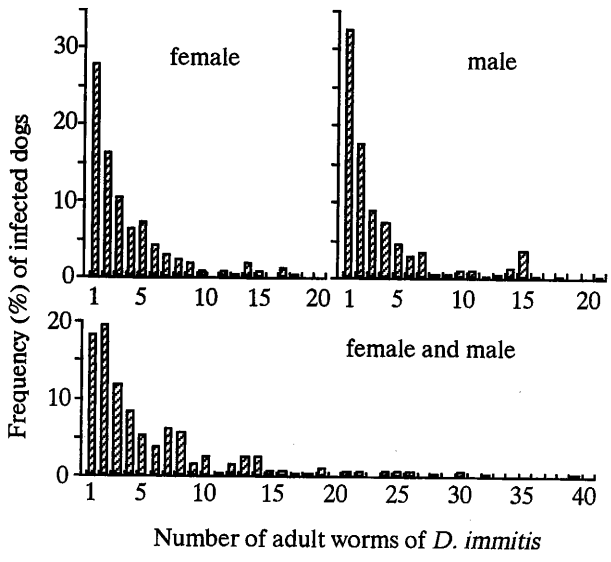

Figure 1 Frequency distributions of the number of adult worms of *D. immitis* detected in stray dogs.

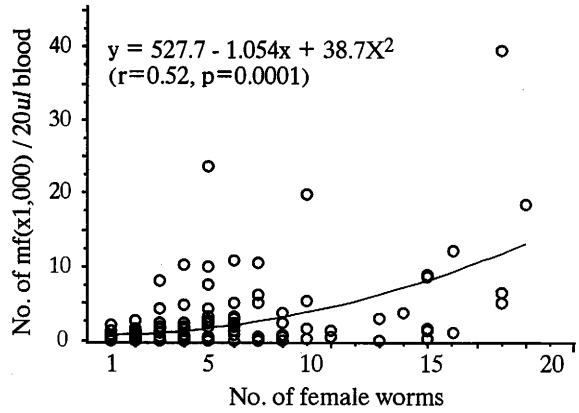

Figure 2 Relationship between the number of female worms of *D. immitis* in a dog and microfilarial density.

(Keegan *et al.*, 1967; Suenaga *et al.*, 1971; Tanaka *et al.*, 1966; Ohshima *et al.*, 1973; Uga *et al.*, 1982).

The present study reveals high infection of dogs with *D. immitis* in Okinawa, Kume and Ishigaki Isls. In Ishigaki Is, the incidence of adult dogs with *D. immitis* was 40.1%, a marked increase over the 6.5% reported from 1981 to 1984 (Asato *et al.*, 1985). This may be reasonably attributed to the frequent movement of personnel and transportation of animals and materials between Ishigaki and Okinawa Isls., and mainland Japan. The low incidence of dogs with *D. immitis* in Miyako Is., 8.3% in the present survey, was similar to that reported by Asato *et al.* (1985). The incidence is expected to be higher if older dogs were examined as the incidence has been demonstrated to increase with age of the dogs (Oda *et al.*, 1995). This is the first survey in Kume Is. in which a small number of stray dogs was examined for infection. In Hyogo Prefecture, Uga *et al.* (1982) reported that the incidence of stray dogs with only *D. immitis* mf was 3.1% among 96 dogs. In our survey, the incidence was 0.7% for 577 dogs which did not harbor the adult worms. *D. reconditum* and *D. repens* were not detected in the dogs examined in the present survey.

The number of worms in a dog was quite different between Okinawa and mainland Japan. The average number of adult worms in a dog was 6.1 (2.9 males and 3.2 females) with a maximum number of 39 in our survey, compared to 18.1 worms (8.7 males and 9.4 females) with a maximum number of 178 (77 male and 101 female) in Hyogo Prefecture (Uga *et al.*, 1982). Likewise, the frequency of 1-10 worms and more than 10 worms detected in a dog was different between Saitama and Okinawa. In Okinawa, 83.5% had 1-10 worms and 16.5% had 11-40 worms, in contrast to 37.5-51% with 1-10 worms and 49-62.5% with 11-200 worms in Saitama Prefecture (Tanaka *et al.*, 1966; Oda *et al.*, 1995). The number of mf correlated with that of adult worms of *D. immitis* in a dog. The number of mf number of worms detected in this study was also smaller than those reported by Tanaka *et al.* (1966). The difference might be due to the mean age of dogs examined in this study.

The incidence of house dogs with mf of *D. immitis* was also examined in Kume Is. It is noteworthy that only dogs reared outdoor were infected (25.5%). Oda *et al.* (1995) reported that the incidence of the house dogs with *D. immitis* had recently decreased in northern and southern parts of Nagasaki City, being 46.3% in 1968 and 8.1% in 1994, due to decrease of the breeding places of *Cx. p. pallens* as a result of the spread of public sewage system. In the house dogs of Hiroshima, reduction of infection was also reported by Saito *et al.* (1995), being 45.8% in 1972 and 18.4% in 1992. This was attributed to the dog owners having good knowledge about parasitological diseases of dog and often giving medicine for prevention or treatment.

Human infection of *D. immitis* in subcutaneous tissue of breast was first reported in Japan in 1964 (Nishimura *et al.*, 1964). In Okinawa, infection of *D. repens* within a subcutaneous nodule was reported by Maclean *et al.* (1979) and *D. immitis* in cheek telasubmucosa by Hibino *et al.* (1981). Up to 1986, one case with *D. repens* and 55 cases with *D. immitis* had been reported in Japan; 69.6% of the cases involved the lungs (Makiya *et al.*, 1987). From laboratory and field studies on vector mosquitoes (Keegan *et al.*, 1967; Intermill and Frederick, 1970; Suenaga *et al.*, 1972; Suenaga, 1972a: Suenaga and Itoh, 1973; Konishi, 1987a,b), *Aedes albopictus, Culex p. pallens, Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* have been incriminated as important vectors in natural infections of *D. immitis* in Japan. Our study shows that about 40% of stray dogs in Okinawa Prefecture were infected with *D. immitis*. As Okinawa Prefecture is located in the subtropical area, *Cx. quinquefasciatus* as well as *Ae. albopictus* were abundant throughout the year (Toma *et al.*, 1978, 1982, 1990; Miyagi *et al.*, 1992), natural transmission of *D. immitis* to dogs by these mosquito species will occur throughout the year even in small islands.

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COMPARATIVE STUDIES FOR DEVELOPMENT OF THREE DIFFERENT GEOGRAPHIC STRAINS OF *SCHISTOSOMA JAPONICUM* CERCARIAE IN FIVE SUBSPECIES OF *ONCOMELANIA HUPENSIS*

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Abstract: Cercarial maturation time, duration of cercarial emergence and number of cercariae released were studied in five subspecies of laboratory-reared *Oncomelania hupensis* (*Oncomelania hupensis nosophora, O. h. hupensis, O. h. formosana, O. h. chiui* and *O. h. quadrasi*) infected with three different geographic strains of *Schistosoma japonicum*. *O. h. nosophora, O. h. hupensis* and *O. h. formosana* were the most suitable host snails for the Japanese, Chinese and Taiwanese strains of *S. japonicum,* respectively. The cercarial maturation time in the most suitable host snails were 8 weeks for the Taiwanese strain of *S. japonicum* and 10 weeks for the Japanese and Chinese strains after exposure to 5 miracidia. The duration of cercarial emergence were 9 weeks for the Taiwanese strain, 17 weeks for the Chinese strain and 16 weeks for the Japanese strain. The duration of cercarial emergence was similar in the remaining subspecies, but the maturation time of cercariae was longer. For numbers of cercariae emergenced from snails, the most suitable host snails had a tendency to released more cercariae than the remaining snails.

Key words: *Schistosoma japonicum, Oncomelania hupensis,* Cercarial development

INTRODUCTION

Schistosomiasis japonica is an important disease in East Asian countries. Diagnosis of the disease, especially the chronic cases, depends mainly on immuno-serological methods. Therefore, a large amount of antigen is needed for immuno-serological diagnosis of schistosomiasis japonica. To obtained a large amount of antigen, it is necessary to establish the life cycle of *Schistosoma japonicum* in the laboratory. In the laboratory maintenance of *S. japonicum* life cycle, the cercarial maturation time, duration of cercarial emergence and the number of cercariae are some of the indicators for its success. There have been few detailed observations on these problems and it is well known that different geographic strains of *S. japonicum* show different degrees of infectivity to *Oncomelania hupensis* subspecies (Hunter *et al.,* 1952; DeWitt, 1956; Moose and Williams, 1963; Iwanaga, 1976 a,b; Iwanaga *et al.,* 1979 a,b; Lo *et al.,* 1993). This study was conducted to answer the above problems using three different geographic strains of *S. japonicum* and five subspecies of laboratory-reared *Oncomelania hupensis*.

MATERIALS AND METHODS

1. Parasites and snails

In this study, the Yamanashi (Japanese), Shanghai (Chinese) and Changhua (Taiwanese) strains of *S. japonicum* were used. The Japanese strain was obtained from the Yamanashi Prefectural Institute of Hygiene and the other two strains were originated from infected snails collected in the endemic areas. These strains have been maintained in the Department of Immunology and Parasitology, Hiroshima University School of Medicine, Japan by passing through most suitable host snails and Swiss albino mice.

Five subspecies of laboratory-reared *Oncomelania hupensis* (Iwanaga and Tsuji, 1972; Iwanaga, 1975) were employed in this study. They were *O. h. nosophora* from Yamanashi, Japan, *O. h. hupensis* from Shanghai, China, *O. h. quadrasi* from Leyte, Philippines, *O. h. formosana*

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from Changhua, Taiwan and *O. h. chiui* from Alilao, Taiwan. The snails used for the study were abult snails.

2. Maturation time of cercariae

For each strain of *S. japonicum*, 30 snails were individually exposed to five miracidia in a small vial for 18 to 20 hours. These snails were kept in inner soil-filter circulating tanks (Iwanaga and Tsuji, 1972) for 6 weeks, then maintained in petri dishes with a moistened filter paper. The emergence of cercariae was determined by shedding for overnight every week for 6 to 25 weeks after miracidial exposure. The negative snails were dissected and examined for sporocysts and cercariae two weeks later.

3. Duration of cercarial emergence and the number of cercariae

For each strain of *S. japonicum*, 80 to 100 snails were individually exposed to one miracidium. Snails proven to be positive for mature cercariae were examined once a week for 20 weeks by cercarial emergence test for overnight to determine the number and duration of cercarial production. Snails were dissected and examined for sporocysts and cercariae at 24 weeks after the first cercarial emergence.

RESULTS

1. Cercarial maturation time

Weekly and total numbers of infected *O. hupensis* subspecies to different geographic strains of *S. japonicum* are shown in Figure 1. With the Taiwanese strain of *S. japonicum*, all five subspecies of *O. hupensis* were susceptible. But shorter cercarial maturation time and higher infection rates were observed in *O. h. formosana* and *O. h. chiui*, e.g. 90% (18/20) of *O. h. formosana* started to shed cercariae between 8 to 11 weeks and 83% (10/12) of *O. h. chiui* between 11 to 13 weeks.

O. h. formosana and *O. h. quadrasi* were not susceptible to the Chinese strain of *S. japonicum*. The cercariae were first detected from *O. h. nosophora, O. h. hupensis* and *O. h. chiui* at 12, 10 and 15 weeks postexposure, respectively. These *O. hupensis* released cercariae up to 20 to 22 weeks postexposure. Especially, 91% (10/11) of the infected snails for *O. h. nosophora* and 82% (14/17) of the infected snails for *O. h. hupensis* were obtained 12 to 17 weeks and 13 to 17 weeks after exposure to miracidia, respectively. *O. h. hupensis* showed the earliest cercarial maturation time and had the highest infection rate.

O. h. formosana was not susceptible to the Japanese strain of *S. japonicum*. Cercariae were detected from the remaining snails from 10 to 15 weeks after exposure to miracidia. 90% (18/20) of the infected snails for *O. h. nosophora* was detected 10 to 15 weeks after exposure to miracidia. *O. h. nosophora* had a shorter cercarial maturation time and higher infection rate than those of the other snails.

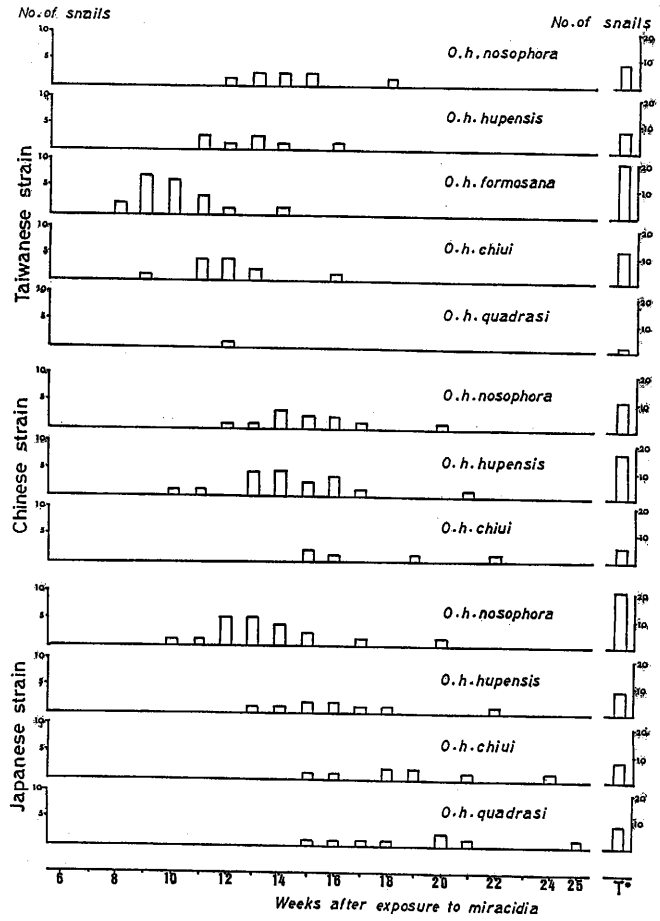

Figure 1 Weekly and total numbers of infected *Oncomelania hupensis* subspecies for different geographic strains of *Schistosoma japonicum*

* Total numbers of infected *Oncomelania hupensis* subspecies at the end of experiments

The maturation time of cercariae in the most suitable host snails for each strain of *S. japonicum* were 8 weeks for the Taiwanese strain and 10 weeks for the Chinese and Japanese strains. And the cercariae were formed at the latest by 14 weeks for the Taiwanese strain, 21 weeks for the Chinese strain and 20 weeks for the Japanese strain after the exposure to 5 miracidia. The Taiwanese strain had a shorter maturation time than those of the Japanese and Chinese strains.

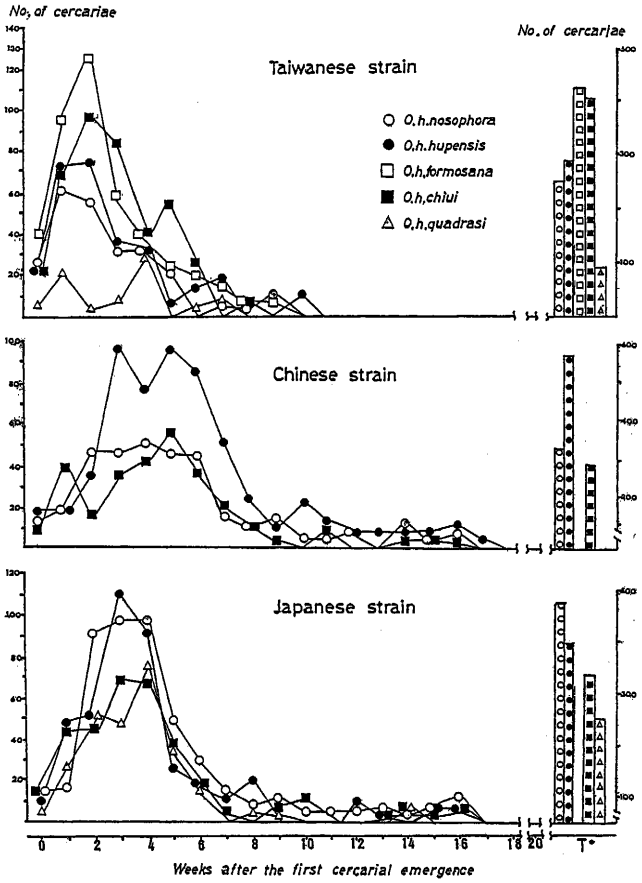

Figure 2 Weekly and total mean numbers of cercariae obtained from *Oncomelania hupensis* subspecies infected with different geographic strains of *Schistosoma japonicum*

* Total mean numbers of cercariae obtained from *Oncomelania hupensis* subspecies at the end of experiments

2. Duration of cercarial emergence and the number

Weekly and total numbers of cercariae obtained from *O. hupensis* subspecies infected with different geographic strains of *S. japonicum* are shown in Figure 2. These snails had been infected with one miracidium and the number of each snail examined was done with 10 infected snails expect *O. h. quadrasi* exposure to Taiwanese strain. The number of *O. h. quadrasi* was done with one snail.

The emergence of cercariae continued for 7 to 10 weeks after the first emergence in the five subspecies of *O. hupensis* for the Taiwanese strain of *S. japonicum* and 79-85% of the mean total number of cercariae per snail had emerged by 4 weeks after the first shedding. The mean total number of cercariae per snail obtained from each snail at the end of experiment ranged from 86 to 432. *O. h. formosana* produced more cercariae than the others.

The duration of cercarial emergence from *O. hupensis* subspecies for the Chinese strain of *S. japonicum* ranged from 16 to 17 weeks after the first emergence. Seven weeks after the first emergence, 82-91% of the mean total number of cercariae had emerged from the snails hosts. The mean total number of cercariae per snail from *O. h. nosophora, O. h. hupensis* and *O. h. chiui* were 340, 585 and 280 cercariae at the end of experiment, respectively.

For the Japanese strain of *S. japonicum*, the duration of cercarial emergence from *O. h. quadrasi* was slightly shorter than that of other subspecies, in which the duration were 14 weeks. The mean total numbers of cercariae per snail were 475 for *O. h. nosophora*, 398 for *O. h. hupensis*, 327 for *O. h. chiui* and 258 for *O. h. quadrasi* at the end of experiment. Although the mean total number of cercariae obtained from *O. h. quadrasi* was less than those from other subspecies, 95% of the mean total cercariae from *O. h. quadrasi* were detected by 6 weeks after the first emergence.

All snails were dissected and examined for sporocysts and cercariae 24 weeks after the first cercarial emergence. The presence of both sporocysts and cercariae were detected in only one specimen of *O. h. nosophora* and *O. h. quadrasi* with the Japanese strain (Fig. 3). The remaining subspecies contained only sporocysts (Fig. 4).

For the duration of emergence and the numbers of cercariae in the most suitable host snails for each strain of *S. japaoicum*, the Taiwanese strain produced the cercariae for 9 weeks after the first emergence, but other strains continued to produce for 16 to 17 weeks after the first emergence. The mean total numbers of cercariae per snail obtained from each snails at the end of experiment were 432 for the Taiwanese strain, 585 for the Chinese strain and 475 for the Japanese strain and they were not significantly different. In order to study the morphological features of the cercariae, 10 cercariae of each strain were fixed with 10% hot formalin and examined under a light microscope. The difference in size of the cercariae was not significant among the different geographic strains. Moreover, there were no significant difference in morphological features (data not shown).

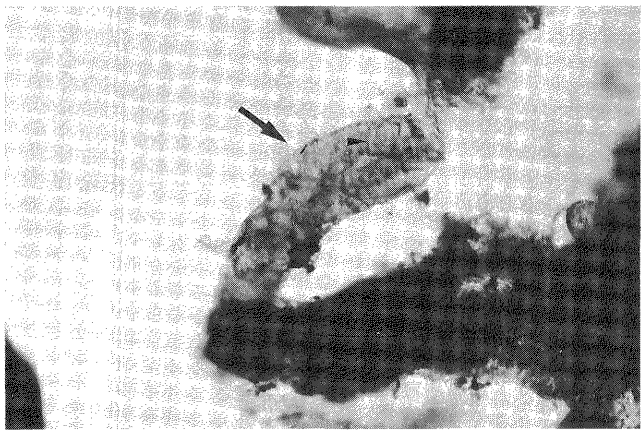

Figure 3 Sporocyst (included cercariae) found in mid-gut region of *Oncomelania hupensis nosophora* on 24 weeks after the first cercarial emergence with the Japanese strain of *Schistosoma japonicum*. Original magnification: x16.
Arrow show sporocyst and arrow-head, cercaria

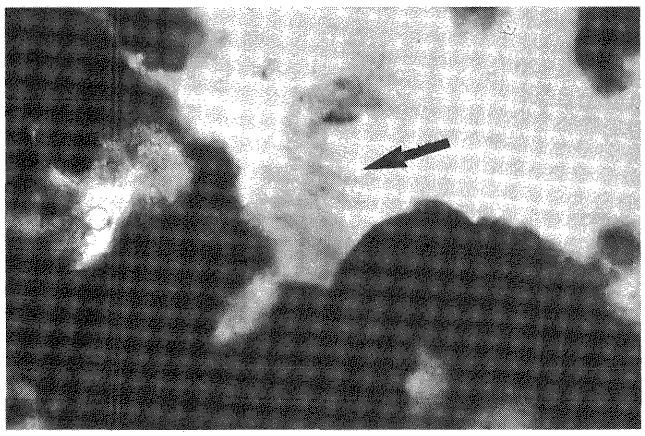

Figure 4 Sporocyst (arrow, non-included cercariae) found in mid-gut region of *Oncomelania hupensis formosana* on 24 weeks after the first cercarial emergence with the Taiwanese strain of *Schistosoma japonicum*. Original magnification: x16.

Table 1 Infection rates of *Oncomelania hupensis* subspecies to different geographic strains of *Schistosoma japonicum**

Snail subspecies	Strains of *Schistosoma japonicum*		
	Japanese	Chinese	Taiwanese
O.h. nosophora	70-80+ (72.5)±	42-46 (43.7)	27-35 (30.3)
O.h. hupensis	30-32 (31.5)	59-68 (62.2)	23-35 (30.8)
O.h. formosana	0-0.8	0	64-70 (68.4)
O.h. chiui	33.3 (33.3)	34-38 (36.2)	35-57 (51.3)
O.h. quadrasi	38.1 (38.1)	0	0-5 (3.7)

* Infection rates as obtained by exposure to 5 miracidia.
The data are extrated from reports of Iwanaga (1976a, b); Iwanaga and Tsuji (1982a, b); Iwanaga *et al.* (1984) and Iwanaga *et al* (1995).
+ Range(%)
± Mean(%)

DISCUSSION

Infection rates of laboratory-reared of *O. hupensis* subspecies exposed to different geographic strains of *S. japonicum* miracidia have been reported by Iwanaga (1976 a,b), Iwanaga and Tsuji (1982 a,b) and Iwanaga *et al* (1984, 1995) (Table 1). The most suitable host snails for the Japanese, Chinese and Taiwanese strains of *S. japonicum* were *O. h. nosophora, O. h. hupensis* and *O. h. formosana*, respectively, as reported by the above authors. This study revealed that the five subspecies of laboratory-reared *O. hupensis* showed different maturation times of cercariae for strains of *S. japonicum*. In the most suitable host snails, the maturation time of cercariae was shorter than that of the other snails. It is suggest that there is a correlation between infection rate and the maturation time of cercariae in *O. hupensis* subspecies with *S. japonicum*. Therefore, there are basic physiologic differences among these snails. In this study, the maturation time of cercariae for the Taiwanese strain of *S. japonicum* in *O. h. chiui* was 9 weeks postexposure to five miracidia. Our results do not agree with those of Chiu (1967) who reported 95 days maturation time when exposed to six miracidia. Ota (1957) and Okamoto (1963) reported that 13 and 18 weeks respectively, were required for the cercariae of *S. japonicum* (Japanese strain) to mature in *O. h. nosophora*, the most suitable host snail. In the present study, the time was also much shorter.

In general, the most suitable host snails released more cercariae than the remaining races. Tomimatsu and Takano (1949) reported that 29 to 10247 cercariae of the Japanese strain of *S. japonicum* were harbored in *O. h. nosophora*. In the present study, the number of cercariae harbored per snail after exposure to one miracidium were 426 to 504 (mean: 475). Our results are quite different from previous reports. This is perhaps due to the fact that Tomimatsu and Takano (1949)

conducted their study under natural conditions and the number of miracidia given was not known.

The duration of cercarial emergence obtained for the Taiwanese strain of *S. japonicum* was found to be shorter than those of the Japanese and Chinese strains. On the other hand, the former strain matured more rapidly to cercariae than the Japanese and Chinese strains. Otori *el al* (1952) reported that the duration of cercarial emergence in *O. h. nosophora* with the Japanese strain was 20 weeks after the first emergence. However, in the present study, the duration of cercarial emergence was much shorter.

Chiu (1967), Ota (1957), Okamoto (1963) and Otori *et al* (1952) kept snails in simple breeding systems (clay flower-pot and dish with soil) after exposure to miracidia. Iwanaga (1976 a,b) found that the snails kept in the inner soil-filter circulating tank had higher infection rates than those in the petri dish with filter paper or the petri dish with agar medium. Therefore, it is likely that the maturation times of the cercariae in *O. hupensis* subspecies also differ under different breeding conditions. And it is probably due to a combination of physiological and biochemical differences in both snails and parasites.

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