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NATURAL INFECTIONS OF *SIMULIUM BIDENTATUM* (DIPTERA: SIMULIIDAE) WITH LARVAE OF *ONCHOCERCA* SPP., IN RELATION TO A HUMAN ZOONOTIC ONCHOCERCIASIS IN OITA, JAPAN

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Abstract: A total of 579 wild female flies belonging to eight species of *Simulium* were collected on human baits, in relation to the transmission of a human zoonotic onchocerciasis found in Oita, Japan. *Simulium bidentatum* was the most dominant species. Natural infections with filarial larvae were found in 11 of 449 *S. bidentatum* (or 6.5% of the parous flies) and in 1 of 23 *S. aokii* (or 10% of the parous flies). All the infected flies had 1-4 either first-, second- or early third-stage larvae in the thorax. Examination of third-stage larvae shows that *S. bidentatum* is infected with at least two species of *Onchocerca*. Our result represents the first record of natural *Onchocerca* infections in Japanese Simuliidae, and also suggests the possibility of *S. bidentatum* as the potential vector of zoonotic *Onchocerca* spp. to humans.

INTRODUCTION

Four human cases of zoonotic onchocerciasis have been reported from various areas of the world (Azarova *et al.*, 1965; Siegenthaler and Gubler, 1965; Beaver *et al.*, 1974; Ali-Khan, 1977). An additional case was recently found in Oita, southern Japan (Hashimoto *et al.*, 1990). Examination of histological sections of a female nematode found suggested that, as in all the previous cases, its morphological features resemble those of *Onchocerca gutturosa* Neumann, 1910 and *O. cervicalis* Railliet et Henry, 1910 infecting cattle and horses respectively (*Beaver et al.*, 1989).

Several *Onchocerca* spp. including *O. gutturosa* and *O. cervicalis* have been reported from Japan (Sato *et al.*, 1954; Suzuki *et al.*, 1982). However, no transmission studies of these filarial nematodes have been carried out, although biting midges (*Culicoides* spp.) or blackflies (*Simulium* spp.) have been known as the vectors of certain *Onchocerca* spp. elsewhere (Bain and Chabaud, 1986).

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The objective of this study is to investigate the transmission of zoonotic onchocerciasis which may be acquired by humans. In this paper, we report natural *Onchocerca* infections of *S. bidentatum* Shiraki collected in one of the suburban residential areas of the city of Oita, where a human zoonotic onchocerciasis occurred.

Materials and Methods

The patient infected with a zoonotic *Onchocerca*, a two-year-old Japanese girl, was an inhabitant of Tabaru, one of the suburban residential areas (altitude ca. 30 m above sea level; coordinates 131°35′E; 33°10′N), situated in the western part of the city of Oita. It was presumed that the zoonotic *Onchocerca* infection might have been acquired by this child at or near her residential area, because, living in one of the housing complexes (ca. 0.01 km^2), she used to spend her time with her mother during the daytime at the recreation ground (ca. 1,200 m^2) located on the west within their premises, and had never left the city, according to her mother. It was therefore attempted to collect biting midges or blackflies at the corner of this recreation ground. Adjacent to this place on the west lies another huge housing area recently developed (with an extension of about 0.4 km^2). There is a low hill with a shrubbery just behind these living areas on the south. On the north, a flat farmland mainly used for rice cultivation widely opens and in its center the Oita River runs eastwards which is, at the nearest, about 400 m away from the collection site. There are several cattle sheds but no horses are raised within a 4-kilometer radius from the collection site.

Collections were made between 16.00 and 18.00 hours during September-December 1988. Flies landing on or flying around the body surface of the human baits were captured by an aspiration tube or by an insect net, and held overnight in small plastic containers. The following day, flies were dissected in saline on a glass slide, and parity was determined by the presence of follicular relics (Detinova, 1962). Parous females were microscopically searched for filarial larvae. The larvae found were preserved in formalin-glycerol (Wharton, 1959) for morphometric observations.

About 170 blackflies collected in December were individually maintained with sucrose solution at 25°C for seven days, and dissected for the presence of third-stage filarial larvae. Generic diagnosis of third-stage larvae followed that of Bain and Chabaud (1986).

Results

Four collections carried out from September to November 1988 yielded a total of eight blackfly species, but no *Culicoides* species was captured. The majority of blackfly species were *Simulium bidentatum*, followed by *S. arakawae* Matsumura, *S. quinquestriatum* (Shiraki) and *S. aokii* (Takahasi) (Table 1).

Natural infections with filarial larvae were found in 2.5% of 449 *S. bidentatum* collected (or 6.5% of the parous flies), and in 4.3% of 23 *S. aokii* captured (or 10% of the parous flies). Eight of 11 infected *S. bidentatum* had 1 to 4 first-stage larvae, two flies each harbored one second-stage larva, and one fly had two third-stage larvae probably just after the second moulting (Fig. 1). One infected *S. aokii* had one first-stage larva (Fig. 2). All the larvae were found in the thorax of the flies.

One of the two third-stage larvae (Fig. 1) recovered from September collection showed

Table 1 Species composition, parity and natural infections of blackflies collected in Tabaru, one of the suburban residential areas, where a human case of zoonotic onchocerciasis occurred, in Oita, Japan

Simulium spp.	Date of collection														
	Sept. 23			Oct. 6			Oct. 13			Nov. 17			Total		
	No. col.	No. par.	No. inf.	No. col.	No. par.	No. inf.	No. col.	No. par.	No. inf.	No. col.	No. par.	No. inf.	No. col.	No. par.	No. inf.
aokii	0	—	—	4	3	1	7	4	0	12	3	0	23	10	1
arakawae	33	11	0	0	—	—	0	—	—	3	1	0	36	12	0
bidentatum	88	30	4	43	9	0	174	69	4	144	62	3	449	170	11
japonicum	0	—	—	0	—	—	1	0	0	1	0	0	2	0	0
nikkoense	0	—	—	0	—	—	0	—	—	1	0	0	1	0	0
quinquestriatum	0	—	—	1	0	0	2	0	0	30	10	0	33	10	0
rufibasis	1	0	0	3	2	0	7	2	0	1	1	0	12	5	0
takahasii	0	—	—	2	1	0	0	—	—	21	1	0	23	2	0

the following measurements: total body length (BL) 743 μm, body width (BW) 26 μm, length of oesophagus (LE) 464 μm, length of tail (LT) 35 μm, width of tail (WT) 21 μm.

In dissections of flies maintained for seven days after collection, one of 144 *S. bidentatum* examined had one third-stage larva (Fig. 3) in the thorax, but all of the 22 *S. aokii* were negative. The third-stage larva recovered (Fig. 3) showed following measurements: BL 1,280 μm, BW 25 μm, LE 620 μm, LT 45 μm, WT 20 μm. This larva, as well as two early third-stage larvae mentioned above, seemed to belong to the genus *Onchocerca* by possessing the very small, almost indiscernible caudal lappets.

It is generally impossible to identify the young third-stage larvae. However, the early third-stage larva found in September seemed to be distinct from the longer third-stage larva found in December because the ratio of LE/BL, as well as body length, was clearly different (i. e., 0.62 vs. 0.48). This conclusion is supported by our observations (unpublished data) that early third-stage larvae of the latter (long type *Onchocerca*) bear the short oesophagus (less than 1/2 of body length) like mature third-stage larva, and measure over 850 μm in body length.

It remained to be determined whether the filaria infecting *S. aokii* belongs to *Onchocerca*.

Discussion

Beaver *et al.* (1989) suggested that the human zoonotic onchocerciasis found in Oita was probably caused by *O. gutturosa* or *O. cervicalis* which have been commonly known in cattle and horses respectively around the world. Our result shows the least possibility that the causative worm was *O. cervicalis*, because its transmitter, *Culicoides*, was not captured during the daytime in the study area. This is also supported by the fact that horses, the definitive host of this parasite, were not raised around this area.

On the other hand, it is demonstrated that there were several anthropophilic blackfly species in the study area, and that *S. bidentatum*, the most dominant species, was naturally infected with larvae of *Onchocerca*. Examination of the third-stage larvae recovered shows

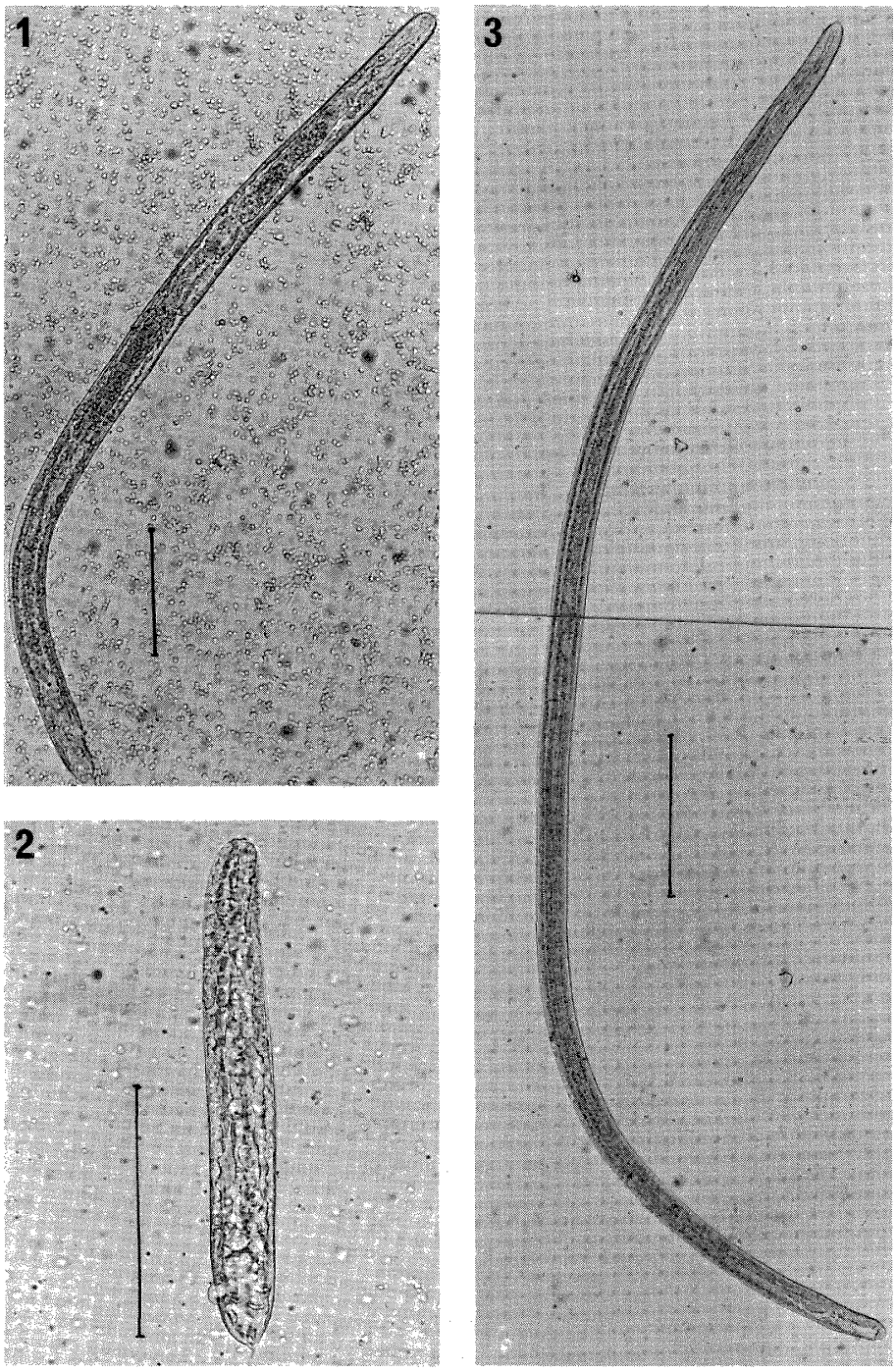

Figure 1 Early third-stage larva of *Onchocerca* sp. found in *S. bidentatum* dissected soon after collection.
Figure 2 First-stage larva of unidentified filaria found in *S. aokii* dissected soon after collection.
Figure 3 Third-stage larva of *Onchocerca* sp. found in *S. bidentatum* maintained for seven days after collection.
All scale bars indicate 100 μm

that *S. bidentatum* was infected with at least two *Onchocerca* species. One of these two fits in with the body length of third-stage larva of *O. gutturosa* given by Bain (1979), but more specimens of mature stage are needed to compare with the bovine parasite. The other one, though only one specimen is available, may belong to a new species because it has a very long body, as compared to those of other known *Onchocerca* species (Bain and Chabaud, 1986). At the present, it is thus difficult to conclude that the zoonotic *Onchocerca* acquired by a Japanese child was *O. gutturosa,* although it might be one of the two *Onchocerca* species found in *S. bidentatum.*

There were many cattle (both Holstein and Japanese Black) at several sheds set up in the farmland near the study area, and microfilariae of *Onchocerca* spp. were already found in blood-fed females of *S. bidentatum, S. aokii* and *S. arakawae* collected at one of five cowsheds examined. Further, three types of third-stage larvae of *Onchocerca* spp. were commonly discovered in the same blackfly species collected at the same cowshed (Takaoka and Bain, unpublished data). One of these three types of *Onchocerca* appears to be the same species as the longer third-stage larva found in this survey, and the remaining two types resemble third-stage larvae of *O. gutturosa* and *O. lienalis* Stiles, 1908 respectively, although both of the latter two bovine filariae have been not yet recorded in Oita. Whether all these types of *Onchocerca* spp. recovered from *Simulium* spp. are parasites of cattle or of other animals should await future studies.

In summary, our findings indicate that there exists a situation, under which the zoonotic *Onchocerca* infection may be acquired by humans through biting of the local blackfly species, in the western suburbs of Oita.

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大分におけるキアシツメトゲブユの *Onchocerca* 幼虫自然感染
—特に動物寄生性 *Onchocerca* による人への伝播に関して—

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大分市の西部郊外において見いだされた，動物寄生性 *Onchocerca* 線虫による人体例の伝播に関して，患者の居住区において人囮法によって吸血ブユの採集を行い，フィラリア幼虫の感染の有無を調べた。その結果，8種のブユが採集され，そのうちキアシツメトゲブユが優先種であることが分かった。また本種ブユでは，調べた449個体のうち11個体の雌に，フィラリア幼虫の感染が見つかった。第3期幼虫の形態的観察により，*Onchocerca* 属に属する2種が含まれていることが分かった。これらの結果から，動物寄生性 *Onchocerca* の人体への感染について考察を行った。

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INTENSITY, INCIDENCE AND CONVERSION/REVERSION RATIO OF *SCHISTOSOMA HAEMATOBIUM* INFECTION

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Abstract: The changes in the intensity of *Schistosoma haematobium* infection, incidence and conversion/reversion ratio in a community were observed over a period of 1.5 years. The intensity of infection was judged by two indices: egg counts per 10 m*l* of urine and egg counts per hour. During the study period, 4 urine examinations were conducted at 6-month intervals. Subjects were included in the analysis of the changes in the intensity of infection in a community if they took all 4 urine examinations and were positive for eggs. The changes in the intensity of *S. haematobium* infection differed considerably according to the indices used. The changes in the intensity of infection were compared with the changes in the level of transmission expressed by incidence and conversion/reversion ratio or with the changes in the urine volume. The changes in the intensity of infection expressed by egg counts per hour closely paralleled the changes in both incidence and conversion/reversion ratio in a community, regardless of urine volume. By contrast, when the intensity of infection was expressed by egg counts per 10 m*l* of urine, it did not correlate with the changes in the level of transmission, and was negatively related to the changes in urine volume. These results suggest that egg counts per hour is a reliable index that accurately reflects the changes in the intensity of *S. haematobium* infection in a community.

INTRODUCTION

Although various indices have been proposed for the measurement of the intensity of *S. haematobium* infection in individual patients (Clarke, 1966; Wilkins, 1977; Stephenson *et al.*, 1984), egg counts per 10 m*l* of urine at midday has been used most frequently thus far. However, one of the shortcomings of this index is that it is affected by daily changes in urine volume, so that the intensity of infection of individual patients varies from day to day.

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This study was conducted under the Kenya-Japan Communicable Diseases Research and Control Project, with support from the Kenya Medical Research Institute and Japan International Cooperation Agency. The data analysis was carried out at the Nagasaki University Information Processing Center and at the Computer Center, Kyushu University.

Therefore, we recommended the use of an index, that is, egg counts per hour, that is not affected by changes in urine volume. This serves as a stable index for the measurement of the intensity of infection at an individual level (Shimada *et al.*, 1986).

For community-based longitudinal studies, particularly cohort epidemiological studies or control projects, the change in the intensity of infection is a key variable. At a community level, however, the intensity of *S. haematobium* infection has also usually been expressed by mean egg counts per 10 m*l* of urine from infected people. Urine volume varies not only from day to day at an individual level but also from season to season, owing to environmental factors such as temperature and humidity. Therefore, it is highly probable that the mean intensity of *S. haematobium* infection in a community differs from season to season even though there is not a true change in the intensity of infection.

In the present study, we attempted to examine the possible changes in the mean intensity of *S. haematobium* infection in a community judged by two indices, that is, egg counts per 10 m*l* of urine and egg counts per hour, over a period of 1.5 years. The results were then compared with the changes in the level of transmission expressed by incidence and conversion/reversion ratio, and with the changes of the mean urine volume.

Materials and Methods

The present study was conducted at Mwachinga village, Kwale district, Kenya. The intensity of infection of the patients was expressed by two indices: egg counts per 10 m*l* of urine and egg counts per hour. The details were described elsewhere (Shimada *et al.*, 1986). Briefly speaking, urine was collected 1 hour after the previous urination during midday (11:30-13:30). The volume of each specimen was measured. The nuclepore filter method of Peters *et al.* (1976) was used to filter and count the number of eggs per 10 m*l* of urine, and then the total number of eggs in the urine was calculated. Egg counts per hour was obtained by dividing total egg counts by the time between two urinations.

The intensity of infection in a community was expressed by the mean egg counts of subjects in logarithm. Urine examinations were conducted 4 times during a study period of 1.5 years. The 1st examination was done in May/June 1982, the 2nd in November/December 1982, the 3rd in May/June 1983 and the 4th in November/December 1983. The number of villagers taking each urine examination was 699, 710, 869 and 682 respectively. Among these, 219 completed all urine examinations and were positive for eggs. These 219 patients were subjected to the analysis of the changes in the intensity of infection.

Four urine examinations done during a period of 1.5 years allowed us to calculate the incidence and conversion/reversion ratio in our study area at 6-month intervals. Incidence was expressed as percentage of persons who had been negative at the first survey but were positive for eggs at the second of two surveys. Conversions were the egg positives at a urine examination who had been negative at the preceding examination, while the reversions were the egg negatives at a urine examination who had been positive at the preceding examination.

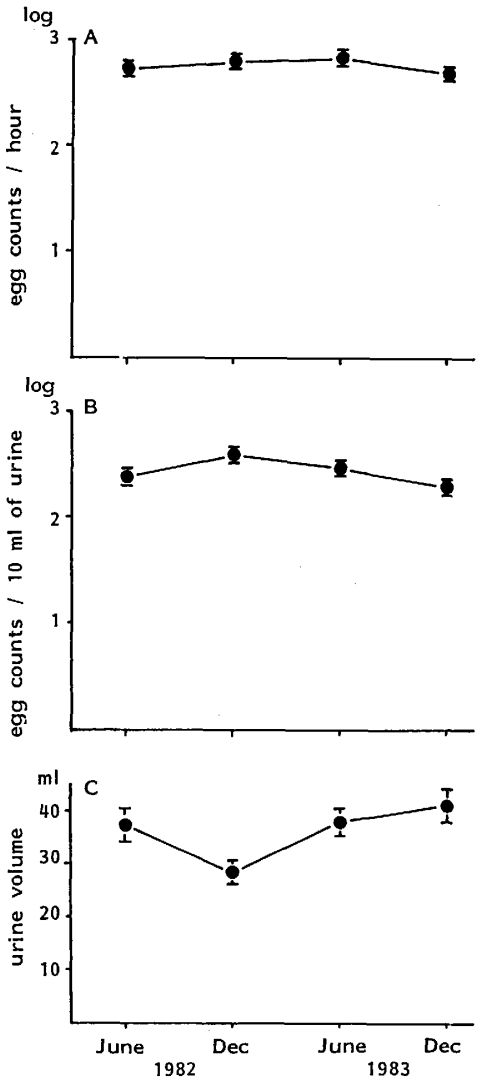

Figure 1 Changes in the intensity of *S. haematobium* infection in a community (A, B) and fluctuation of urine volume (C) over a period of 1.5 years. Intensity of infection was expressed by egg counts per hour (A) and egg counts per 10 m*l* of urine (B).
I represents mean±standard error.

Results

Intensity of Infection

When egg counts per hour was used as an index, the intensity of infection in a community fluctuated slightly (Fig. 1A). The mean log egg counts per hour increased from 2.732 to 2.804 during the first 6-month period between June and December 1982. During the second 6-month period, December 1982-June 1983, the intensity of infection again increased slightly to 2.839. Egg counts then decreased to 2.696 during the third 6-month period. The changes in the intensity of infection over a period of 1.5 years were not statistically significant ($p=0.3674$).

When egg counts per 10 m*l* of urine were used, the pattern of the changes in the intensity of infection was different from that expressed by egg counts per hour (Fig. 1B). Egg counts increased from 2.394 to 2.585 during the first 6-month period, then decreased to 2.474 in the second and to 2.289 in the third 6-month period. The difference in the intensity of infection during the study period was statistically significant ($p=0.0138$). The mean egg counts per 10 m*l* of urine were significantly different between the second and the fourth urine examinations by Scheffe's test ($p<0.05$).

Incidence and Conversion/Reversion Ratio

The results are shown in Table 1. Although the incidence was not significantly different from season to season, the incidence among children under 14 years of age during the first and second 6-month periods was approximately 2 times greater than that during the third period.

Conversion/reversion ratios during the first and second 6-month periods exceeded 1, and ratios during the third 6-month period were smaller than 1, but the difference was not statistically significant. The changes of the intensity of infection expressed by egg counts per hour closely paralleled the changes in incidence or conversion/reversion ratio.

Urine Volume

The results are shown in Fig. 1C. The mean volume of urine obtained at each examination showed significant changes; 37.51 m*l* for May/June 1982, 28.34 for November/December

Table 1 Incidence and conversion/reversion ratio in the study area

Study period	Incidence		Conversion/Reversion ratio	
	0-14 yrs	15 yrs or more	0-14 yrs	15 yrs or more
June 1982-Dec. 1982	16/73*(21.9)†	21/81 (25.9)	16/8‡(2.0)§	21/14 (1.5)
Dec. 1982-June 1983	13/68 (19.1)	23/75 (30.7)	13/9 (1.4)	23/23 (1.0)
June 1983-Dec. 1983	7/80 (8.8)	15/70 (21.4)	7/10 (0.7)	15/19 (0.8)

* : No. of egg positives at the second of two surveys who had been negative at the first survey/No. of egg negatives at the first survey

† : (%)

‡ : conversion/reversion: conversion=new infection (− to +), reversion=spontaneously lost infection(+ to −)

§ : ratio

1982, 38.11 for May/June 1983, and 41.23 for November/December for 1983 ($p=0.0097$). The mean volume of urine at the second urine examination was significantly lower than that at the fourth examination (Scheffe's test, $p<0.05$). The fluctuation pattern of the intensity of infection expressed by egg counts per 10 m*l* of urine was mirrored by that of the urine volume.

Discussion

The data presented in our study show that it is important to reconsider the precision of the indices by which the mean intensity of *S. haematobium* infection in a community has been judged in the past. The changes in the mean intensity of infection in a community during a certain period of time differed considerably according to the index used, that is, egg counts per 10 m*l* of urine or egg counts per hour. When the changes in the intensity of infection were compared with the changes in the level of transmission in our study area, the advantages and disadvantages of the two indices were disclosed.

Incidence and conversion/reversion ratio are considered to indicate the level of transmission in an endemic area of schistosomiasis. When these values are greater during a certain period of time than those during a previous period, the mean intensity of infection in a community is expected to increase during the correnponding period of time, and vice versa. In our study, when the mean intensity of infection was expressed by egg counts per hour, the changes in the intensity of infection in a community during a study period of 1.5 years, although not statistically significant, closely paralleled the changes in incidence and conversion/reversion ratio in that community. By contrast, when it was expressed by egg counts per 10 m*l* of urine, the intensity of infection did not correlate with the changes of the level of transmission.

Variation and stability in *S. haematobium* egg output have long been cosidered (Wilkins and Scott, 1978). In discussions on the changes in egg output, urine volume has usually been indentified as a factor which may affect egg output (Clarke, 1966; Wilkins, 1977; Stephenson *et al.*, 1984). However, there has been no report describing the actual changes in mean urine

volume and their effect on the measurement of the mean intensity of infection in a longitudinal study of a community. Recently, we reported that at an individual level, egg counts per 10 m*l* of urine are affected by the changes of urine volume (Shimada *et al.*, 1984). The present study revealed once more that, at a community level, the changes in the intensity of infection expressed by mean egg count per infection expressed by mean egg count per infection expressed by mean egg count per 10 m*l* of urine were negatively correlated to the changes of mean urine volume ($R=-0.908$, $0.1>p>0.05$, $n=4$). By contrast, the mean egg count per hour was not affected by the changes of mean urine volume.

Although the results of the present and previous studies do not conclusively prove that egg count per hour is the best index for the measurement of the intensity of *S. haematobium* infection, it is undoubtedly a reliable index which can be used as a field technique at both the individual and community levels. Egg count per 10 m*l* of urine measures the concentration of eggs in urine, but not the real intensity of infection.

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ビルハルツ住血吸虫症の感染の強さ，罹患率と陽転・陰転比

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ビルハルツ住血吸虫症の感染の強さ，罹患率，陽転・陰転比の変化を1年半にわたって観察した。感染の強さは2つの指標，尿10 m*l* 中の虫卵数と1時間当たりの排泄虫卵数で表した。調査期間中，6カ月毎に4回尿検査を調査地で行った。すべての調査を受け，かつすべて虫卵陽性であった者を，部落における感染の強さの変化の解析の対象者とした。

感染の強さの変化は，用いる指標によって全く異なった。この変化を罹患率，陽転・陰転比で示した感染の圧力の変化，あるいは尿量の変化と比較した。感染の強さを時間当たりの排泄虫卵数で表すと，その変化は罹患率，陽転・陰転比と同様に変化した。この変化は，尿量の変化とは無関係である。一方，尿10 m*l* 中の虫卵数で表すと，感染の強さの変化は，感染の圧力とは全く関係せず，尿量とは逆相関した。

これらの結果は，調査地でビルハルツ住血吸虫症の感染の強さを表す指標としては，1時間当たりの排泄虫卵数の方が信頼できることを示している。

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EPIDEMIOLOGICAL STUDY OF *SCHISTOSOMA HAEMATOBIUM* INFECTION IN A COASTAL AREA OF KENYA - THE IMPORTANCE OF WATER CONTACT PATTERNS IN RELATION TO *S. HAEMATOBIUM* INFECTION

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Abstract: Water contact observation and urine examination were carried out on a population in an endemic area of *Schistosoma haematobium* infection, Mwachinga, Kwale district, Kenya. The change in the mean level of water contact with age and the age-prevalence or age-intensity distribution of *S. haematobium* infection showed similar trends, but not a direct quantitative relationship. Therefore, a mathematical model was applied in the attempt to analyze the relationship between the intensity of *S. haematobium* infection and the level of water contact. The worm burden, number of paired worms and proportion of hosts with paired worms were estimated from the level of water contact, annual incidence, and the proposed life-span of *S. haematobium* by using an immigration-death model on the basis of simple assumptions. These estimated values were compared with the observed prevalence and intensity of infection. The results showed a linear correlation between observed data and estimated values. We conclude that the characteristic shape of the age-egg output curve for *S. haematobium* in the community is satisfactorily explained solely in terms of the change in water contact with age.

INTRODUCTION

The relationship between the age and the prevalence and intensity of *S. haematobium* infection is similar in most endemic areas. The characteristic shape of the age-prevalence and age-intensity curve for *S. haematobium* is a peak between the age of 10 and 14 years and a relatively rapid decline in older people (Warren, 1973; Mott, 1982). Intestinal schistosomiasis also shows a similar age-related pattern. With regard to influences upon immigra-

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tion and emigration of worms, two major factors have been considered in explaining of the characteristic relationship between age and infection (Warren, 1973). These are 1) the development of protective immunity by the age of 10 years (Bradley and McCullough, 1973) and 2) the variation in water contact with age (Dalton and Pole, 1978; Kvalsvig and Schutte, 1986).

However, no convincing evidence has been presented on the role of protective immunity in the decline of the intensity or prevalence of infection after adolescence. Most of the evidence of protective immunity recently reported was observed after chemotherapy (Sturrock *et al.*, 1983; Butterworth *et al.*, 1985; Wilkins *et al.*, 1987). Meanwhile, epidemiologists concluded on the basis of human water contact studies that the characteristic shape of the age-egg output curve for *S. haematobium* is satisfactorily explained solely by the variation in water contact with age (Dalton and Pole, 1978; Kvalsvig and Schutte, 1986). Again, however, shortcomings exist in their analysis, one of which is that the death of worms was not taken into account.

To further discuss the possible role of the level of water contact in the characteristic shape of the age-prevalence and/or intensity curve, not only a quantitative analysis of human water contact but also an estimation of worm burden is necessary. This is because the egg output reflects accumulated worm burden resulting from contact with infested water, not the level of water contact itself. On the basis of the level of water contact and the proposed life-span of *S. haematobium* (Wilkins *et al.*, 1984), we attempted to estimate the worm burden in the community by using an immigration-death model. Since schistosome needs to mate to lay eggs, the probability of pairing was also calculated. The comparison between the observed data and estimated worm pair burden allows us to determine whether or not the patterns of *S. haematobium* infection in a community can be adequately explained solely in terms of the patterns of water contact.

Materials and Methods

Urine examination

The present study was conducted at Mwachinga village, Kwale district, Kenya. Information concerning the geography, population, and prevalence and intensity of *S. haematobium* infection in the study area was compiled during an initial survey done in June 1982 and has been published elsewhere (Shimada *et al.*, 1987). The method of urine examination was also published previously (Shimada *et al.*, 1986). Briefly speaking, urine was collected 1 hour after the previous urination during midday. The intensity of infection was expressed as the number of eggs excreted per hour assessed by the filtration method of Peters *et al.* (1976). The geometric mean was obtained by using the $n+1$ transformation for a series of egg output including zeros.

Water contact study

Water contact observation was carried out on the population over a period of one year (June 1982 to May 1983) by the direct observation method of Dalton and Pole (1978). The study sites were 16 major points which had been identified as busy sites by a questionnaire. The observers were two villagers who have lived there for many years. In order to reduce the effect of site and seasonal variation in our study area, a 4×4 Latin square with three replicates was used. The study sites were divided at random into two groups of 8 sites. Each

Table 1 Water contact observation schedule

	Week 1				Week 3			
	Tue	Fri	Sat	Sun	Tue	Fri	Sat	Sun
June	1	2	3	4	5	6	7	8
July	2	3	4	1	6	7	8	5
August	3	4	1	2	7	8	5	6
September	4	1	2	3	8	5	6	7

Numbers indicate the study sites.

Table 2 Percentage of parts of body to total body surface

	Children (0-14 yrs)	Adults (15 yrs or more)
1. One hand	2	2
2. Two hands	4	4
3. One foot	2	2
4. Two feet	4	4
5. One arm	10	9
6. Two arms	20	18
7. One leg	15	18
8. Two legs	30	36
9. Hands and feet	8	8
10. Hands and legs	34	40
11. Arms and feet	24	22
12. Arms and legs	50	54
13. Whole body	100	100

Cited from Behrman and Vaughan (ed.). Nelson Textbook of Pediatrics, 1986.

group of 8 sites was observed by one observer. Water contact was observed at each site for 4 days in each of the 3 "seasons" of 4 months' duration. Table 1 shows the date and sites of water contact observation made by one observer during one season of 4 months. The observation was carried out from 06 h 00 to 18 h 30. The collected information included names of inhabitants, time of day, duration of contact (in minutes), and submerged parts of the body. The level of water contact of an individual during a single submersion was expressed by the duration of contact (minutes) multiplied by the percentage of submerged parts to total body surface, which was calculated from the burn chart shown in Table 2. Then the level of water contact of a person for one year was calculated as the sum of each contact. The level of water contact of a certain age group was expressed by the geometric mean. A population census was conducted in June 1983.

RESULTS

Water contact observation

Out of 1,195 villagers, 617 came in contact with water during the one year study period. The overall geometric mean of the level of water contact among observed persons was 1,510.

Table 3 shows the relationship between the level of water contact and sex or age. The geometric mean of the levels of water contact of male and female was 1,580 and 1,450, respectively. The difference between sexes was not significant ($t=0.48$, $p=0.63$).

To express the level of water contact of each age group, the mean level of water contact for the observed person was multiplied by a ratio of the number of observed people to the total number of people of the group registered. The reason for this modification of the level of water contact is that the proportion of observed people differed much by sex and age, and non-observed persons probably came scarcely or less frequently in contact with water.

The modified level of water contact showed that, for males, the level of water contact reached a peak among those aged 10-14 years and then declined rapidly to lower levels, with the exception of a relatively high level of water contact at the age of 40-49 years. For females, the level of water contact reached a peak at the age of 5-9 years, decreased rapidly to a low level at the age of 15-19 years, and remained low in older people.

Estimation of worm burden, number of paired female worms and proportion of hosts with paired worms

The estimation of worm burden, number of paired female worms and proportion of hosts with paired worms was based on simple assumptions for immigration and emigration of

Table 3 Observed level of water contact and modified level of water contact

Age	Level of water contact of person observed -geometric mean- (A)	No. of persons observed (B)	Population (C)	Modified level of water contact (A)×(B)/(C)
Males				
0 - 4	1,230	18	82	270
5 - 9	3,720	56	102	2,040
10-14	3,980	64	81	3,150
15-19	1,700	41	56	1,240
20-29	1,070	44	75	630
30-39	500	24	52	230
40-49	1,410	27	32	1,190
50-59	230	17	32	120
60-	370	16	38	160
total	1,580	307	550	880
Females				
0 - 4	1,550	28	121	360
5 - 9	1,900	52	110	2,320
10-14	1,910	52	78	1,270
15-19	980	29	55	520
20-29	1,350	55	94	790
30-39	780	38	60	490
40-49	710	20	43	330
50-59	1,450	21	46	660
60-	240	15	38	90
total	1,450	310	645	690

schistosomes. To estimate the worm burden, we used a immigration-death model (Anderson, 1976). The number of worms in a host (N) at time t can be expressed as

$$N=\{(A\times I)/D\}\times\{1-\exp(-D)\}+\{N_{t-1}\times\exp(-D)\}$$

where N_{t-1} is the number of worms at $t-1$, A is a constant, and I is the relative level of water contact. Thus, $A\times I$ is immigration rate and D is death rate of a worm per year. The immigration rate ($A\times I$) can be calculated from the following equation proposed by Hairston (1965), $m=-\log(1-\sqrt{B})$, where m is the mean number of female worms acquired by a person per year and B is the annual incidence. The annual incidence in our study area was about 0.28 (Shimada *et al.*, 1989). Solving the equation, therefore, 0.75 female worms were acquired per person per year and there are 2m or 1.5 worms acquired per person per year. We assumed that the number of cercariae invading a host reflects the level of water contact. Therefore, a person with a level as high as the mean annual level of water contact of the villagers in our study area is expected to acquire 1.5 worms per year. To obtain the immigration rate of each age group, we gave the constant A the value 1.5 and I the ratio of the level of water contact of each age group to the mean level of water contact of all the villagers. The death rate (D) was assumed to be 0.3 per worm per year. This was calculated from the estimated life-span of *S. haematobium* (3.4 years) proposed by Wilkins *et al.* (1984).

In addition to the estimate of worm burden in a host, an attempt was made to estimate the number of paired female worms which reflects the intensity of infection or egg output, and also the proportion of hosts with paired worms which represents the prevalence of infection. The probability of male and female worm mating was given as $(0.88\times N)/(N+1.73)$ as proposed by Goddard (1978). Thus, the number of paired female worms (F) is expressed mathematically as

$$F=N\times\frac{0.88\times N}{N+1.73}\times\frac{1}{2}$$

The proportion of hosts with paired worms (P) is expressed as

$$P=\{1-\exp(-N/2)\}^2$$

This is based upon the random distribution of worms among people (Macdonald, 1965). A calculation program for these formulae in the NEC computer is written by BASIC language.

Table 4 Estimated number of paired worms and proportion of hosts with paired worms

Age	Males		Females	
	No. of paired worms (mean)	Proportion of hosts with paired worms (%)	No. of paired worms (mean)	Proportion of hosts with paired worms (%)
0 - 4	0.09	8	0.20	18
5 - 9	2.43	90	3.79	97
10-14	5.47	100	4.19	99
15-19	4.16	99	2.28	91
20-29	1.65	82	1.85	88
30-39	0.48	39	1.28	76
40-49	1.76	84	0.73	55
50-59	0.59	42	1.28	76
60-	0.14	13	0.41	33

The estimated number of paired worms and the proportion of hosts with paired worm by sex and age is shown in Table 4. The number of paired worms reached a peak at the age of 10-14 years and declined to a lower number in older people. In males, however, another peak was observed at the age of 40-49. The proportion of hosts with paired worms showed a similar pattern.

Parasitological study

Of the inhabitants registered, 694 provided urine specimens in December 1982. Table 5 shows the prevalence of infection. The difference by sex was not statistically significant as a whole (Mantel-Haenszel $\chi^2=3.374$, $p=0.066$). The prevalence of infection reached a peak at the age of 10-14 years in both sexes. However, the shape of the age-prevalence distribution after the peak differed by sex. Males showed a relatively sharp decline in prevalence from the peak to a lower level in older people with exceptionally lower prevalence at the age of 30-39 years. In contrast, the prevalence in females decreased from the peak gradually to

Table 5 Prevalence of infection by age and sex

Age	Males			Females		
	No. examined	No. of egg positive	Prevalence (%)	No. examined	No. of egg positive	Prevalence (%)
0 - 4	36	5	14	36	11	31
5 - 9	74	56	76	71	59	83
10-14	54	52	96	47	44	94
15-19	30	27	90	37	33	89
20-29	19	15	79	64	54	84
30-39	17	9	53*	42	35	83*
40-49	22	15	68	41	26	63
50-59	22	14	64	32	21	66
60-	24	15	63	36	17	65
Total	298	208	70	396	300	76

*p=0.015, Chi-square value=5.896

Table 6 Intensity of infection by age and sex

Age	Males		Females	
	No. examined	Egg counts/hour (geometric mean)	No. examined	Egg counts/hour (geometric mean)
0 - 4	36	1.0	36	2.6
5 - 9	74	130.8	71	228.1
10-14	54	1,777.3	47	999.0
15-19	30	435.5	37	425.6
20-29	19	56.5	64	122.0
30-39	17	4.1*	42	52.7*
40-49	22	36.2	41	16.4
50-59	22	14.8	32	23.5
60-	24	19.0	36	4.4
Total	298	74.8	396	76.6

*p=0.0007, t test

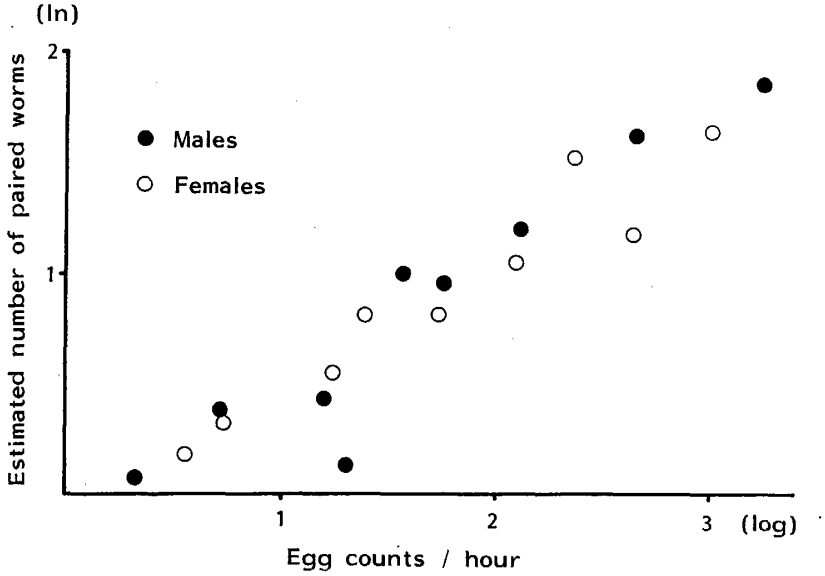

Figure 1 The correlation between estimated number of paired worms and observed egg counts. The estimated number of paired worms and the observed mean egg counts were plotted by age and sex. The number of paired worms was transformed to logaritnm. Egg output was expressed by egg counts per hour and transformed to logarithm. Pearson correlation coefficient; for males 0.945 ($n=9$, $p<0.001$), for females 0.961 ($n=9$, $p<0.001$).

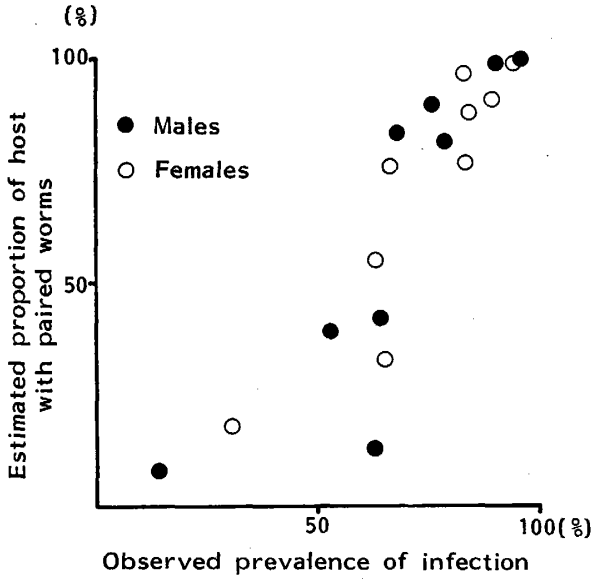

Figure 2 The correlation between estimated proportion of hosts with paired worms and observed prevalence of infection. The estimated proportion of hosts with paired worms and the observed prevalence of infection were plotted by age and sex. Spearman rank-order correlation coefficient; for males 0.93 ($n=9$, $p<0.001$), for females 0.90 ($n=9$, $p<0.005$).

a lower level in older people. The prevalence of males aged 30-39 years was significantly lower than that of females ($\chi^2=5.896$, $p=0.015$).

The overall intensity of infection expressed by a geometric mean egg count was 76 eggs per hour, that of males being 75 eggs per hour and that of females 77 eggs per hour. No statistically significant difference was observed. The age-intensity distribution was similar to that of the age-prevalence curve in both males and females (Table 6). The egg output increased with age and reached a peak at the age of 10-14 years in both sexes. In males, egg counts declined rapidly from the peak to a lower level in older people with lowest counts at the age of 30-39 years. In females, by contrast, the intensity decreased gradually from the peak to a lower level in older people. The mean egg count of males aged 30-39 years was significantly lower than that of females ($t=3.1923$, $p=0.0023$)

Correlations between observed and estimated data

The estimated number of paired worms was compared with the observed egg output. The result is shown in Fig. 1. The estimated values and observed data were plotted by sex and age. A linear correlation between observed and estimated data was observed. The coefficient was statistically significant. The estimated proportion of hosts with paired worm was compared with the observed prevalence in our study area (Fig. 2). Again a linear correlation was shown between the observed and estimated data. The coefficient was statistically significant.

When the level of water contact was compared directly with observed egg output, the correlation was not as prominent as the above.

Discussion

The main aim of the present study is to explain quantitatively the characteristic shape of the age-egg output distribution for *S. haematobium* by the change in the level of water contact with age. Dalton and Pole (1978) and Kvalsvig and Schutte (1986) observed the duration and frequency of water contact among the villagers and considered the possibility of a relationship between water contact and infection of *S. haematobium*. They concluded that there was a close relationship between water contact activity and the intensity or prevalence of infection. In their studies, however, important factors influencing the intensity or prevalence of infection- that is, the accumulation and death of worms- were not taken into account. Changes in prevalence and intensity of infection result from an imbalance in the opposing processes of immigration and emigration of worms.

To overcome the shortcomings in the previous analysis of water contact observations and to examine carefully the relationship between the level of water contact and the intensity of *S. haematobium* infection, we estimated the worm burden, number of paired worms and proportion of hosts with paired worms from the water contact observation and annual incidence in our study area and the proposed life span of *S. haematobium* by using a simple immigration-death model and other equations. The estimated values were compared with the observed data. There was a quantitatively close relationship between both the estimated number of paired worms and the observed intensity of infection, and between the estimated proportion of hosts with paired worms and the observed prevalence. The age-prevalence and age-intensity distribution of males in our study area showed an unexpectedly low prevalence and intensity in the 30-39 year age group. The present study revealed that this unexpectedly

low prevalence and intensity of males 30-39 years of age could also be explained by the estimated proportion of hosts with paired worms and the number of paired worms. All these results imply that in this area the decline in intensity and prevalence of infection is satisfactorily explained solely by the decline in water contact after adolescence.

When we directly compared the level of water contact with the observed prevalence or with the intensity of infection, the variation in level of water contact by age did not correlate well with the change in the prevalence or intensity of *S. haematobium* infection. For example, the highest level of water contact in females was observed at the age of 5-9 years although the intensity of infection showed a peak at the age of 10-14. This is not surprising, though, because the level of water contact only reflects the degree of immigration of worms.

Our analysis is based on a one year-long observation of water contact and a cross-sectional examination of villagers for *S. haematobium* infection. We presumed that the water contact behavior of villagers had not changed for decades, and therefore that the infection of inhabitants was the result of behavior patterns and stayed more or less the same. Indeed, no major changes such as the installation of piped water had occurred in this area for several decades. The direct method of observation for water contact might influence the water contact behavior of villagers, especially of women. Thus, the level of water contact of women might be underestimated.

The assumptions made for the estimation of worm burden from the data of water contact may also be too simple. We assumed that the degree of water contact defines the degree of exposure, that the number of worms infected is proportional to the degree of exposure, that the death rate and fecundity of worms are constant throughout the life of the people, and so on. Although some studies have been carried out on these issues, especially with regard to *S. mansoni* (Upatham and Sturrock, 1973; Cheever, 1968; Medley and Anderson, 1985; Crombie and Anderson, 1985; Wertheimer *et al.*, 1987), still little is known. It is undoubtedly necessary to further clarify whether or not these assumptions are applicable to our analysis.

In the present study, no account was taken of other factors which may influence the acquisition of worms. These include the distribution of snails and their infection rates, the concentration of cercarial density in water and its diurnal fluctuation, and the time of day when villagers come in contact with water, etc. In our study area, children tended to come in contact with water at midday when the concentration of cercariae in the water was high, while adults were likely to come in contact with water in the morning and evening when the concentration of cercariae was low (unpublished data). When these factors are incorporated in the model, the difference in the worm burden gained by children and adults may be much wider.

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ケニア，沿岸州におけるビルハルツ住血吸虫症の疫学的研究
—ビルハルツ住血吸虫感染における水との接触行動の重要性

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ヒトと水との接触行動の観察と尿検査（虫卵検査）をケニア，クワレ地区，ムワチンガ村というビルハルツ住血吸虫症流行地で行った。年齢による住民と水との接触量の変化は，住血吸虫症患者の年齢による感染の強さの変化と，全く同じではないがよく似る。そこで感染の強さと水との接触量を，数学モデルを介して，定量的に比較してみた。寄生虫体数，雌雄抱合虫体数，雌雄抱合虫体を有す住民の割合を，水との接触行動観察データ，年罹患率，ビルハルツ住血吸虫成虫の推定生存期間より，虫体の侵入死亡に関する数学モデル，immigration-death model，その他の式を用いて算出した。これらの推定値を，調査地住民の感染率と感染の強さと比較した。その結果，推定値は観察値と直線関係を示した。これらの結果より，我々はビルハルツ住血吸虫症流行地で普遍的にみられる，年齢—虫卵排泄曲線の特長的型は，単に年齢による住民と水との接触量の変化によって，うまく説明出来ると結論する。

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AGE SPECIFIC ASEXUAL PARASITE AND GAMETOCYTE DENSITY IN HIGHLY ENDEMIC MALARIA IN NORTH SUMATRA, INDONESIA

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Abstract: A mass blood examination was carried out in a coastal village of Perupuk, Asahan Regency, North Sumatra, Indonesia in August 1982. The parasite rate in all ages revealed remarkably high (44%). This was caused by an explosive outbreak of *Plasmodium falciparum* malaria. The parasite rate in young children (0-9 years) was very high around 60% whereas low less than 20% in adult (over 34 years). This indicates a possibility of outbreak of malaria mostly in children posing public health subject in a village of tropical rural area. Gametocyte carriers of *P. falciparum* were detected only in the age group less than 25 years. The high parasite density of *P. falciparum* with both asexual form (more than 1,000 ring forms/mm^3) and gametocyte (more than 100 gametocytes/mm^3) was detected only in the age group less than 12 years. Falciparum malaria of high intensity repeatedly occurs mostly in younger age group in *Anopheles sundaicus* breeding coastal area in north Sumatra. A possible control method aiming at gametocyte carrier of young age group is discussed.

INTRODUCTION

Although malaria is still a very important disease especially in rural area of most tropical coutries, detailed epidemiological information is not always provided for malaria control activities. This was also true in Sumatra Island. It has been told that in North Sumatra malaria is endemic less than 2% in average. Administration officials considered this is lower than the level which needs any anti-malarial action. Previous reports indicated also low malaria endemicity in Sumatra (Carney *et al.*, 1974, 1975; Stafford *et al.*, 1976).

Kanbara and Panjaitan (1983) reported the patchy distribution of malarious foci in the coastal area of Asahan Regency, North Sumatra. Entomological studies revealed *Anopheles sundaicus* is a responsible vector in brackish water of the coastal area (Ikemoto, 1982). *A. sundaicus* is well known as a common vector of malaria in the coastal areas in southeast Asia. In Jawa Island, hyperendemic malaria was caused by *A. sundaicus* once ago in Tanjung Priok district of Jakarta and Tingal town. Recently in 1984, *A. sundaicus* transmitted malaria

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attacked Kampung Laut in Cilacap, Jawa and caused not less than hundred death as a result of increase of vectors due to construction of artificial fishing ponds (Kurihara, 1970).

The endemicity of *A. sundaicus* transmitted malaria in Sumatra has not been well investigated so far. We report here a result of intensive malaria surveys including mass blood examinations in the coastal malarious foci. This was conducted as an activities of the project for "The Promotion of Health in North Sumatra" which is an international cooperation between the Republic of Indonesia and the Government of Japan. The results of age-specific parasite rates and parasite density of both asexual and sexual form of *P. falciparum* showed patchy highly endemic areas in coastal villages presenting some public health problems and a possibility of strategic malaria control approach to younger age group.

Materials and Methods

A community village, Perupuk, kecamatan Lima Puluh, kabupaten Asahan of North Sumatra was selected as the pilot study village, for it was found as a highly endemic focus in the project area (Kanbara and Panjaitan, 1983). It is located on the sea side facing to Malacca Strait and aproximately 150 km southeast of Medan, the capital of North Sumatra.

The mass blood examination was carried out in high prevalence season (August, 1982) when malaria endemicity was reported the highest in a year in the village from the results of former malariometric surveys (Kanbara and Panjaitan, 1983). In the village, most houses were built gathered along the road near the harbour as drawn in geographical reconaissance (Fig. 1). Most inhabitants engage in fishery. The population size was about 6,000 in Perupuk and that of Lorong (block) 2, where the mass blood examination was conducted, was 372. House to house visits to collect blood samples were conducted during night time when adults returned home from their work. Blood specimens were obtained from finger tips, then thick and thin smears were made on microscope slides. Thick smears were air dried for 12 hours and hemolysed in distilled water. The smears were stained for 30 min with 5% Giemsa solution diluted with phosphate buffer (pH 7.2). The thick films were examined counting more than 1,500 white blood cells to secure positivity. Parasite density was calculated in relation to the number of white blood cells assuming 8,500 white blood cells in 1 mm^3 of blood (Bruce-Chwatt, 1980).

Results

The age specific spleen rates were as follows: 21.2% (7/33) for 1-4 years, 20.5% (8/39) for 5-9 years, 29.4% (10/34) for 10-14 years, and 14.1% (18/128) for over 14 years old age groups.

The results of mass blood examination are shown in Table 1. In total, 239 persons were examined in the survey with coverage rate of 64.2% (239/372). The parasite rate was 44.4% in all and 60.2% in the age group of 0-14 years. Two species of malaria parasite, *Plasmodium falciparum* (P. f.) and *P. vivax* (P. v.) were found to exist and the ratio of P. f. and P. v. was roughly 3 : 1. Gametocyte rate of *P. falciparum* was 12.6% in all age groups and 20.0% in the age of 0-24 years. Age specific prevalence curve is shown in Fig. 2. The curve of *P. falciparum* infection draws high plateaux in the age group of 0-9 years and begin to slope down after that. It reaches to the bottom in 35-44 years. The curve of gametocyte rate moves

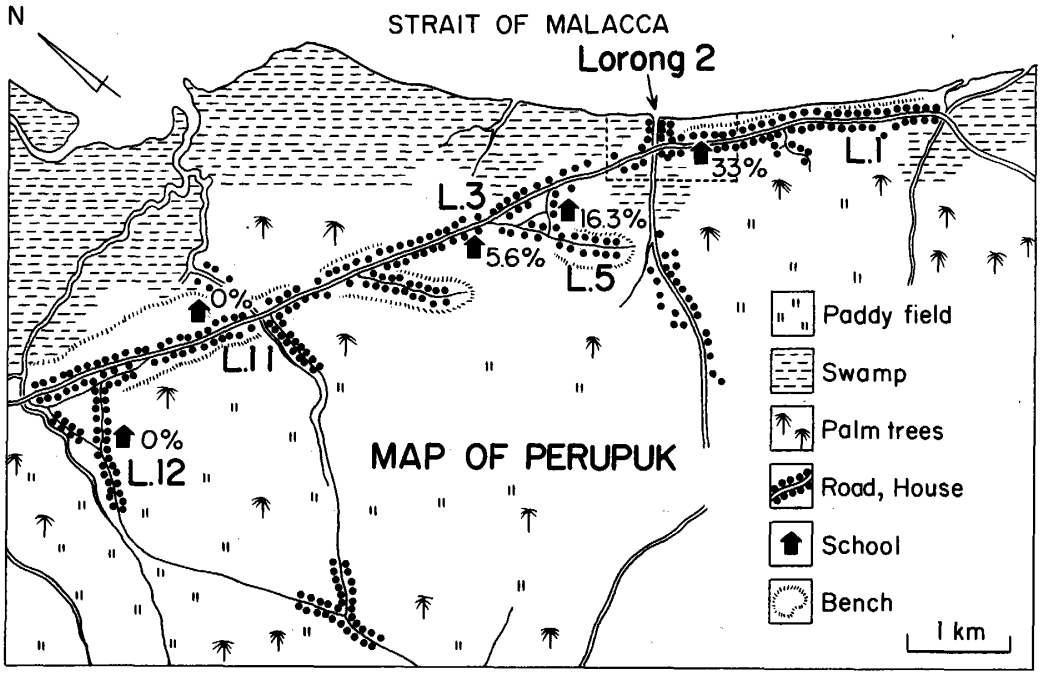

Figure 1 A malaria survey sketch map of village Perupuk, north Sumatra, Indonesia. Spleen rate (%) is shown in every school.

Table 1 Age specific distribution of malaria in Perupuk village, North Sumatra, Indonesia in August 1982

Age (years)	Number population (registered)	Number examined	Number positive cases *P. falciparum*		*P. vivax*	Mix	Total
1	7	4 (57.1%)	2 (50.0%)		0	0	2 (50.0%)
1-4	44	33 (75.0%)	17 (51.5%)	#g(8)	2 (6.1%)	2 (6.1%)	21 (63.6%)
5-9	56	39 (69.6%)	19 (48.7%)	#g(9)	5 (12.8%)	0	24 (61.5%)
10-14	58	35 (60.3%)	14 (40.0%)	#g(9)	7 (20.0%)	0	21 (60.0%)
15-24	73	38 (52.1%)	10 (26.3%)	#g(4)	5 (13.2%)	0	15 (39.5%)
25-34	45	29 (64.3%)	9 (31.3%)		3 (10.3%)	0	12 (41.4%)
35-44	28	18 (64.3%)	1 (5.6%)		1 (5.6%)	0	2 (11.1%)
45-54	33	24 (72.7%)	4 (16.7%)		0	0	4 (16.7%)
55	28	19 (67.9%)	3 (15.7%)		2 (10.5%)	0	5 (26.3%)
All ages	372	239 (64.2%)	79 (33.1%)		25 (10.5%)	2 (0.8%)	106 (44.4%)

#g number of gametocytemia cases in falciparum infections

in parallel with the curve for *P. falciparum* infections and reaches to "zero" level in over 24 years. The curve of age specific prevalence rates and parasite density of *P. vivax* did not show so typical pattern like those of *P. falciparum*.

The parasite density of asexual form in *P. falciparum* infections is shown in Fig. 3. High parasite density more than 1,000 ring forms/mm^3 blood was found only in young children under 12 years. All cases in the age of 25-45 years were less than 100 ring forms/mm^3. Gametocyte density in *P. falciparum* infections is shown in Fig. 4. Six out of 8 cases in the

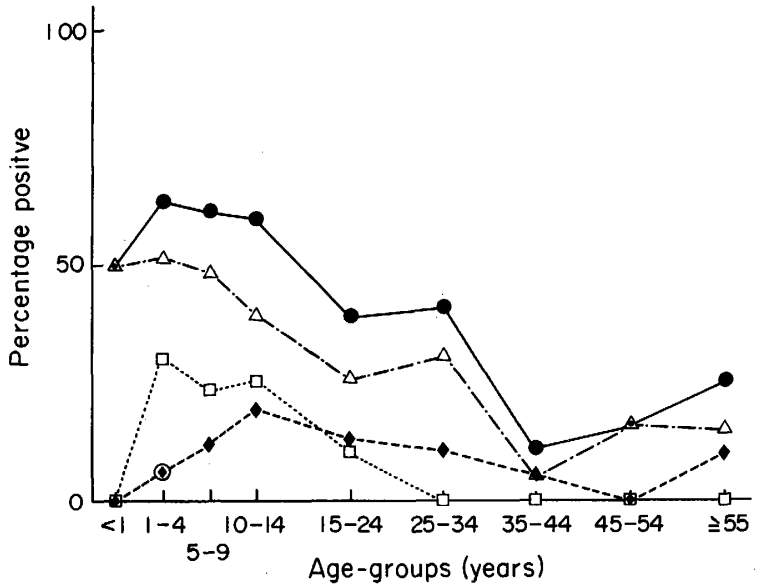

Figure 2 Age specific distribution of malaria parasite positive cases in village Perupuk. ●——● : total malaria parasite rate, △-·-·-△ : *P. falciparum* asexual form, □·····□ : *P. falciparum* sexual form (gametocyte), ◆----◆ : *P. vivax* asexual form. ○ mixed infection.

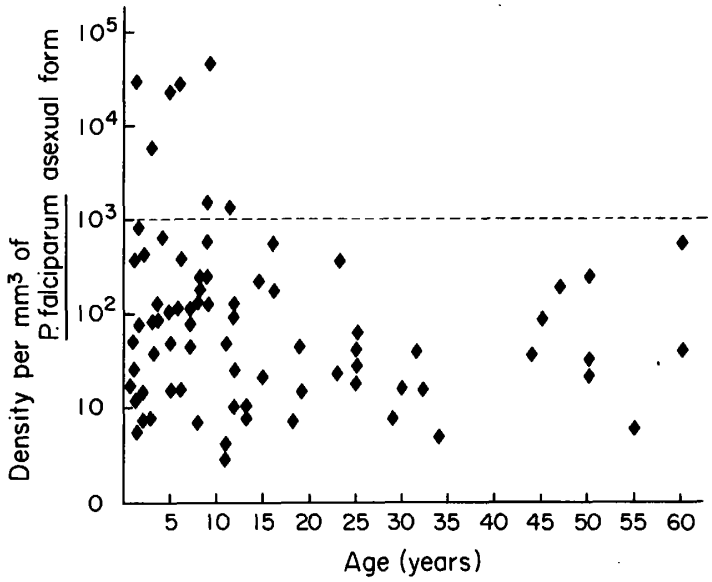

Figure 3 Age specific distribution of asexual form parasite density in *P. falciparum* infections in village Perupuk.

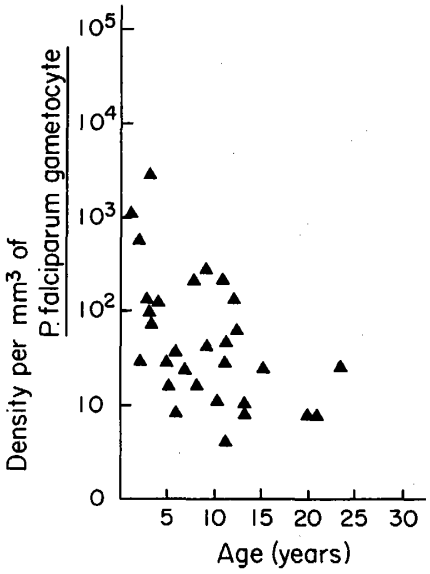

Figure 4 Age specific distribution of sexual form gametocyte density in *P. falciparum* infections in village Perupuk.

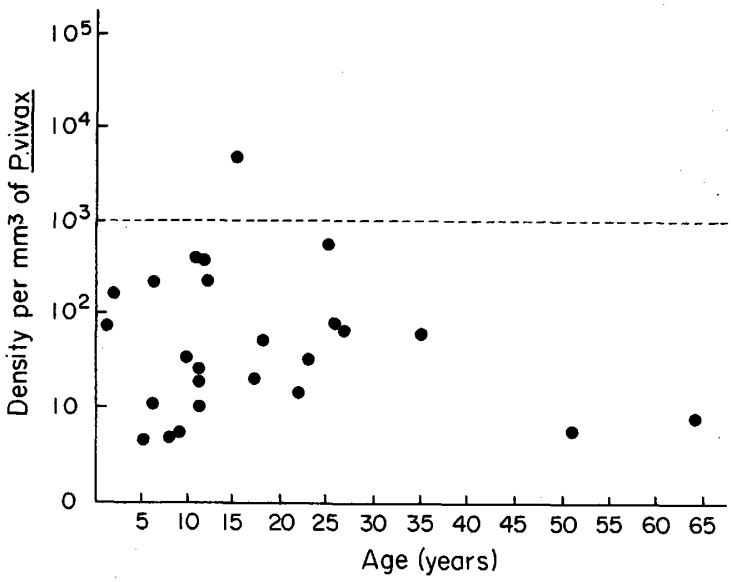

Figure 5 Age specific distribution of parasite density in *P. vivax* infections in village Perupuk.

age of less than 5 years old were found with more than 100 gametocytes/mm³ and two of them showed more than 1,000 gametocytes/mm³. On the other hand, most cases over 14 years of age showed low density gametocytemia less than 100/mm³, the parasite density of *P. vivax* infections is shown in Fig. 5. Only one case showed parasitemia higher than 1,000/mm³.

Discussion

From the figures of spleen rate in this study, it was considered that the surveyed area was mesoendemic in malaria endemicity. The parasite rate in young children was remarkably high being around 60%. This high parasite rate was a result of epidemic outbreak of *P. falciparum* infections. It has been reported in north Sumatra that malaria infection rate is less than 2% according to the existing reporting system through local health centers. This is because of uneven distribution of malaria cases in whole north Sumatra. However, as Kanbara and Panjaitan (1983) reported, malaria endemic areas were found in coastal villages. The result of this study indicated that there could be a malaria outbreak with public health problem even when available statistics show low rate of malaria prevalence. This poses a public health subject not to be overlooked because especially high infection rate was observed in younger age groups without enough immunity to malaria. The cause of this explosive outbreak of *P. falciparum* was not clear although increased man-biting rate of *Anopheles sundaicus* was reported before this survey (C. Imai, personal communication).

The age specific distribution of asexual parasite and sexual gametocyte of *P. falciparum* made us consider of the importance of younger age group in malaria transmission. Parasite density higher than 1,000 ring forms/mm^3 blood as well as gametocyte density higher than 100/mm^3 were found only in the age of less than 12 years. The number of gametocyte carriers decreased by age and the density of gametocyte decreased also by age. The background of the phenomena may be explained by the progress of immunity (Boyd, 1949), however this remained to be elucidated in further studies. From the practical point of view, this is very important when one considers to interrupt malaria transmission by any method of intervention. Children constitute the most important reservoir of malaria infection for the transmission of the agent. Transmission blocking immunity and vaccine have been studied in some laboratories (Targett and Sinden, 1985). Another method of transmission blocking is the use of gametocyticidal drugs such as 4-aminoquinoline, primaquine (Matsuoka *et al.*, 1987).

Infectivity of human population with malaria to mosquitoes has been studied by some workers (Jeffery and Eyles, 1955; Graves *et al.*, 1988). According to Rieckmann *et al.* (1968), gametocytemia higher than 100/mm^3 is usually necessary to make mosquitoes infective. Their feeding experiment on volunteers suggested this critical point. Carter and Gwadz (1980) also wrote that density of gametocyte at 1,000/mm^3 mosquito infection was the highest and majority of cases are infectious to mosquitoes at density of 100 gametocytes/mm^3.

In this study, gametocytemia higher than 100/mm^3 was found mostly in children. The present results showed that the target population of the transmission blocking is younger age group especially that of under 12 years. For that purposes primary school children and preschool children are very important. School health activities and MCH (mother and child health) activities would constitute the most rational and useful strategic element for the intervention of malaria.

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インドネシア国北スマトラにおけるマラリア高度浸淫地と年齢別生殖母体血中濃度分布について

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インドネシア国，北スマトラ州のマラッカ海峡沿岸村落において，夏期にマラリア集団検血を実施した。従来，マラリア感染率は全般的に２％以下とされていたにもかかわらず，沿岸村落に44％と高い感染率が検出された。特に０－９歳小児においては60％にも及び，公衆衛生上の問題となる事が指摘された。マラリア原虫の年齢別分布ならびに血中濃度の算定成績から，熱帯熱マラリアと三日熱マラリアが約３：１に存在し，熱帯熱マラリアにおいて学齢前幼児，小中学校生徒に高い感染率が認められ，無性原虫血中濃度も高かった。特に生殖母体の血中濃度は25歳以下の年齢の者に高く，$10^2/mm^3$以上の者は15歳以下の者に検出され，マラリア伝播に中心的役割を果している事が判明した。この事はマラリア伝播を切断して，マラリア対策を行う際に重要な点を示唆している。三日熱マラリアでは感染率は比較的低く，感染は高年齢者層に及んでおり，$10^3/mm^3$以上の原虫濃度は稀であった。

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日熱医会誌., 第17巻 第4号 1989 311-321頁

ブタ回虫感染マウスにおけるサイトカインとNK活性の動態

陶　　道

平成元年7月10日受付/平成元年9月5日受理

はじめに

蠕虫感染によって，宿主の免疫機構はなんらかの影響をうけ，液性免疫や細胞性免疫に様々な変化が起こるが，特に抗体産生では蠕虫と無関係な抗原に対する免疫応答が抑制されることが知られている（Crandall and Crandall 1971, 1976; Crandall *et al*., 1978; Faubert and Tanner 1971; Faubert, 1976; Good and Miller, 1976; Komatsu *et al*., 1979)。

ブタ回虫をマウスに感染させると，その感染時期や程度に応じて，マウスの抗体産生に各免疫グロブリン（Ig）クラスについて特異的な変化をきたすことが明らかにされてきた（Crandall and Crandall, 1971, 1976; Crandall *et al*., 1978)。しかしながら，こういった免疫学的変調には，単なるB細胞のみならず，マクロファージ，T細胞，またはナチュラルキラー（NK）細胞などの免疫担当細胞の，量的あるいは質的変化も関与していると考えられるが，これらについての解析は乏しく，いまだ不明な点が多い。

免疫応答はT細胞，B細胞，マクロファージなどの細胞間相互作用によって成り立っているが，これらの免疫担当細胞より産生される各種サイトカインは，免疫応答の誘導相や反応相において各細胞相互間のシグナルの伝達因子として，その応答の発現や調節機構に重要な役割を演じている（Aarden *et al*., 1979; Gorden and McLean, 1965; Kasakura and Lowenstein, 1965; Morgan *et al*., 1976)。他方，NK細胞は自然に備わった細胞性の生体防御因子として，抗腫瘍活性やウイルス（Bukowski *et al*., 1985; Mcintyre and Welsh, 1986)，細菌および寄生虫などの感染に対する防御活性を示す（Murphy and McDaniel, 1982; Nabavi and Murphy, 1985; Hatcher *et al*., 1980; Hatcher and Kuhn, 1982; Orago and Solomon, 1986; Solomon, 1986）のみならず，自己の免疫応答の調節にも関与するものとされている（Callewaert, 1985)。

本研究では，これら宿主寄生虫相互関係の一局面を，免疫学的観点から細胞レベルで理解するために，ブタ回虫感染マウスにおける各種免疫担当細胞によるサイトカインの産生状況や，NK活性の経時的変動を検討した。

材料と方法

1）実験動物

以下の純系マウス（日本チャールズ・リバー社より購入）を用いた。7週齢雌のBDF_1（C57BL/6NCrj×DBA/2NCrj）マウス6-8匹を1群として感染実験に用いた。またIL-1活性の測定には，5-6週齢雌のC3H（C3H/HeNCrj）マウスを用いた。

2）回虫幼虫包蔵卵の感染

大阪食肉センターにおいて屠殺されたブタより採取したブタ回虫の子宮内卵を，2%ホルマリン液中にて28°C，30日間培養して得た幼虫包蔵卵の10^3あるいは10^4個を含む0.1m*l*の生食浮遊液を，マウス用ゾンデを用いて経口的に胃内に注入した。

3）培養液および細胞の培養

培養液として5×10^{-5}Mの2-メルカプトエタ

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ノール，10%の牛胎児血清（FCS）を含むRPMI 1640を用いた。各種細胞の培養は5%の炭酸ガス存在下，37℃で行った。

4）腹腔滲出細胞の分離および培養上清の調整

幼虫包蔵卵感染後のマウスの腹腔内に5 mlの5%FCS-RPMI 1640を注射したのち，それを回収することにより，経時的に腹腔滲出細胞を得た。得られた細胞をさらに同培養液で2回洗浄し，トリパンブルー染色液で生細胞を数え，1×10^6/mlの細胞浮遊液を作成した。この浮遊液をプラスチックシャーレに入れ，37℃，5%の炭酸ガスの存在下に1時間静置して，非付着細胞を除いたのち，付着細胞をセルスクレイパーで回収し，1×10^6/mlになるように調節した。

上記の方法で得た付着性腹腔滲出細胞（腹腔マクロファージ）に，lipopolysaccharide（LPS, 半井化学薬品）を最終濃度10 μg/mlになるように加え，T-25型プラスチック培養フラスコで24時間培養した後，その培養上清を回収し，−20℃にて凍結保存して，これをIL-1活性およびPGE_2測定（後述）用腹腔マクロファージ培養上清サンプルとした。

腹腔マクロファージは，非特異的エステラーゼ染色法で同定し，好酸球はギムザ染色法で同定した。染色後200個の細胞を数え，マクロファージあるいは好酸球の比率を算定した。

5）脾細胞および培養上清の調整

マウスの脾臓を無菌的に摘出し，培養液中で注射針にてほぐした後，ステンレスメッシュを通して単細胞浮遊液を作成した。培養液で2回洗浄した後，トリパンブルー染色液にて生細胞数を数え，細胞密度を調節した。

5×10^6/mlの脾細胞単細胞浮遊液に，Concanavalin A（ConA, Miles社）を最終濃度2 μg/mlになるように加え，24時間培養した後，その培養上清を回収し，−20℃にて凍結保存し，これをIL-2活性測定（後述）用脾細胞培養上清サンプルとした。

付着細胞の単離と培養上清の調整は1×10^7/mlの脾細胞について，腹腔マクロファージと同様な方法で行い，回収した上清をIL-1測定（後述）用脾細胞培養上清サンプルとした。

6）IL-1活性の測定

IL-1活性の測定はMizel *et al.*（1978）の方法に準じた。すなわちC3Hマウスの胸腺を無菌的に摘出し，10%FCS-RPMI 1640を用いて2×10^7/mlの単細胞浮遊液を作成して，96穴マイクロプレートの各ウェルに50 μlずつ分注し，これに5 μg/mlのphytohemagglutinin-P（PHA, 和光純薬）を50 μl加えた。さらに段階希釈した前述の各種培養上清サンプルを10 μl加えて72時間胸腺細胞を培養し，その回収6時間前に^{3}H-TdR 0.5 μCiを添加してその取り込みを測定した。なお，培養上清サンプルの20，60，180および540倍の希釈濃度では，その希釈と^{3}H-TdRの取り込みの間に直線関係が見られたので，IL-1の測定にはすべて180倍希釈培養上清サンプルを用いた。

7）IL-2活性の測定

IL-2の活性は，CTLL-2細胞（C57BL/6マウス由来Friend virus induced leukemia）のIL-2依存性増殖反応を用いて測定した。すなわち，2倍連続希釈した培養上清サンプル100 μlを入れた96穴マイクロプレートの各ウェルに，5×10^3個のCTLL-2細胞を含む細胞浮遊液0.1 mlを分注して，20時間培養後，ウェル当たり0.5 μCiの^{3}H-TdRを加え，さらに4時間培養した。培養終了後，セルハーベスターで回収し，^{3}H-TdRの細胞への取り込みを，液体シンチレーションカウンターで測定した。

8）IL-2活性に対する抑制の測定

IL-2活性に対する抑制の測定は，Lelchuk and Playfair（1985）の方法に準じた。すなわち，回虫感染マウスの腹腔マクロファージ，あるいは脾臓付着細胞の培養上清50 μlを，1×10^5/mlのCTLL-2細胞浮遊液100 μlおよび10 unit/mlのリコンビナントIL-2（武田薬品）50 μlを含む96穴のマイクロプレートのウェルに加えて20時間培養後，ウェル当たり0.5 μCiの^{3}H-TdRを加え，さらに4時間培養して，^{3}H-TdRの取り込みを測定し，培養上清の添加による取り込みの減少をIL-2活性の抑制とした。

抑制因子の分子量を概算するために，透析用セルロースチューブ（分画分子量12,000-14,000）を用いて，上記の培養上清をRPMI 1640で透析した

り，モルカット10,000（Millipore）で分子量10,000以上を除去した後のIL-2活性への抑制を測定した。

9）IL-2産生に対する抑制の測定

まず，5×10⁶/m*l*の正常脾細胞浮遊液5m*l*に前項で用いた培養上清サンプルを25%あるいは50%になるよう，またConAを最終濃度2μg/m*l*になるように加えて10m*l*とし24時間培養した。この培養上清を前項と同じ条件で予め透析し，その中に含まれる低分子のIL-2活性抑制因子を除去したのち，前述と同じ方法で培養上清中のIL-2活性を測定した。培養上清サンプルのかわりに，培養液を加えた対照よりの減少をIL-2産生の抑制とし，抑制率(%)を以下の式で算出した。

$$抑制率(\%)=(A-B)/A\times100$$

ここではAは対照におけるCTLL-2細胞の，^{3}H-TdRの取り込み値を示し，Bは培養上清サンプルとConAを加えた正常脾細胞の培養上清によるCTLL-2細胞の^{3}H-TdRの取り込み値を示す。

10）NK活性の測定

脾細胞単細胞浮遊液1×10⁷/m*l*を3倍連続希釈し，96穴マイクロプレートの各ウェルに0.1m*l*を加え，^{51}Crで標識した0.1m*l*のYAC-1細胞（A/Snマウス由来Moloney virus-induced lymphoma）と共に4時間培養後，標的細胞から^{51}Crの遊離を測定し，NK活性とした。Specific lysisは常法により算定した。

なお，NK抵抗性を示すP815細胞（DBA/2マウス由来Mastocytoma）についても同様の測定を行った。

また，polyinocinic polycytidylic acid（Poly I•Poly C）による脾臓内NK活性や血清中IFN活性の誘導を見る場合には，その測定の前日に，Poly I•Poly C（シグマケミカル社）100μgをマウスの腹腔内に投与した。

11）インターフェロン(IFN)活性の測定

NK活性の測定に用いたマウスの血清を，96穴マイクロプレートにて2倍連続希釈した。この0.1m*l*に同量の10⁶/m*l*のL細胞（マウス由来のfibroblast)を加え，24時間培養した後，0.1m*l*当たり100プラークフォーミングユニット(PFU)のvesicular stomatitis Virus（VSV）を感染させた。血清添加によるVSVのL細胞細胞障害の抑制をもって，血清中のIFN力価とした。

IFNの型は抗マウスIFN-α，β抗血清を用いて判定した。

結　果

1）回虫感染によるマウス腹腔滲出細胞の変動

回虫感染によりマウスの腹腔滲出細胞数は，図1に見られる如く，15日目を峰として一過性に増

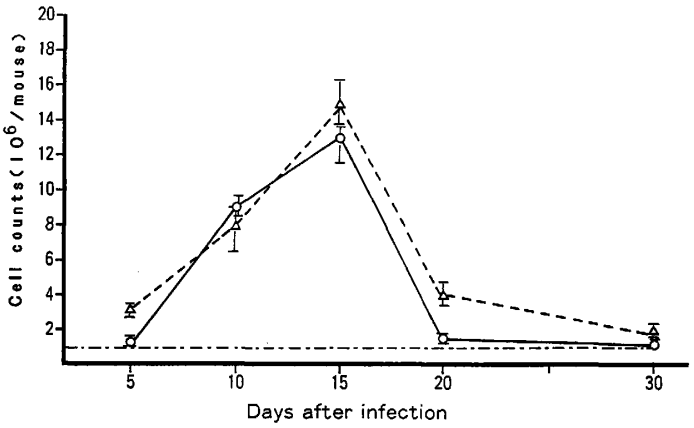

Figure 1 Change of peritoneal cell counts in mice during infection with 10³(○) or 10⁴(△) eggs of *Ascaris suum*.
Mean of six mice with vertical bars as the S.D. is shown.

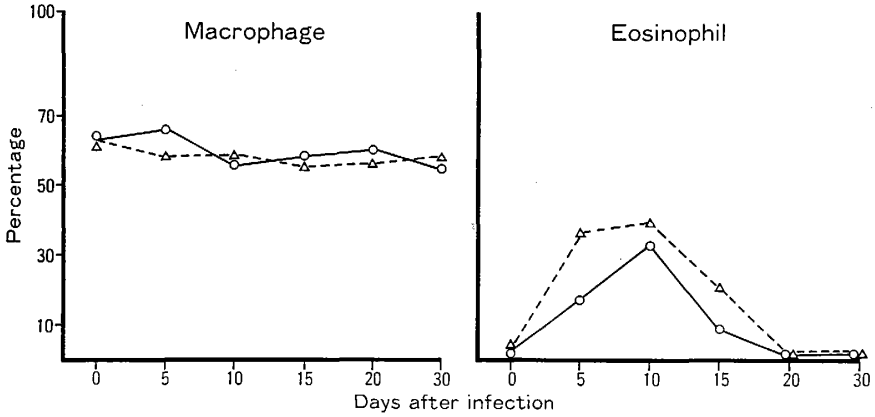

Figure 2 Percentage of macrophages and eosinophils in the peritoneal cavities of mice infected with 10^3(○) or 10^4(△) eggs of *A. suum*. Two hundred cells were counted at least, and percentage of macrophages and eosinophils were calculated after that cells were stained with nonspecific esterase or Giemsa solutions. Results were obtained from three mice.

加することが観察された。10^3あるいは10^4個の虫卵感染によりマウスの腹腔滲出細胞数は，正常マウスのそれに比べ，感染後5日目において，それぞれ1.2と3.4倍，10日目において9.1と8.1倍，15日目において13.8と15.3倍と著明に増加した。しかし，20日目に1.5と4倍と増加率は大幅に減少し，30日目には，10^3の感染群で正常マウスと同程度となり，10^4の感染群で正常マウスの2倍以下までに減少した。

この腹腔滲出細胞に含まれるマクロファージと好酸球を，非特異的エステラーゼ染色法とギムザ染色法を用いて同定し，その細胞数を計測したところ，図2に示すように，マクロファージの総細胞数に対する比率は正常マウスのそれと相異なく，感染時期による明らかな変動を示さなかったが，図1に示したように総細胞数が顕著に増加したことから，腹腔内のマクロファージの総数は感染後15日目を最高として，一過性に増加することが示された。好酸球の比率は，5日目に増加し始め，10日目に最高に達し，15日目より減少する傾向を示し，30日目に対照群と同程度に復元したが，両者の比較から感染マウスの腹腔内で好酸球はマクロファージに比して，約5日間先行して一過性に増加することが明らかとなった。

2）回虫感染による腹腔滲出細胞のIL-1産生の変化

回虫感染マウスから経時的に採取した腹腔マクロファージを，そのまま培養してもIL-1産生は認められないが，LPSで刺激すると10^3あるいは10^4個のいずれの感染群においてもIL-1の産生が起

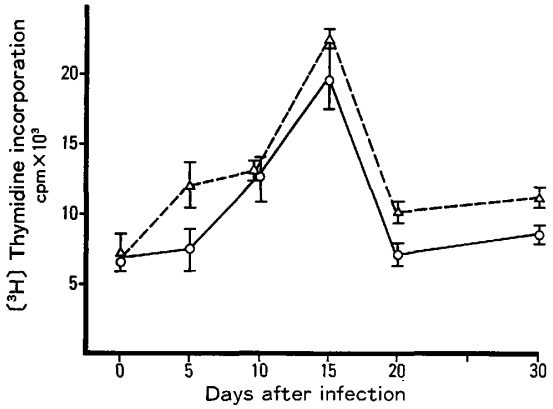

Figure 3 Time course of LPS-induced IL-1 production of peritoneal adherent cells of *A. suum* infected mice. The culture supernatants of peritoneal adherent cells from mice infected with 10^3(○) or 10^4(△) eggs of *A. suum* were assayed on PHA-stimulated thymocyte cultures at a dilution of 1:180. Each point represents the mean±S.D. of three experiments. Day 0 stands for the value of uninfected mice.

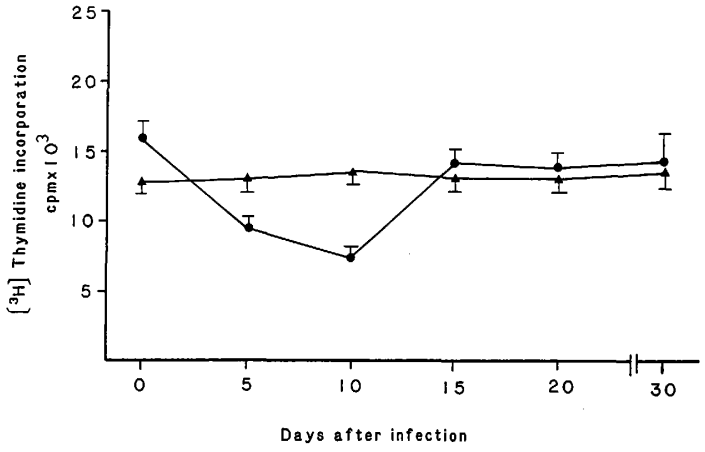

Figure 4 Kinetics of suppression of IL-2 activity by culture supernatants of peritoneal adherent cells from mice infected with 10^4 eggs of *A. suum*.
Before (●) or after (▲) dialysis, 50 μl of the culture supernatants were added, together with 50 μl of 10 unit/ml recombinant IL-2 to 1×10^4 CTLL-2 cells contained in 100 μl. After 24 hr-culture, proliferation of CTLL-2 cells were measured by ^{3}H-TdR incorporation. Each point represents the mean±S.D. of three experiments.

こり，図3に示すように培養上清中のIL-1活性は感染後5日目より漸増し，15日目に最高に達したあと，20日目に向って次第に減少したが，この動態は腹腔内マクロファージの総数の動態とほぼ同調していた。

以下いずれも図表に示さないが，上述の培養上清中のPGE_2を測定したところ，10^4の感染群では対照群に比し，5日目，10日目において1.1倍，15日目に1.2倍であったが，感染による有意な増強とは認められなかった。また同上清中には，いずれの時期においてもTNF活性は認められなかった。

3）回虫感染マウスの腹腔滲出細胞の培養上清中のIL-2抑制因子

前述の感染各時期の腹腔滲出細胞のLPS刺激培養上清について，IL-2活性に対する作用を調べたところ，図4に見られるように正常マウスのLPS刺激培養上清の場合に比し，感染10日目における53%を最高とする顕著な抑制作用を認めた。次に，この培養上清中の正常マウス脾細胞のConA刺激によるIL-2産生に，どのような影響を与えるかを調べた。図5に見られるように，正常マウス脾細胞のConA刺激で産生されるIL-2活性は，正常マウスの腹腔滲出細胞のLPS刺激培養上清の50%添加により約11%抑制されたのに対し，

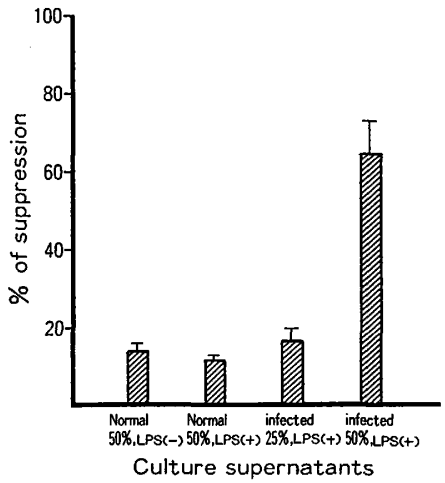

Figure 5 Suppressive activity on IL-2 production of normal spleen cells.
The culture supernatants of peritoneal adherent cells from mice 10 days after infection with 10^4 eggs of *A. suum* and from normal spleen cells (cultured with or without LPS) were incubated with ConA activated normal spleen cells at indicated concentration. After 24 hr culture, the remaining activity of IL-2 in the cell-free supernatants were assayed using CTLL-2 cells. Results represents the mean±S.D. of triplicated cultures.

感染10日目の腹腔滲出細胞の培養上清は，25%あるいは50%の添加により正常脾細胞のIL-2産生をそれぞれ15%，64%抑制した。

このような培養上清中に含まれる抑制因子の分子サイズを調べるために，上清を透析用セルロースチューブを用いて透析し，12,000-14,000以下の分子量の物質を除去したところ，図4に見られるように，感染後いずれの時期の上清についてもIL-2活性への抑制活性は検出できなくなった。次いで，同じ培養上清をモルカット10,000で濾過し，その濾液についてIL-2活性抑制を調べたところ，データは示さないが，濾過前と同じ程度の抑制活性が確認された。

なお，感染マウスの脾臓付着細胞のLPS刺激培養上清については，いずれの感染時期においても上記のような抑制作用が認められなかった。

4）回虫感染によるマウス脾細胞のIL-2産生

次に，マウス脾細胞のConA刺激によるIL-2産生能の回虫感染による変化を調べた。その結果は，図6に示すように，10^3個の感染では主として10日目に，10^4の感染では，5日と10日目の両時期において，IL-2産生は強く抑制されていた。いずれの群においても，その抑制は15日から回復する傾向を示したが，20日以降まで持続した。

5）脾細胞のNK活性

脾細胞のNK活性の感染による変化を，YAC-1細胞に対する細胞障害作用を指標として調べたところ，データは示さないが，10日目に若干の低下が見られたものの，有意の変化は認めなかった。そこで，その差を明らかにするために，脾細胞採取前日に100μgのPoly I・Poly Cを投与することにより，積極的に誘導したNK活性の増強が，感染によって変化するか否かを検討した。その結果，図7に見られるように，感染マウス脾細胞のNK活性は，10^3と10^4個のいずれの感染群においても上述と同じく感染10日目に有意に低下し，15日目には回復した。なお，データは示さないが，P815細胞に対する細胞障害作用も同時に調べたが，両群いずれも対照群に比べ，明らかな変化

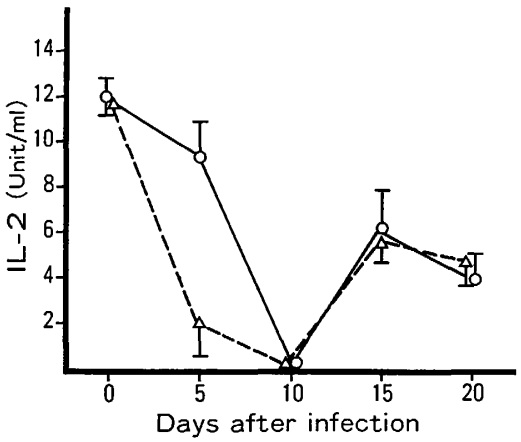

Figure 6 Time course of IL-2 production.
The spleen cells from mice infected with 10^3(○) or 10^4(△) eggs of *A. suum* were stimulated with ConA for 24 hr and culture supernatants were assayed using CTLL-2 cells. Each point represents the mean±S.D. of three experiments.

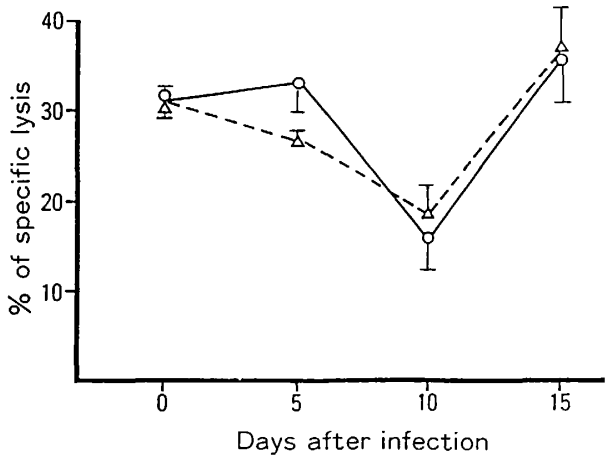

Figure 7 Anti-YAC-1 cytotoxic activity induced by Poly I•Poly C in mice infected with 10^3 (○) or 10^4(△) eggs of *A. suum*.
Spleen cells were removed on day 5, 10 and 15 after infection from mice which were intraperitoneally received 100 μg of Poly I•Poly C one day before sacrifice. Cytotoxicity was measured by release of ^{51}Cr from labeled YAC-1 target cells after 4 hr incubation at 100:1 E/T ratio. Day 0 stands for the value of uninfected and Poly I•Poly C injected mice. Each point represents the mean±S.D. of three experiments.

はなかった。

6）血中IFNの活性

Poly I•Poly C刺激マウスについては，その血清中IFN活性の感染による影響をも検討した。

図8に示すように，いずれの感染群においても，感染5日目の血清中IFN活性は対照群に比べ増強したが，10日目には逆に低下し，15日目には復元した。

これらIFN活性は，抗マウスIFN-α，β抗血清の処理で中和されたので，Poly I•Poly Cによって誘導された血清中IFN活性は，α，β型であることが確認された。

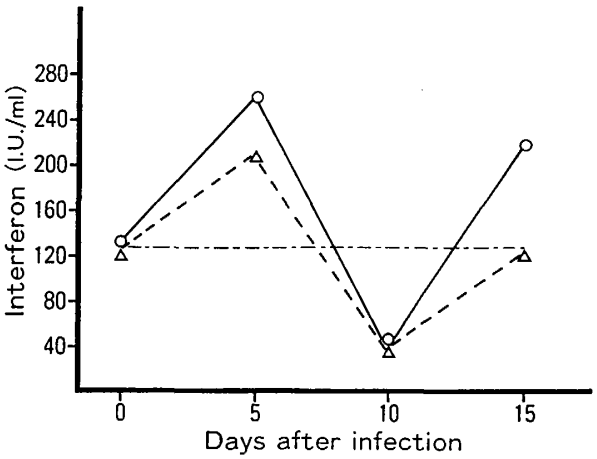

Figure 8 Induction of IFN by Poly I•Poly C in *A. suum* infected mice. The serum samples originated from the same donor mice infected with 10^3(○) or 10^4(△) eggs of *A. suum* just as described in Fig. 7. Each value represents the average of six samples.

考　察

蠕虫感染時における宿主の免疫系の変化の特徴として，非特異的IgE産生の増強や好酸球増多があげられるが（Jarrett and Miller, 1982），同時に液性あるいは細胞性免疫能が非特異的に低下することもよく知られている。この免疫低下は，宿主寄生虫相互関係を免疫学的観点から理解する上で重要な課題であり，すでに多くの虫種について，その機構の解析がなされて来たが，その様相は虫種によって一様でなく不明な点が多く残されている。

この点に鑑み，本研究では，ブタ回虫の経口感染をうけたマウス（非固有宿主）の腹腔内滲出細胞の量的変動や，モノカインの産生状況を調べるとともに，脾内のT細胞の機能やNK細胞活性，さらには血中IFN活性に対する感染の影響を検討し，この感染系における免疫低下の成因をさぐろうとした。

すでに，著者らはブタ回虫幼虫包蔵卵10^4個をマウスに経口感染させたのち，小腸で孵化した幼虫の体内移行状況を調べ，肝臓では5日目，肺臓では7日目で回収幼虫の数が最大となり，約14日以後には，いずれの臓器においても検出できなくなることから腸，肝，肺の移行経路を確認するとともに，マウスの体重が感染6日目より減少しはじめ，10日目には最低に達し対照群の75%となるが，その後漸増し，約16日目に復元することなどを見出し，幼虫の体内移行に伴って一過性に全身状態が悪化することを示して来た（未発表）。今回は，回虫感染によって腹腔内滲出細胞数が感染15日目を最高として，一過性に著しく増加することを見出すとともに，この滲出細胞のうち好酸球数は10日目を，マクロファージ数は15日目を最高とする経時的変動を示すことを明らかにした（図1，2）。次に，これら腹腔マクロファージを*in vitro*でLPS刺激して産生されてくるIL-1活性の経時的変動を調べたところ，マクロファージ数の増加にほぼ同調して，感染後15日目を最高として一過性に増加するのを認めたが（図3），このような好酸球やマクロファージ数の増加や活性化は腹腔内を移行する幼虫に対する防御反応の一面を反映するものと思われる。

他方，回虫感染後の脾内ヘルパーT細胞の機能の経時的変動を知るために，感染後の各時期において，脾細胞を*in vitro*でConA刺激して産生されてくるIL-2活性の動態を調べたところ，感染後5日目より脾細胞のIL-2産生は減少し始め，10日目には最低に達したあと，15日目以後回復に向うことが明らかになった（図5）。これは，ブタ回虫感染後11-12日目において，マウス脾細胞のConAや

PHA刺激による増殖反応や遅延型過敏反応の低下を見出したCrandall *et al.* (1978) の報告と一致し，この時期にヘルパーT細胞の潜在的IL-2産生能が障害されていることが示された。次にIL-2によって誘導される，NK活性の感染マウス脾内の変動を調べたところ，データは示していないが，無処置の感染マウスの脾細胞では，正常マウスのそれとの差は認めなかった。しかし，NK活性の誘導物質であるPoly I•Poly Cをマウスに予め投与して，NK活性を積極的に誘導させると，感染と非感染の両マウス間に脾内NK活性の差が見られ，上記IL-2産生が低下する感染10日目においてNK活性の誘導が抑制されていたが，15日目には復元していた（図7）。Poly I•Poly C投与によって血中に誘導されるIFN活性についても同様な変動が見られ，感染5日目では活性が若干上昇するが，10日目には低下し，15日目には復元していた（図8）。一般にNK活性はIFNを介して増強されるので，感染マウスの両活性が同調して低下するのは当然と考えられるが，感染5日目にIFN活性のみが対照群より上昇した原因は不明である。なお，データには示していないが，脾内におけるLymphokine activated killer（LAK）細胞の存在を，P815細胞を標的細胞とする障害活性で調べたところ，感染による変化を認めなかった。

以上のように，回虫感染による脾内ヘルパーT細胞のIL-2産生能の低下は，NK活性やIFN活性の誘導にも影響を及ぼしつつ，感染マウスの免疫応答性の低下をもたらすのであろうと考えられるが，IL-2産生能の低下を惹起する機序については不明のままである。すでに種々の蠕虫感染における免疫低下の原因として，虫体自身あるいは虫体成分によるT細胞の直接的な障害や，抑制性T細胞やマクロファージの誘導などが報告されているが（Barriga, 1975; Faubert and Tanner, 1975; Lubiniecki and Cypess, 1975），これまでの実験系ではこれらの検討はまだなされていない。そこで，本研究ではIL-2活性に影響する因子を検索するため，感染マウス腹腔内滲出細胞をLPS刺激して得た培養上清のIL-2産生に対する影響を調べたところ，既存のIL-2活性を抑制するとともに正常脾細胞によるIL-2産生（感染10日目の培養上清）をも明らかに抑制した（図4，5，6）。この培養上清中には上述のようにIL-1活性も含まれているので，回虫感染によって腹腔マクロファージが活性化されIL-1を産生すると同時に，抑制因子をも産生していることが明らかとなった。この抑制因子は，透析および濾過実験の結果から，分子量10,000以下であることが推定されたが，更にこの因子の経時的変動を調べたところIL-1の産生と同調して，感染5日目より増加しはじめ10日目に最高に達したあと減衰し，15日目には復元した（図4）。

マクロファージに由来する低分子の抑制因子としてはPGE_2が挙げられるが（Davis *et al.*, 1984; Goodvin and Ceuppens, 1983; Kunkel *et al.*, 1986），本実験では，データは示していないが，感染マウス腹腔細胞のLPS刺激によって誘導されるPGE_2の量は正常マウスにくらべ，明らかな変化を示さなかった。また，TNF活性はいずれの群においても上清に認められなかった。同様な抑制因子が，感染マウスの脾臓付着細胞のLPS刺激によっても産生されるかどうかを検討したが，その培養上清にはIL-2活性，あるいはIL-2産生に対する抑制作用を認めなかった。これはCrandall *et al.* (1978) の系で，感染により免疫低下を示すマウスの脾細胞中に抑制性細胞の存在を認めなかったことと相通じるかも知れない。幼虫の移行経路に当たる腹腔内のマクロファージが，虫体，あるいは虫体成分による直接刺激によって活性化され抑制因子を産生するのであれば，幼虫が移行しない脾臓では付着細胞がその因子を産生しないのは当然であろうが，もし感染による免疫低下が，主として上記の抑制性腹腔マクロファージの産生する抑制因子によるものとすれば，多分循環を介して遠隔の脾の免疫系に影響を与えるのであろうが，その機序は今後の課題として残されている。

今回の研究により，ブタ回虫感染初期におけるT細胞の機能低下には，腹腔マクロファージより産生されるIL-2抑制因子が関与している可能性があり，回虫の宿主体内移行部位にある腹腔内マクロファージは生体防御および免疫抑制という両面で，感染に対応していることが示唆された。また，回虫感染はNK細胞やIFN活性の変動を介し

て，宿主の免疫機構に複雑な影響を及ぼしている可能性も示唆された。

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CHANGE OF CYTOKINE AND NATURAL KILLER ACTIVITIES IN *ASCARIS SUUM* INFECTED MICE

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In order to analyze the cellular mechanism of immunosuppression during acute infection by *Ascaris suum*, cytokine and natural killer activities in *A. suum* infected mice were investigated.

Ten days after infection with 10^3 or 10^4 embryonated *A. suum* eggs, Concanavalin A induced interleukin 2 (IL-2) production by splenocytes were reduced. At the same time, NK activity of splenocytes and the levels of serum interferon induced by Poly I•Poly C were also reduced. On the other hand, culture supernatants of peritoneal adherent cells from *A. suum* infected mice showed significantly high interleukin 1 activity which reached peak level on day 15 following lipopolysaccharide stimulation. Increase in the number of macrophages in the peritoneal cavity were also observed in this period. Furthermore, the culture supernatants of peritoneal adherent cells from 10-days *A. suum* infected mice exhibited a suppressive effect on both IL-2 production by normal splenocytes and IL-2 activity assayed using CTLL-2 cells. Dialysis and molecular filtration studies indicated that the factor with molecular weight below 10,000 related to the suppressive effect. But there was no significant increase of prostaglandin E_2 in the culture supernatants.

These results suggest that activated macrophages may play an important part in the immunosuppression by producing a soluble suppressive factor during acute infection by *A. suum*.

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MONOCLONAL ANTIBODIES RECOGNIZING THE MAIN SURFACE ANTIGENS OF NEWLY EXCYSTED METACERCARIA OR ADULT FLUKES OF *PARAGONIMUS OHIRAI*

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Abstract: This study was undertaken to prepare monoclonal antibodies which recognized the tegumental antigens of newly excysted metacercaria (NEM) and adult flukes of *Paragonimus ohirai*. From splenic cells of mice immunized with either the Triton X-100 extract of adult fluke surface or NEM sonicate, monoclonal antibodies were produced by ELISA screening followed by trial immunostaining of fluke sections. We prepared a monoclonal antibody (AS-Mab) recognizing the tegumental antigen of the adult fluke and a monoclonal antibody (MS-Mab) for the NEM tegumental antigen. AS-Mab also bound to the tegument of 1-week-old juveniles but not to that of NEM. MS-Mab bound the tegument from the metacercaria to adult stages, but the degree of immunostaining appeared to decrease with maturation of the flukes. By the double immunodiffusion technique, the two monoclonal antibodies formed crossing precipitin lines against a mixture of the adult surface antigen extract and the NEM antigen extract. MS-Mab and AS-Mab gave precipitin line against the incubation fluid from NEM and adult flukes, respectively.

INTRODUCTION

The surface of a parasite plays a crucial role in the immunological interaction between host and parasite. This is well-known in African trypanosomes, which undergo antigenic variation of their surfaces according to the host immune response (Cross, 1979). In parasitic helminths, changes of the surface antigens occur during development (Philipp *et al.*, 1980; Snary *et al.*, 1980; Maizels *et al.*, 1983) and might be one of the ways in which they evade the host's immune attack. There have been a number of reports about the stage-specific surface antigens of helminths, as reviewed by Philipp and Rumjaneck (1984), but most of the studies went no further than identification of the surface antigens using surface-labelling and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). It is important for understanding fully the immunobiological phenomenon occurring in helminth infections to investigate the various immunochemical and biochemical characteristics of surface antigens.

In the lung fluke, an anti-surface antibody response has been observed to occur during the course of *Paragonimus ohirai* infection (Ikeda and Tani, 1985; Ohara *et al.*, 1985), the surface antigens have not yet been immunochemically and biochemically analyzed. Therefore, we

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planned to use monoclonal antibodies to characterize as well as identify the individual antigens. The present study was undertaken to produce monoclonal antibodies against the surface antigens of newly excysted metacercariae (NEM) and adult flukes.

Materials and Methods

Crude NEM antigen was prepared as described previously (Ikeda and Tani, 1988). In brief, NEM obtained by culturing metacercariae were sonicated twice for 30 sec in an ice bath. After the sonicate was centrifuged at 20,000×*g* for 30 min, the supernatant was dialyzed against 10 mM phosphate buffered saline (PBS, pH 7.2) and used as the NEM antigen. Adult surface antigen was prepared as follows. Living adult flukes obtained from infected rats were incubated in several changes of saline for 5 h at room temperature. The washed flukes were incubated for 10 min at 4°C in PBS containing 0.5% Triton X-100. The extract was centrifuged at 20,000×*g* for 30 min, dialyzed against PBS, and used as the adult surface antigen.

BALB/c mice were immunized with either NEM antigen or adult surface antigen. Splenic cells from the immunized mice were fused with P3U1 myeloma cells by using polyethylen glycol. Hybridoma cultures were screened by enzyme-linked immunosorbent assay (ELISA) and then positive cultures were assessed by immunoperoxidase staining of sections of NEM or adult flukes embedded in paraffin. The hybridomas which secreted antibodies against the surface antigens of NEM or adult flukes were cloned by limiting dilution. Monoclonal cells were injected into the peritoneal cavities of pristane-primed mice and the ascitic fluid was collected as the source of monoclonal antibodies.

ELISA was performed in microtiter plates using horseradish peroxidase-conjugated rabbit anti-mouse IgG according to the conventional procedure. Microtiter wells were sensitized with 100 μl of NEM or adult fluke antigen (10 μg protein/ml) in 0.1 M $NaHCO_3$ buffer (pH 9.6). As the substrate for peroxidase, 2, 2'-azino-di-(3-ethylbenzthazoline sulfonic acid) diammonium salt (ABTS) was used. Immunoperoxidase staining was performed as follows. Flukes were fixed with cold 95% ethanol for from 5 h to 1 day, dehydrated in absolute ethanol, and then embedded at 60°C with paraffin. Fluke sections were cut and stained essentially by the indirect immunoperoxidase technique of Nakane and Pierce (1966). The sections were deparaffinized with xylene and ethanol. After washing with PBS, sections were incubated with diluted monoclonal antibody or normal mouse serum for 1 h, washed in PBS, and then incubated for 1 h with peroxidase-conjugated anti-mouse IgG antibody (Cappel Laboratories, Westchester, PA) diluted to 1/200. After washing, the sections were developed in substrate of 0.02% 3, 3'-diaminobenzidine and 0.003% hydrogen peroxide in 0.05 M Tris-HCl buffer (pH 7.6).

Results

The monoclonal antibody (MS-Mab) prepared from NEM antigen-immunized mice gave a high ELISA level on the NEM antigen-coated plate but a low level on the adult antigen-coated plate. The monoclonal antibody (AS-Mab) prepared from adult surface antigen-immunized mice gave a high ELISA level on the adult antigen-coated plate but a low level on the NEM antigen-coated plate.

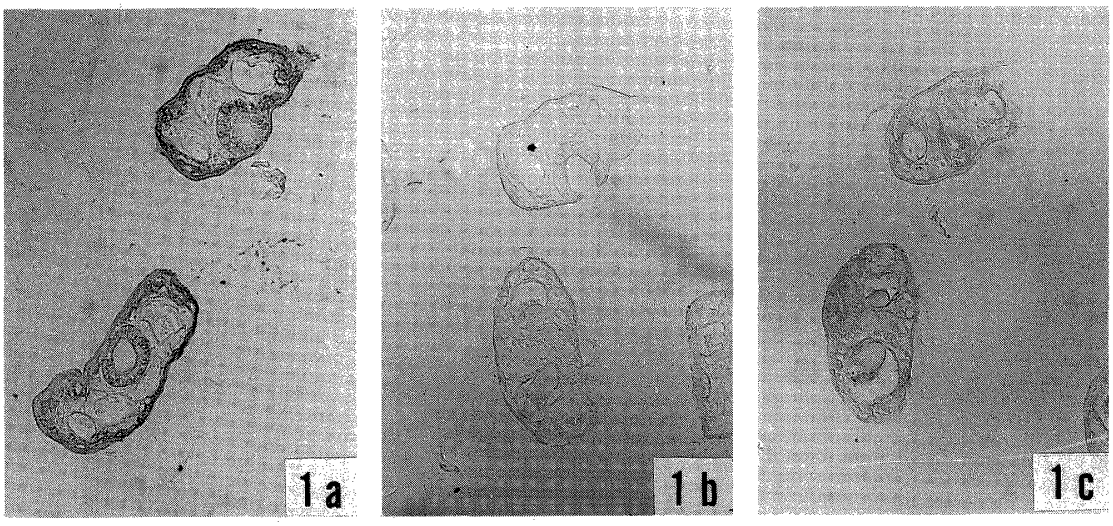

Figure 1 Immunostaining of *P. ohirai* NEM sections. Sections were treated with the monoclonal antibodies MS-Mab (a) or AS-Mab (b), or with normal mouse serum (c). The surface syncytium, the tegumental cells, and the ventral sucker were stained by MS-Mab, but not by AS-Mab or normal mouse serum.

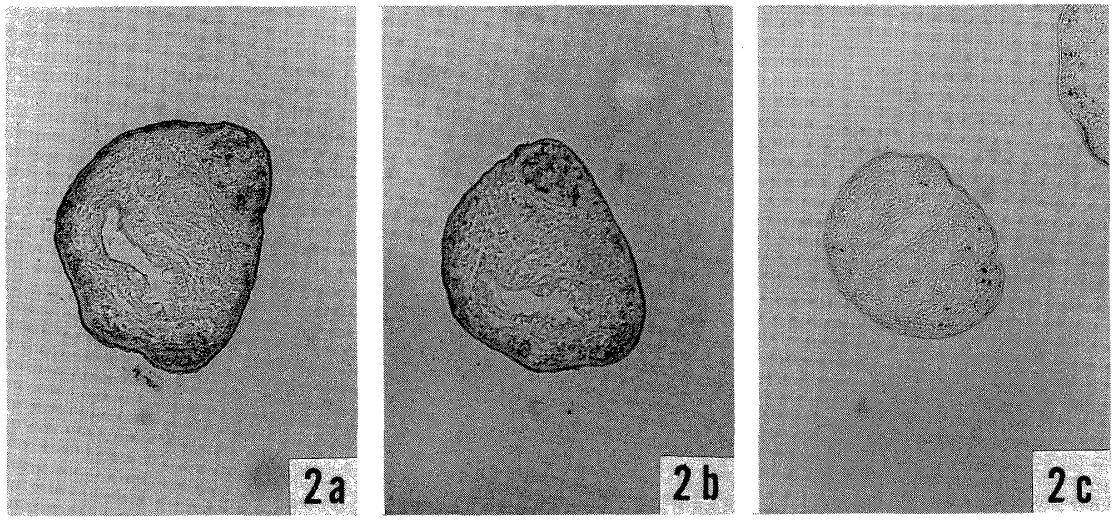

Figure 2 Immunostaining of sections of 1-week-old *P. ohirai* juveniles obtained from the peritoneal cavities of infected rats. Sections were treated with the monoclonal antibodies MS-Mab (a) or AS-Mab (b), or with normal mouse serum (c). The surface syncytium and the tegumental cells were stained by MS-Mab and AS-Mab, but not by normal mouse serum.

Fig. 1 shows the immunostaining of NEM sections. MS-Mab diluted to 1/800 produced strong immunoperoxidase staining of the surface syncytium and the tegumental cells of NEM. When MS-Mab diluted to 1/100 was used, nonspecific staining was observed over the whole body. AS-Mab and normal mouse serum did not stain the NEM sections even at a dilution of 1/100.

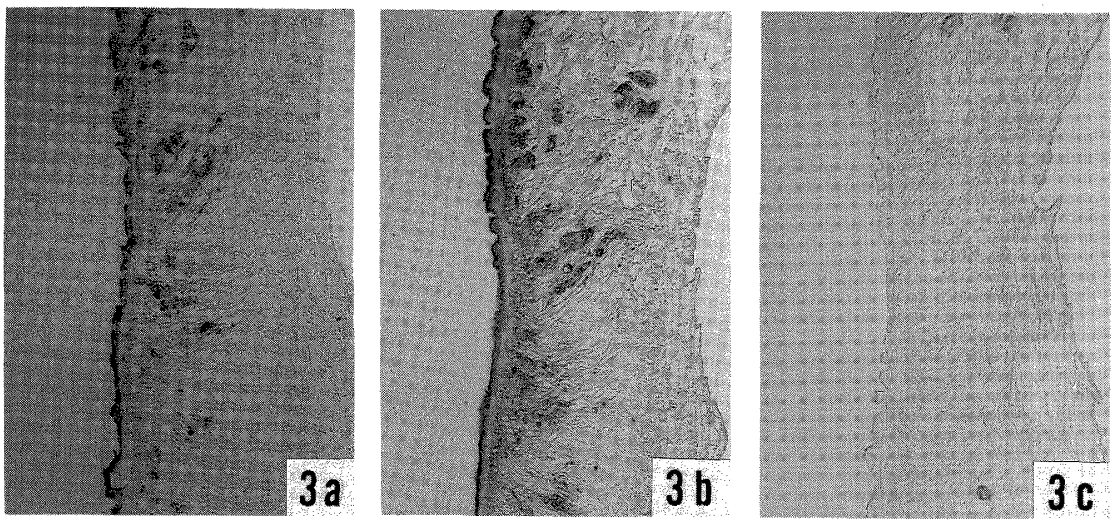

Figure 3 Immunostaining of sections of adult *P. ohirai* flukes obtained from the lungs of infected rats. Sections were treated with the monoclonal antibodies MS-Mab (a) or AS-Mab (b), or with normal mouse serum (c). The surface syncytium and the tegumental cells were stained by MS-Mab and AS-Mab, but not by normal mouse serum.

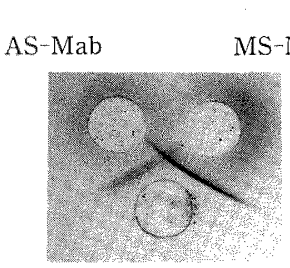

Figure 4 Double immunodiffusion in agar between the two monoclonal antibodies and a mixture of the antigen preparations from NEM and adult flukes. MS-Mab and AS-Mab produced independent precipitin lines.

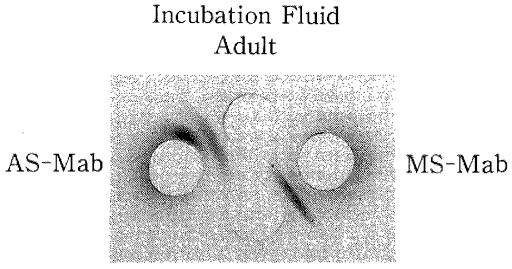

Figure 5 Double immunodiffusion in agar using the two monoclonal antibodies and incubation fluid (2 mg protein/m*l*) from either NEM or adult flukes. MS-Mab formed a precipitin line against NEM incubation fluid, and AS-Mab did so against adult fluke fluid.

In sections of 1-week-old juveniles from the peritoneal cavities of infected rats, the surface syncytium and the tegumental cells were stained by both MS-Mab and AS-Mab diluted to 1/100 (Fig. 2). In adult sections, the same sites as in the juveniles were stained by the two antibodies diluted to 1/100 (Fig. 3). The immunostaining with AS-Mab was relatively stronger than with MS-Mab. Throughout the development of the fluke, the strongest staining was observed on the tegumental apical surface.

Double immunodiffusion of MS-Mab and AS-Mab against a mixture of the adult surface antigen extract and the NEM sonicate produced crossing precipitin lines (Fig. 4). When the

two monoclonal antibodies were diffused against incubation fluid of the flukes, MS-Mab and AS-Mab formed precipitin line against the fluid from NEM and adult flukes, respectively (Fig. 5).

Discussion

We prepared the two monoclonal antibodies, MS-Mab and AS-Mab. Both of them recognized the P.o. tegumental antigens present in the tegumental syncytium and cells and on the apical tegumental surface, and both showed the strongest immunostaining of the apical surface which may have glycocalyxes. MS-Mab bound to the tegument throughout development of the flukes from metacercaria to adult, while AS-Mab bound to the tegument of adult flukes and 1-week-old juveniles but not to that of NEM. The intensity of immunostaining with MS-Mab decreased with the development of the flukes, whereas it increased with AS-Mab. Thus, the two monoclonal antibodies appeared to recognize different tegumental antigens, and this was verified by the formation of independent precipitin lines on double immunodiffusion.

Bennett and Threadgold (1975) observed at the electron microscopic level that the glycocalyx of *F. hepatica* was formed by the discharge of tegumental granules which they morphologically divided into 3 types; T0, T1, and T2 granules. Hanna (1980) proposed that the antigenicity of the glycocalyx changed during the development of *F. hepatica* and that the change seemed to be related to the appearance of T2 granules. In *Paragonimus* species, Fukuda (1986) reported the presence of tegumental granules resembling those of *F. hepatica*. We have observed at the ultrastructural level that MS-Mab bound to some types of tegumental granules and to the glycocalyx (Fujino *et al.*, submitted for publication), but we have not yet studied AS-Mab. From the similarity in immunostaining features and the presence of antigens in the fluke incubation fluid, AS-Mab is predicted to recognize the antigen present in the other type of tegumental granules.

Hanna and Trudgett (1983) prepared six monoclonal antibodies recognizing a tegumental antigen present in T1 granules and the glycocalyx of *F. hepatica*. However, the monoclonal antibodies also strongly labelled the glycocalyx of gut cells and the excretory duct in juvenile and adult flukes. Our monoclonal antibodies showed little binding to any antigens except the tegumental antigen, especially in juvenile and adult flukes. It is interesting that the epitope of their antibodies appeared to be a polypeptide while ours appeared to be sugar chains (unpublished data). Neither MS-Mab and nor AS-Mab reacted with crude *F. hepatica* adult antigens.

The two monoclonal antibodies prepared in this study should aid investigation of the characterization and bioimmunological functioning of these surface antigens.

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大平肺吸虫の脱嚢メタセルカリア主要外皮抗原と成虫主要外皮抗原を認識するモノクローナル抗体

及川陽三郎・池田　照明

大平肺吸虫（P.o.）の脱嚢メタセルカリア（NEM）抗原あるいは成虫トリトンX-100抽出表層抗原でマウスを免疫し，それらマウスより，それぞれP.o.主要外皮抗原を認識するモノクローナル抗体，MS-MabおよびAS-Mabを作製した。ELISAでは，MS-MabはNEM抗原と極めて高い反応性を示し，成虫抗原との反応性は低かった。一方，AS-Mabは成虫抗原のみに高い反応性を示した。

P.o.のNEM，1週目幼虫，および成虫のパラフィン包埋切片を作製し，2種モノクローナル抗体が認識する抗原局在部位を，酵素抗体法で観察した。NEM切片では，MS-Mabのみが外皮および外皮細胞に免疫染色性を示した(図1)。1週目幼虫および成虫切片では，両種モノクローナル抗体とも，外皮および外皮細胞に免疫染色性を示した(図2，3)。成虫切片では，両種抗体に染色性の差が見られ，AS-Mabがより強い染色性を示した。いずれの発育期でも，両種抗体とも外皮最外層に最も強い染色性が見られた。NEM抗原と成虫表層抗原の混合液に対してオクタロニー反応を行うと，両種抗体はそれぞれ交差する沈降線を形成した（図4）。また，NEMあるいは成虫の排泄・分泌（E･S）抗原に対するオクタロニー反応では，MS-MabはNEM E･S抗原に，AS-Mabは成虫E･S抗原にそれぞれ沈降線を形成した（図5）。免疫染色像，およびE･S抗原における認識抗原の存在などの結果は，これら2種のモノクローナル抗体がP.o.外皮glycocalyxを抗原として認識していることを示唆し，この外皮抗原の主要抗原が発育期に伴って変化することを示した。

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EPIDEMIOLOGICAL SURVEY OF LEISHMANIASIS USING SKIN TEST AND ELISA IN ECUADOR

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Abstract: The present study was designed to evaluate the intradermal skin test (ST) and the ELISA as diagnostic tools in the screening for Ecuadorian cutaneous and mucocutaneous leishmaniasis. The antigen for skin testing was prepared from ruptured promastigotes of *Leishmania braziliensis*. The ST and ELISA positive rates among 72 subjects with active dermal lesions were 81.8% (36/44) and 81.3% (52/64), respectively, while parasites were observed in 31 (44.9%) of 69 subjects presenting active lesions. In the parasites positive cases, all subjects proved to be positive for the two tests except for one in ST and two in ELISA. In 35 healed cases, the ST and ELISA positive rates were 86.2% (25/29) and 72.4% (21/29), respectively. On the other hand, the positive rate in subjects without clinical signs was only 3.8% in ST and 8.2% in ELISA. An epidemiological survey in Selva Alegre, Esmeraldas, revealed that among 115 inhabitants 38 were positive for the clinical signs, 10 active and 28 healed cases. Of these subjects 33 (86.8%) showed positive reactions against ST and/or ELISA. Based on the results obtained, therefore, we concluded that the present skin testing antigen and ELISA were very useful for the screening of leishmaniasis in the endemic areas of Ecuador.

INTRODUCTION

Since the first human case of leishmaniasis was described in Ecuador in 1920, many additional cases of the disease have been reported (Rodriguez, 1974; Hashiguchi *et al.*, 1984). The causative agent has been considered *L. braziliensis* complex based mainly on their clinical manifestations in humans and epidemiological features (Rodriguez, 1974). Recently, we characterized six strains of *Leishmania* isolates in Ecuador, using isoenzyme electrophoresis and monoclonal antibodies. Three strains, isolated from cutaneous lesions of humans, were identified as *L. b. panamensis,* while the remainings from wild animals were identified as *L. mexicana amazonensis* (Mimori *et al.*, 1989). Thus, the species of *Leishmania* most commonly found in our study areas endemic for leishmaniasis in humans was considered to

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be *L. braziliensis* complex.

Little information, on the other hand, has been available on epidemiological features of the disease in this country (Hashiguchi *et al.*, 1984). Such insufficient epidemiological information has partly been due to the lack of reliable diagnostic tool in field studies. Recently, it has been reported that a soluble leishmanial extract prepared from *L. donovani chagasi* promastigotes was highly sensitive and specific for an intradermal skin test for American visceral leishmaniasis patients (Reed *et al.*, 1986). In order to obtain a better understanding of the epidemiology of leishmaniasis in Ecuador, we therefore designed a preliminary survey to evaluate a similarly prepared skin testing antigen.

The present paper deals with the finding that the soluble antigen prepared from *L. braziliensis* promastigotes can be readily employed in skin testing for the screening of cutaneous and mucocutaneous leishmaniasis in areas of Ecuador endemic for these diseases.

Materials and Methods

Study areas and subjects

The present examinations were carried out during the dry season from July to August, 1986 in Ecuador. A preliminary study using skin test (ST) and enzyme-linked immunosorbent assay (ELISA) was made on 63 subjects with active dermal lesions who visited the Instituto Nacional de Higiene y Medicina Tropical (INHMT), Guayaquil, and rural health centers and hospitals in several endemic areas of Ecuador. An epidemiological survey was conducted on 115 inhabitants in Selva Alegre, Esmeraldas, Ecuador by performing ST and ELISA. Thirty four school children in Gramalote Chico, Los Rios, were also examined by ST alone. Thus, a total of 212 subjects were tested by leishmanial ST and/or ELISA in this study.

ELISA

L. b. braziliensis (MHOM/BR/75/M 2904) obtained from Dr. P. Desjeux, PDP, WHO (formerly Instituto Boliviano de Biologia de Altura, Bolivia) was cultured with Pan's medium (Pan, 1984). Soluble extract prepared from the harvested promastigotes was used as an antigen. The ELISA procedure was performed according to the method described by Mimori *et al.* (1987). All the serum samples tested were diluted 1:10. Absorbance values of more than OD 0.25 at 500 nm were employed as criteria for evaluating positive serum, since the absorbances in tested control sera from 66 healthy individuals in Ecuador were less than OD 0.25.

Intradermal skin test

A soluble antigen used for skin testing in this study was prepared by the method of Reed *et al.* (1986). The promastigotes of *L. braziliensis* were harvested and washed five times with a balanced salt solution. After the final washing the parasite pellet was resuspended in 5 volumes of distiled water, and a freeze-thawing procedure with aceton-dry ice and tepid water was repeated 10 times. The disruped parasites were diluted in PBS and centrifuged at $10,000 \times g$ for 30 min at 4°C. The supernatant was adjusted to 250 μg protein concentration per ml before filtration with a 0.45 μ sterile filter. One-tenth ml of the antigen solution was injected intradermally in the flexor surface of the forearm. Induration size of more than 5 mm at the site 48 hours after the injection was considered to be a positive reaction based on the criteria employed by Reed *et al.* (1986).

Biopsy smear specimens

Smear samples were taken from the margin of ulcerated lesions, stained with Giemsa or Wright solutions, and examined with an oil immersion objective. At least 100 fields were examined.

RESULTS

In our initial study, an evaluation of ST and ELISA was made on 63 subjects with active dermal lesions who visited INHMT, rural health centers and hospitals in Ecuador (Table 1). In this case 45.5% were positive for leishmanial amastigotes in smear specimens. The ST and ELISA positive rates were significantly high in the parasite positive group compared with the parasite negative one ($P<0.1$ in ST, $P<0.05$ in ELISA, and $P<0.05$ in the both tests). However, a relatively high positive rate for ST and ELISA was observed in the parasite negatives. This high rate suggests that a considerable number of true leishmanial patients may be included in those parasite negatives. No significant difference in average induration size between parasite positives (17.4 ± 9.0) and negatives (13.6 ± 5.7) was observed. These data indicate that the ST and ELISA are very suitable tools in the screening of Ecuadorian leishmaniasis.

Using these diagnostic tools, an epidemiological survey was conducted in Selva Alegre, Esmeraldas (Table 2). Of 115 subjects examined dermatologically for leishmanial ulcers, nodules and scars, 10 active and 24 of 28 healed cases were discovered from the physical examination and interview prior to the skin testing. The remaining 4 of the healed cases were detected by re-examination after these had shown ST-positive results. All the leishmanial scars in such cases were observed on the usually unexposed areas of the body. We went through the same experience in another endemic area, Gramalote Chico, Los Rios. In the survey, typical leishmanial scars were confirmed in 6 out of 8 ST-positives among 34 examinees after skin testing.

In Selva Alegre, a parasitological examination was performed on 7 out of 10 subjects with active dermal lesions. Only one patient was positive for leishmanial amastigotes, and he also showed positive responses to ST and ELISA. The ST and ELISA positive rates among these subjects were 80.0% (8/10) and 66.7% (6/9), respectively. In the subjects with healed dermal lesions, the positive ST and ELISA rates were 79.2% (19/24) and 71.4%

Table 1 Results of a preliminary evaluation of intradermal skin test and ELISA in 63 subjects with active cutaneous (leishmanial) lesion in Ecuador

Smear specimens*	No. examined		Positive rate per examinees†		
	female (age)	male (age)	skin test	ELISA	skin test & ELISA
+	14 (2-66)	16 (1-66)	93.3(14/15)	92.3(24/26)	90.9(10/11)
−	18 (1-66)	15 (7-61)	73.7(14/19)	75.7(22/29)	56.3 (9/16)
Total	32	31	82.4(28/34)	83.6(46/55)	70.4(19/27)

*Materials from ulcer lesions were fixed, stained and then examined microscopically.

† Examinees were not the same number in each test, mainly because of follow-up difficulties.

Table 2 Correlation between clinical diagnosis and immunodiagnosis in 115 inhabitants in Selva Alegre, Esmeraldas, Ecuador, using skin test and ELISA

Reaction to		No. with signs		No. without signs	Total
skin test	ELISA	active*	healed†		
+	+	5	15		20
+	−	2	4	2	8
−	+	1	1	6	8
−	−	1	4	64	69
(sub-total)		9	24	72	105
+	ND‡	1			1
ND	+		4		4
−	ND			4	4
ND	−			1	1
(sub-total)		1	4	5	10
Total		10	28	77	115

*Persons with leishmanial ulcers.
†Persons with leishmanial scars.
‡Not done.

Table 3 Summary of the results of clinical, parasitological and immunological examinations in 212 subjects in Ecuador

Reaction to		No. with signs					
		active*			healed†	No. without signs	Total
skin test	ELISA	smear specimens‡					
		+	−	ND§			
+	+	11	12	1	15		39
+	−		4		4	2	10
−	+	1	5	1		6	13
−	−			1	4	64	69
(sub-total)		12	21	3	23	72	131
+	ND§	4	4		6	2	16
ND	+	13	8		6		27
−	ND					30	30
ND	−	2	5			1	8
(sub-total)		19	17		12	33	81
Total		31	38	3	35	105	212

*Persons with leishmanial ulcers.
†Persons with leishmanial scars.
‡Materials from ulcer lesions were fixed, stained and then examined microscopically.
§Not done.

Table 4 Frequency distribution of induration size in leishmanial skin test positive subjects with active or healed cutaneous (leishmanial) lesions

Induration size (mm)	No. positive subjects	Active lesions		Healed lesions	
		No.	%	No.	%
5-10	11	9	25.0	2	8.0
11-15	10	10	27.8		
16-20	18	7	19.4	11	44.0
21-25	16	6	16.7	10	40.0
26-30	2	2	5.6		
≧ 31	4	2	5.6	2	8.0

(20/28), respectively. Twenty subjects who showed positive reactions for both tests had either active or healed lesions. It was found that five subjects with those signs were negative for both the ST and ELISA, while eight without active or healed lesions were positive for one of the two tests. Coincidence rates of ST and ELISA among 105 subjects, 33 with and 72 without the signs, who received both tests in Selva Alegre, were as follows: both positive, 19.0%; both negative, 65.7%; and positive against one of the two, 7.5%.

Table 3 summarizes the results of clinical, parasitological and immunological examinations of 212 examinees in the current study. Coincidence rates between both tests among 59 subjects who had clinical signs and received the two tests were as follows: positive and negative for the two tests, 66.1% and 8.5%, respectively; positive against one of the two, 13.5% in ST and 11.9% in ELISA. Furthermore, 64 (88.9%) out of 72 subjects who had no clinical signs and received the two tests did not react against these tests, completely. Thus, a close agreement between dermal clinical signs and immunodiagnosis was demonstrated with a few exceptions.

Frequency distribution of the ST induration size in positive subjects with active or healed dermal lesions is summarized in Table 4. Average induration size was 15.7 ± 7.7 in active, and 20.0 ± 5.4 in healed cases. The size was significantly different between both cases ($P < 0.05$), showing a strong ST reaction in the healed one. No significant difference was found in the induration size between the patients with active and healed cutaneous lesions, when considered with regard to either sex or age.

Discussion

The present study was carried out to evaluate two immunodiagnostic tools, ST and ELISA, in the screening of cutaneous leishmaniasis in the endemic areas of Ecuador. In the epidemiological survey in endemic areas, a presumptive diagnosis is often made on the basis of clinical diagnosis and Montenegro skin test (Mayrink *et al.*, 1979; Werner and Barreto, 1981). A definitive diagnosis of leishmaniasis requires demonstration of the etiologic agent from the lesions. However, the visualization of protozoa in tissue samples and the isolation of parasites from active lesions by culture methods were fraught with difficulties. Weigle *et al.* (1987) recommended two diagnostic procedures based on their studies, i.e., dermal scraping smears for immediate diagnosis and culture of aspirates for a definitive para-

sitological diagnosis of cutaneous lesions. In the present survey, parasites were observed in 31 (44.4%) of 69 subjects presenting active lesions (Table 3).

As shown in Table 3, ST-positive rate was 81.8% (36/44) of the total skin tested examinees with active lesions. In the parasite positive cases, furthermore, all subjects but one proved to be positive for the ST. On the other hand, the positive rate in subjects without clinial signs was only 3.8% (4/104). It is well recognized that the immunological methods, especially ST and ELISA, are useful tools for diagnosis of New World cutaneous leishmaniasis (Bray, 1980). The reliability of these tests, however, has been hampered by the problem of cross reactivity with various species within the family, Trypanosomatidae, as well as with other microorganisms such as mycobacteria (Mauel and Behin, 1982). We have no data with regard to cross reactivity of the present *L. braziliensis* antigen preparation. Reed *et al.* (1986), however, reported that their similarly prepared *L. d. chagasi*-soluble extract produced no positive responses in either normal controls, tuberculosis patients, or schistosomiasis patients, and less than 5% positive responses in persons with Chagas' disease. From this information and our results, it is assumed that the present preparation also shows a lesser degree of cross reactivity.

Table 3 suggests that there may be close agreement between clinical signs and results of immunodiagnosis, except for 12 subjects in Selva Alegre and 2 in Glamalote Chico. Four subjects in Selva Alegre, showing negative reactions against both tests, may have had other dermatological lesions mistaken for leishmanial scars. The remaining cases suggest the possibility of either cross-reactions with other infectious agents or oversight of leishmanial lesions.

Diagnosis using ST and ELISA is commonly used against New World cutaneous leishmaniasis. However, the standardization of skin test antigen has not yet been done sufficiently to have a satisfactory results. In this paper, we evaluated the soluble extract obtained by the method of Reed *et al.* (1986) from ruptured *L. braziliensis* promastigotes, as a ST antigen. Our results obtained showed that the extract was found to be highly sensitive and specific against Ecuadorian cutaneous and mucocutaneous leishmaniasis patients. It was concluded that the use of the present ST, together with ELISA, would be useful in diagnosis for patients with active and healed leishmanial lesions in the endemic areas of the disease.

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南米エクアドル共和国リーシュマニア症の皮内反応と ELISA 法による疫学調査

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V.V. Coronel・川端　真人・橋口　義久

南米エクアドル共和国における，皮膚型および皮膚粘膜型リーシュマニア症の疫学調査での皮内反応（ST），および ELISA 法の有用性について検討した結果を報告する。

皮内反応抗原は，*Leishmania braziliensis* promastigote 型原虫から作成した。潰瘍病変（leishmanial ulcers）保有者72名における ST と ELISA の陽性率は，それぞれ81.8%（36/44）および81.3%（52/64）であった。これらの患者の内，原虫が認められた者は検査が実施できた69名中31名（44.9%）であった。原虫が証明された患者において，ST で1名，ELISA で2名が陰性であった以外は，全ての者が両検査に陽性であった。治癒病変（leishmanial scars）保有者35名における ST と ELISA の陽性率は，それぞれ86.2%（25/29）と72.4%（21/29）であった。一方，病変が認められない者における ST と ELISA の陽性率は，3.8%（ST）および8.2%（ELISA）と非常に低率であった。

同国 Esmeraldas 地方の Selva Alegre 村で，リーシュマニア症の罹患状況を調べた。その結果，115名中38名に何らかの病変（潰瘍病変10名・治癒病変28名）が認められ，その内の33名（86.8%）が ST と ELISA 両検査法に陽性であった。

以上の結果から，同国リーシュマニア症流行地域での疫学調査に，今回の両検査法が有効であることが示された。

アマゾン流域日系移住地，トメアスー地区における回虫およびトキソプラズマ感染状況についての考察

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はじめに

日本国内における寄生虫感染症は，戦後の混乱期にあたる1945年から1950年にかけて急激に増加したが，1950年を境に急速に減少の傾向を示した（Kobayashi, 1983)。厚生省および日本寄生虫予防会の報告による都道府県別寄生虫感染状況によれば，最近の蠕虫類感染者は蟯虫を除くと0.5%にも満たない（予防医学ジャーナル，1980—1989)。しかし，国外，特に衛生状態などに問題の多い発展途上国では，蠕虫感染者はかなりの数に昇っている。一方原虫類感染については，我が国では全国的な調査は行われておらず，したがってその感染の実態については必ずしも明らかではないが，トキソプラズマについては日本においても感染者が多数報告されている（中山，1968；伊勢ら，1979；白坂ら，1972)。我々はこのたびブラジル，アマゾンのトメアスー地区（図1）で，マラリアに関する疫学調査を行う機会を得たが，その際に，住民における抗回虫抗体の高い保有率が見いだされた。さらに，その地域における比較的濃厚なネコの棲息密度から，同地域には高率にトキソプラズマ感染者が存在することも予想された。そこでこれら住民におけるトキソプラズマの特異抗体保有状況について調査するとともに，比較のため日本国内における一般人の抗回虫抗体および抗トキソプラズマ抗体保有の実状をも調査したので，それらの結果を併せて報告する。調査は何れも，免疫学的手法による抗体検査によって行った。

方　法

調査地域

調査地トメアスーは258 km^{2}の開拓地であり，州都ベレン市の南，直線距離にして125 kmに位置する(図1)。1962年より入植が開始され，熱帯雨林地帯の気候の中，現在70家族350人余りの日系人たちが，胡椒，カカオ，コーヒーの栽培や，畜産により生活を営んでいる。今般の調査対象となったトメアスーの住民のうち，日系人は102名，現地労働者は119名であった。

被検血清

被検血清は1988年8月にブラジル，アマゾンのトメアスー地区において5歳から85歳までの男女から採取した。分離した血清にはNaN_3を添加して低温で空輸し，−20°C下に保存した。また比較対象とした日本人の血清としては，群馬大学医学部付属病院の供血者，17歳から59歳までの健康人男女から交差試験用に採血し，低温に保存した血清を使用した。

回虫抗原特異 IgE 抗体の測定

回虫抗原としてヒト回虫と抗原性がほとんど同じといわれている豚回虫 *Ascaris suum* を前橋屠場より入手し，虫体をリン酸緩衝液（pH 7.2）中

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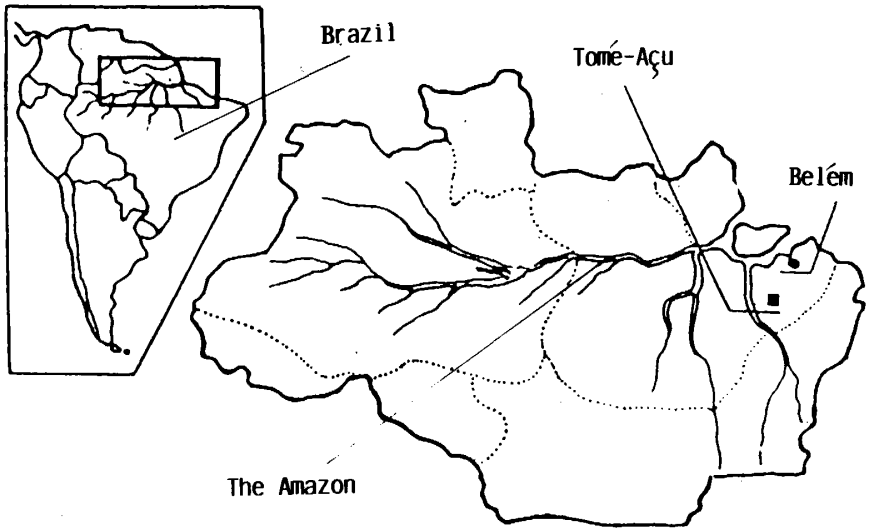

Figure 1 Tomé-Açu is a Japanese settlement covering 10 by 25.8 km space, located 125 km to the south of Belém (latitude 2°31′ S, longitude 48° 22′ W). The temperature of Tomé-Açu is 21-33°C and the rain fall in a year amounts 2,635 mm on the average. The place makes highly vegetated flat area at 11-30 m above sea level and with natural water networks by Amazon branches.

でヒスコトロンを用いて磨砕し，その遠心上清を蛋白量25 μg/ml に希釈して使用した。特異 IgE 抗体の測定は，佐藤ら（1986）がすでに報告した fluorescence enzyme immunoassay（fluorescence-EIA）法に準じて行った。特異 IgE 抗体量は，Micro FLUOR reader の示す relative fluorescence unit（RFU）をそのまま使用した。抗原蛋白量の測定は，フェノール試薬を用いた Folin 法の Lowry らの改良法によって行い，標準蛋白として牛血清アルブミンを使用した（Lowry *et al.*, 1951）。

トキソプラズマ特異 IgG 抗体の測定

トキソプラズマ特異抗体の測定は，平井ら（1977），坪田ら（1977）の報告に従ってトキソテスト-MT（栄研化学 KK）を用いたラテックス凝集反応のマイクロタイター法によって行った。判定はトキソテスト-MT の判定基準に従って抗体価で表し，32倍陽性以上を陽性者と判定した。

結　果

回虫抗原特異 IgE 抗体保有状況

図2にトメアスー地区の人を対象として調査した，回虫抗原特異 IgE 抗体保有状況のヒストグラムを示した。被検者221名の，特異 IgE 抗体 RFU 値は，10-924まで幅広く分布していた。ここで，ヒストグラムより，通常我々が本法に適用している陽性判定算定基準（林，玄番，1986）に従って，RFU 値≧73.5を示す例を陽性者と決定した。その結果，抗回虫 IgE 抗体陽性者は221名中99名で，抗体陽性率は44.8%であった。

次に，群馬大学医学部付属病院供血者血清の，抗回虫抗体保有状況について図3にまとめた。被検者182名の特異 IgE 抗体 RFU 値は，11-123までに分布しており，上記の陽性判定基準に従えば，抗体陽性者は182名中4名であり，抗体陽性率は2.2%であった。

トキソプラズマ特異 IgG 抗体の保有状況

トメアスー地区の人を対象として，ラテックス凝集反応により検査した抗トキソプラズマ抗体の抗体価と，その出現頻度を図4に示した。また，

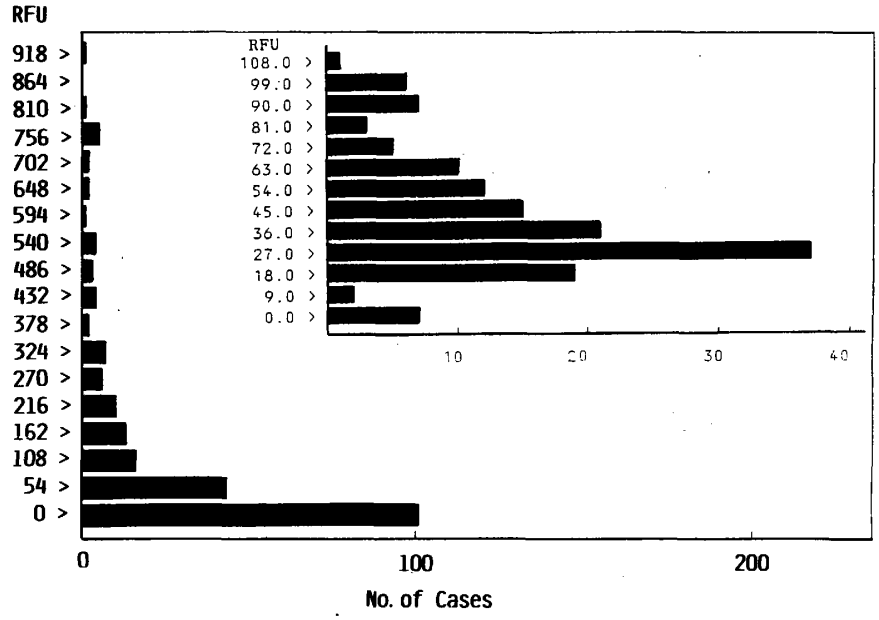

Figure 2 Histogram of the frequencies of *Ascaris*-antigen-specific-IgE antibodies (RFU values) in Tomé-Açu inhabitants.

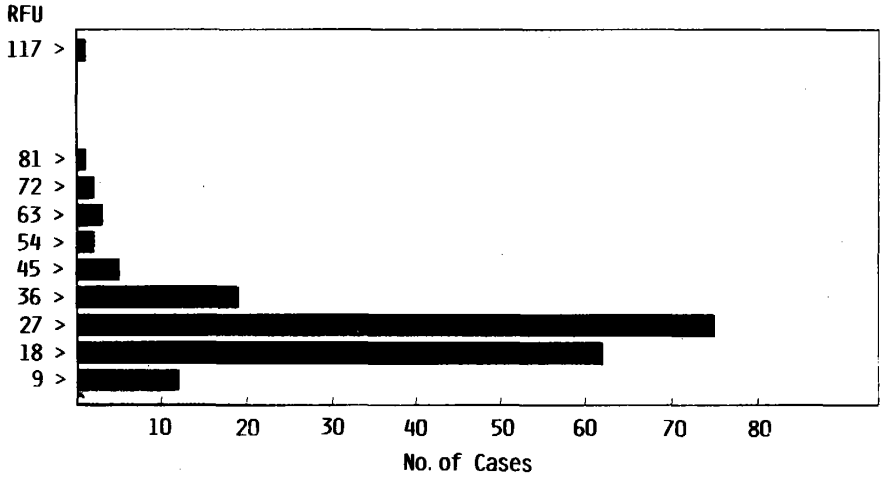

Figure 3 Frequencies of the *Ascaris*-antigen-specific-IgE antibodies (RFU values) of Japanese donors of Gunma University Hospital.

群馬大学医学部付属病院供血者400名についての結果もあわせて示した。トメアスー地区住民の被検者218名中抗体陽性者は164名で，抗体陽性率は75.2%であった。男女別にみると，男は118名中90名陽性であり陽性率は76.3%，女は100名中74名陽性であり陽性率は74%とほぼ等しい値を示した。これに比較して，群馬大学医学部付属病院供血者400名中の抗体陽性者は65名で，陽性率は16.3%であった。次にトメアスー地区住民の年齢別の抗体価を検討した結果を表1に，年齢別の抗体陽性率を表3に示した。また，群馬大学供血者の年齢別抗体価の分布は表2に，年齢別の抗体陽性率を表3にまとめた。この結果から，トメアスー地区のトキソプラズマ感染者はどの年齢層にも広がっ

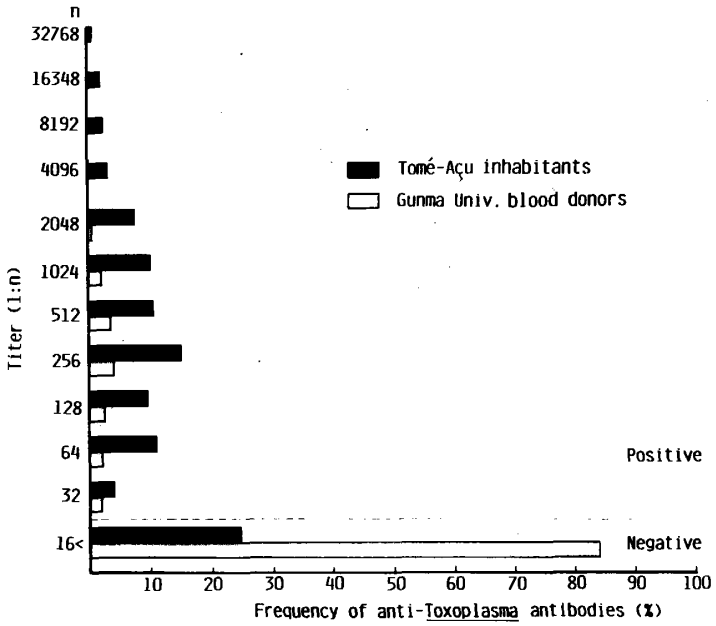

Figure 4 Comparative frequencies of anti-*Toxoplasma* antibodies between Tomé-Açu inhabitants and blood donors in Gunma University Hospital.

Table 1 High anti-*Toxoplasma* antibody titers in age groups of Tomé-Açu inhabitants

Age group	Titer (No. of cases)											
	<16	32	64	128	256	512	1,024	2,048	4,096	8,192	16,384	32,768
<10	1*			1‡	1†							1‡
10-19	22	2	6	5	6	3	4	4	4	2	2	
20-29	14		7	4	9	7	5	4		2		
30-39	8	1	4	3	7	2	3	3	2		1	
40-49	4	1	3	2	8	6	6	3		1		
50-59	3			3	1	2	2	1			1	
60-69	1	2	4	3	1	3	1	1	1			
70≦	1	2					1					

*2 years old †8 years old ‡9 years old

Table 2 Distribution of anti-*Toxoplasma* antibody titers in age groups of blood donors in Gunma University Hospital

Age group	Titer (No. of cases)							
	<16	32	64	128	256	512	1,024	2,048
10-19	32							
20-29	133	1	2	1	4	1	2	1
30-39	110	4	4	4	8	5	4	
40-49	55	2	3	5	4	7	1	
50-59	5					1	1	

Table 3 Comparison of positive rates of anti-*Toxoplasma* antibody titers in each group

Age group	Positive rate, % (No. of examined)	
	Tomé-Açu inhabitants	Gunma Univ. blood donors
<10	75.0 (4)	ND
10-19	63.3 (60)	0 (32)
20-29	73.1 (52)	8.3 (145)
30-39	76.5 (34)	20.9 (139)
40-49	88.2 (34)	28.6 (77)
50-59	76.9 (13)	28.6 (7)
60-69	94.1 (17)	ND
70≦	75.0 (4)	ND

ND : Not done

ており，5歳-10歳に75%の陽性率，10歳代でも63.3%の抗体陽性率を示した。また，抗体価は32-32,764倍陽性まで分布していたが，高い抗体価を示す人が若年から老年にわたってほぼ同じ比率みられた。一方，群馬大学供血者では，10歳代の被検者32名中に抗体陽性者はみられず，20歳代以上ではどの年齢層でも32-1,024倍の抗体価を示し，抗体陽性率は年齢が高くなるにつれて上昇した。

ネコの飼育状況

ペットとしてネコを飼っているかどうかを，個人別に採血時にアンケート調査した。解答のあった278名中，211名（77.6%）がネコを飼っている家庭に居住していた。

食肉嗜好

ブタ肉と牛肉とどちらを食べるか，さらに，生焼けを好むかどうかの質問を採血時に行った。牛肉の生焼けを好む人は10%にすぎず，ブタ肉を生焼けで摂食する人はいなかった。

考　察

日本国内における回虫感染率は，多くの府県で今や0.5%にも満たない感染状況であるが，アジア，アフリカ諸国における寄生虫検査成績を見ると，回虫感染者は多数である（建野ら，1984；横川ら，1983)。

熱帯地調査においては，採取材料の種目を限らざるを得ない制約があるため，回虫感染調査を行う場合でも，検便というオーソドックスな方法はしばしば困難である。従って，マラリア調査などに伴い採取される血清を使って，免疫学的方法により感染率を算出することが時として現実的となる。免疫学的方法の中でも，本報告において用いた特異的 IgE 抗体を測定する手法は，感染との相関がかなり高いものと想定される。これまでに述べた日本国内の感染状況から考えると，群馬大学病院の検体での陽性者が182名中4名であることは，周辺環境が農村であることから十分にうなずける数字であり，このことから考えてトメアスー地区での抗体陽性率が43.4%という事実は，この地区の回虫感染者が相当数に上ることを意味するものと考えられる。いうまでもなく，本法によった場合，他の線虫類内の交差反応による偽陽性反応を含むことを，考慮しなければならない。また，特異的 IgE 抗体は，虫体排出後も長期間陽性を維持することをも計算にいれなければならない。

対象となったトメアスー地域の日系人は，幼児を除いてそのほとんどが戦後移住した一世農民であり，食習慣等をはじめとする一般生活形態は，本邦の日本人に類似している。しかし，上下水道設備や汚水，し尿処理等，生活に基本的な衛生条件が現在の日本とは依然として大きくかけ離れており，回虫の陽性者が高いことはその反映と思われる。戦後の日本においては，駆虫薬を集団投与することにより，環境衛生状態の改善が一般化する以前に，回虫，鉤虫等の駆逐に成功している

(Kobayashi, 1983)。トメアスーという比較的閉鎖した地区では，戦後日本の回虫・鉤虫駆除計画を応用して，一定の方針のもとに集団駆虫を繰返すことが効果的と考えられる。

日本におけるトキソプラズマ感染状況については，これまでにも多数の報告がなされ，感染者数は成人のおよそ1/3に見られると成書に記されている(吉田，1987)。また，年齢の値%，即ち20歳代ならば20%の感染があるともいわれてきた。最近，平井ら (1989) は東京における感染状況を調査し，一般集団のトキソプラズマ抗体保有率は25.7%であり，年齢の増加に従って保有率も上昇したと報告した。また，妊娠中の女性の抗体保有率は1982年-1986年までほとんど変らず，平均15.9%の保有率であったという。我々も1987年に群馬大学医療短大学生と群馬大学病院供血者について調査したところ，学生の抗体保有率はおよそ4.0%であり，20歳代から50歳代にわたる病院供血者では20.8%であることを見出している (佐藤，小河原，1987)。今回の調査結果でも，若年層におけるトキソプラズマ感染率は低く，高齢者になるに従って感染率が上昇する傾向が確認された。一方，国外でのトキソプラズマ感染状況についても多数の報告が見られ (Arene, 1986; Shenone *et al.*, 1982-1986)，地域を問わず全世界的規模で多数の感染者が存在しているようであり，日本国内より高感染率を示す地方も多い。ブラジルにおける Lamb, Feldman (1968) の報告によると，抗トキソプラズマ抗体陽性率は，全土で52%，アマゾン地域で56%である。今回の我々の調査では，トメアスー地区住民の抗体陽性率はおよそ70%に達し，また若年者から老年者にかけてどの年齢層の感染率も等しく高く，定量的にみた場合，抗体価が日本国の感染者の平均を大きく上回っている事実が証明された。特に，10歳以下の小児4人中3人までが陽性例であった点が注目される。陽性例は8歳児2名，9歳児1名の内訳となるので，母体から胎盤を経て移入された抗体が残存した結果とは考えられず，いずれも後天性感染の結果と判断される。トメアスーの小児の間での感染率については，今後改めて調査を行う必要がある。

トキソプラズマ感染が高率に見られることは，特別の要因を考えなければならない。本調査での問診により，当地の77.6%の人がネコを飼っている事実が判明している。しかし，ほとんどの人は生肉を食べる習慣は無い。小林ら (1976) による養豚地帯における調査によれば，ブタのトキソプラズマ抗体陽性率の高い地域においては，ヒトもまた高い陽性率を示し，両者がよく相関する事実が見出されている。これは恐らく，ブタもヒトもネコから排出されるオーシストによる感染を受ける結果であろうと推測される。即ち，ヒトもブタも高い陽性率を示す地域においては，ネコが高率にトキソプラズマに感染し，オーシストを排出する例が多いことが想定される。トメアスーにおいても，ネコのトキソプラズマ感染率，オーシスト排出率などについて調査を進め，対策をはかる必要がある。

ほぼ同一の生活習慣を営む同一民族が，移民などによる異なった環境におかれた時，ある特定の寄生虫病に対して著しく異なった感染率を示すことがあり得る。このような場合には，寄生虫感染の背後にある生態系とのかねあいを研究する最もよい機会となるだろう。トメアスーにおけるトキソプラズマ感染例は，本邦の日本人に比較して異常に多い事実が証明された。トメアスーは上記の事例の1つとして，トキソプラズマ感染の生態をさらに解明する上に，有益な野外研究の場を提供することと考える。

ま と め

ブラジル，アマゾン流域の日系移住地トメアスー地区住民について，回虫特異 IgE 抗体およびトキソプラズマ特異 IgG 抗体保有状況を，免疫学的手法により調査した。比較対象として，群馬大学付属病院健康人供血者血清について，同時に検査した。トメアスー地区の抗回虫抗原特異 IgE 抗体陽性者は，被検者221名中99名で，陽性率は44.8%であった。これに比較して行った群馬大学供血者の結果は，被検者182名中抗体陽性者4名で，陽性率は2.2%であった。トメアスー居住者の抗体陽性率が高いのは，その地域の上下水道設備や汚水，し尿処理等の衛生条件が一因となってい

ると考えられた。一方トキソプラズマ感染者についての調査は，トメアスー地区住民218名中，抗体陽性者は164名で，陽性率は75.2%，群馬大学供血者400名中抗体陽性者は65名，陽性率は16.3%であった。トメアスー地区住民陽性者の抗体価は，若年者から高齢者まで異常に高い値を示した。問診の結果，77.6%の人が家庭においてネコを飼っていることが判明した。しかし肉を生，または生に近い状態で摂食する習慣は認められなかったため，ネコが高い感染率の原因であることが推定された。

謝　辞

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HIGH ANTIBODY POSITIVE RATES OF ASCARIASIS AND TOXOPLASMOSIS IN AN AMAZONIAN JAPANESE SETTLEMENT

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Seroepidemiological studies on ascariasis and toxoplasmosis were conducted in a Brazilian Japanese settlement, Tomé-Açu, in August, 1988. The specific IgE antibodies against *Ascaris* antigen were assayed and 99 individuals out of 221 inhabitants in the area were found positive (44.8%), while only 4 out of 182 blood donors in Gunma University Hospital, Japan, were positive (2.2%). The poor general sanitary conditions such as water supply or sewerage system in Tomé-Açu may be the cause of the high positive rate of anti-ascaris antibody. The specific IgG antibodies against *Toxoplasma* were assayed, 164 out of 218 were positive in Tomé-Açu (75.2%), while 65 out of 400 were positive in Gunma University Hospital (16.3%). The results of the questionnaire showed that 77.6% of the inhabitants in Tomé-Açu kept cats in their houses. Very few of them answered the custom of taking under-cooked meat. The role of cats was assumed to be attributable to the high prevalence rate of *Toxoplasma* infection.

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症例報告

ヒトクイバエ *Cordylobia anthropophaga* による ハエ症の輸入例

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人体ハエ症は我が国でも数多くの症例が報告されているが，その殆どは偶然の寄生によるハエ症である。ところが熱帯地域では，ある種のハエ幼虫がその発育の為に宿主を必要とするものがあり，まれに人もその宿主にされるため，その際に病状の発現を見ることがある。その様なハエ幼虫感染は，その土地での生活環境に無知な旅行者に対して起こっても決して不思議ではなく，Kagei *et al.* (1974) が，ブラジルで感染し，日本で発見されたヒトヒフバエ (*Dermatobia hominis*) の患者を報告してから後，同種のハエによる感染例が藤原ら (1977)，大滝ら (1978) によって中南米からの帰国者から報告され，更に堀ら (1984) により3人のアフリカからの帰国者から *Cordylobia* 属ハエ幼虫寄生例が発見され報告されている（1人はロダインコブバエ *C. rodhaini* の寄生例，他の2名はヒトクイバエ *C. anthropophaga* の寄生例である)。

本報告は，同じくアフリカから帰国した人でのヒトクイバエによる，ハエ症の患者の追加症例報告である。

症　例

25歳　男子　ミュージシャン

1989年1月14日から国際交流基金による「ジャズおよび和太鼓」の公演の為，アフリカのセネガルを皮切りにナイジェリア，ザイール，ケニア，タンザニア，ザンビアを訪問，同年2月20日帰国した。

旅行の途中，何回か睡眠中に，チクッとした虫刺され感を経験したという。その後右腹側に5×5cm大の発赤を伴った腫張が見られ，中央部は潰瘍を形成した。

帰国翌日の2月21日午前9時頃，本人自身の指圧により膿汁排出を試みたところ，無色の液と共に，体長1.5cmの虫体が飛び出してきたので，本人自ら東京都立衛生研究所に持参した。

寄生虫学的検索

持参時の摘出虫体はまだ生きており，写真1に示す様に，体長1.5cm，円柱状で体節は12節に分かれ，2本の鉤型の口部硬皮 (labial sclerites) が突出し，それぞれの側に6本の小さな歯を持ったSclerotized integmentがある。各体節には後方に向かって曲がる棘が密生し，後端には気門が見られる。以上の形態から，本虫はヒトクイバエ *Cordylobia anthropophaga* (Blanchard, 1893) の3令幼虫と同定された。

なお，本幼虫のさなぎ化，更に羽化させての成虫での同定を考えて，しばらくその飼育を試みたが，途中で幼虫のまま死亡した。

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Photo. 1 Dorsal view of third larval stage of *Cordylobia anthropophaga* expelled from the lesion of right abdomen of a patient.

考　察

ヒトクイバエはサハラ砂漠以南の西アフリカ一帯に広く分布し，その宿主としてチンパンジーやサル，ヒョウ等極めて多くの野生動物が報告されているが（Zumpt, 1965 は16種の野生動物と数種の家畜を挙げている），主たる保虫宿主はネズミ類といわれる。人体も宿主になりうる事が過去より知られているが，その土地の者でない旅行者は，本症の存在を知らない。従ってアフリカで感染して，帰国後自国で発見されても不思議ではない。その様な症例は現在迄に，我が国では堀ら（1984）によってアフリカのコートジボワールのオフィス街マンションに居住していて感染し，虫体が自然脱出した1例と，母親の手で押し出して虫体を摘出したという症例の2例が報告されている。その他，人体症例ではないが，ヒトクイバエの輸入例は加納（1967）により，東アフリカより千葉県血清研究所に実験動物として搬入されたミドリザルからも報告されており，恐らく今後も本虫の我が国における人，および動物での輸入例が見出されるであろうことは十分示唆される。

本虫輸入に伴っての問題点は，人による輸入時の確実な診断と治療についてである。幸いにも，堀ら（1984）の症例も今回の著者らの例も，幼虫自身が自然に脱出したり，患者自身が虫体を押し出したりして，大きな症状の出現までには至らなかったが，先に Kagei *et al.*（1974）が報告したヒトヒフバエ寄生例に見られた様に，確実な診断のつかないまま，良性の肉芽腫疑いで大きく組織を切除されることもあるので，臨床医は本虫に対する十分な知識を必要とする。

一方，人の感染例ではさして問題はないと考えられるが，感染動物の輸入例では，3令幼虫が輸入動物から脱落，さなぎ化，羽化，産卵して，再び人を始めとする動物へ寄生する可能性がある事は否定できないし，あるいは我が国に土着するかも知れないという問題点を持っている。

ヒトクイバエの成虫は，動物の排泄物で汚れた乾いた砂や，着物の上に産卵し，そこで孵化した幼虫が人を含む動物を襲い，皮下に侵入するので，本症例もアフリカ公演中に幼虫との接触があったものと考えられる。そのことは日時は定かではないが，旅行中に何回か睡眠中に虫刺されを経験したと本人も述べている事も，これを裏付けている。摘出時の虫体が，既に3令幼虫まで発育していた事は，幼虫侵入後5－6日で3令に達し，8日目位で脱落する事を考えると（Zumpt, 1965），本公演旅行の日程のかなり後の方の行程で感染した事が窺えるが，本虫の分布域と行動の上からは若干のずれが見られ，もう少し早かったのかも知れない。

ま と め

1989年1月から1カ月以上にわたるアフリカでの音楽公演旅行を終えて帰国した患者が，帰国直後に，右腹側部に形成された潰瘍部を，患者自身の手指によって圧迫したところ，1匹の虫体が脱出するのを見た。その虫体についての寄生虫学的検索を行った結果，ヒトクイバエ *Cordylobia anthropophaga* (Blanchard, 1893) の3令幼虫である事が解り，我が国における本虫寄生輸入症例の第3例として報告した。

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AN IMPORTED CASE OF MYIASIS CAUSED BY A LARVA OF *CORDYLOBIA ANTHROPOPHAGA* FROM AFRICA

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A 25 year-old Japanese man (musician) had been in Middle-Africa for the concert-tour from 14 January to 20 February 1989. On 21 February after homecoming to Japan, a lesion with a severe pain was found in the right abdomen. He expelled a white larval worm (1.5 cm in length) from this lesion by pressure of his fingers. This larval worm taken out from the lesion was identified as the third-stage larva of *Cordylobia anthropophaga* (Blanchard, 1893), by their pair of toothed, spade-like oral hooklets and by several pairs of posteriorly directed fleshy processes arising from the twelve abdominal segments, as well as by the character of the posterior spiracles, which lack a distinct chitinous rim.

The disease caused by the larva of *C. anthropophaga* is a familiar form of myiasis in Africa. This fly lays its eggs on dry sand polluted with the excrement of animals, or clothing. The hatched larva invade the skin of human and animals. This patient may be contacted with a larva of this fly in Africa.

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