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IS THE MALE *AEDES ALBOPICTUS* ATTRACTED TO THE BLOOD SOURCE?

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Abstract: The responses of male *Aedes albopictus* to a bait mouse were examined under laboratory conditions using the cage-test method. In most of the tests fewer males were recovered in the bait cage than the control cage. There was no evidence that male mosquitoes were fond of the mouse or attracted to the animal.

During daytime mosquito collections we often experience male *Aedes* (*Stegomyia*) mosquitoes landing on the human body. This phenomenon has been noted by several researchers in different locations. During their human bait collection in Bangkok, Yasuno and Tonn (1970) observed that as many *Ae. aegypti* males as females are attracted to the human bait. Following this, Hartberg (1971) in Tanzania, and Gubler and Bhattacharya (1972) in Calcutta also reported, respectively, on the responses of male *Ae. aegypti* and *Ae. albopictus* mosquitoes to the human body. Does the male really perceive a blood source and approach the body?

The cage-test method is useful in examining the behavioral responses of blood sucking flies in the laboratory (Kurihara *et al.*, 1991). With some modification of this method, male behaviour, particularly in its response to a blood source was studied.

MATERIALS AND METHOD

Three wire cages were used (30×30×30 cm), each with two round holes 15 cm in diameter on opposite walls, and a piece of 6 mm mesh net. In the first series, the three cages were set side by side with the holes aligned, and the net was stretched between the holes of two adjoining cages as a physical barrier. The mosquitoes were, however, able to pass through the netting easily if they wished. In the second series the two holes of the middle cage were covered with the net, and each hole was then connected to the hole of the neighbouring cage by a transparent plastic pipe, 15 cm in diameter and 30 cm in length. Where it contacts the hole of the neighbouring cage the end of the pipe was covered with a flap to prevent mosquitoes from passing through.

The first series of seven tests utilized different combinations of males and females as shown in Table 1. In the second series, three tests were carried out with different age males. In both series, mosquitoes were released at the middle cage. One of the connecting two cages was a bait cage containing a mouse, and the other was an empty control cage, the only

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exception being test No. 7 in which a small net container (15×15×15 cm) holding 20 virgin females was placed in the cage.

In the first series, mosquitoes were released at 3 p.m. and recovered at 10 a.m. in the following morning. In the second series, mosquitoes were lightly anesthetized with ether and released at around 2 p.m. One hour later, the flaps were opened. Observation was made for 15 min, the observer himself also acting as an attractant, and watching the mosquitoes, that flew into the bait cage.

These cage tests were carried out in a room at 25°C, 80% RH, without circulation of air, and on a constant condition of surroundings such as illumination and colour of the background. Each cage was supplied with cotton soaked with 5 m*l* of a 10% sucrose solution. The *Ae. albopictus* used in the experiments were a laboratory colony, maintained in an insectarium at 23-25°C, 80% RH, and with 16 : 8 (hr. L:D) photoperiod. In the pupal stage or just after emergence the sexes were separated for each test. The number of mosquitoes was checked before releasing them. After recovery, the number of each sex from each cage was also recorded.

Results and Discussion

The number of mosquitoes examined is shown in Table 1 together with the results of each test. Each test was replicated 5 times, and the results of recovery are shown as mean number with standard deviation. In all tests in which females were released together with males, more females were recovered in the bait cage than the control cage. In most of the tests fewer males were recovered in the bait cage than the control cage. Significant difference in the number of males recovered from the bait cage was found in two tests only: in Test No. 3 the number was unexplaindly increased in control cage, and in Test No. 7 the number was

Table 1 Number of mosquitoes released in the cage test, and mean number recovered and standard deviation in each test

Series	Test	No. released		No. recovered			
				Bait cage		Control cage	
		♂	♀	♂	♀	♂	♀
1.	No. 1	50	0	6.8±2.7		9.4±5.1	
	2	70	25	9.7±2.4	14.2±4.5*	13.7±5.7	3.8±1.4
	3	70	25m	7.2±3.8*	10.8±2.8	21.0±2.9	6.5±2.6
	4	50m	0	9.1±4.1		8.6±1.3	
	5	70m	25	7.7±3.8	5.7±1.2*	9.5±1.6	3.0±2.1
	6	70m	25m	7.0±1.7	14.0±3.0*	9.0±4.6	5.6±4.4
	7	50†	0	7.6±1.8*		11.0±2.1	
2.	No. 8	50‡	0	3.6±1.2		4.0±2.1	
	9	50§	0	4.3±1.2		3.0±0.8	
	10	50	0	4.6±1.6		4.3±2.6	

m : Mated mosquitoes; others were unmated.
* : Significant difference with number in control cage.
† : 20 virgin females were held in control cage.
‡ : Age at days 3 after emergence, and § : day 4. All others were day 5.

also greater when virgin females were held in the control cage.

In the second series, visually checked was the number of mosquitoes that flew into the bait cage. However, during a 15 min period, there were almost no males actively approaching the bait cage and hovering above the bait. No significant difference was found within a particular age group.

There was no evidence that male mosquitoes were fond of the mouse or attracted to the animal. On the contrary, more unmated males were in the control cage than in the bait cage in series 1; they appeared to avoid entering the bait cage.

Gubler and Bhattacharya (1972) found that during periods of low density in the study area, males were seldom attracted to the observers' bodies. Thus, it seems that the phenomenon of "males landing on a human body" is an accidental happening due to their high density as they rest in vegetation. They may not have been attracted to the body but have had to move from blades of grass or plant leaves to another object which had entered the area.

Unquestionably, many males were also attracted by female mosquitoes which came to bite. We often overlook the presence of females and notice only males which are slower in movement. The males were usually active in approaching the females as shown in Test No. 7.

In a field experiment, however, McIver *et al.* (1980) observed that the *Mansonia* males were significantly more numerous at a distance 3 m from the bait than at 7 m-15 m. Therefore, it is still early to conclude that males are not attracted to a mouse. It is possible that the size of the present test apparatus was somewhat small and the males could not orientate to the mouse from the release cage. It is also possible that the bait mouse was not appropriate for attracting the males. Grant (1969) found that certain chemicals attracted male *Ae. aegypti*, and Fay (1968) observed the swarming of male *Ae. aegypti* to objects of certain colours and shapes. The mouse may not have had the appropriate attractancy in colour and/or shape.

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雄ヒトスジシマカの吸血源への誘引飛来についての室内試験

栗原　毅

昼間屋外で蚊の人おとり採集法を実施していると，ヒトスジシマカの雄が人体へ飛来接近する現象を観察することがある。こうした経験を共有するためか，既に雄蚊の人体誘引現象についての野外観察成績が，2，3報告されている。この現象を室内試験で再現するべく実験を行った。供したケージテスト法は，30 cm 角の二面に開孔部をもつ金網ケージを3つ，相互に蚊の往来を可能なように一線に並べ，中央ケージに蚊を放ち，1つはベイトケージでマウスをいれ，他にはコントロールケージとして空のままで，どちらに選択飛来をするか観察するものである。午後3時から翌朝10時までのどの試験でも，雌蚊はベイトケージに多数集まるが，雄のヒトスジシマカが特にマウスのいるケージを選択して集まるような現象は観察できなかった。ただコントロールケージに20匹の未交尾雌を容した場合は，有意に多数集まった。次にケージ間を口径15 cm のパイプで連ねて，観察者自身もマウスのいる側におとりとなりながら雄蚊の飛翔を観察しても，やはり雄蚊がマウスのいるケージに近づく様子が見られなかった。今回の成績から考え，従来の野外での観察成績は，本種の著しい高密度に由来する機械的な飛来現象か，あるいは雌蚊に伴っての雄の飛来を注目したものではないかと思わせる。

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AFRICAN ENDEMIC-TYPE KAPOSI'S SARCOMA — A HISTOPATHOLOGIC STUDY AND FLOW CYTOMETRIC DNA ANALYSIS OF NINE CASES —

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Abstract: Flow cytometric DNA analysis using formalin-fixed, paraffin-embedded tissues were performed on nine cases of African endemic-type Kaposi's sarcoma (KS). Histologically, these cases were classified into the following three types; granulation tissue-like (one case), angioma/angiosarcoma-like (four cases) and spindle cell (four cases). Although these three types showed a variety of cellularity and cellular atypism, there were no fundamental differences in the view point of proliferation of spindle cell. Mitotic figures were not prominent. All cases were exclusively diploid and lacked an aneuploid population by flow cytometric measurement of DNA content. These results suggest that African endemic-type KS is a less aggressive disease rather than a malignant neoplasm.

INTRODUCTION

Kaposi's sarcoma (KS) was first described by Kaposi (1872) as an "Idiopathic multiple pigmented sarcoma of the skin". Since then, many cases of KS have been reported in European, American and African countries. Recently, it has been observed frequently in patients with acquired immune deficiency syndrome (AIDS).

KS is broadly classified into four categories; classical (European) type, African endemic-type, AIDS-related type and the type associated with immunosuppressive therapy. The clinical manifestations of these types are not necessarily same (Safai, 1985; Itakura *et al.*, 1986). Furthermore, African endemic-type KS is divided into cutaneous form and lymph node form by the affected site (Toriyama *et al.*, 1987a, b). Generally, cutaneous KS is long standing, spontaneously regressive, and not fatal. On the other hand, lymph node KS mainly occurs in children, and shows an aggressive clinical course similar to that of adult AIDS patients (Olweny *et al.*, 1976; Bayley, 1983). However, it has been reported that twenty-seven years was the longest duration of lymph node KS following initial diagnosis in childhood (Dutz and Stout, 1960). The true nature of KS including histogenesis has been widely disscussed, but still remains obscure.

Flow cytometric measurement of DNA content has been increasingly used as an objec-

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tive determinant of biological behavior and prognosis in certain solid neoplasms. Abnormal DNA ploidy appears to indicate poor prognosis in various malignant tumors (Hedley *et al.*, 1985; Merkel *et al.*, 1987; Kiyabu *et al.*, 1988; Stanley *et al.*, 1988). However, there have been few flow cytometric studies of KS. We have performed flow cytometric DNA analysis of paraffin-embedded tissues of African endemic-type KS, and discussed the relationship between histological findings and DNA ploidy.

Materials and Methods

Materials:

Nine cases of KS obtained from 1986 to 1989 in Provincial General Hospitals in Nakuru and Kisumu, the Republic of Kenya, were examined. Clinical data and relevant information were recorded as accurately as possible.

Histopathologic studies:

For light microscopic examination, each specimen was prepared with hematoxylin-eosin stain (H.E.), periodic acid-Schiff reaction, Azan-Mallory's stain and silver impregnation for reticulin fibers. Histological growth pattern, cellularity, cellular atypism and mitotic rate (per 10 high power fields; ×400) were determined.

Flow Cytometry:

The technique of Schutte (1985) for DNA analysis was employed using the formalin-fixed, paraffin-embedded tissues. In brief, single cell suspension was obtained by mechanical and enzymatic treatment of three or four 50 μm paraffin sections of each specimen, and stained with propidium iodide. Cellular DNA content was measured by a FACScan equipped with an argon laser. The excitation wavelength was 488 nm. The number of cells in each measurement was at least 2×10^4. The coefficient of variation (CV) of the diploid peak ranged from 4.0 to 8.7. To confirm the presence of the lesion in sections used for DNA analysis, we cut further 4 μm sections adjacent to the analyzed sections from each specimen. They were stained with hematoxylin-eosin and examined to reconfirm the histological diagnosis and features.

Results

The clinical manifestation, histologic features and DNA ploidy in the nine cases were summarized in Table 1. The age of these patients ranged from 18 to 56 years (mean 40 years). All patients were male. Eight of the nine cases occurred in the skin of the lower extremities. One case occurred in the lymph nodes of the upper arm.

According to the predominant histological features, three main types of growth were recognized. One case was classified as the granulation tissue-like type. This was characterized by an angioproliferative process with an inflammatory cell infiltration, but was less cellular than the other two types (Fig. 1). Four cases were angioma/angiosarcoma-like type. The lesions were composed predominantly of well-formed vascular spaces, with slit-like or anastomosing vasculature similar to that seen in angiosarcoma, but with minimal cellular atypism (Fig. 2). Four cases were spindle cell type. This type showed a high cellularity, but its cellular atypism and mitotic figures were not prominent (Fig. 3). Transitional zones and intermingling of these three types were also frequently observed in the same case. Mitotic

Table 1 Clinical manifestation, histologic features and DNA ploidy of African endemic-type Kaposi's sarcoma

Case	Age(yr)	Sex	Site of location	Histological type	Cellularity	Cellular atypism	Mitotic rate (per 10 HPFs)	DNA ploidy
1	55	M	Skin(leg)	S	+++	++	4	D
2	32	M	Skin(limb)	S	+++	+	2	D
3	U	M	Skin(leg)	A	++	+	1	D
4	39	M	Skin(leg)	A	++	+	3	D
5	53	M	Skin(limb)	S	+++	+	5	D
6	56	M	Skin(foot)	A	++	+	1	D
7	18	M	Skin(knee)	A	++	++	0	D
8	38	M	Skin(leg)	G	+	+	0	D
9	30	M	Lymph node (upper arm)	S	+++	++	8	D

U:unknown; G:granulation tissue-like type; A:angioma/angiosarcoma-like type; S:spindle cell type; HPF:high power field; D:diploid

rate ranged from 0 to 8 per 10 high-power fields (×400).

All cases were diploid by flow cytometric measurement of DNA content.

Discussion

Many studies have been performed about the histogenesis of KS, such as vascular endothelial cell (Hashimoto *et al.*, 1987; Scully *et al.*, 1988), lymphatic endothelial cell (Beckstead *et al.*, 1985; Dictor and Anderson, 1988) and mesenchymal cell (Komuro and Toriyama, 1991). Some immune factors with angiogenic activity, such as thymosin, interferon, lymphokines and prostaglandin, are thought to be crucial to the development of KS (Levy

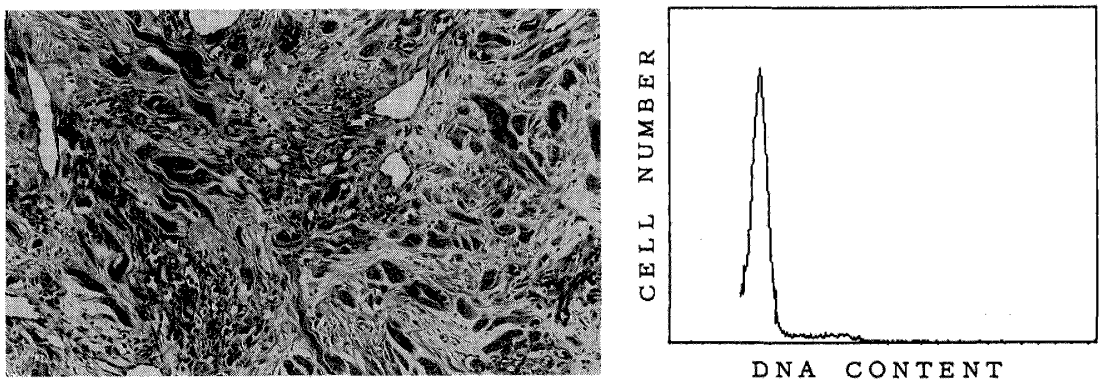

Figure 1 (left) Case 8(granulation tissue-like type). The lesion is characterized by an angioproliferative process with an inflammatory cell infiltration (H.E., ×100). (right) DNA histogram showing diploidy (CV 8.2).

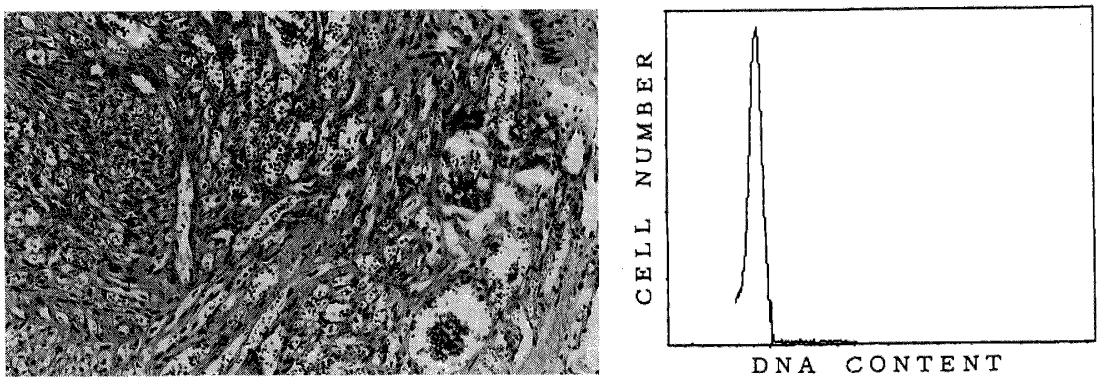

Figure 2 (left) Case 6(angioma/angiosarcoma-like type). The lesion is composed predominantly of anastomosing vascular channels and well-formed vessels (H.E., ×100). (right) DNA histogram showing diploidy (CV 7.7).

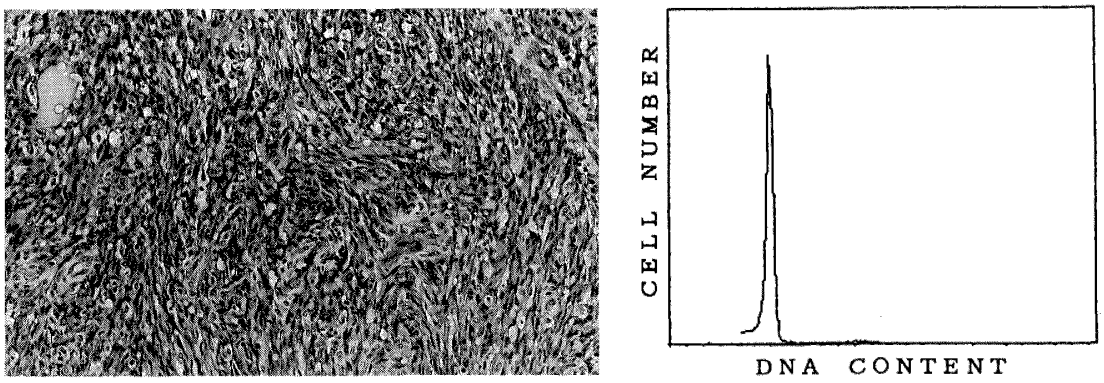

Figure 3 (left) Case 9(spindle cell type). The lesion is formed by interlacing bundles of spindle shaped cells (H.E., ×100). (right) DNA histogram showing diploidy (CV 4.0).

and Ziegler, 1983). However, the true nature of KS, whether hyperplasia or neoplasia, remains unknown (Costa and Rabson, 1983; Brooks, 1986; Mirra, 1986).

African endemic-type KS is usually divided into cutaneous form and lymph node form by the affected site (Toriyama *et al.*, 1987a, b). Generally, cutaneous KS usually occurs in lower extremities of adults, and is long standing, growing slowly and occasionally regressive (Itakura *et al.*, 1986). On the other hand, lymph node KS mainly occurs in children without cutaneous lesions, and shows an aggressive clinical course with generalized lesions often involving the visceral organs similar to that of adult AIDS patients (Olweny *et al.*, 1976; Bayley, 1984). However, it has been reported that twenty-seven years was the longest duration of lymph node KS following initial diagnosis in childhood (Dutz and Stout, 1960).

In this study, African endemic-type KS was histologically classified into the following three types; granulation-tissue like type, angioma/angiosarcoma like type and spindle cell type. Transitional zones and intermingling of these three types were also frequently observed

in the same case. Although these three types showed a variety of cellularity and cellular atypism, there were no fundamental differences in the view point of proliferation of spindle cell. Mitotic figures were not prominent.

Recently, DNA ploidy analysis by flow cytometry has shown that DNA aneuploidy is common in several malignant tumors and is a useful prognostic indicator (Hedley *et al.*, 1985; Merkel *et al.*, 1987; Kiyabu *et al.*, 1988; Stanly *et al.*, 1988). However, there has been no flow cytometric studies of African endemic-type Kaposi's sarcoma. In our study, all cases of African endemic-type KS were exclusively diploid and lacked an aneuploid population by flow cytometric measurement of DNA content. Histological appearance and DNA ploidy of lymph node KS were identical with cutaneous KS. These results are consistent with the reported findings in AIDS-related type KS cases (Fukunaga and Silverberg, 1990). Although there are no sufficient follow-up studies, our results suggest that African endemic-type KS is a less aggressive disease rather than a malignant neoplasm.

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アフリカ風土病型カポシ肉腫
── 9例における病理組織学的検討ならびにフローサイトメトリー法による核DNA量解析 ──

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カポシ肉腫 (Kaposi's sarcoma, KS) は一般には，欧米古典型，アフリカ風土病型，AIDS関連型，その他の免疫不全型の4型に大別され，さらにアフリカ風土病型KSは，発生部位により皮膚型とリンパ節型に分けられている。それぞれの型において若干の病態的な差異が認められているが，その組織発生を含め本態は未だ不明のことが多い。最近ではフローサイトメトリー (FCM) 法による核DNA量解析が腫瘍，あるいは腫瘍様病変の悪性度評価に，広く用いられている。今回9例のアフリカ風土病型KSにおいて，病理組織学的検討，ならびにホルマリン固定，パラフィン包埋ブロックを用いたFCM法による，核DNA量解析を行った。組織学的には次の3型に分けられた。すなわち肉芽組織類似型（1例），血管腫あるいは血管肉腫類似型（4例）と紡錘形細胞型（4例）である。これら3型においては，細胞密度や細胞異型に程度の差はあるものの，紡錘形細胞の増殖という点からは，基本的な差は見られなかった。核分裂像も目立たなかった。FCM法による核DNA量では，全例がdiploidを示しており，aneuploid例は見られなかった。これらの結果より，アフリカ風土病型KSは悪性腫瘍というより，むしろless aggressiveな病変であると思われた。

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A NEW BLACKFLY SPECIES OF *SIMULIUM* (*GOMPHOSTILBIA*) FROM JAVA, INDONESIA (DIPTERA: SIMULIIDAE)

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Abstract: A new blackfly species, *Simulium* (*Gomphostilbia*) *parahiyangum* sp. nov. is described based on the female, male, pupal and larval specimens collected from Java. This new species is very distinctive among the *Gomphostilbia* species in possessing prominent dorsal protuberances on abdominal segments 1-5 and a deep postgenal cleft reaching the hypostomium in the larval stage. By the number of antennal segments (11 vs. 10) the male of this species is easily distinguished from that of *S.* (*G.*) *varicorne* Edwards, 1927 reported from Sumatra, to which the larva and pupa of this species had been once thought to be conspecific.

INTRODUCTION

The Simuliidae of the Sunda Islands has not been studied since Edwards (1934) described 11 new species from Sumatra, Java and Bali, making a total of 19 taxa (including two subspecies) for this archipelago.

Our recent preliminary survey on the blackflies in East and West Java yielded a total of 16 species of *Simulum* Latreille s. l. including several new species, of which two belonging to the subgenus *Simulium* Latreille s. str. have already been described (Takaoka and Hadi, 1991). This paper describes one new blackfly species of the subgenus *Gomphostilbia* Enderlein.

The classification follows that of Crosskey (1969). Collecting and rearing methods, as well as dissection of anatomical parts for description, were mentioned in Takaoka (1983).

DESCRIPTION

Simulium (*Gomphostilbia*) *parahiyangum* sp. nov.

Female. Body length 1.8 mm. *Head* slightly narrower than width of thorax. Frons brownish, whitish grey pruinose, densely covered with whitish yellow recumbent pubescence except middle longitudinal portion narrowly bare; frontal ratio (i.e., ratio of the greatest width at vertex, the narrowest near antennal base, and the height of the frons) 1.7:1.0:1.8.

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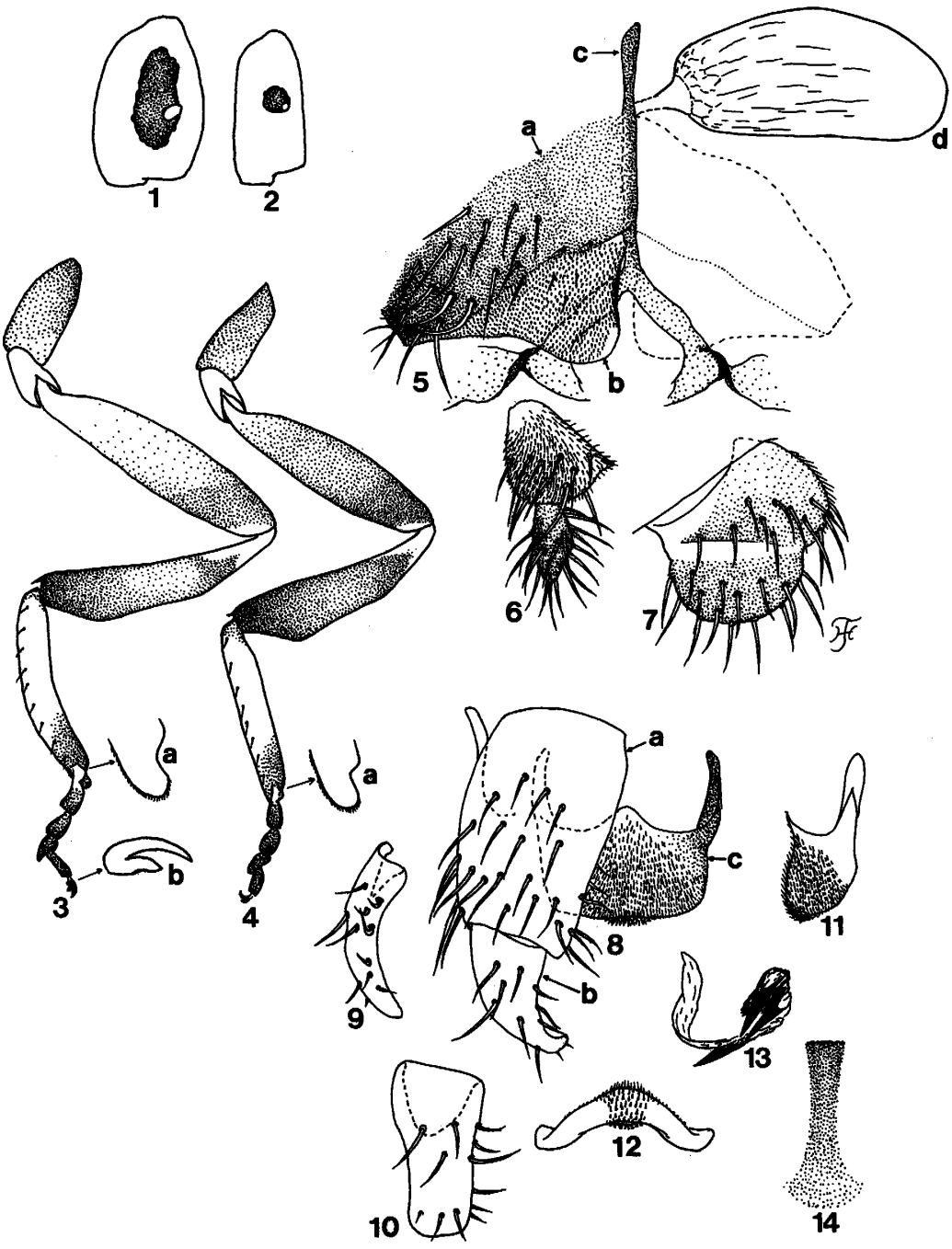
Frons-head ratio (i.e., ratio of the greatest width of the frons against that of head) 1.0:3.9. Clypeus brownish black, whitish grey pruinose, and densely covered with whitish yellow pubescence interspersed with several dark hairs. Antenna composed of 2+9 segments, brownish black except scape and pedicel (in one female, base of 1st flagellar segment too) yellow. Maxillary palp composed of 5 segments with proportional length of 3rd, 4th and 5th segments being 1.0:1.1:2.3; 3rd segment not so enlarged, but sensory vesicle (Fig. 1) enlarged, 0.6× as long as 3rd segment and with its opening on distal 1/3. Maxilla with 14 inner and 15 outer teeth. Mandible with ca. 30 small inner teeth and devoid of outer ones. Cibarium with heavily sclerotized arms but without any denticles medially. *Thorax.* Scutum brownish black in ground colour, thinly whitish grey pruinose, with three dark longitudinal lines which are distinct when viewed anterodorsally; scutum densely covered with whitish yellow recumbent pubescence. Scutellum brown with whitish yellow pubescence as well as long upstanding dark hairs along posterior margin. Postscutellum brownish black, whitish grey pruinose and bare. Pleural membrane bare. Katepisternum brownish black, whitish grey pruinose, with numerous pale and dark hairs, and longer than deep; sulcus distinct. *Legs.* All coxae and trochanters yellow except mid and hind coxae brown. All femora yellow, somewhat darkened distally and brown on distal cap. All tibiae yellow to dark yellow on basal 2/3, brown on distal 1/3, and with subbasal dark spot. Fore tarsi brownish black. Mid and hind tarsi brown except basal 1/3 of mid basitarsus, basal 2/3 of hind basitarsus and basal 1/2 of hind 2nd tarsal segment yellowish. Fore basitarsus slightly dilated, ca. 5.9× as long as its greatest width. Hind basitarsus (Fig. 3) slender, parallel-sided. Calcipala (Fig. 3a) moderately developed, nearly as long as wide, and ca. 0.6× width of basitarsal tip. Pedisulcus also distinct at basal 1/3 of 2nd tarsal segment. Claws (Fig. 3b) each with large basal tooth which is 1/2× as long as claw. *Wing.* Length 1.7 mm. Costa with spinules as well as hairs. Subcosta haired. Tuft hairs at base of stem vein dark brown. Basal portion of radius fully haired. *Abdomen.* Basal scale yellow with a fringe of pale yellow hairs. Dorsal surface of abdomen brown to brownish black except that of 2nd segment entirely pale yellow; tergite of 2nd segment whitish pruinose; tergites of 6th, 7th and 8th segments wide and shiny. *Genitalia.* Sternite 8 (Fig. 5a) bare medially, and with ca. 18 dark macrosetae on each side. Anterior gonapophyses (Fig. 5b) thin, membraneous, rounded posteriorly, covered densely with microsetae except posterointernal margins narrowly bare, and with a few short setae near anterior border; inner margins well sclerotized. Genital fork (Fig. 5c) of usual reversed-Y form, with arms each produced inwards to some extent but lacking any projection directed forwards. Paraproct (Figs. 6 and 7) of usual form, and with ca. 12 macrosetae ventrally and laterally. Cercus (Fig. 7) short, 1/2× as long as wide, rounded posteriorly, when viewed laterally, and covered with ca. 14 macrosetae on outside surface. Spermatheca (Fig. 5d) oblong, well sclerotized except small adjacent area near tubal juncture unsclerotized.

Male. Body length 1.8 mm. *Head* slightly wider than width of thorax. Upper eye consisting of 17 vertical colums and 15 horizontal rows of large facets. Clypeus brownish black, whitish pruinose, and covered densely with yellow pubescence interspersed with dark hairs. Antenna composed of 2+9 segments, dark brown except scape and pedicel yellow; 1st flagellar segment elongated, 2× as long as 2nd flagellomere. Maxillary palp with 5 segments; proportional length of 3rd, 4th and 5th segment 1.0:1.1:2.3; sensory vesicle (Fig. 2) small, ca. 0.17× as long as 3rd segment and with very small opening distally. *Thorax.* Scutum brownish

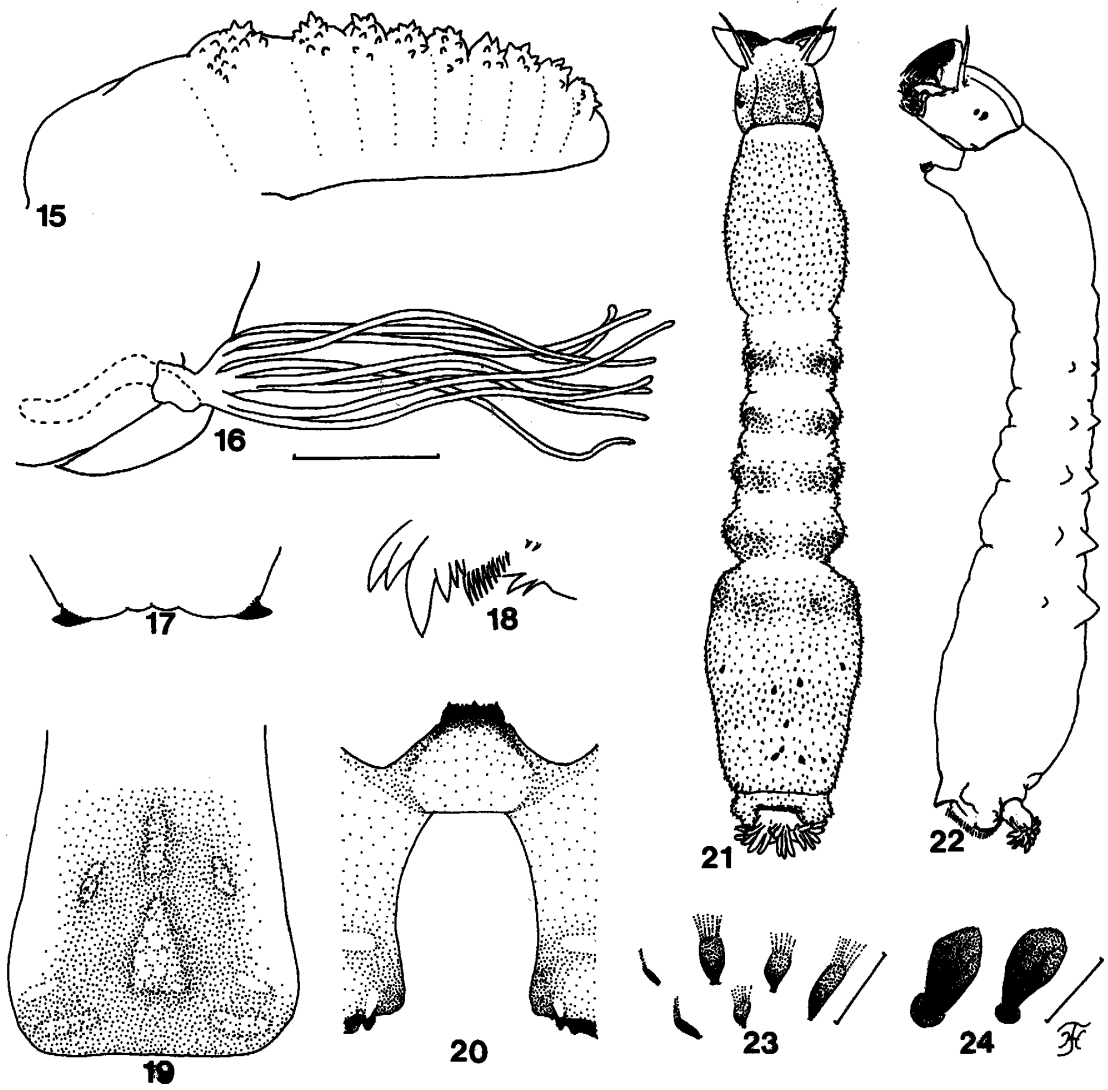


Figs. 1-14 Adult of *S.* (*G.*) *parahiyangum* sp. nov.: 1 and 2, 3rd segments of maxillary palp showing sensory vesicle in front view (1, female; 2, male); 3 and 4, hind legs (3, female; 4, male—a, calcipala; b, claw); 5-7, female genitalia {5, 8th sternite (a), anterior gonapophysis (b), genital fork (c) and spermatheca (d) in ventral view; 6 and 7, paraprocts and cerci in ventral view (6) and in lateral view (7)}; 8-14, male genitalia {8, coxite (a), style (b) and ventral plate (c) in ventral view; 9 and 10, styles viewed mediolaterally (9) and ventrolaterally (10); 11 and 12, ventral plates viewed laterally (11) and posteriorly (12); 13, paramere with 3 distinct parameral hooks; 14, median sclerite}.

black, entirely whitish pruinose in certain angle of light and densely covered with bright yellow recumbent pubescence. Scutellum brownish black, with bright yellow pubescence and several long marginal hairs. Postscutellum brownish black, whitish pruinose, and bare. Pleural membrane and katepisternum as in female. *Legs.* Mostly brown to brownish black except hind trochanter, base of hind femur and tibia, basal 2/3 of hind basitarsus and basal 1/2 of hind 2nd tarsal segment whitish yellow. Fore basitarsus slender, ca. 6.9× as long as its greatest width. Hind tibia (Fig. 4) subequal to hind femur in greatest width. Hind basitarsus (Fig. 4) slender, parallel-sided. Calcipala (Fig. 4a) well developed, 1.2× as long as wide, and 0.65× as wide as basitarsal tip. Pedisulcus well developed. *Wing.* Length 1.6 mm. Other features as in female except subcosta bare. *Abdomen.* Basal scale dark brown and its hair fringe dark basally and pale distally. Dorsal surface of abdominal segments brownish black except that of 2nd segment brown, and covered with short hairs; a pair of dorsolateral whitish pruinose patches on segments 2, 6 and 7. *Genitalia.* Coxite (Fig. 8a) nearly rectangular in ventral view, ca. 1.6× as long as wide. Style (Figs. 8b, 9 and 10) much shorter than coxite, gently curved inwards and without apical spine. Ventral plate (Fig. 8c, 11 and 12) flat, with microsetae almost entirely on ventral surface but only medially on posterior surface. Paramere (Fig. 13) with 3 long parameral hooks and a few small, indistinct ones. Median sclerite (Fig. 14) plate-like, with widened tip.

Pupa. Body length (excluding gill filaments) 2.0 mm. *Head* and *thorax.* Integument yellowish brown, covered densely with cone-shaped tubercles. Antennal sheath (Fig. 15) along its length with 9 well marked ridges on outer margin, each covered with several cone-shaped tubercles. Head with 1 facial and 3 frontal pairs of simple long trichomes. Thorax with 5 pairs of simple long trichomes on anterior 1/2. Gill (Fig. 16) with 8 slender greyish brown filaments arranged in 3 groups, i.e., upper and middle triplets and 1 lower pair; all filaments very short (ca. 0.7 mm), subequal in length and thickness to one another, with numerous transverse furrows becoming indistinct towards apex, and covered with minute tubercles. *Abdomen.* Terga 1 and 2 slightly darkened, and without tubercles; tergum 1 with a long simple seta on each side, and tergum 2 on each side with 6 simple minute setae, of which 1 seta is longer than others. Terga 3 and 4 each with 4 hooked spines directed forwards along posterior margin, and a short seta medially on each side. Tergum 5 with 5 very minute setae but devoid of spine combs. Terga 6-8 each with spine combs in transverse row, and comb-like groups of very minute spines on each side; terga 6-8 each also with a pair of minute setae on each side; tergum 9 with comb-like groups of minute spines and a pair of simple terminal hooks (Fig. 17). Sterna 4-8 each with comb-like groups of minute spines scattered all over. Sternum 4 with 1 simple slender hook on each side. Sternum 5 with a pair of bifid or trifid hooks situated close together on each side. Sterna 6 and 7 each with a pair of inner bifid or trifid and outer simple hooks widely spaced on each side. Sternum 9 with 3 grapnel-shaped hooks on each side. Cocoon simple, slipper-shaped, moderately woven, extending ventrolaterally, and with thick anterior margin which has no anterior projection.

Mature larva. Body length 4.0 mm. Body colour greyish yellow to yellowish brown. Head moderately covered with minute setae; cephalic apotome (Fig. 19) pale on anterior 1/2, somewhat darkened on posterior 1/2 and with negative head spots. Antenna longer than cephalic fan stem, with 4 segments, proportional length of 1st, 2nd and 3rd segments 1.0:0.9:



Figs. 15-24 Pupa and larva of *S.* (*G.*) *parahiyangum* sp. nov. 15-17, pupa (15, antennal sheath showing tuberculate projections; 16, respiratory filaments; 17, terminal hooks in dorsal view); 18-24, larva (18, apical tip of mandible; 19, cephalic apotome showing negative head spots; 20, ventral surface of head capsule showing hypostomium and deep cleft; 21, dorsal view of larva showing the distribution of spinules and spines; 22, lateral view of larva showing dorsal and dorsolateral protuberances on abdominal segments 1-5 (spinules and spines omitted); 23, flat spinules of various sizes on thorax and abdomen—2 spinules from left are seen laterally; 24, black spines on abdominal segments 6-8). Scale bars indicate 0.2 mm for 16 and 0.02 mm for 23 and 24.

0.9. Cephalic fan with ca. 30 main rays. Mandible (Fig. 18) with 2 mandibular serrations; comb teeth decreasing in length and thickness from 1st to 3rd tooth. Hypostomium with a

row of 9 apical teeth, of which corner and median teeth are moderately developed; lateral serration absent; 4 hypostomial setae lying parallel to lateral margin on each side. Postgenal cleft (Fig. 20) deep, widely reaching posterior margin of hypostomium. Histoblast of pupal gill with 8 slender filaments, arranged in 3+3+2. Thoracic segments densely covered, on dorsal and lateral surfaces, with small dark flat spinules which have several transparent slender branches apically (Fig. 23). Abdominal segments 1-5 each with a dorsal pair of conical protuberances and a dorsolateral pair of rather smaller protuberances (that of segment 5 not well defined) (Figs. 21 and 22) which are covered with a few colorless setae and numerous small dark spinules similar to those on thoracic cuticle; abdominal cuticle, besides on protuberances, covered densely with similar spinules dorsally and laterally except on intersegment spaces; there are also 12-20 black distinct spines scattered dorsally on segments 6-8 (Figs. 21 and 24); colorless setae also present on dorsal and lateral surface of last segment. Rectal gill lobes compound, each lobe with 8-10 finger-like lobules. Anal sclerite of usual X-form, posterior arms a little longer than anterior ones; basal portion of arms widely sclerotized. Ventral papillae well developed. Posterior circlet with ca. 74 rows of up to 13 hooks per row.

Type specimens. Holotype ♀ slide-mounted together with its pupal skin and cocoon on glass slide, reared from pupa, taken at foot of Mt. Tangkubanperahu, 6 km north of Bandung, West Java, Indonesia, 30.XII.1990, H. Takaoka. Allotype ♂, slide-mounted, same data as holotype. Paratypes, 3 ♀♀, 1 ♂, reared from pupae, and 3 mature larvae (all preserved in alcohol except 1 larva slide-mounted), same data as holotype. Holotype ♀, allotype ♂, 1 paratype ♀ and 1 larval paratype will be deposited at the Department of Parasitology and Pathology, Faculty of Veterinary Medicine, Bogor Agricultural University, and 1 ♀ and 1 larval paratype at the Department of Entomology, Bogor Museum of Zoology, Bogor, and other paratypes (1♀, 1♂ and 1 larva) at the Natural History Museum, London.

Distribution. Java.

Ecological note. The pupae and larvae of this species were found on banana leaves and on slender plant roots trailing in the water of a small shaded stream (ca. 1 m wide; ca. 1,000 m in altitude) running down through the sloped field cultivated for tea plantation not far from the northern exit of National Park of Mt. Tangkubanperahu, along the road to Segalaherang. Collected together with this species were *S.* (*G.*) *batoense* Edwards, 1934 and *S.* (*S.*) *sigiti* Takaoka and Hadi, 1991.

Discussion

This new species was named after the Sundanese "parahiyanga" (=remarkable) because its larva is very distinct in possessing prominent dorsal protuberances (Fig. 22) on the abdomen and the deep postgenal cleft (Fig. 20) reaching the hypostomium, a combination of these characters being unusual among the known species of the subgenus *Gomphostilbia*. There has been no report of any *Gomphostilbia* species with these peculiar characters except *S.* (*G.*) nr. *varicorne* (only larval stage known) which was collected from Assam (Datta, 1975). The larva of *S.* (*G.*) *parahiyangum* resembles that of *S.* (*G.*) nr. *varicorne*, from which

it is differentiated by the branching method of respiratory filaments as seen in the histoblast (i.e., two triplets plus one pair vs. four pairs). The pupa of *S.* (*G.*) *parahiyangum* is characterised by the short slender filaments. In this aspect, this species is similar to *S.* (*G.*) *montiblense* Takaoka, 1983 from Palawan Island of the Philippines (Takaoka, 1983), and *S.* (*G.*) *darjeelingense* Datta, 1973 from India (Datta, 1973). However, there is a clear difference in the branching method of the respiratory filaments.

The larva and pupa of *S.* (*G.*) *parahiyangum* are probably conspecific to those thought as the immature stages of *S.* (*G.*) *varicorne* Edwards, 1925 which was known only from the type male specimen collected from Sumatra (Edwards, 1934). Our study showed that Edwards' tentative association was incorrect since the reared adults of *S.* (*G.*) *parahiyangum* had a normal antenna composed of 11 segments {not 10 as seen in *S.* (*G.*) *varicorne*}. The immature stages of *S.* (*G.*) *varicorne* may be of different form. In this connection, it should be noted that two Malayan specimens of male adults labelled as *S. varicorne* at the Natural History Museum, in London, had their pupal exuvia with long-stalked respiratory filaments which were apparently different from those of *S.* (*G.*) *parahiyangum* (Takaoka, unpublished data).

The male of this new species is similar to *S.* (*G.*) *friederichsi* Edwards, 1934 known only from male type specimen taken from Java in having the dark leg coloration and the slender hind basitarsus. The male of *S.* (*G.*) *parahiyangum* is easily distinguished from the latter by lacking a distinct terminal spine on the style as well as any pruinose scutal pattern.

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ジャワ島産ナンヨウブユ亜属の1新種について

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1990年12月から1991年1月にかけて，ジャワ島において吸血性昆虫ブユの採集調査を行った。得られた標本を検討した結果，数種の新種が含まれていることが分かった。本論文では，ブユ属ナンヨウブユ亜属に属する，1新種の記載を行った。本種は，幼虫の頭部腹面の postgenal cleft が深く，腹部第1節から5節の背面に顕著な瘤様の突起を持つなど，これまでこの亜属のなかでは見られない珍しい形態的特徴を有する。

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EFFECTS OF NUTRITIONAL DEFICIENCY ON PARASITIC INFECTION OF THE INFANT IN GREATER ACCRA, GHANA

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Abstract: In Accra City and five rural villages near Accra, human parasites and nutritional status were examined in 269 preschool children. Nine species parasites were recognized. Nutritional status was determined by the measurement of weight and height. Malnutrition was frequently found in the children belonged to the age group 0-5 years. The infective rates of parasites in children were 58.0% in the normal nutritional status and 25.0% in the malnutritional status. Infective rate was higher for the well-nourished children and was lower in the malnutritional children. We also recognized that infective rates of parasites differed from species of parasites.

INTRODUCTION

Both parasitic infection and human malnutrition are important health problems in the developing countries today. These problems are investigated independently, but relations between human parasites and nutritional status of hosts are not known. Stephenson and Holland (1987) reviewed how helminth parasites had influenced the nutrition of human host. Five helminthes, *Schistosoma, Ascaris, Trichuris, Strogyloides* and hookworms are common human parasites to cause malnutrition of the developing world's young children (Stephenson and Holland, 1987). Odugbemi *et al.* (1982) reported that these parasites caused diarrhoea, that increased nutrient-losses and induced malnutrition, in Nigerian children. Nevertheless, these parasites were not important diarrhoeal agents in Indonesian children (Soenarto *et al.*, 1983) and in Ghanaian children (Minakami *et al.*, 1991). If parasites are important causation to induce malnutrition in children, parasites might be found high-frequently in the malnutritional children. But, malaria (*Plasmodium falciparum*) was found higher in the children that did not have malnutrition and lower in the other children that had malnutrition (Vázquez and Sânchez, 1988). Present results demonstrate the relations between parasitic infection and malnutrition in Ghanaian children.

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Materials and Methods

The survey was conducted in Accra City and five villages (Ayikai-Doble, Akraman, Okashibiade, Yaoman and Onyasanu) of the northwest Accra along Densu River in Ghana. Samples of stool, urine and blood smear were collected from 269 infants up to seven years old children. Body weight (kg) and height (cm) were also measured.

Nutritional status (weight for height) were determined according to the procedure of Jelliffe (1966). Each stool specimem was mixed with a drop of water and a drop of Lugol solution (5% iodine, 10% KI and make 100 m*l* with distilled water) on a microscopic slide in the laboratory and examined for the presence of protozoan and helminth parasites. Formal-ether method (Ritche, 1948) also was applied to parasitological investigation of the same stool specimen. Urine specimen was centrifuged at 1,500 rpm for 10 min and the sediment was examined for the presence of *Schistosoma* eggs and other parasites. Blood smear specimen was stained with Giemsa solution and examined for evidence of malaria parasites.

Results

Thirty nine per cents of examined children were infected with parasites (Table 1). Mixed infection was common. *Ascaris* was the most prevalent parasite and was identified in 70 children (26.0%). *Schistosoma* eggs in urine of children were found in 35 persons (17.7%). This high incidence rate of *Schistosoma* due to the Densu River where is an endemic area of *Schistosoma haematobium*. In the malaria infection, *P. falciparum* was predominant in this study area.

The nutritional status of children was divided into five ranks according to the Jelliffe (1966). In the normal healthy 81 children with the nutritional status revealed 100%, parasites were found in 47 cases (58%). On the other hand, the parasite positive rate in the most malnourished (revealed with 60% nutritional status) children was 25% (Table 2). The relations between parasitic infection and nutritional status in human host indicated that the infective rate decreased according to the change for the worse of the nutritional status.

Table 1 Parasitic infection rates among 269 infants in Accra

Parasites	Number of infected infants	Infective rates (%)
Helminths		
Ascaris lumbricoides	70	26.0
Schistosoma haematobium	35	17.7
Strogyloides stercolaris	10	3.7
Hookworm	8	3.0
Protozoa		
Plasmodium falciparum	19	12.0
Plasmodium malaria	1	0.4
Giardia lumblia	4	1.5
Entamoeba coli	1	0.4
Dientamoeba fragills	1	0.4
Parasite carrier	107	39.8

Table 2 Parasitic infection rates on each nutritional status

Degree of Nutritional status (%)	Parasite positive rates (%)	Number of parasites carrier	Number of invest.
100 (Standard)	58.0	47	81
90	37.0	30	81
80	30.4	17	56
70	29.0	9	31
60	25.0	4	16

Fig. 1 shows that the species specificity of parasites was recognized. Although the number of carriers suffered from *Ascaris, Schistosoma* or *Plasmodium* was reduced by the degree of malnourished status, the number of *Strongyloides* carriers was not interfered with nutritional status of host.

Discussion

Ascaris, Strongyloides and hookworms were found as major parasites of the residents in rural Accra (Wurapa *et al.*, 1975) and in semi-slum of Accra City (Anteson *et al.*, 1981). Present study revealed similar results with the previous studies. Compared with the previous studies, we found that *Schitosoma* infection rate was higher. This is due to the reason that the rural survey area of this study was an endemic area of *S. haematobium.*

Five species of parasites, *Ascaris, Strongyloides, Schistosoma, Trichuris* and hookworms

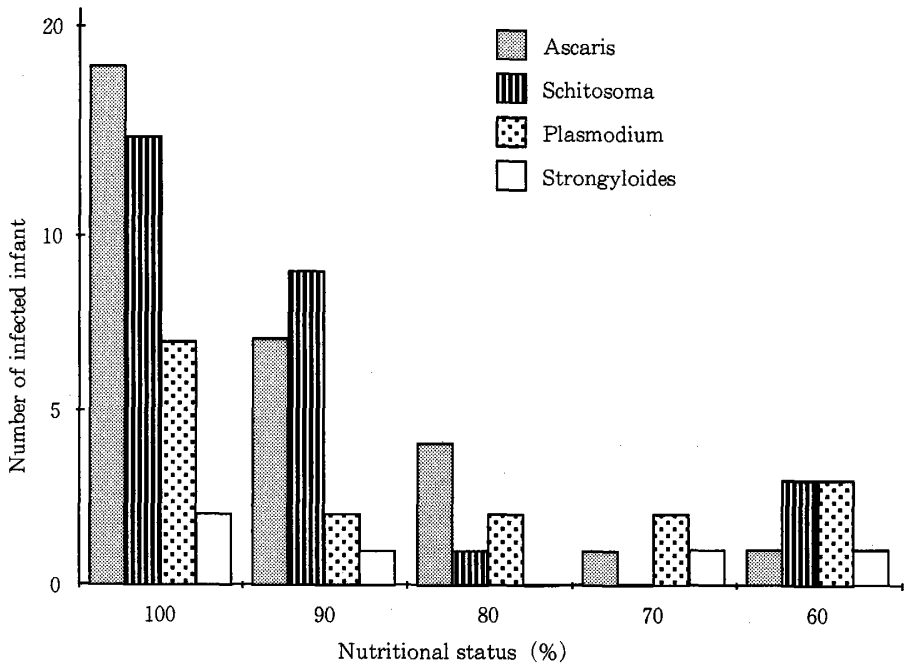

Figure 1 Distribution of infection and nutritional status among 107 infants carried with parasites.

cause malnutrition in the developing world's children because of the nutritional deficiency induced by parasites (Stephenson and Holland, 1987). Most parasitological studies had been examined the effects on human hosts induced by parasites and few information regarding to the malnutritional effects to parasites in human hosts are known. Vázquez and Sánchez (1988) found that *P. falciparum* was found higher in the well-nourished children and lower in the malnutritional children. We got similar results in the helminth parasites (Table 2). The positive infection rates decrease dues to the decrease of the nutritional status.

Malnutrition induces various changes of body in the early developmental stage of both human and animal. In the malnourished young rats, the level of biogenic amines in brain and periperial tissues (Stern *et al.*, 1975) and periperal blood ammonia levels (Stevens *et al.*, 1975) increased. In human, Johnson *et al.* (1972) found the hyperammonemia in newborn infants accompanying parenteral nutrition; Abo-Hussein *et al.* (1984) found highly significant increase of blood- and cerebrospinal fluid-ammonia in the children with protein energy malnutrition. In malnourished children (Stephan and Waterlow, 1968) and malnourished rats (Schimke, 1962), low protein diet caused a reduction in the activity of urea-cycle enzyme and therefore hyperammonemia was induced.

The present study demonstrated that the nutritional deficiency decreased the positive infection rate of parasites in the children up to seven years old. The body condition of the malnutritional status might be unconfortable for human parasites, because the concentration of blood ammonia is significantly higher than in the well-nourished children.

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ガーナ，アクラにおける乳幼児の栄養状態が寄生虫感染率に及ぼす影響

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アクラ市とアクラ市近郊農村5部落において，7歳以下の子供269名について，人体寄生虫と栄養状態についての調査を行った。寄生虫の感染率は39.0%で，9種類の寄生虫が認められた。栄養失調は5歳以下の子供に多かった。寄生虫感染率と栄養状態との関係は，栄養状態が正常な子供では感染率58.0%，栄養失調の子供では25.0%であった。栄養状態が悪いほど，寄生虫感染率は低かった。5歳以下の栄養失調症の子供は，高アンモニア血症を起こすので，血中のアンモニア濃度の上昇が寄生虫の人体寄生を困難にしていると考えられる。

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A SEROEPIDEMIOLOGICAL STUDY OF DENGUE AND JAPANESE ENCEPHALITIS VIRUS INFECTIONS IN VIENTIANE, LAO PDR

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Abstract: A total of 141 serum specimens from inhabitants in eight age groups in Vientiane, Lao PDR was examined to estimate the neutralizing anitibody levels to dengue (DEN) and Japanese encephalitis (JE) viruses. From the analyses of the results of neutralization (N) tests, the following conclusions were drawn about the prevalences of DEN and JE viruses in Vientiane: (1) The percentage incidences of N antibodies to DEN 1-4 viruses increased with age, but the incidences of N antibodies to DEN-3 and DEN-4 were lower than those to DEN-1 and DEN-2, which reached 100% by the age of 21-30 years old. (2) The percentage incidence of N antibody to JE virus was similar to those to DEN-3 and DEN-4. (3) The geometric mean titer of N antibody to DEN-2 was highest among four serotypes in every age group, indicating that, in the past, DEN-2 virus was most prevalent. (4) By the age of 15 years old, the majority of the inhabitants (88.2%) in Vientiane were infected with two or more serotypes of DEN viruses and most children seem to be exposed first to DEN viruses and later to JE virus.

INTRODUCTION

The Lao People's Democratic Republic (PDR), a landlocked country located in Indochina, has a total population of 3.8 millions with an area of 146.8 thousand square kilometers (Fig. 1).

In 1987, a large epidemic of dengue fever (DF)/dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS) occurred during the rainy season (May-September) in Vientiane. A total of 6,567 hospitalized cases with a high morbidity rate (1,530/100,000) was reported to the Ministry of Public Health. Most of them were children under 15 years old. In the same year, large outbreaks of DH/DHF were also reported in Thailand and Vietnam.

In Lao, the virological and serological diagnoses of DEN virus infections are being performed by the Laboratory Services of the National Institute of Hygiene and Epidemiology (NIHE) in Vientiane. Since 1987, attempts were made to isolate virus from DF/DHF patient sera by using a mosquito cell line (Igarashi, 1978), but were unsuccessful probably due to the lack of experience. Although hemagglutination-inhibition (HI) tests (Clarke and Casals,

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1958) were carried out for the serological diagnosis of DF/DHF, the results at most were not interpretable due to the lack of serum specimens in the convalescent phase. For JE, no diagnostic test had been performed in the NIHE. No epidemic of JE-like encephalitis was reported so far in the Lao, though sporadic JE cases are believed to exist. Thus, no reliable data of these viral infections had been available. In order to establish appropriate measures to prevent outbreaks of these infections, it is essential to clarify the seroepidemiological status of the inhabitants in Lao.

The purpose of this study was to estimate the extents of past exposures of inhabitants in Vientiane to DEN and JE viruses. This paper describes the seroepidemiological data of DEN and JE infections in the inhabitants of Vientiane, Lao as tested by focus reduction neutralization (N) test.

Materials and Methods

Serum Specimens

A total of 141 serum specimens were obtained at the Mahosot Hospital, NIHE, and blood bank in Vientiane, during a period of February to March, 1990. They were collected by vein puncture. The subjects were divided into eight age groups. The number of specimens of each

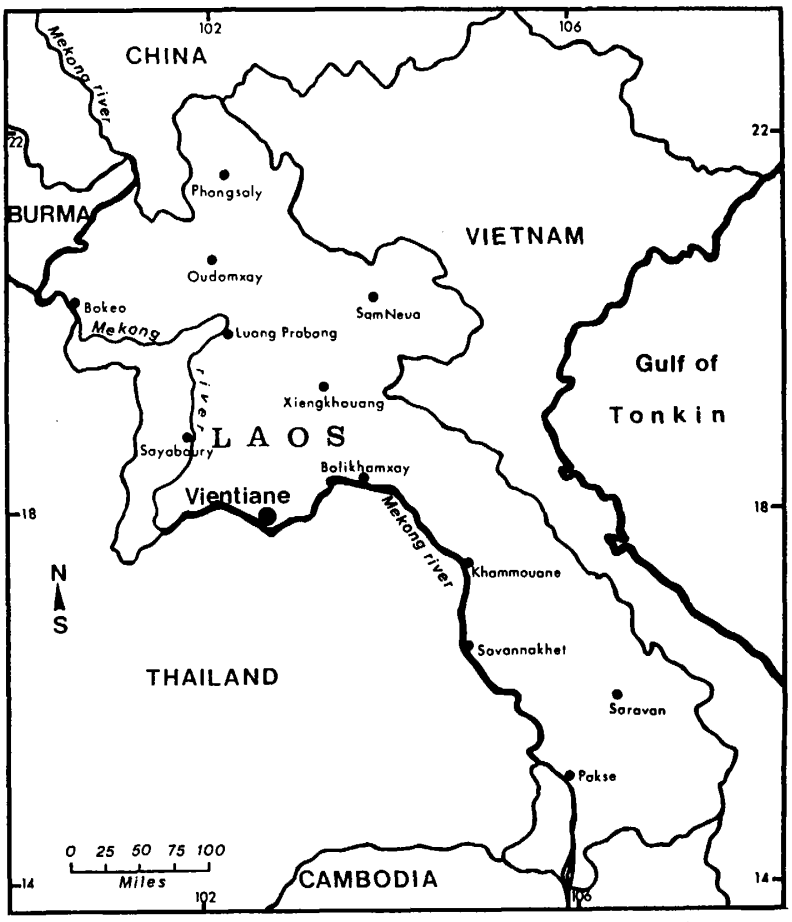

Figure 1 Map of the Lao People's Democratic Republic.

group was between 12 to 20 (Table 1). The sera were diluted with Eagle's minimum essential medium supplemented with 2% fetal calf serum and antibiotics (EMEM-2FCS), and heat-inactivated at 56°C for 30 min and stored at −20°C until used.

Virus strains

The strains of DEN and JE viruses used for N tests were Hawaiian (DEN-1), New Guinea B (DEN-2), H-87 (DEN-3), H-241 (DEN-4), and JaGAr # 01 (JE). The stock viruses were prepared from 10% homogenates of infected suckling mouse brains and stored at −70°C.

Neutralization test

Focus reduction neutralization test using peroxidase-anti-peroxidase (PAP) staining method (Okuno *et al.*, 1985; Ishimine *et al.*, 1987) was employed. The serum specimens were first screened for the presence of N antibodies against DEN and JE viruses. Briefly, 1:20 dilution of serum specimens were made in EMEM-2FCS and mixed with an equal volume of virus suspension containing about 80 focus-forming units and incubated for 90 min at 37°C. The virus-serum mixture was then inoculated onto BHK-21 cell monolayers in 96-well microplate (Sumilon, Sumitomo Bakelite, Japan). After appropriate incubation periods, the foci were visualized by 3-step PAP staining method using anti-JE or anti-DEN rabbit serum (diluted 1:2,000 or 1:1,000 respectively); sheep anti-rabbit IgG (1:1,000 dilution, Cappel, U.S.A); rabbit PAP complex (1:5,000 dilution, Jackson Immunoresearch Lab., U.S.A) and 3, 3'-diaminobenzidine tetrahydrochloride (0.3 mg/m*l*, Sigma, U.S.A. plus 0.03% H_2O_2). The sera with a focus reduction rate of 75% and more were, then, serially diluted and reacted with virus as above to determine N antibody titer. The serum dilutions and the percentages of the focus reduction were plotted on the probit chart. The serum dilution giving 50% focus reduction from the probit regression line was defined as the N antibody titer.

Results

The incidences of JE- and DEN-antibodies in the serum specimens increased with age as shown in Fig. 2, indicating that JE virus and all four serotypes of DEN viruses were circulating in the Vientiane. The incidences of N antibodies to DEN-1 and DEN-2 rose to 82-88% by the age of 11-15 years old and reached 100% by the age of 21-30 years old. In comparison with those to DEN-1 and DEN-2, the incidences of N antibodies to DEN-3, DEN-4 and JE appeared to be lower and did not reached 100% in any age group.

Table 1 Number of serum specimens collected in each age group

Age group (years old)	Number of specimens
0- 5	12
6-10	18
11-15	17
16-20	17
21-30	20
31-40	20
41-50	18
51-	19
Total	141

Fig. 3 shows the geometric means of N antibody titers (GMTs) to four serotypes of DEN viruses and JE virus in age groups. In each age group, the GMT of N antibody to DEN-2 virus was highest among those to DEN and JE viruses, which rose up to more than 300 by the age of 21-30 years old and remaining at a similar-level in the older age groups. The GMTs of N antibodies to DEN-1 and DEN-3 showed middle level, and those to JE and DEN-

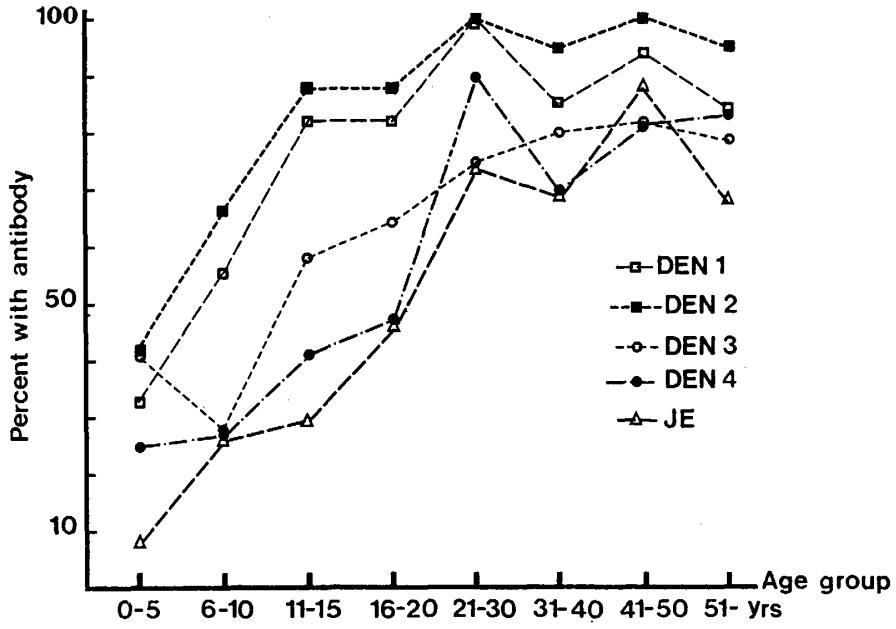

Figure 2 Percentage incidences of N antibodies to DEN and JE viruses.

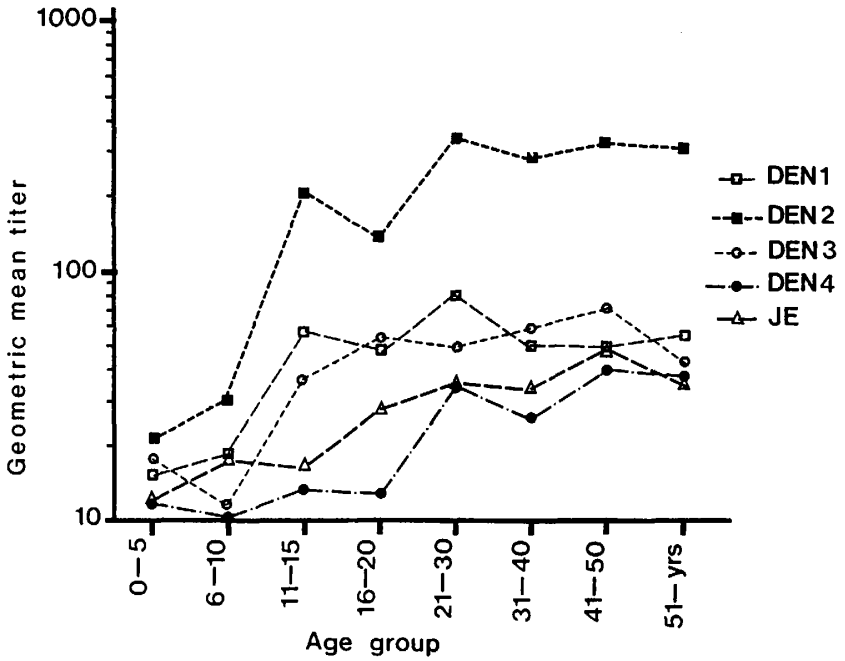

Figure 3 The geometric mean titers of N antibodies to DEN and JE viruses in age groups. Negative samples are supposed to have titers equal to 1:10.

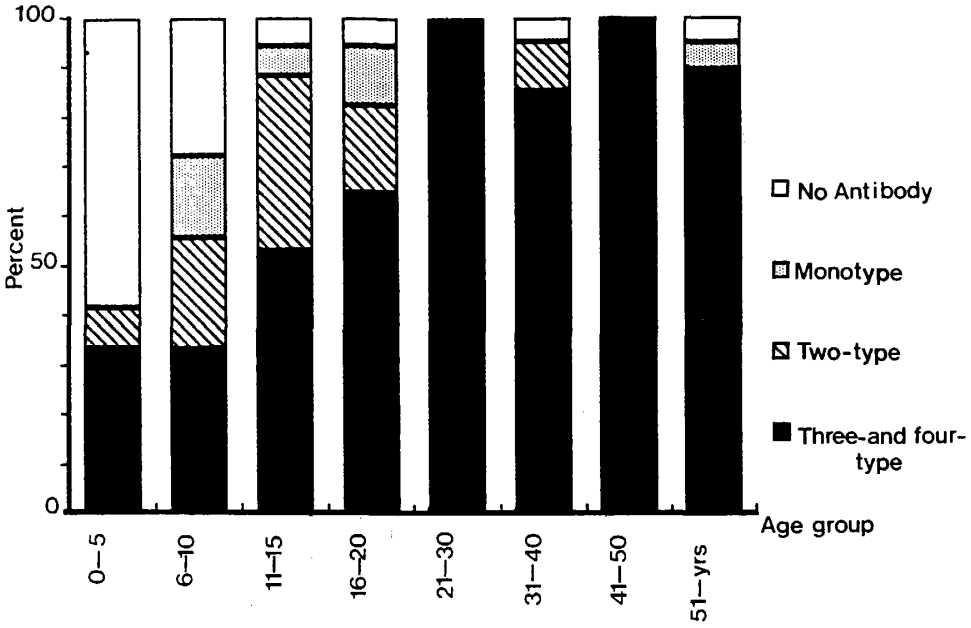

Figure 4 Relative frequency of specimens possessing no N antibody, monotype, two-, three-, and four-types of N antibodies to DEN viruses.

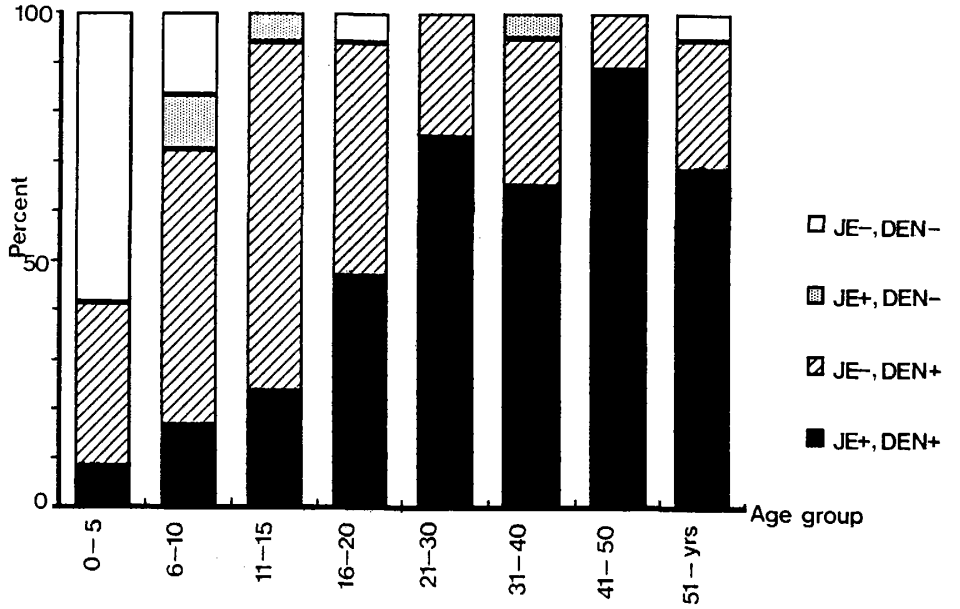

Figure 5 Relative frequency of specimens possessing N antibodies to JE and/or DEN viruses.

4 were at the lower levels. The findings indicate that in the past, DEN-2 virus was most prevalent in Vientiane among four serotypes of DEN viruses and JE virus.

In order to examine the multiple DEN serotype infections in individuals, the specimens examined were grouped with respect to their N antibodies to four serotypes of DEN viruses: The specimens possessing (1) no antibody to either type, (2) monotype antibody, (3) antibodies

to two different serotypes, and (4) antibodies to three or four different serotypes. The results are shown in Fig. 4. The percentage of the groups with multitype antibodies (group 3 and group 4) rose to about 90% by the age of 11-15 years old, suggesting that heavy infections with two or more serotypes of DEN viruses had been taken place in the area. Within the monotypic group (i.e., group 2), 50.1% were antibody to DEN-2, 33.3% were to DEN-1 and 16.6% were to DEN-4. This indicates a higher prevalence of DEN-2 virus than other serotypes in the late epidemics.

The relative frequencies of N antibodies to DEN and JE viruses were analyzed by rearranging the specimens into four groups: (1) absence of antibody to either type of DEN viruses and to JE virus, (2) presence of antibody to JE virus but not to either type of DEN viruses, (3) presence of antibodies to either type of DEN viruses but not to JE virus, and (4) presence of antibodies to either type of DEN viruses and to JE virus. The proportion of the specimens possessing antibodies to JE virus and either type of DEN viruses (group 4) increased with age reached 75% by the age of 21-30 years old (Fig. 5), indicating the co-existence of DEN and JE viruses in the area. On the other hand, the percentage of the group possessing antibodies to either type of DEN viruses but not to JE virus (group 3) was markedly predominant in the younger three age groups: 0-5, 6-10 and 11-15 years old with the percentages of 33.3%, 55.5% and 70.5%, while the percentages of the group possessing antibody to JE virus but not to DEN viruses (group 2) were only 0.0%, 11.1% and 5.8% respectively. The results suggest that most children in Vientiane were exposed first to DEN viruses and later to JE virus.

Discussion

A seroepidemiological study on DEN and JE infections in Vientiane was carried out by N test. The results obtained indicate that DEN and JE viruses have been co-circulating in the area with higher prevalences of DEN viruses. The N antibodies to all four serotypes of DEN viruses were found in the specimens and their percentage incidences increased with age. About 2/3 of 6-10 years old and 9/10 of 11-15 years old children had been infected with at least 2 different serotypes of DEN viruses. Thus, Vientiane was found to be a high endemic area for DEN viruses, as previously suggested by Halstead (1980).

During the DHF epidemic of 1987, the number of hospitalized DHF/DSS cases was beyond the admission capacity of the pediatrics wards in two main hospitals of Vientiane Municipality, and many extra beds had to be added in the corridors. This fact and the result of high percentage incidences of N antibodies to multiple DEN serotypes in children under 15 years old, seem to support the role of enhancing antibodies in the pathogenesis of DHF (Halstead *et al.*, 1980; Halstead 1988, Kliks *et al.*, 1989). Of four serotypes of DEN viruses, the geometric mean of N antibody titers to DEN-2 was highest in each age group (Fig. 3), indicating the highest prevalence of DEN-2 virus in the past in the area. Previously, Burke *et al.* (1983) reported that infections with DEN-2 and DEN-4 viruses were regularly associated with anamnestic responses to flavivirus antigens in patients with DHF in Bangkok. The result of the GMT mentioned above, together with the results of the highest percentage of monotypic DEN-2 antibody, may give an explanation to the large outbreak of the DHF in 1987.

In Vientiane, JE virus is co-circulating with DEN viruses but no outbreak of JE-like

encephalitis has been reported. In the Chiang Mai Valley, located in the north of Thailand, JE epidemics have been reported since 1969 (Yamada *et al.*, 1971). The incidence of JE N antibody in the Chiang Mai area was much higher (more than 70% at 10-14 years old) (Fukunaga *et al.*, 1984) than that in Vientiane area (30% at 11-15 years old). The low level of N antibody incidence of JE virus may reflect the epidemiological situation in Vientiane.

DEN viruses were found to be more prevalent than JE virus and most children seemed to be exposed to DEN viruses first and later to JE virus. It is unclear whether the children having DEN antibodies can overcome JE virus infection. It is also not clear whether JE vaccination can protect against DHF or increase the risk of DHF, or has no effect on it (Hoke *et al.*, 1988)

In this study, serum specimens were collected only in Vientiane. Further sero-epidemiological studies on the arbovirus infections should be carried out in other provinces of the Lao PDR.

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ラオス国ビエンチャンにおけるデング熱および日本脳炎ウイルス感染症の血清疫学的研究

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ラオス国ビエンチャンにおける住民の，デング熱ウイルス（DEN）および日本脳炎ウイルス（JEV）に対する年齢階層別抗体保有状況を調べた。1990年2-3月の期間，ビエンチャン市内の病院，国立衛生疫学研究所および血液銀行で，各年齢層毎に12-20検体の血清，合計141検体を採取し，フォーカス減少中和試験により，中和抗体価を測定した。DEN 1-4型のすべてに対する抗体保有率は，年齢と共に増加した。DEN 1，2型に対する抗体保有率は，21-30歳までに100%に達するのに対し，DEN 3，4型およびJEVに対する抗体保有率はやや低く，どの階層においても100%となることはなかった。DEN，JEVに対する抗体価の幾何平均値は，DEN 2型に対する抗体がどの年齢層においても最も高かった。また住民の多く（88.2%）は，15歳頃までに複数の型のDENに対する抗体を保有するようになっていた。JEVに対する抗体は，低年齢層（0-5歳）ではDENに対するもの（25.0-41.7%）より低い（8.3%）ことから，この地域では始めにDENに感染した後，JEVに感染するパターンが多いことが考えられた。

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プラジカンテルによる治療後短期間に起こったビルハルツ住血吸虫の再感染

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緒　言

今日，住血吸虫症の地域村落に於けるコントロール法としては化学療法が最も効果的であると言われている。しかし，これまで行われたほとんど全てのコントロールプログラムにおいては集団治療後，再感染や重感染が起こり，繰り返し集団治療を行うことが必要となってくる（Wilkins, 1989; World Health Organization, 1985）。

再感染率およびその強さは種々の要因により変化し，各村落により大きく異なっている（Wilkins, 1989）。このことから，それぞれの村に於ける再感染の状況を把握し，それに関与する要因を見いだすことは，より効果的な集団治療の方法の研究に大いに役立つものと思われる。著者らはビルハルツ住血吸虫症の対策研究を，ケニア共和国コースト州Kwale地区で行っている。今回Kwale地区のある村でプラジカンテル（PZQ）を用いて選択的集団治療を行い，治療後の住民の感染の変化を1年間にわたり観察した。本研究はこの村に於ける再感染の特徴を把握することを目的として，これまで得られたデータを解析したものである。

材料と方法

調査場所と人口：

この研究は，ビルハルツ住血吸虫の濃厚な流行地として知られている，ケニア共和国Kwale地区のMwachinga村で行った。この村の住血吸虫症の流行の様子は既に発表され（Shimada *et al.*, 1987），抑制対策としてメトリフォネイト（1984年2，3月）による治療と水道水の供与が実施されている。そのため，本研究の行われた時点での住民の感染率，感染の強さ，血尿の頻度，伝播地の中間宿主貝の感染率はプロジェクト開始時より幾分減少していた（Sato *et al.*, 1988; Noda *et al.*, 1988）。1986年のセンサスで登録された住民の数は1,491名（男性681名，女性810名）であった。そしてこれらの人々の内，約半数の人は15歳未満の子供であった。

集団治療：

PZQによる集団治療は，1986年7，8月に実施した。投薬予定者は1986年6，7月の検尿による虫卵陽性者と5-15歳の子供全員であった。しかし実際には612名の虫卵陽性者では，その内の515名（84.2%）にしか投薬できなかった。5-15歳の子供は全員投薬を受けた。PZQは体重当たり40 mg/kg投与した。

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Table 1 Change in the prevalence and intensity of infection and frequency of gross haematuria of urinary schistosomiasis before and after selective mass chemotherapy with praziquantel

Urine examination	Before treatment	After treatment	
		3 months	1 year
S. heamatobium eggs			
No. of examined	868	533	676
No. of infected	445	89	211
Prevalence (%)	51.2†	16.7†	31.2†
Intensity (Eggs/hr *, Mean±SD)	1.10±1.28‡	0.26±0.72‡	0.58±1.01‡
Gross haematuria			
No. of examined	857	530	668
No. of with haematuria	104	13	28
Frequency (%)	12.1§	2.5§	4.2§

* A $\log_{10}$ (n+1) transformation was used for calculating the mean (Mean) and standard deviation (SD).
Prevalence and intensity of infection and frequence of gross haematuria 3 month and 1 year after treatment were significantly lower than those before treatment. † Chi-squared test ($\chi^2=181{,}000$, Df=2, $P<0.001$), ‡ F-test ($P<0.01$), § Chi-squared test ($\chi^2=59.160$, Df=2, $P<0.001$).

検尿：

治療前（1986年6，7月），治療3カ月後（1986年10，11月）および1年後（1987年7月）に全村民を対象に検尿を実施した。検尿の方法および感染の強さの表わし方（排泄虫卵数/時間）は，Shimada *et al.* (1986, 1989a) に準じ，血尿の判定は肉眼によった。

統計学的差の検定：

治療前後の感染の変化は，各時期の感染率および平均排泄虫卵数（感染の強さ：対数変換，排泄虫卵数0を含む）で示した。尚，再感染の解析における感染の強さは，再感染者のみの排泄虫卵数（排泄虫卵数0は含まない）を対数変換後平均値を求めて表わした。集団間の感染率はχ^2検定，また感染の強さは平均値の差をF検定により比較した（大崎，1984）。

結　果

部落全体の感染率および感染の強さの変化

治療前に868名，治療3カ月後に533名および1年後に676名について尿検査を実施し，受検率はそれぞれ58.2%，35.7%，45.3%であった。各検査時における感染率，感染の強さ，血尿の頻度を表1に示した。感染率，感染の強さ，血尿の頻度，全てはPZQの治療により急激に減少した。しかし，1年後には感染率は治療前の61%，感染の強さは53%，血尿の頻度は35%にまで増加した。これらの事から治療1年後まではこの村において，感染の元への復帰は見ないものの，伝播は継続しているものと考えられた。

治療を受けた住民の感染率，感染の強さの変化

治療前，治療3カ月および1年後の計3回の全ての検査と，PZQ投与による治療を受けた住民199名を対象に，治療後の感染の変化を解析した。これらの人の治療前から，治療1年後にかけての

感染の変化を表2に表わした。即ち，治療前の検査で虫卵陽性を示した人は151名で，その内，125名がPZQ投与により3カ月後に虫卵陰性となった。しかしながら，この虫卵陰性は一部の人たちでは持続せず，38名は1年後の検査で虫卵の排泄が見られた。一方，PZQにて虫卵陰性にならなかった者は，26名であった。その内11名は1年後の検査で虫卵陰性となり，他の15名は，1年後でも虫卵の排泄が確認された。残りの48名は，治療前の検査で虫卵陰性を示していたが，その内8名（16.7%）には1年後に虫卵の排泄が見られた。治療前，虫卵陽性でPZQ投与により虫卵陰性となった125名は，PZQにより治癒したと考えると，その内1年後に陽性となった38名（30.4%）は再感染を受けたことになる。そこで，再感染を受け易い要因を解析する一手段として，これら125名を対象に性，年齢，治療前の感染の強さの3因子と再感染の関係を検討した（表3）。

性：再感染率および再感染者の治療前の感染の強さには，男女間に差は見られなかった。しかし，再感染の強さは，男性が女性よりも有意に高かった。

Table 2 Change of *S. haematobium* infection among before, 3 months and 1 year afer treatment with praziquantel

Result of examination for *S. haematobium* eggs			No. of subjects
Before treatment	After treatment		
	3 months	1 year	
+	−	−	87
+	−	+	38
+	+	+	15
+	+	−	11
−	−	+	8
−	−	−	40
Total			199*

* The villagers attending the three round urine examinations and being treated with praziquantel, were selected for this analysis.

年齢：年齢層別に分けて見ると，年齢によって再感染率は明らかに異なっている。20歳以上では再感染する人の率は低いが，10-14歳の年齢では

Table 3 Reinfection of *S. haematobium* 1 year after treatment

Groups	No. of subject	Reinfected villagers			
		No.	Prevalence (%)	Mean egg counts (Mean±SD)	
				Before	1 year after
All	125*	38	30.4	2.500±0.889	1.831±1.041
Sexes					
Male	59	19	32.2	2.624±0.790	2.204±1.123‡
Female	66	19	28.8	2.370±0.982	1.457±0.818‡
Ages					
≦9	25	5	20.0†	2.208±0.978	2.308±1.055
10—14	50	24	48.0†	2.576±0.832§	1.842±0.975
15—19	22	5	22.7†	3.143±0.455‖	2.112±1.261
≧20	28	4	14.2†	1.570±0.923§‖	0.819±0.770

* The villagers showing both egg positive before and egg negative 3 months after treatment in Table 2 (+ to −), were selected for this analysis.

A $\log_{10}(n+1)$ transformation was used for calculating the mean (Mean) and standard deviation (SD).

There were significant differences, † Chi-squared test ($\chi^2=12.643$, Df=3, P=0.005), ‡; §; ‖ F=test ($P<0.05$).

48.0%の者が再感染を受けていた。治療前の感染の強さには，年齢による差異が観察されたが，治療1年後の感染（再感染）の強さでは年齢による差は見られず，ほぼ同様の値が示された。

治療前の感染の強さ：再感染率が年齢により異なることから，治療前の感染の強さと，易再感染性との関係の解析は年齢による違いを除外して行う必要がある。このため，再感染観察対象者125名のうち10-19歳に属する72名を選び，治療前の感染の強さにより3グループ—L（＞0，＜100），M（≧100，＜1,000），H（≧1,000）—に分け，各グループの再感染状況を観察した（図1）。これらのグループの平均年齢は13.4±2.7歳（L），13.2±1.1歳（M）および14.0±2.3歳（H）を示し，それら

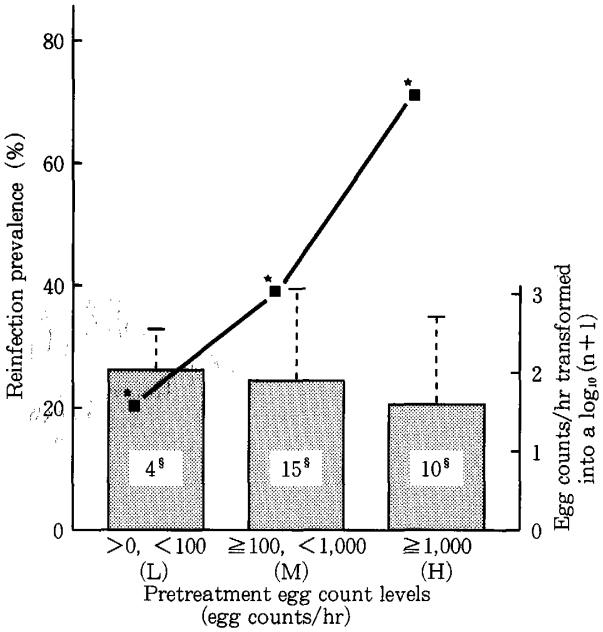

Figure 1 Distribution of reinfection prevalence and intensity in reinfected villagers aged 10-19 years 1 year after treatment, according to pretreatment egg count levels.

■—■ , Prevalence of reinfection

T , Mean egg counts in reinfected villagers (Mean and standard deviation)

§ No. of samples used for calculating the mean and standard deviation

There were statistical differences among the reinfection prevalences, * chi-squared test ($\chi^2=9.007$, Df=2, P=0.001)

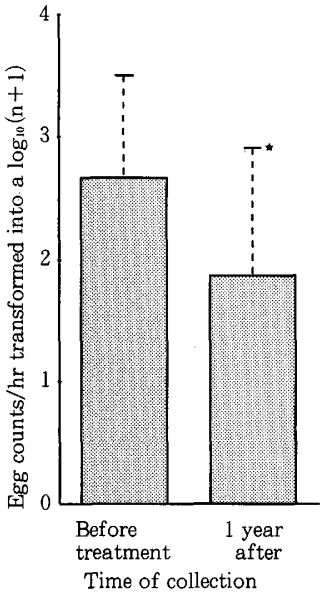

Figure 2 Egg counts/hr in 29 reinfected villagers aged 10-19 years before and 1 year after treatment.

T Mean and standard deviation

Egg counts 1 year after were significantly lower than that before treatment, *F-test (P<0.01)

の間には統計学的有意の差は見られないことから（P＞0.05，F検定），グループ間の比較において，年齢に依存する偏りは除外し得たと考えられる。再感染率は治療前の感染の強さに依存して大きく，治療前に感染の強さが一番高いH群では71.4%の人に再感染が起こっていた。しかし，再感染の強さは治療前の強さに関係なく，各群ほぼ同様であった。図2は10-19歳に属する再感染者の治療前の感染の強さと，再感染の感染の強さを比較したものである。両者の間には有意の差が見られ，1年後に於ける再感染の強さは治療前の強さまでには達していないことが明らかとなった。

考　察

ビルハルツ住血吸虫症のコントロールをめざして，駆虫薬を用いて集団治療を行った場合，しばしば再感染が問題になってくる。再感染を起こす

要因はいくつか報告されているが（Bensted-Smith *et al*, 1987; Tinglery *et al,* 1988; Wilkins, 1989; Etard *et al*., 1990; Chandiwana *et al*., 1991），それらの要因は各流行地によって異なるとされている。本研究で，我々はケニアのビルハルツ住血吸虫症流行地で行った選択的集団治療（PZQ 使用）の結果を解析し，集団治療後住民がどのように再感染を受けているかを追求してみた。住民の協力程度が比較的低い我々の調査地でも（受検率46.4%，受治療率84.2%），PZQ はその優れた治療効果により，部落全体の感染率を一時低下させたが，治療後1年たつと感染率は再び上昇した。我々の調査地では住民の新感染，再感染の危険性は高く維持されていることが解る。

そこで我々は，調査地でどのような住民が再感染を受け易いかを解析した。まず，治療前，治療後3カ月，1年の検尿および治療を受けた住民199名について，尿中の排泄虫卵数の変化に基づき治療後の感染の変化を解析した。その結果，治療により治癒し（寄生虫学的治癒：尿中虫卵陰転），1年後に再感染するものが30.4%あることが明らかとなった。そこで我々の調査地に於ける易再感染性を性，年齢，治療前の感染の強さについて調べた結果，1）10-14歳の子供が再感染を受け易いこと，2）治療前において排泄虫卵数が多いグループほど，再感染を起こす人が多いことが観察された。尚，性別に於て，男性が強く再感染するとの結果を得ているが（表3），これは男性と女性の年齢構成の違いにより生じたもので，同一年齢群（19歳以下）で比較すると，両者の間で差異は見い出されなかった（男性，2.20±1.12；女性，1.62±0.76）。この結果はビルハルツ住血吸虫の易感染性は，年齢と治療前の感染の強さによって決まるとした，Etard *et al*.（1990）の結果と一致する。

治療後の再感染は，水との接触レベルと獲得免疫による感染防御効果に左右されると考えられている（Wilkins, 1989）。この免疫による再感染防御効果は，既にマンソン住血吸虫およびビルハルツ住血吸虫の集団治療後の再感染に於いて，再感染抵抗者が出現することで示されており，10歳を過ぎると抵抗性が強まる傾向があるとしている（Butterworth *et al*., 1985; Wilkins *et al*., 1987）。しかし，我々の結果ではこれと異なり，10-14歳群の感染率は9歳以下の群の2倍以上を示した。我々の調査地では，子供が頻回に水と接触し，それが数年間蓄積され，感染の強さの年齢分布として現われることは既に知られており（Shimada *et al*., 1987, 1989b），男性では10-14歳で，女性では5-9歳で水との接触が最も激しい。治療後の再感染に於いては，防御免疫よりも水との接触行動が大きな役割を果たしていると考えられる。

我々の調査地では，水道水を対策方法として導入し，住民の水との接触頻度は全体的には半減した事が観察されている（Sato *et al*., 1987）。しかし，今回の PZQ 投与前後では住民の水との接触行動は，あまり変化していない状態にあった。従って，治療前の感染が強かった人達は，それまで水との接触量が多かったことを意味し，この人達は治療後も同様に接触量を変えることなく，汚水との接触を行うため，再感染が高い頻度で起こったものと考えられる。我々の結果では，治療前に高い感染の強さを示した者の治療後の易再感染性は，感染率では示されたものの感染の強さでは示されなかった。これは治療前虫卵排泄が多いものは再感染後も虫卵を多く排泄するとしたBensted-Smith *et al*.（1987）の結果とは異なる。この違いは対象となった住血吸虫の種の違いによるものか，計算方法が異なるためか，標本数の違いによるものか不明であるが，我々の結果は，治療前の感染の強さはそれまでの感染の積み重ねを表現しているのに対し，治療1年後の再感染の強さは，積み重ねがまだ少ないことを示していると考えてよい。

本研究は，再感染し易い住民の特性を明らかにした。より効果的なコントロールを行うためには，これらの人々への主として水との接触行動習慣を変更すべき衛生教育を，導入することが必要である。また一方，治療方法では，Bensted-Smith *et al*.（1987）や Tingley *et al*.（1988）がマンソン住血吸虫症において，治療前に感染の強い人は再感染に対する素因があるとし，Targeted treatment を行う対象者として選択したように，我々

の調査地に於いても，集団治療を行った後，前述した易再感染性を持つ住民に対しては，早期にTargeted treatmentを行えば，再感染を低く抑制することが出来るものと考えられる。

結　論

1986年7-8月に，ケニア共和国コースト州Mwachinga村に於いてプラジカンテル（PZQ）を用いてビルハルツ住血吸虫症の選択的集団治療を実施した。集団治療により部落全体の感染率および感染の強さは大幅に低下したが（治療前の32.6%および23.4%），1年後には住民の感染率および感染の強さは治療前の60.9%，および52.7%まで上昇し，新感染および再感染が継続して起こっていることが示された。PZQで治癒したものの内，30.4%が1年以内に再感染した。再感染者の大部分は19歳以下の子供であり，特に10-14歳の年齢群に於いては，他の年齢群の子供に比し，2倍以上の再感染が示された。この再感染は，治療前の感染の強さが高いグループほどよく起こっていた。再感染した人たちの再感染の強さは治療前の感染の強さよりも低く，また年齢，および治療前の感染の強さによる差異は観察されなかった。

我々は先の研究で（Shimada *et al.*, 1987, 1989b），子供達は汚水とよく接触し，そして水との接触量が感染率，およびその強さを反映することを明らかにしている。そして，また本研究の結果は，我々の調査地の人々の水との接触行動を反映していた。

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SCHISTOSOMA HAEMATOBIUM REINFECTION OCCURRED SHORTLY AFTER TREATMENT WITH PRAZIQUANTEL

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In Mwachinga Village, Kwale district, Kenya, selective mass chemotherapy (SMC) of schistosomiasis haematobium with praziquantel (PZQ) was conducted from July 1986 through August of the same year. "SMC" caused a marked reduction in the overall prevalence (32.6%) and intensity (23.4%) of infection of the pretreatment level three months later. However, prevalence and intensity of infection rose again to 60.9% and 52.7% of their respective levels one year after treatment.

Of individuals cured by "PZQ", 30.4% became reinfected with *S. haematobium* within 1

year. Most of the reinfected individuals were children aged less than 19 years, especially, in the 10-14 years old age bracket. The prevalence of reinfection was two times higher than that in the other children. The reinfection occurred frequently in individuals who showed heavy infection before treatment.

The egg counts in reinfected individuals were lower than their pretreatment levels and didn't differ by sex, age nor pretreatment egg count levels.

Our previous communication (Shimada *et al.*, 1987, 1989b) had been revealed that children were frequently in contact with infested water and levels of water contact reflected a prevalence and intensity of infection in our study population.

The results of the present study were probably again reflected by human water contact in our study area.

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Short communication

TWO CASES OF HUMAN INFECTION WITH *BERTIELLA STUDERI* IN NORTH SUMATRA, INDONESIA

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Bertiella studeri, a cyclophyilidean tapeworm of the family Anoplocephalidae is commonly found in the intestine of the monkey and other primates in Asia, Europe, Africa and Puerto Rico (Yamaguti, 1959). The life cycle of this tapeworm was elucidated with mites as the experienced intermediate host (Stunkard, 1940), and human infection was believed to occur by accidental ingestion of mites containing cysticercoid larvae of this tapeworm. Although human infections have been reported from many parts of Asia and Africa (Faust *et al.*, 1971), the present cases are the 11th and 12th human infections with *B. studeri* reported from Indonesia.

Case 1: A 3-year-old female from Langkat district, Sumatra, Indonesia, passed out a piece of white tapeworm with her stools, and after fixation with formalin solution this tapeworm was sent from the Langkat Health Center to us for identification. However, her mother and doctor did not explain to the clinical symptoms and the results of clinical examination tests.

Case 2: Case 2 is an adult male living in Medan, North Sumatra, Indonesia. However, data from the clinical examination and symptoms in this case was not reported by the doctor. A specimen was merely sent to us for identification.

Upon the examination, the white specimens that were brought to the authors were apparently parts of tapeworms without scolex. The tapeworms measured 0.8 mm (1st case) and 0.76 mm (2nd case) in length, and 5 and 10 mm in width respectively.

Each proglottid owing to the transverse- and flatten-section (Figs. 1 and 2) of the tapeworm was found to be wider than long (1st case 7.98×0.61 mm; 2nd case 7.63×1.00 mm). There was a single set of reproductive organs per proglottid (Fig. 1).

In mature proglottids, the many follicular testes were seen distributed in the anterior part and almost the whole width of the proglottid. The genital pores alternated irregularly. The cirrus sac was a strong muscular organ, cylindrical or spindle-shaped containing a narrow canale cirrus. The ovary could not observed clearly in the section with proglottids. The funnel shaped vagina was found to be weakly developed. The uterus was a single transverse tube having the appearance of outpocketings towards the anterior and posterior margins.

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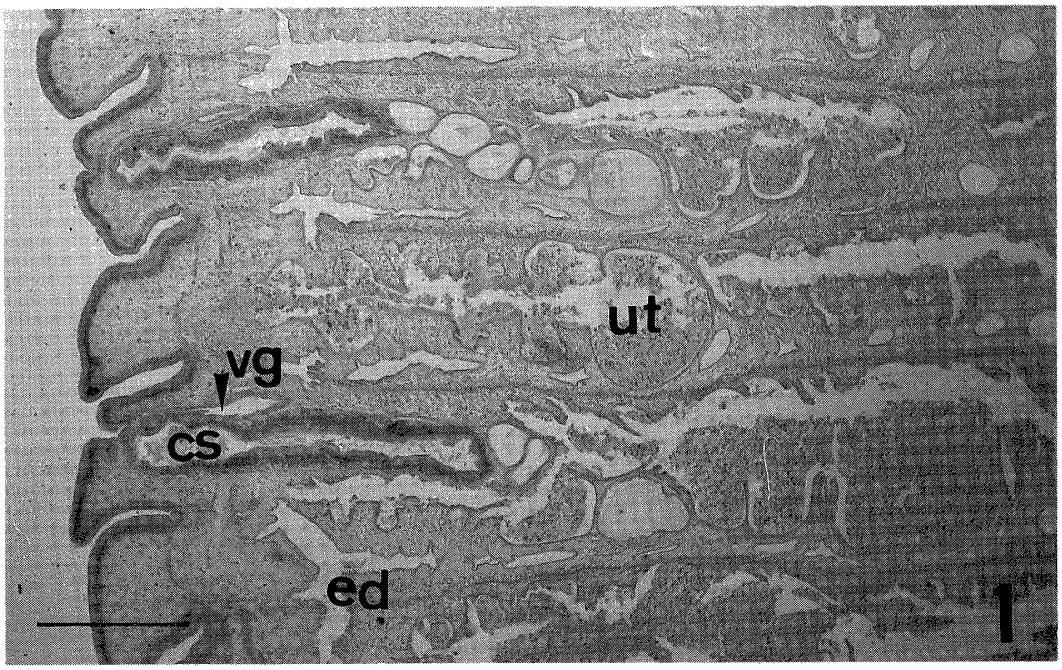

Figure 1 Flatten section of the proglottid of *B. studeri* (Scale bar: 0.46 mm)

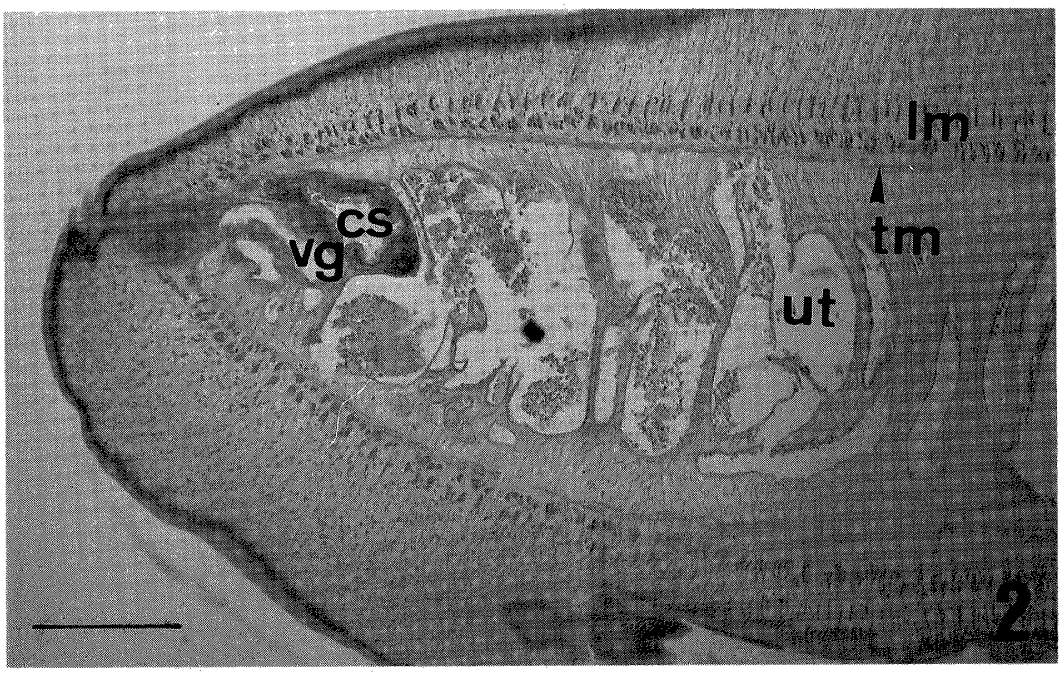

Figure 2 Transverse section near posterior border of the proglottid of *B. studeri* (Scale bar: 0.46 mm).
Abbreviations: cs: cirrus sac; ed: excretory duct; lm: longitudinal muscle bundles; tm: transverse muscles; ut: uterus; vg: vagina.

Eggs in the uterus were (33-41) × (36-41) μm.

About 22 species of Genus *Bertiella* have been reported (Yamaguti, 1959), but only two species, *B. studeri* and *B. mucronata* are known to infect man (D' Lessandro *et al.*, 1963). *B. studeri* differs from *B. mucronata* in possessing the following features: strongly developed muscular cirrus sac and weakly developed funnel shaped vagina. Accordingly these present tapeworm should be therefore identified as proglottids of *B. studeri*.

B. studeri is not commonly encountered in man, as evidented by only 47 cases have been documented by Bandyopadyay and Manna (1987). The first report of human infection from Indonesia was in 1931 by Joyeux and Dollfus. After that the additional nine cases were recorded (Bonne, 1944; Lie Kian Joe, 1961; Kwo and Koh, 1969). Although the pathogenicity of *B. studeri* in the current patients could not be determined, a few researchers (Richard-Lenoble *et al*, 1986; Bandyopadhyay and Manna, 1987) reported that patients suffered from various symptoms, including abdominal pain, loose bowels movements, and loss of body weight.

B. studeri, for which human infections have been reported by many authors, is primarily a primate parasite. There is a close association with infecting agents (free-living mites) which carry the cysticercoid stage of the parasite. Actually, the patients in this study told us that people in the village were breeding monkeys or apes. These breeding sites may be a possible source of infection.

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インドネシア，北スマトラにおける条虫 *Bertiella studeri* の人体感染の2症例

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本来は猿やその他の霊長類の腸管内に寄生する条虫，*Bertiella studeri*（Anoplocephalidae）の北スマトラにおける人体感染の2症例を見出し，インドネシアにおける第11例目（3歳，女性）と第12例目（成人男性）として報告した。

共に詳細な病歴は不明であったが，排出虫体の形態によって *B. studeri* と同定された。現在，世界的には総計47例の本虫感染例報告のある中で，インドネシアにおける本虫感染例が，極めて目立って多い事が解った。

本虫の感染は自由生活性のダニ類の経口摂取にある所から，人体への感染は偶然の機会に行われ，その感染予防の為には，終宿主となる猿類を椰子の実取り等の労働に使用したり，ペットとして飼育する際に十分な注意を行う事が，最も重要と考えられた。

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Short communication

CURRENT STATUS OF *STRONGYLOIDES* INFECTION IN OKINAWA, JAPAN

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Okinawa Prefecture is located in the southwestern part of Japan, belonging to the subtropical zone. It has been known as one of the areas with high prevalence of *Strongyloides stercoralis* infection (Asato *et al.*, 1990; Sato, 1985). Okinawa has also been recognized as an endemic area of Human T-cell Lymphotropic Virus 1 (HTLV-1) infection (Kohakura *et al.*, 1986a, b), and *S. stercoralis* has attracted attentions as an important opportunistic pathogen in adult T-cell leukemia patients (Nakada *et al.*, 1984). However, the incidence of fatal dissemination among *Strongyloides* carriers has not been yet known. In order to obtain basic data for estimation of the incidence of fatal case, the prevalence of *Strongyloides* infection has been surveyed in almost all regions of Okinawa since 1988. This paper presents the results of the surveys and a distribution map of *Strongyloides* infection.

Twenty three farming localities were surveyed: 15 localities on Okinawa Island, 5 in Miyako Islands; 3 in Yaeyama Islands. Fecal samples from 3,943 inhabitants of aged under 40 years old (1,780 males and 2,163 females) and 23,816 inhabitants aged over 40 years old (9,661 males and 14,155 females), were examined using the agar-plate culture (Arakaki *et al.*, 1988, 1990).

The overall prevalence of *Strongyloides* infection by sex and age is shown in Table 1. Prevalence of *Strongyloides* infection was 16.0% in males and 7.7% in females. Among the inhabitants aged over 40 who were born before the end of the World War II mostly, the prevalence was 18.7% in males and 8.8% in females. However, the prevalence in the age groups under 40 years was only 1.6% in males and 0.8% in females. The prevalence recorded in the presest survey was definitely higher than those in previous studies (Asato *et al.*, 1990), confirming that the agar-plate culture is highly sensitive compared with the traditional methods (Arakaki *et al.*, 1988, 1990). The low prevalence in the younger age groups and significant sex difference have been noticed in all surveyed areas. As has been already discussed (Asato *et al.*, 1990), the aged people apparently acquired the infection during or

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Table 1 Prevalence of *Strongyloides* infection among the inhabitants of Okinawa, Japan, by sex and age

Age	Male		Female	
7-19	2/221	(0.9)	1/155	(0.7)
20-29	4/373	(1.1)	2/442	(0.5)
30-39	22/1,186	(1.9)	14/1,566	(0.9)
40-49	126/1,329	(9.5)	59/1,889	(3.1)
50-59	459/2,450	(18.7)	321/3,674	(8.7)
60-69	685/3,182	(21.5)	425/4,630	(9.2)
70-79	430/2,067	(20.8)	327/2,971	(11.0)
80-	106/633	(16.8)	113/991	(11.4)
Total	1,834/11,441	(16.0)	1,262/16,318	(7.7)

No. positive/No. examined(%)

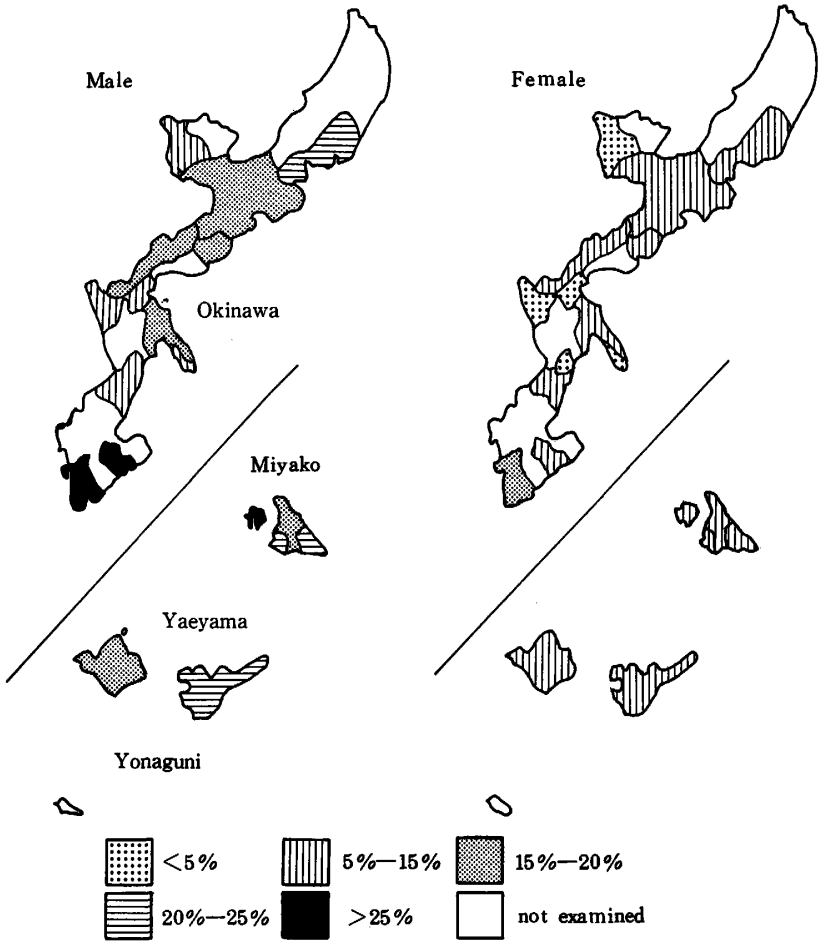

Figure 1 Prevalence of *Strongyloides* infection among the inhabitants aged over 40 in Okinawa, Japan.

immediately after the World War II when the environmental sanitary was very poor (Asato *et al.*, 1990).

The prevalence among the inhabitants aged over 40 years old by sex in surveyed localities is shown in Table 2 and Fig. 1. Males on Miyako Island, the southern part of Okinawa Island and Yonaguni Island, were highly infected. The southern part of Okinawa Island showed the highest prevalence among females. The middle part of Okinawa Island was low endemic. A marked difference in prevalence rate was observed between neighboring areas in the northern part of Okinawa Island. There are no reasonable explanations on the difference in the prevalence among the localities. It has been once supposed that the ground battles fought on Okinawa Island during the war resulted in high prevalence of *Strongyloides* infection. However, it was not the case because Miyako and Yaeyama Islands, where ground battle was not existed, had also high prevalence. It probable that some difference in farming style was one of the major factors which determined the prevalence. Unfortunately, no

Table 2 Regional prevalence of *Strongyloides* infection among the inhabitants aged over 40 years by sex

Localities	Male		Female	
Okinawa Island				
Northern Part				
Higashi	46/217	(21.2)	30/264	(11.4)
Motobu	17/211	(8.1)	14/305	(4.6)
Nago	203/1,250	(16.2)	109/1,654	(6.6)
Ginoza	44/274	(16.1)	36/392	(9.2)
Onna	35/211	(16.6)	28/316	(8.9)
Middle Part				
Ishikawa	32/338	(9.5)	22/611	(3.6)
Yomitan	100/951	(10.5)	73/1,647	(4.4)
Gushikawa	24/125	(19.2)	18/213	(8.5)
Katsuren	19/210	(9.1)	15/313	(4.8)
Kitanaka	17/247	(6.9)	13/443	(2.9)
Nakagusuku	4/40	(10.0)	6/52	(11.5)
Ginowan	4/47	(8.5)	8/89	(9.0)
Southern Part				
Tamagusuku	76/363	(29.9)	63/494	(12.8)
Ohzato	63/233	(27.0)	46/380	(12.1)
Itoman	171/623	(27.5)	152/848	(17.9)
Miyako Islands				
Gusukube	164/743	(22.1)	98/1,039	(9.4)
Shimoji	57/231	(24.7)	43/357	(12.0)
Ueno	42/255	(16.5)	26/347	(7.5)
Irabu	35/103	(34.0)	13/186	(7.0)
Hirara	51/257	(19.8)	38/428	(8.9)
Yaeyama Islands				
Ishigaki	456/2,056	(22.2)	308/2,972	(10.4)
Taketomi	85/465	(18.3)	51/548	(9.3)
Yonaguni	61/211	(28.9)	35/257	(13.6)

No. positive/No. examined(%)

detailed information on farming before 1960 exist. Some researchers claimed an association between strongyloidiasis and HTLV-1 infection (Nakada *et al.*, 1984). However, the present epidemiological data do not support this assumption, at least in some location. For example, *Strongyloides* infection is highly endemic on Miyako Island (Table 1, Fig. 1), where HTLV-1 infection is considerably rare (Kohakura *et al.*, 1986a, b). Moreover, on Okinawa Island the prevalence of *Strongyloides* infection is rather higher in northern part than in middle part, whereas HTLV-1 infection shows a reversed situation (Kohakura *et al.*, 1986a, b)

The detection of *S. stercoralis* from feces is often difficult (Grove, 1989). It has been known that even with the agar-plate culture, about 20% of carriers may be overlooked (Arakaki *et al.*, 1990). If the feces of several consecutive days are examined by the agar-plate culture or if some immunological diagnostic methods are applied in addition, the prevalence may become slightly higher than the present survey. From the present results, the number of *Strongyloides* carriers in Okinawa is estimated approximately to be about thirty thousands. Further surveys should be carried out to evaluate accurately the risk of fatal dissemination, and to decide whether a mass treatment is really necessary for all of the carriers of which majority are asymptomatic or apparently healthy.

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沖縄県における糞線虫感染の現状

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沖縄県のほぼ全域における糞線虫感染の状況を，寒天平板法で調べた。全調査地域の感染率は40歳未満の年齢層では男性1.6%，女性0.8%であるが，40歳以上では男性18.7%，女性10.0%であった。若年齢層に少なく，性別では男性に多い傾向は全ての地域で認められた。40歳以上の男女の地域別の感染率は男性では6.9%-29.9%，女性では2.9%-17.9%と明らかな地域差があった。今回の調査結果から，沖縄県内の糞線虫保有者は約3万人と推定された。糞線虫保有者の大多数を占める無症状者に対して，どのような対処が実際に必要であるかについては，今後の研究課題である。

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Short communications

FURROW-LIKE IMAGES MADE BY SOME MICROORGANISMS ON THE SURFACE OF AGAR-PLATE FOR *STRONGYLOIDES* DETECTION

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The agar-plate culture has been applied for the detection of *Strongyloides stercoralis* from human feces (Arakaki *et al.*, 1988, 1990). In this technique, the presence of *S. stercoralis* is easily known by the furrows left by its larvae on the agar surface (Fig. 1). This technique has been proved to be highly effective by other researchers too (Koga *et al.*, 1990, 1991). It is necessary to distinguish the furrows by *S. stercoralis* larvae from those by other nematodes such as hookworms and free-living rhabditids. We have already reported that the furrows formed by *Necator americanus* larvae were readily distinguished from those by *S. stercoralis* (Arakaki *et al.*, 1990).

On the routine examinations in two hospitals of Okinawa, Japan, we recently experienced that two kinds of microorganisms from human feces made furrows-like images on the agar-plate. In this paper, their characteristics are reported in order to contribute the differential diagnosis of strongyloidiasis by the agar-plate culture.

Smooth rotating tracks were noticed on the agar surface in the 2nd day of stool cultures (2 cases at outpatient department) (Fig. 2). At the end of the double-line tracks, the wandering mass was found. The mass was revealed to be a moving bacterial colony (Fig. 3). The direction of rotation of these colonies observed were clockwise or counterclockwise. The track made by the rotating colony was not a real furrow, but was parallel lines of bacteria left behind. The bacteria were demonstrated to be gram negative rods in one case and gram positive rods in another case. Gram positive rod was identified a *Bacillus* spp., but genus of the gram negative rods has not been identified. A gram positive rod such as *Bacillus circulans* and some gram negative rods have been known to form motile colonies on the agar plate as in the present case (Muto, 1904; Smith and Clark, 1938; Turner and Eales, 1941; Fuller and Norman, 1943), although the mechanism of the movement has not been clarified yet.

A lot of dots around the edge of the stool were found on the agar surface in 2 days of stool cultures from three outpatients (Fig. 4). Irregular tracks with rough contours were found on

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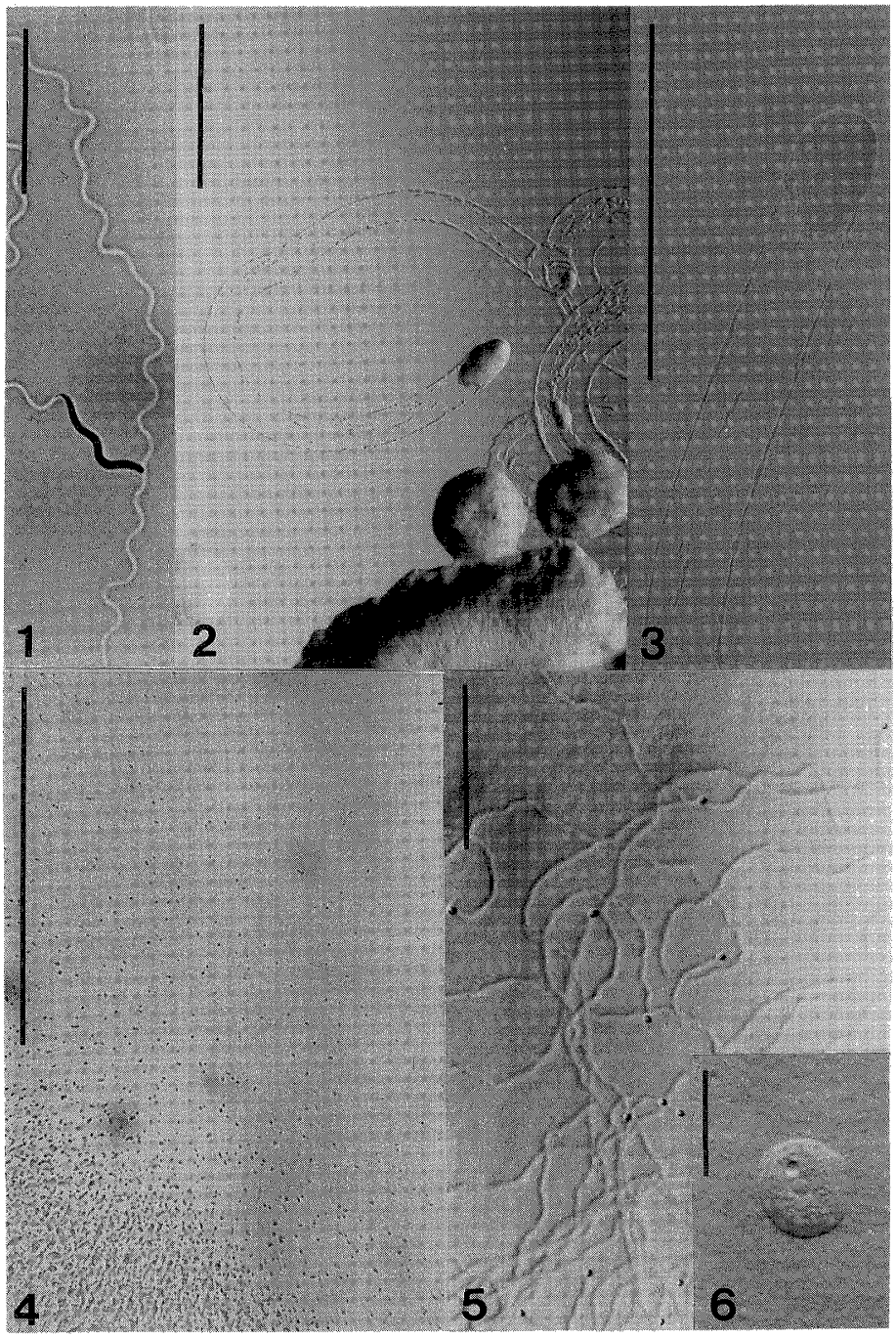

Figure 1 Furrows left on agar surface by *Strongyloides* larvae.
Figure 2 Double-line track left by moving bacterial colony.
The colony consists of gram positive rods. Motility of the colony was confirmed under microscopical observation.
Figure 3 High magnification of Fig. 2.
Figure 4 Amoebae (look like a lot of dots) coming out from the stool mass on the agar-plate.
Figure 5 Furrows left by amoebae. The amoebae (black dots) were seen at the end of furrows.
Figure 6 Amoeba observed under the differential interference microscopy.
Scale bar: Figs. 1-4; 0.5 mm, Fig. 5; 0.1 mm, Fig. 6; 0.02 mm

the agar surface, and at the end of each track a minute protozoan was observed (Fig. 5). Higher magnification revealed that the protozoan had a nucleus and moved with pseudopod (Fig. 6). It has been well documented that non-pathogenic free-living amoebae are occasionally contaminated in human stools (Faust *et al.*, 1970; Thompson and Robertson, 1929). Although no further identification has not been made, the present protozoan was presumably a free-living species.

The tracks by both microorganisms were easily distinguished from those by *S. stercoralis* larvae. However, attentions should be paid on the fact that non-nematode microorganisms make furrow-like image on the agar-plate culture of human feces.

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寒天平板法による糞線虫の検出
—寒天平板上に見られる様々な遊走痕について—

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糞線虫検査の際に，寒天平板上に見られた様々な遊走痕について報告した。それらは細菌の運動性コロニー，および自由生活性アメーバの栄養型によるものであった。運動性コロニーを有する細菌には，グラム陰性桿菌および陽性桿菌が認められ，コロニーは回転運動によって移動し，後に菌体が線状配列した2本の平行遊走痕を残した。自由生活性アメーバは偽足によって移動し，やや不規則な遊走痕を形成した。これらの遊走痕は，糞線虫成虫および幼虫のものとは，大きさや形で容易に区別することができた。このように人糞便内に混入する線虫類以外の微生物によっても，寒天平板上に遊走痕が形成されることがあるので，糞線虫検査にあたっては注意すべきものと考えられる。

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