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FINE STRUCTURE OF GAMONT OF *HEPATOZOON CANIS*

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Abstract: Gamont of *Hepatozoon canis* was observed by light, phase contrast and transmission electron microscopies. In a thin blood film the gamont was contained within a pale-blue capsule in the leukocyte. By phase contrast microscopy, the gamont was seen to be surrounded by a contrasty substance. Transmission electron microscopy revealed the presence of a parasitophorous vacuolar membrane, two layers in the pellicle of the gamont and a fine fibril-like structure surrounding the parasitophorous vacuole.

INTRODUCTION

Hepatozoon canis infection is a protozoan parasitic disease of dogs found worldwide. The various host animals have been infected with *Hepatozoon* spp. and a human case of *Hepatozoon* has been reported in the Torrid Zone (Craig, 1990). *H. canis* infection occurs mainly in India, South Africa, Nigeria, Israel, the Philippines, Malaysia, North America and Japan (Bentley, 1905; McCully *et al.*, 1975; Ogunkoya *et al.*, 1981; Elias and Homans, 1988; Carlos *et al.*, 1971; Rajamanickam *et al.*, 1984/85; Craig *et al.*, 1978; Murata *et al.*, 1991).

Little information is available concerning the fine structure of the gamont of *H. canis*. We therefore attempted to observe the gamont by light, phase contrast and transmission electron microscopies.

MATERIALS AND METHODS

A dog infected with *H. canis* in Fukuoka Prefecture was employed in this study. Thin blood films were prepared as follows. One milliliter of peripheral blood was mixed with 1 or 2 units of heparin sodium (Green Cross Co., Japan). The film was then fixed with methanol for 1 min and stained with Giemsa stain for 40 min. An unstained film was observed with a phase contrast microscope (Nikon Optiphoto, Tokyo).

Preparation for the transmission electron microscopy (TEM) was made as follows: the heparinized blood sample was centrifuged at 2,000 rpm for 10 min and the buffy coat was separated. The specimen was fixed with a mixture of 2.5% glutaraldehyde and 1% osmic acid and suspended in 4% agarose gelatin. The solidified specimen was dehydrated in a graded series of ethanol and embedded in Quetol 812 epoxy resin. The ultrathin sections were stained with uranyl acetate and lead citrate and examined in JEOL 1200EX electron microscope at 80 kV.

RESULTS

The blood films stained with Giemsa stain revealed that the gamont of *H. canis* had one nucleus dark blue in color in the leukocyte. A capsule with pale-blue color was seen to invest the gamont (Fig. 1, arrow).

By phase contrast microscopy a thick capsule was clearly observed to surround the gamont (Fig. 2, arrow).

TEM of the present examination showed that the gamont contained within a parasitophorous vacuole (PV) possessed one nucleus, apical complex, electrondense and spindle-shaped micronemes, electrondense microbodies and ovoid granules. A thick electronlucid material was observed to invest the PV in the cytoplasm of the host leukocyte (Fig. 3a, arrow).

At high magnification, two layers were found in the

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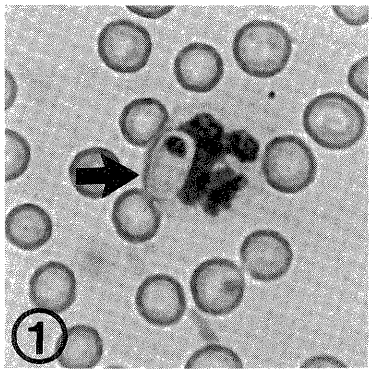

Figure 1 One nucleus of *H. canis* gamont can be seen in the capsule (arrow) in the leukocyte (×1,000, Giemsa stain).

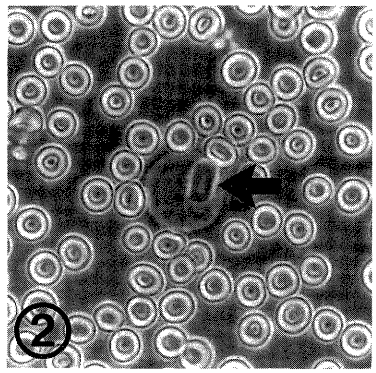

Figure 2 Phase contrast micrograph showing the capsule (arrow) investing the gamont (×400). The capsule forms a striking contrast to the gamont.

pellicle of the gamont and a parasitophorous vacuolar membrane (PVM) was seen over them 50 nm in width (Fig. 3b). These two layers were found to be a thick outer layer and an inner complex of two closely apposed membranes. Subpellicular microtubules coming from the apical complex appeared to be seen beneath the layers. The PV including the gamont was surrounded by the fine fibril-like structure in the cytoplasm of the leukocyte.

Discussion

The present examination on the gamont of *H. canis* seen in the leukocyte showed notable characteristics. Phase contrast microscopy appeared to indicate that the contrasty thick material was situated around the gamont. Little information is available on the fine structures of the capsules surrounding the gamonts of *H. aegypti* and *H. mocassini* (Bashtar *et al.*, 1984; Nadler and Miller, 1985). According to Nadler and Miller (1985), the nonstaining capsule seen by light microscopy was considered to correspond to the electron-lucid space between the gamont pellicle of *H. mocassini* and the PVM. The ultrastructures of the gamonts of *Haemogregarina magna, Haemogregarina adeleine* and *Babesiosoma stableri* are well known, but TEM has not shown the existence of a distinct structure around the PV (Paterson *et al.*, 1988; Baker and Lainson, 1967; Barta and Desser, 1986). Therefore, in this observation, the fine fibril-like structure was found to be situated around the PV containing the gamont of *H. canis*; however, its role in pathogenesis is still unknown.

TEM of the present examination revealed that the pellicle of the gamont of *H. canis* consisted of the thick layer and the double membrane complex. The pellicle of the gamont of an adeleine haemogregarine was reported to show a double membrane complex that lacked a coated membrane (outer limiting unit membrane) and a plasmalemma (Baker and Lainson, 1967). The feature seems to be induced by poor fixation or other procedual differences, as mentioned by Nadlar and Miller (1985). Thus, we consider the thick layer and the double membrane complex in the gamont pellicle of *H. canis* to resemble the outer and the inner layers in that of *H. mocassini* (Nadler and Miller, 1985).

The gamont of *H. canis* possessed the nucleus, apical complex, micronemes, microbodies and ovoid granules, which appeared to be closely similar to those of *H. mocassini, Haemogregarina magna* and *Babesiosoma stableri* (Bashtar *et al.*, 1984; Nadler and Miller, 1985; Paterson *et al.*, 1988; Barta and Desser, 1986; Baker and Lainson, 1967).

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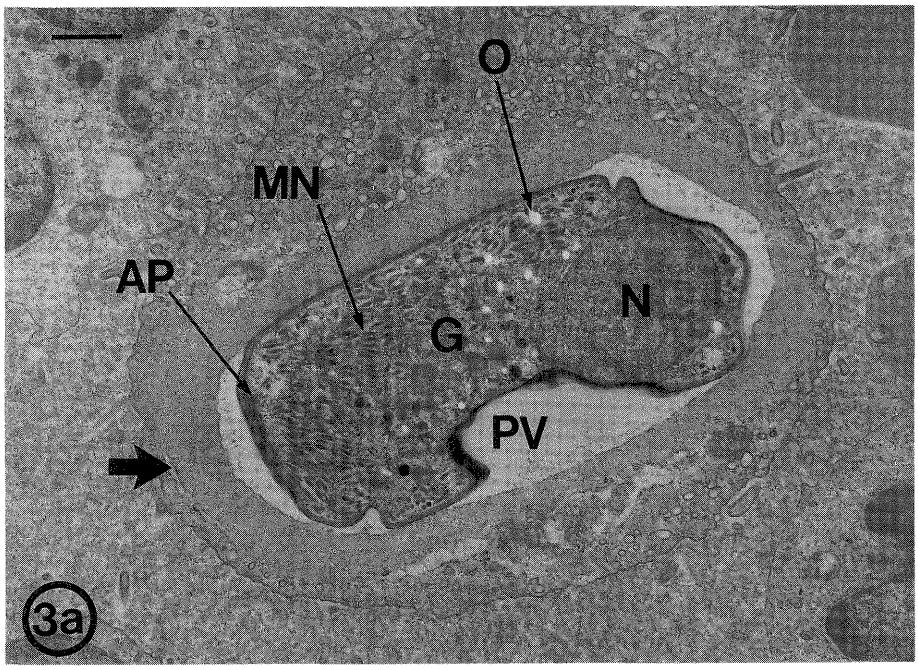

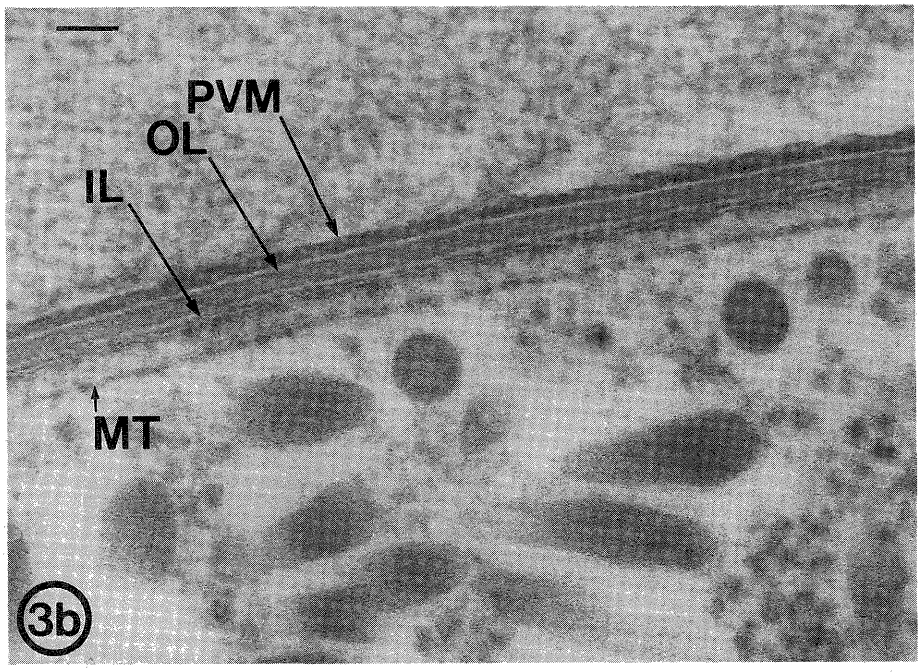

Figures 3a and 3b Transmission electron micrographs. (a): Gamont (G) showing one nucleus (N), micronemes (MN), microbodies, apical complex (AP) and ovoid granules (O) in the cytoplasm. The thick material (arrow) is seen around the PV containing the gamont (×6,000, Bar: 1 μm). PV parasitophorous vacuole (b): Two layers can be seen in the pellicle of the gamont (×100, 000, Bar: 50 nm). The PV is surrounded by a fibril-like structure. PVM parasitophorous vacuolar membrane; OL outer layer; IL inner layer; MT microtubules.

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Jpn. J. Trop. Med. Hyg., Vol. 21, No. 2, 1993, pp. 111-115

CAUSATIVE ORGANISMS OF ACUTE RESPIRATORY INFECTIONS IN NORTHERN THAILAND

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Abstract: We examined the causative organisms of respiratory infections from 1989 to 1990 at Mae Sot General Hospital in collaboration with Chiang Mai University, Thailand. We collected sputum from patient with acute bronchitis and pneumonia to identify the causative organisms by sputum culture and inflammatory sputum cytology. We experienced 72 cases (97 strains of bacteria) of acute broncitis and 17 cases of pneumonia (20 strains of bacteria) in Mae Sot General Hospital. The most frequently identified pathogens in respiratory infections were *Haemophilus influenzae, Streptococcus pneumoniae* and *Branhamella catarrhalis*. This study shows that Gram-stained smears and quantitative sputum culture together have a significant role in enhancing the diagnostic value of expectorated sputum specimens. The causative organisms between Thailand and Japan were very similar in acute respiratory infections. The most important outcome of this study was the detection of *Branhamella catarrhalis* in many adult cases with acute respiratory infection.

INTRODUCTION

It is well recognized that the majority of acute upper respiratory infections are caused by viral infections which are sometimes followed by bacterial infections. Bacterial infections cause complications of common cold: acute sinusitis, otitis media, acute bronchitis and pneumonia. It is sometimes dangerous for young children and aged people to catch cold because of various complications occur due to secondary bacterial infections. Respiratory infections cause much mortality and morbidity (Murray, 1982). This is true both for the developed and developing countries. From different hospitals of developed countries data on bacteria causing respiratory infections are regularly published. On the other hand, such data are not regularly obtained from developing countries. Moreover the foundation of chemotherapy of respiratory infections is first of all to determine pathogens (Matsumoto, 1963). Therefore it is important to study the bacterial pathogens causing respiratory infections. It is not known which pathogenic organisms predominate in Thailand. Therefore, this study was carried out to identify the causative organisms of respiratory infections in a rural district of Thailand. We examined the causative organisms of respiratory infections from 1989 to 1990 at Mae Sot General Hospital in collaboration with Chiang Mai University, Thailand. This type of studies are helpful to understand about the incidence of bacteria causing acute respiratory infection between the two countries, which has difference in climate, nutritional status, economy etc.

MATERIALS AND METHODS

Location: This study was carried out in Mae Sot General Hospital in collaboration with Chiang Mai University (Fig. 1). In Mae Sot, from November to February is winter, from March to May is summer and from June to October is rainy season. This investigation

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Figure 1 The location of Chiang Mai and Mae Sot, Thailand.

was done at three different time interval, i.e. from December 1989 to January 1990 (winter), from July to August 1990 (rainy) and from November to December 1990 (winter).

Collection of sputum samples: Fresh expectorated sputum, from patients with respiratory infections submitted to the Department of Internal Medicine, Mae Sot General Hospital, were used in this study.

Sputum smear: Gram staining and Acid fast staining were done of sputum smear to observe the inflammatory cytology and bacteria. The method for Gram staining of sputum specimens was based on Hucker's modification (Sonnenwirth, 1980). Ziehl-Neelsen's staining was done to recognize acid-fast bacilli (Cowan and Steel, 1974).

Sputum culture: Sputum was cultured either quantitatively or semi-quantitatively on TSA agar containing 7% human blood and on chocolate agar. Agar plates were incubated at 37°C for 18 hr in ordinary incubator. Bacteria were identified by standard methods (Cowan and Steel, 1974) at the Department of Microbiology, Chiang Mai University, Thailand.

Clinical studies: Our standardized criteria of respiratory infection with causative organisms are as follows; (1) Gram stain of sputum smear show plenty of polymorphoneuclear leukocytes or macrophages and dominant bacteria located intracellularly and extracellularly, (2) much growth of these bacteria in semi-quantitative culture or more than 10^7 CFU/m*l* in quantitative culture (Matsumoto *et al.*, 1978), (3) increase in number of these bacteria coincides with clinical and laboratory findings. Clinical data corresponding to the specific data of sputum collection were obtained from patient case records at Mae Sot General Hospital.

RESULTS

Time period: The first and third study period were in the winter and the second study period was in the rainy season.

Patients: There was a total 392 patients and their mean age was 43 yrs. A total 249 patients were male with a mean age of 43.4 yrs and 143 patients were female with a mean age of 42.5 yrs. Details of age, sex and number of patients are shown in Table 1.

Identification of bacterial pathogen: From a total 72 cases of acute bronchitis 97 strains of causative pathogenic bacteria were isolated. The main pathogenic bacteria were *Haemophilus influenzae* (*H. influenzae*) (44.5%), *Streptococcus pneumoniae* (*S. pneumoniae*) (26.8%) and *Branhamella catarrhalis* (*B. catarrhalis*) (14.3%). Main causative bacteria, number of cases, strain isolated in different period of time are shown in Figure 2.

From a total 17 cases of pneumonia, 20 strains of pathogenic bacteria were isolated. These bacteria were, *H. influenzae* (45%), *S. pneumoniae* (40%), *Klebsiella pneumoniae* (*K. pneumoniae*) (10%) and *B. catarrhalis* (5%). The results are shown in Figure 3.

Comparision of causative bacteria of acute bronchitis between Mae Sot General Hospital, Mae Sot,

Table 1 Age, sex and number of patients attended at the Department of Internal Medicine, Mae Sot General Hospital, Thailand in three different study period

Periods	1989. 11-1990. 1		1990. 7-1990. 8		1990. 11-1990. 12	
Sex	M	F	M	F	M	F
No. of patients	79	46	97	44	73	53
Mean age (y.o.)	44.6	36.9	43.4	46.3	42.3	44.4
Total	125		141		126	

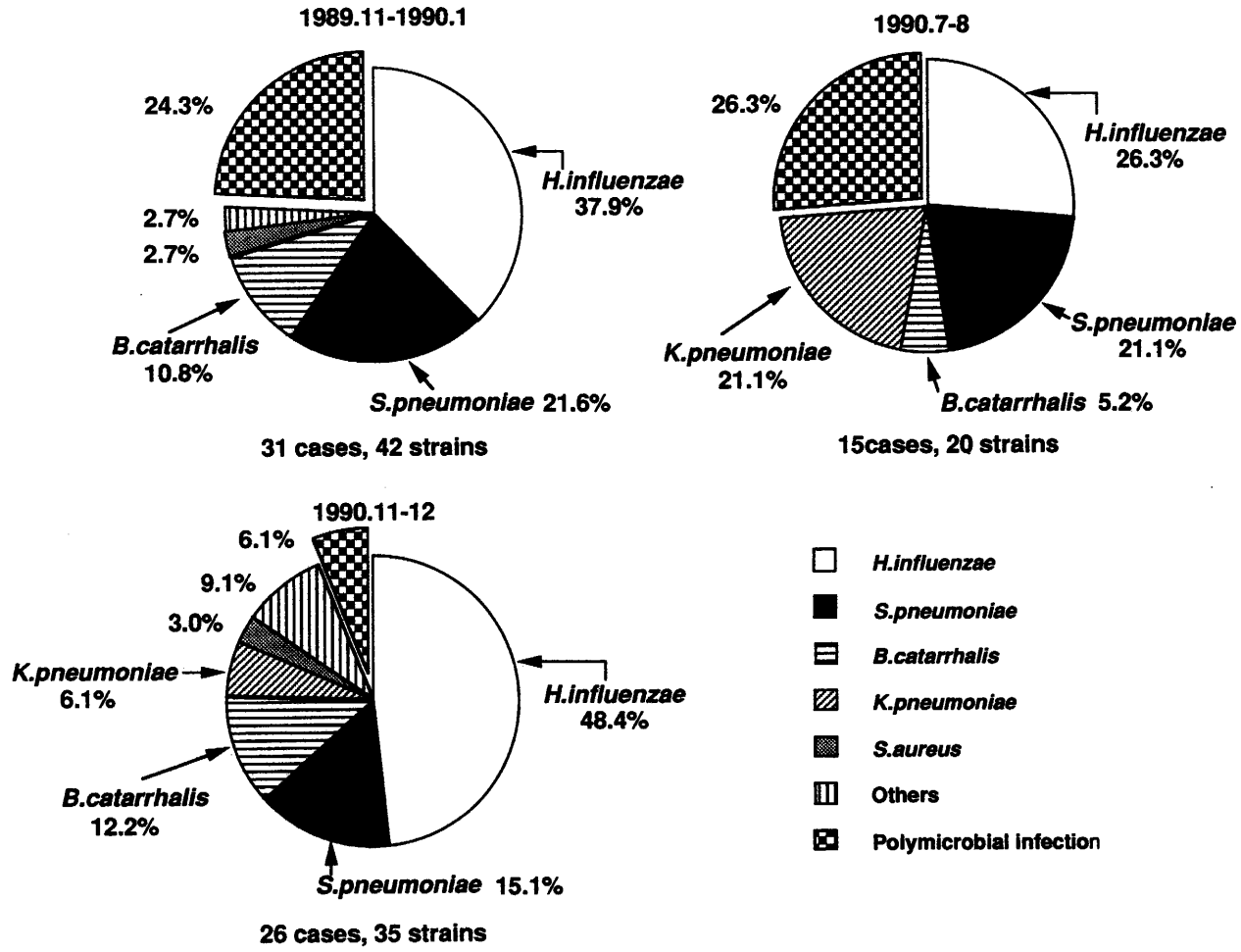

Figure 2 The destribution of pathogenic bacteria isolated from sputum of patients with acute bronchitis in three different study period.

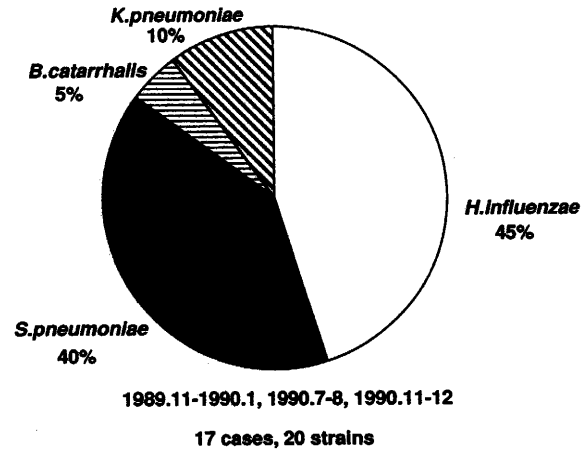

Figure 3 The distribution of pathogenic bacteria isolated from sputum of patients with pneumonia. This represent the combined results of three different study period.

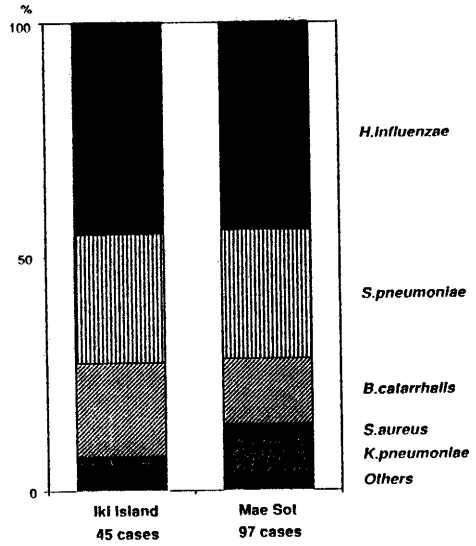

Figure 4 The distribution of pathogenic bacteria isolated from sputum of patients with acute bronchitis. Left column and right column reprensent the result obtained from Iki Island, Nagasaki and Mae Sot, Thailand respectely.

Thailand and Iki Public Hospital, Nagasaki, Japan are shown in Figure 4. The percentage of causative organisms are similar.

DISCUSSION

In regard to the causative organisms of respiratory infection some studies of developing countries showed that the major bacterial pathogens were *S. pneumoniae* and *H. influenzae* (Ghafoor et al., 1990; McIntosh, 1990; Vathanophas et al., 1990).

In Mae Sot General Hospital we experienced a total 72 cases of acute bronchitis and 17 cases of pneumonia. From sputum of acute bronchitis and pneumonia, 97 strains and 20 strains of pathogenic bacteria were isolated respectively. The main pathogenic bacteria were *H. influenzae, S. pneumoniae* and *B. catarrhalis.*

Previously we reported in Japan, *B. catarrhalis* is one of the major pathogenic organism in respiratory infection (Matsumoto *et al.,* 1981; Nagatake, 1985). Subsequently we reported various important aspect of this bacteria to elucidate its mechanism of pathogenicity (Ahmed *et al.,* 1990; Rikitomi *et al.,* 1991; Ahmed *et al.,* 1992). In this study we found in Mae Sot, Thailand, *B. catarrhalis* is one of the major pathogen in respiratory infection. These data show that *B. catarrhalis* infection increased simultaneously in different parts of the world (Mcleod *et al.,* 1986; Bartos *et al.,* 1988). The present data also shows that there is a high incidence of *B. catarrhalis* in winter. This is also similar to our previous observation and we also showed that this increase incidence of *B. catarrhalis* infection in winter is due to the increase attachment of *B. catarrhalis* to respiratory epithelium in winter (Mbaki *et al.,* 1987).

Although *H. influenzae* is usually regarded as a lower respiratory tract pathogen in patients with chronic lung disease. *H. influenzae* was the main pathogen of acute bronchitis and pneumonia in Mae Sot General Hospital. The incidence of *H. influenzae* pneumonia in adults have been increasing in many countries (Levin *et al.,* 1977; Woodhead *et al.,* 1987; Maniji *et al.,* 1990).

It is very interesting that the causative organisms of acute respiratory infections between Thailand and Japan were similar. In Mae Sot, doctors had conception that in that area, respiratory infection occured mostly by *S. pneumoniae.* Because in Mae Sot General Hospital, instead of rabbit blood human blood is used in agar, on which it is difficult to recognize *H. influnezae.* As *B. catarrhalis* is a newly recognized pathogen, in Mae Sot they did not have experience to recognize the bacteria. Our experience showed that in most of the developing countries have similar misconception about the recognition of pathogenic bacteria. This collaboration study helped the recognition of pathogenic bacteria in Mae Sot. We also found that Gram-stained sputum smears can greatly aid clinicians to make presumptive etiological diagnosis, and permit the laboratory to enhance the isolation of pathogenic bacteria by selective culture methods (Odhiambo *et al.,* 1990). This study shows that 'big three' pathogens (*H. influenzae, S. pneumoniae* and *B. catarrhalis*) of the community acquired respiratory infections are observed commonly in the world (Nagatake, 1991; Davies *et al.,* 1990). The criteria used in this study would appear to be more adaptable for routine use in developing countries. Similar type of collaboration studies are very helpful for the developing countries.

ACKNOWLEDGMENTS

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Jpn. J. Trop. Med. Hyg., Vol. 21, No. 2, 1993, pp. 117-121

IMMUNOHISTOCHEMICAL DEMONSTRATION OF *MYCOBACTERIUM LEPRAE* IN THE NERVOUS SYSTEM OF LONG-TERM CURED LEPROSY PATIENTS USING A *M. LEPRAE* SPECIFIC ANTI-PGL ANTIBODY

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Abstract: Leprosy (Hansen's disease) has become a curable disease by the advance of chemotherapy, however, deformities evoked by nerve damages are still a great problem of treatment. We have recently demonstrated a highly sensitive and specific method to identify the leprosy antigen in skin tissue by immunohistochemical staining using a *Mycobacteriam leprae*-specific antiphenolic lipid-I (PGL) monoclonal antibody. Using this method, we have examined the peripheral nerves of lower extremities, spinal cords and brain stems of clinically cured (skin slit smear negative more than 10 years) leprosy autopsy cases (Lepromatous (L): n=6, Tuberculoid (T): n=6), in which ordinary Fite's acid-fast staining did not reveal *M. leprae*. Positive staining was observed as follows: (1) Peripheral nerves: L: 6/6, T: 1/6. (2) Dorsal root ganglia and posterior spinal roots: L: 6/6, T: 2/6. (3) Anterior roots: L: 0/6, T: 0/6. (4) Spinal cord: L: 6/6, T: 2/6, observed in posterior horn cytoplasm and anterior horn neurons. (5) Medulla oblongata: L: 6/6, T: 2/6, observed mainly in ambiguus, facial, hypoglossal, cuneate and gracile nuclei. These findings indicate that *M. leprae* specific antigen remains in the peripheral sensory nerves as well as central sensory and motor nerves long after the clinical cure, especially in lepromatous patients where definitely abnormal cellular immunity against *M. leprae* is noted, which suggest the role of motor neurons in the pathogenesis of quiet nerve paralysis.

INTRODUCTION

Leprosy is a chronic weakly infectious disease evoked by acid-fast bacilli, *M. leprae*. The disease mainly affects peripheral nerves and skin, but internal organs such as testes, liver, spleen and lymph nodes may be involved in severe cases (Jopling, 1988). Lack of host cellular immunity against *M. leprae* is responsible for the onset of the disease.

In Japan, leprosy became a disease of the past. Although about 7,000 persons still live in 13 national and 3 private leprosaria, most of them are bacteriologically inactive (cured) because of effective chemotherapy, yet have physical deformities evoked by leprous neuropathy. Recently only about 20 new cases have been reported every year, and they are usually successfully treated as outpatients. However, leprosy is still a world-wide active disease affecting more than 10,000,000 peoples in the developing countries, and common cause of peripheral neuropathy.

Leprosy shows wide variety of clinical and pathological manifestations from tuberculoid to lepromatous types. In the tuberculoid type, a few dermal macula are associated with peripheral neuritis. Epithelioid granuloma completely replace the nerve fascicles while only small number of *M. leprae* are found. In the lepromatous type, where cellular immunity to *M. leprae* is almost absent, multiple dermal nodules (lepromas) appear. These lepromas show numerous *M. leprae* in the foamy macrophages, but inflammatory reaction is lack-

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ing. In the late stage of this type, glove and stocking type sensory neuropathy results.

We could demonstrate the persistence of mycobacterial antibodies in the skin biopsies of lepromatous leprosy, even in long-treated cases where usual acid-fast staining was negative. Both polyclonal antibody against BCG cross-reacting with many acid-fast bacilli and monoclonal antibody against *M. leprae* specific phenolic glycolipid-I (PGL) (Hunter *et al.*, 1982) showed positive immunohistochemical staining in paraffin sections of such cases (Goto and Izumi, 1991).

We have also summarized our neurological and pathological works of leprosy based on biopsy and autopsy performed in Hoshizuka-Keiaien. Minauchi found persistence of ultrastructurally densely stained bacterial structure in the myelinated Schwann cells of clinically inactive cases as well as demyelination and remyelination. In the longitudinal study of lower extremity nerves, he also showed sensory nerve tropism and upward extension of the bacteria and lesions (Minauchi *et al.* in press). Usually these ultrastructurally proven bacilli did not show positive acid-fast staining, which led us some problems of specificity.

This study was primarily aimed to prove that these bacillary structures have the chemical properties of *M. leprae* using BCG and PGL immunohistochemistry, which is successfully demonstrated here. Moreover, on the contrary to our expectation, PGL positive staining was also demonstrated in the spinal cord and brain stem.

Materials and Methods

Since 1983, autopsy has been permitted in about 2/3 of the deceased individuals in our leprosarium, and systemic examination of nervous system as well as internal organs has been performed. Among them, six cases of lepromatous leprosy and six cases of tuberculoid leprosy were selected, in which skin slit smears to examine the active leprous skin lesions were negative for more than at least ten years and leprosy was considered to be clinically cured. As controls, three lepromatous leprosy with positive skin-slit smear within 5 years before death and two non-leprosy were used. Sural, tibial, peroneal and sciatic nerves, lumbar dorsal root ganglia, anterior and posterior spinal roots at lumbar level, spinal cord (lumbar, thoracic, cervical) and 3-4 mm step serial coronal sections of brain stem (from pyramidal decussation to superior colliculus) were fixed in formalin and embedded in paraffin. In some cases peripheral nerves were fixed in glutaraldehyde, but this fixation did not suppress the immunohistochemistry of PGL. Five micrometer thick sections were stained by hematoxylin and eosin (HE) and Fite's acid fast staining.

As primary antibodies, mouse monoclonal anti-PGL antibody (ml 1-21) raised against synthetic natural trisaccharide (Fujiwara *et al.*, 1984; courtesy of Fuji-Rebio Research Institute, Japan, diluted ×1,000) or rabbit polyclonal anti-BCG (Bacillus Calmette-Gue'rin) antibody (DAKO Japan B124, diluted ×2,000) were used (60 min at room temperature). Immunostaining was done as described by Hsu *et al.* (1981) using biotinylated secondary antibodies and ABC complex (Vectastain PK-4002 for PGL or PK-4001 for BCG), followed by diaminobenzidine-peroxide. Finally, counterstaining was done by hematoxylin.

Results

Peripheral nerve:

All cases (6/6) of lepromatous leprosy showed PGL and BCG antigens in most of the examined sural, tibial, peroneal and sciatic nerves. As there were no essential differences in the staining sites between PGL and BCG, the following results will be focused to PGL staining. In the sciatic nerve, PGL staining was much different among the nerve fascicles. Positive staining distributed sporadically along the axon adjacent to Schwann cell nuclei as granular or foamy pattern. Old large globi (giant foamy macrophage) were perivascularly recognized in some lepromatous cases, but these globi were PGL negative. Except for one case where dense lymphocytic infiltration is noted focally, there were no inflammatory reaction or tissue damages associated with PGL antigen. In tuberculoid leprosy, most cases (5/6) showed PGL negative.

In the lumbar dorsal root ganglia and posterior spinal roots (Fig. 1a, b) similar positive findings (Lepromatous 6/6, Tuberculoid 2/6) were observed. In the ganglia, neuronal soma were also stained. Lumbar anterior roots of both lepromatous and tuberculoid types showed negative PGL staining. Dorsal root ganglia and spinal roots of non-leprosy were PGL negative.

There were no diffeneces of staining location, pattern or intensity between six cured lepromatous cases and three bacteriologically positive cases.

Spinal cord and brain stem:

Generally, PGL antibody showed much stronger staining than BCG. The spinal cord showed PGL positive in nerve fibers of dorsal horn and anterior horn neuron (Fig. 1c, d). The latter was frequently associat-

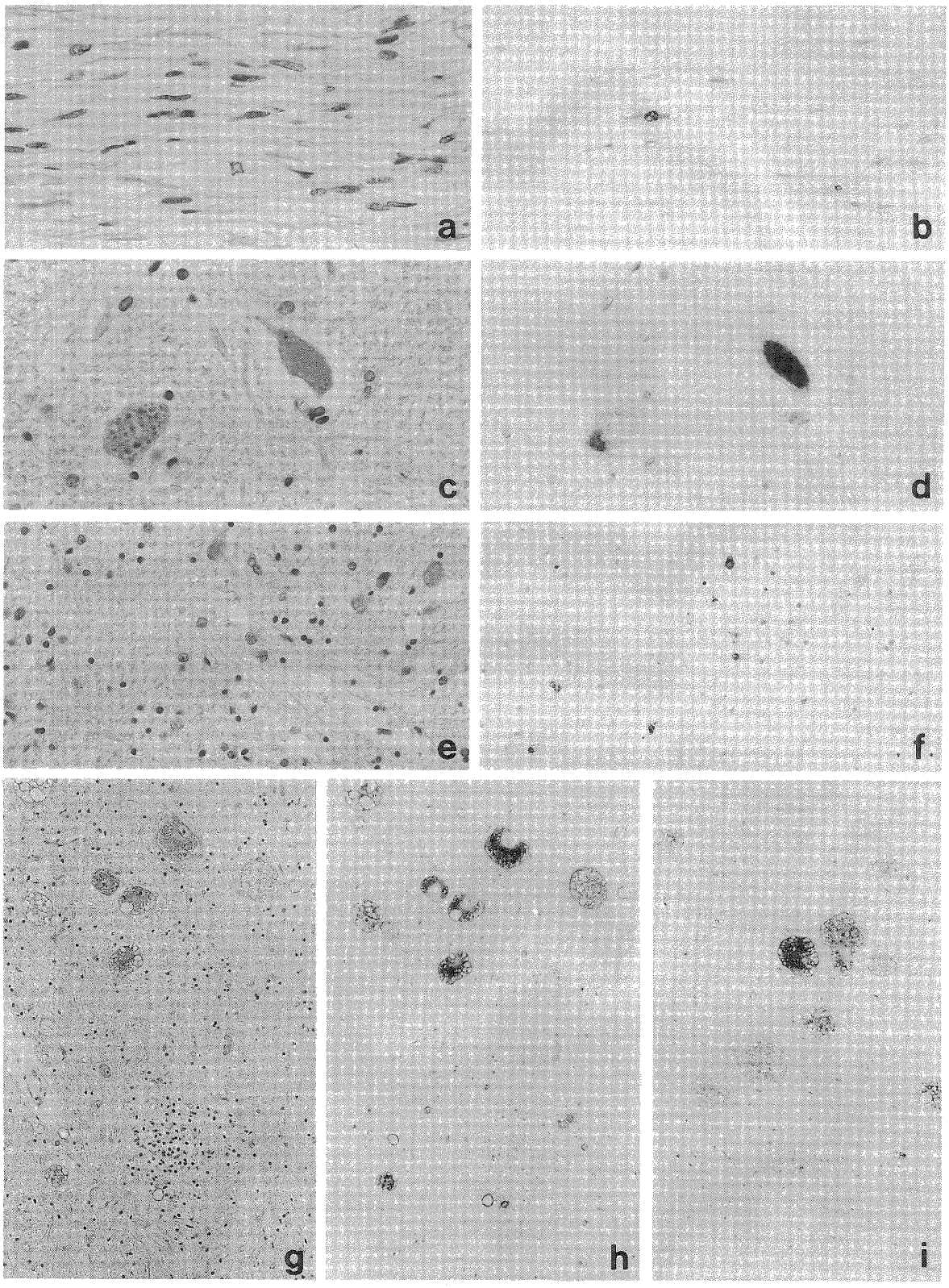

Figure 1 PGL immunohistochemistry in posterior root (a, b ×380), spinal anterior horn cell (c, d ×380), gracile nucleus (e, f ×250), ambiguus nucleus (g, h ×125). (i) shows ambiguus nucleus by BCG immunohistochemistry (×125). Left photographs (a, c, e, g) were taken with red filter for nuclear information, while right ones (b, d, f, h, i) were taken with blue filter for immunostaining.

ed with unusual vacuolation degeneration, but many neurons preserved their normal shape. Sometimes brown immunohistochemical products were difficult to differentiate from lipofuscin.

In the step sections of brain stem of lepromatous leprosy, motor neurons such as (1) ambiguus nucleus, (2) facial nerve nucleus, (3) hypoglossal nucleus, and sensory system such as (4) gracile nucleus and tract (Fig. 1e, f), (5) cuneate nucleus and tract, (6) vestibular nucleus, (7) spinal trigeminal nucleus and tract, (8) solitary nucleus were PGL positive. Among them, (1) to (5) were almost constantly positive, whereas the other were variable from case to case. In the positive nuclei, most strongly stained part is neuronal soma with granular or foamy patterns, but perineural zone also showed small foamy patterns. In two cases, micro-granulomas were detected in close association to PGL positivity (Fig. 1g-i). In tuberculoid leprosy, 2/6 cases were PGL positive, but two non-leprosy control were PGL negative. There were no diffenences between six cured lepromatous and three bacteriologically positive cases.

DISCUSSION

In the peripheral nerves, the PGL antigen (*M. leprae* and its degeneration product) was preserved even long after the clinical cure. Solid *M. leprae* was stained as granular pattern, while non-solid (dead) *M. leprae* as foamy pattern in the skin biopsy study. In the nerves both patterns were observed, which suggest that these bacilli still survive, although these bacilli are usually unstained by conventional Fite's staining. We do not know whether this fact is merely a technical problem or essential problem of *M. leprae* infection.

By the investigation of autopsy cases in Hoshizuka -Keiaien, we have found secondary degeneration of spinal dorsal column due to sensory neuropathy, which is severe in lepromatous leprosy, and degeneration of optic system in blind cases (Goto and Minachi, 1985).

Yamada reported the presence of acid-fast substance in the brain stem of leprosy using periodic acid -pretreated acid-fast staining (Yamada, 1984), but we could not get similar results except for non-specific staining. Thus, until recently we have believed that *M. leprae* invade up to dorsal root ganglia and do not penetrate the central nervous system.

Mitsuda described acid-fast bacilli in the soma of facial nerve nucleus, ambiguus nucleus and spinal anterior horn nucleus (Mitsuda, 1952). Our present study confirm his work from the immunohistochemical standpoint. On the contrary, our results were mostly different from Yamada's study which stresses the acid-fast substance located in the pyramidal and other tracts of tuberculoid leprosy.

Prevention of quiet nerve paralysis, which occurs insiduously without acute neuritic episodes, is an important problem for the successful treatment of leprosy (WHO Expert Committee on Leprosy, 1988). Thickened nerve trunks are usually observed in this condition, but there are also some cases of quiet nerve paralysis without swollen nerves. The results obtained in our present study may add a new viewpoint for the further understanding of pathogenesis of the quiet nerve paralysis.

ACKNKOWLEGDEMENTS

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Research Note

MORPHOMETRIC CHARACTERISTICS OF *GLOSSINA PALLIDIPES* POPULATIONS ON THE SOUTH KENYA COAST

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Received January 18 1993/Accepted February 25 1993

Abstract: In this study both spatial and seasonal variations in the size of tsetse flies from the south Kenya coast were investigated. The work was done at two sites; in Rongomwagandi (in Shimba Hills National Researve) and in Muhaka Forest. Wing length was used as an index of fly size. In a cross-sectional study the size of flies from two study sites was compared. Significant differences were found between the size of flies from Rongomwagandi and Muhaka Forest. In a longitudial study the size of flies from two study sites was compared over a six month period. The population of flies at Rongomwagandi showed larger seasonal variation in size than those in Muhaka. Additionally, all flies collected were aged using the wing fray method. More older flies were observed in dry season than in rainy season.

INTRODUCTION

Etten (1982) demonstrated the existence of diversity in behaviour between tsetse populations. For the effective control of tsetse flies and trypanosomiasis, it is also important to understand the variability of other characteristics between different tsetse populations.

In this study, comparison was made of the sizes of *Glossina pallidipes* in Rongomwagandi (Shimba Hills National Researve) and Muhaka Forest in Kenya south coast.

Furthermore, a longitudinal study of tsetse morphometrics in spatially separated populations was done. Since the larva of tsetse fly develops in uterus, variation in size of adult fly reflects, in part, the favourability of the environment for the parent females, and may be related to the adult mortality rate and vectorial capacity (Dransfield *et al.*, 1989).

MATERIALS AND METHODS

Flies were collected from 2 sites on south Kenya coast. Rongomwagandi in the Shimba Hills National Researve (4°5′S, 39°25′E, alt. 180 m) consists of a moraine of forest and savannah. Only wild animals were present in this area including bushbucks, bushpigs, worthogs and buffalos. Muhaka Forest outside the Shimba Hills National Researve (4°20′S, 39°32′E, alt. 35 m) is a region of forest relicts in rural areas and had both domestic and wild animals including cattle, goats, warthogs, bushpigs and bushbabies (Fig. 1).

Blue and white biconical traps (Challier *et al.*, 1977) were used for sampling tsetses. Flies were collected using 2-5 traps in each place. Traps were operated for 24 hr at sampling stations.

The right wing was removed from each fly and mounted on a glass slide. The length of the middle part of the fourth longitudinal wing vein was then measured (Jackson, 1946). The wing fray category was also recorded (Minter, 1982).

RESULTS AND DISCUSSION

There was a significant difference in the size of flies from Rongomwagandi and Muhaka Forest. It is known that the size of flies is influenced by humidity, temperature and food availability (Jackson, 1952; Dransfield *et al.*, 1989). In Shimba Hill National Researve, wild animals were abundant and especially, Rongomwagandi, a bush area, could be a habitat of animals. On the other hand, in Muhaka Forest, both wild and domestic animals were present, though there were significantly less game

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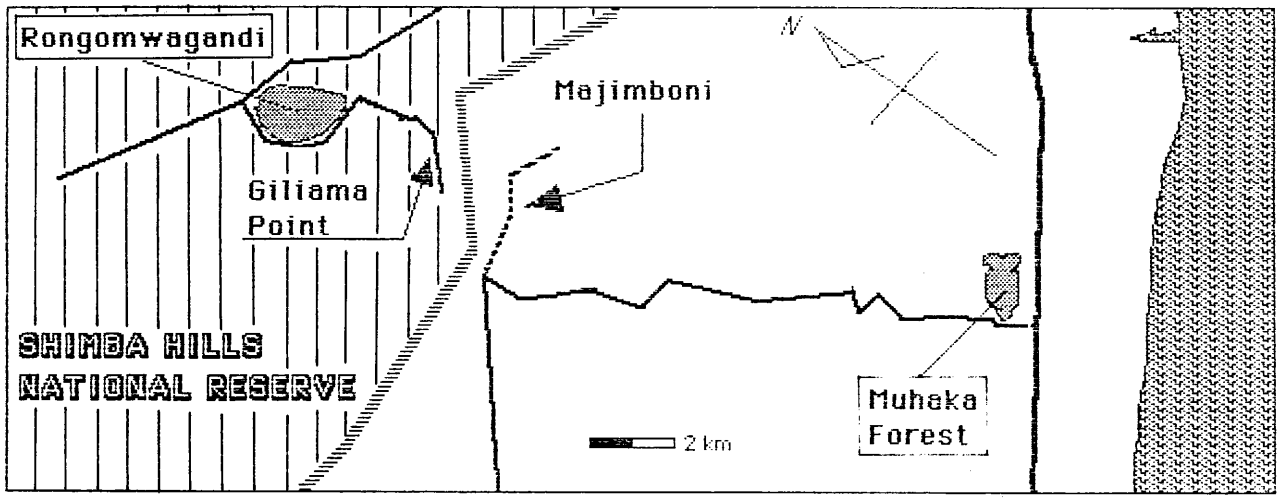

Figure 1 Map of the South Kenya Coast showing Rongomwagandi and Muhaka Forest.

animals there than in Shimba Hills National Researve.

Seasonal changes in the mean size of *G. pallidipes,* as indicated by wing vein length, were monitored in June (rain season), September (dry season) and December (short-rain season), 1989 in two study sites (Fig. 2). Both male and female flies from Shimba Hills were significantly larger in June and December. However, in September flies from Muhaka were larger. Size of tsetses in Shimba Hills was varied, whereas in Muhaka size was rather stable.

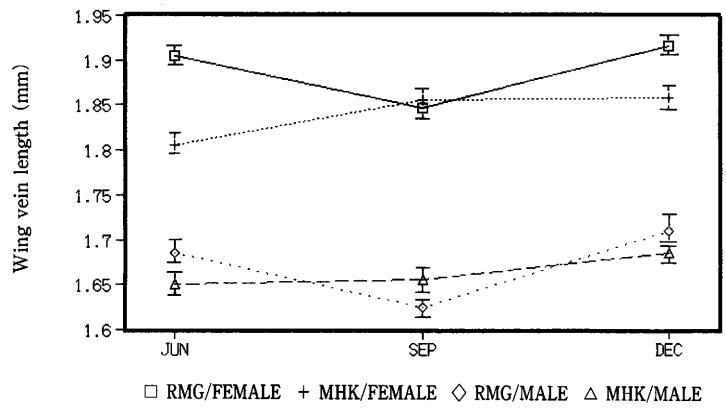

Figure 2 Changes in mean wing vein length (± standard error) of *Glossina pallidipes* in Rongomwagandi (RMG) and Muhaka Forest (MHK).

It seems that there was not much difference in environmental conditions such as temperature and humidity between these two places, although the micro-climatic condition might be different. In the dry season the game animals in Shimba Hills migrate to the other side of the Researve where food was available, but at Muhaka Forest both wild and domestic animals stayed all the year round.

Since size may be related to the mortality rate of flies (Dransfield *et al.,* 1989) these results suggest that the transmission dynamics of trypanosomes in the two sites may be different.

Wing fray age structures of fly populations from the two sites changed seasonally (Fig. 3). About 50% of populations were old flies (>category 2) at both Rongomwagandi and Muhaka Forest in June. In September older flies predominated in both populations (73.4% were old flies at Rongomwagandi and 61.9% at Muhaka Forest). Younger flies appeared in December. This data suggests that fly survival was greater or breeding rates were lower in September than in the other two seasons.

If we assume that wing fray age category of tsetse is related to the number of blood meals they have taken, then populations containing large numbers of older flies will have greater potential for disease transmission. When planning the timing of tsetse control programs it is important to take into account the age structure of fly populations.

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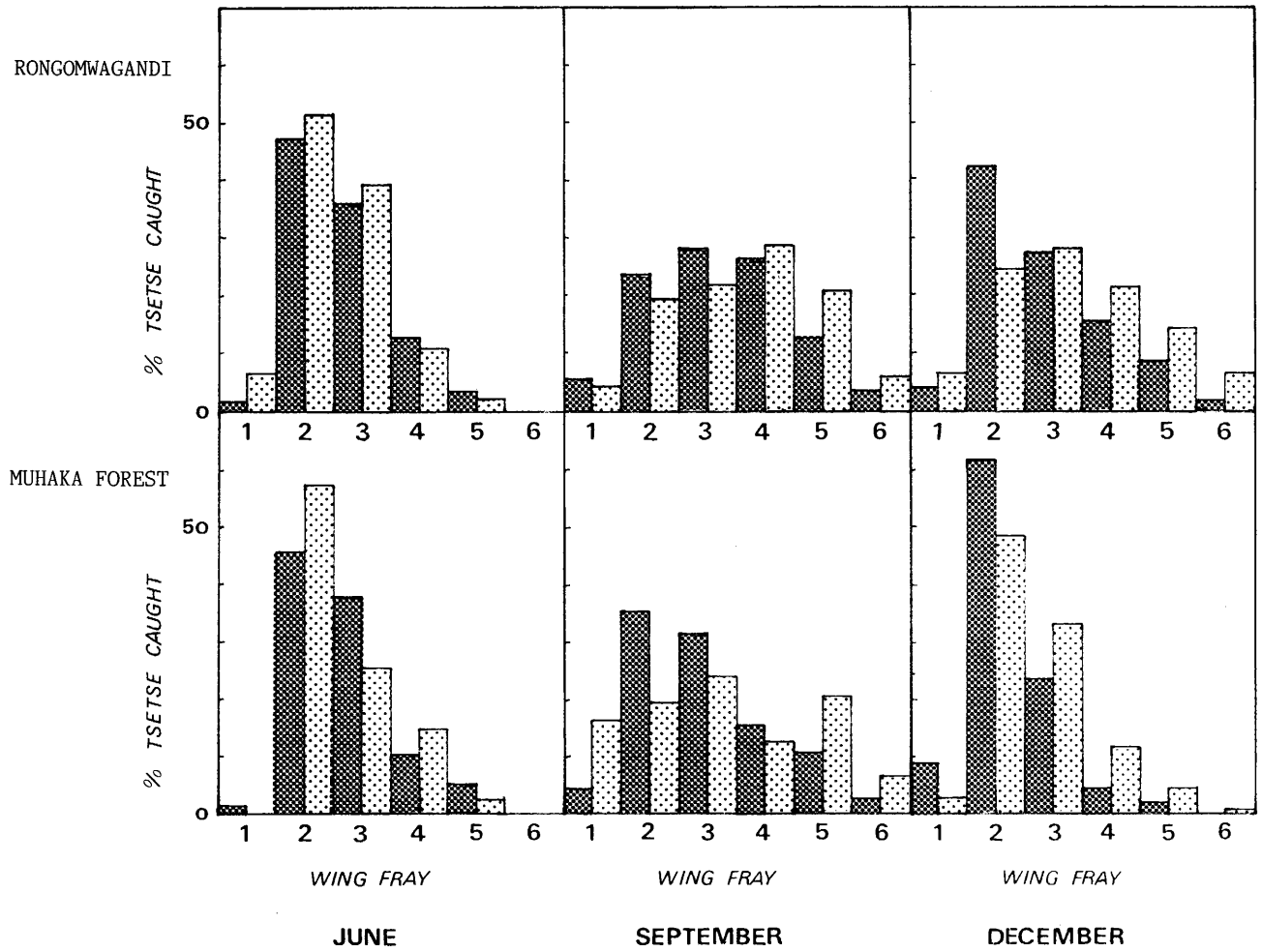

Figure 3 Age structure by wing fray categories of the populations of *Glossina pallidipes* in June, September and December (dark bar=female, light bar=male).

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Clinical Report

A CASE OF IMPORTED *PLASMODIUM MALARIAE* MALARIA

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Abstract: Imported malaria is one of the perplexing problems in the world. Migrants, either refugees or those looking for better opportunites in life, have contributed to the introduction of malaria to previously free areas. The increased incidence of imported malaria is distinctly attributed to a failure of travellers to take appropriate action to prevent infection. Annually up to 100 cases of malaria are presumed to be imported into Japan and most of these cases are *Plasmodium falciparum* and *P. vivax* malaria. The reports of *P. malariae* malaria cases are very few in Japan. The present paper reports a case of one patient with imported *P. malariae* malaria infection accompanied with marked hepato-splenomegaly who has come from Nigeria. And this patient was treated with Fansidar® (sulfadoxine-pyrimethamine) and minocycline which were very effective against this infection.

INTRODUCTION

Recently, there has been an enormous influx of foreigners, who are travellers and workers in Japan, from Southeast Asia, South America, Africa and so on. But these peoples often have health problems which are unfamiliar to Japanese physicians. Some of them harbor parasites from tropical areas, for example *Plasmodium* spp., *Entamoeba histlytica, Giardia lamblia, Ascaris lumbricoides,* hookworm, *Trichuris trichiura, etc.* (Akao *et al.,* 1992).

In recent years, imported malaria cases are reported to be about 48 to 101 cases in Japan annually. The most of these cases were either *P. falciparum* malaria or *P. vivax* malaria. But, cases of *P. malariae* malaria are very rare in Japan (2.6%; Yoshida, 1991). Importantly, the number of Japanese literatures on *P. malariae* malaria was only 8 papers over the past 25 years (Nakabayashi *et al.,* 1988; Yamakami *et al.,* 1990; Amano *et al.,* 1992).

In this paper, a case of one patient who has come from Nigeria with *P. malariae* malaria accompanied with remarked hepato-splenomegaly is reported.

CASE REPORT

A 29-year-old Nigeria-born male who had worked as pharmacist at Ragos in Nigeria was admitted to Utano-Tsujimura Hospital in Nara Prefecture in December 1989, because of a high intermittent fever. He had been travelling and stayed at his friend's lodging in Utano, Nara Prefecture. Already, this patient had known that he had been infected with malaria in Nigeria and whenever he had a malaria infection, he had treated himself with chloroquine.

On admission, although he appeared quite ill but his vital signs were stable and the physical examination was unremarkable except for marked hepatomegaly and splenomegaly (Fig. 1). Routine laboratory examinations confirmed the presence of anemia (red blood cell count $277\times10^4/mm^3$, Hb 7.7 g/d*l*, Ht 23.5%, reticulocyte 1.7%) which was normocytic, but hypochromic. The white blood cell count was $5,400/mm^3$ and platelets count $24.4\times10^4/mm^3$. Peripheral blood smears revealed malarial parasitemia (0.245%). All formes of *P. malariae* were observed in red blood cells (Fig. 2). The serum total proteins, albumin, cholinesterase and total cholesterol were low. LDH was increased because of haemolysis. ZTT, TTT and CRP were increased. A

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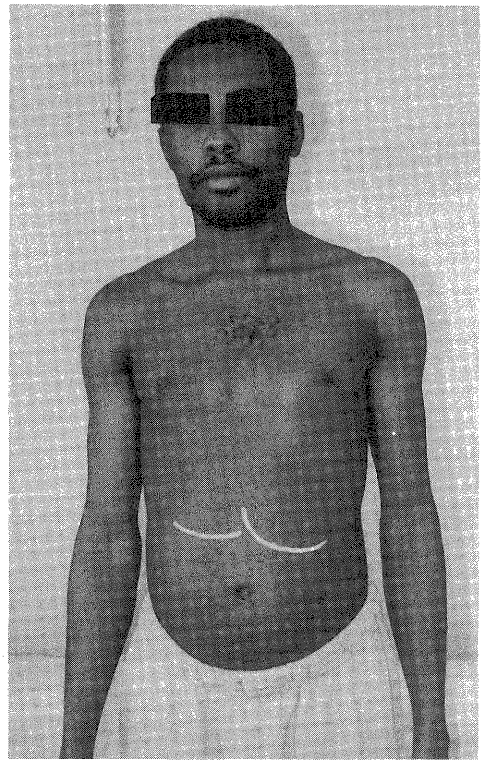

Figure 1 The upper half of this patient. Remarked hepato-splenomegaly was seen.

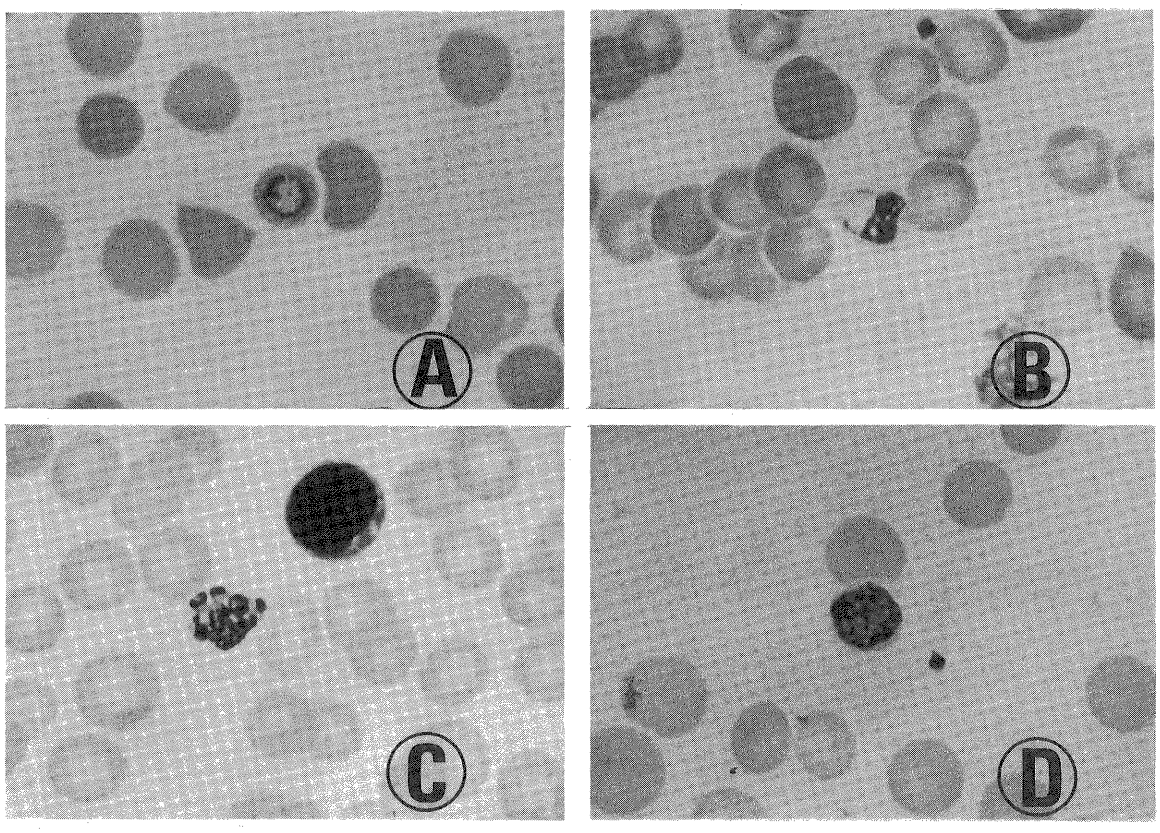

Figure 2 Blood smear of the patient, containing all stages of *P. malariae*. A: slight deformed ring form. B: band form. C: completely matured schizont. D: macrogametocyte.

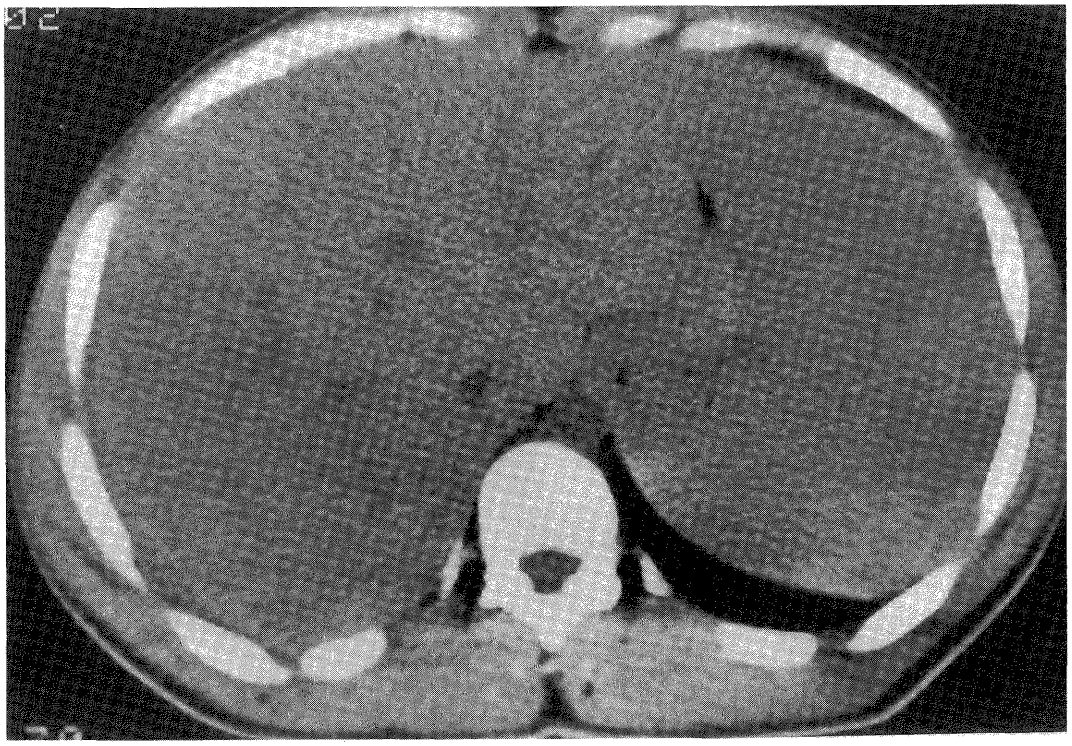

Figure 3 Computed tomography scan of the abdomen of the patient showed swelling of the liver and spleen.

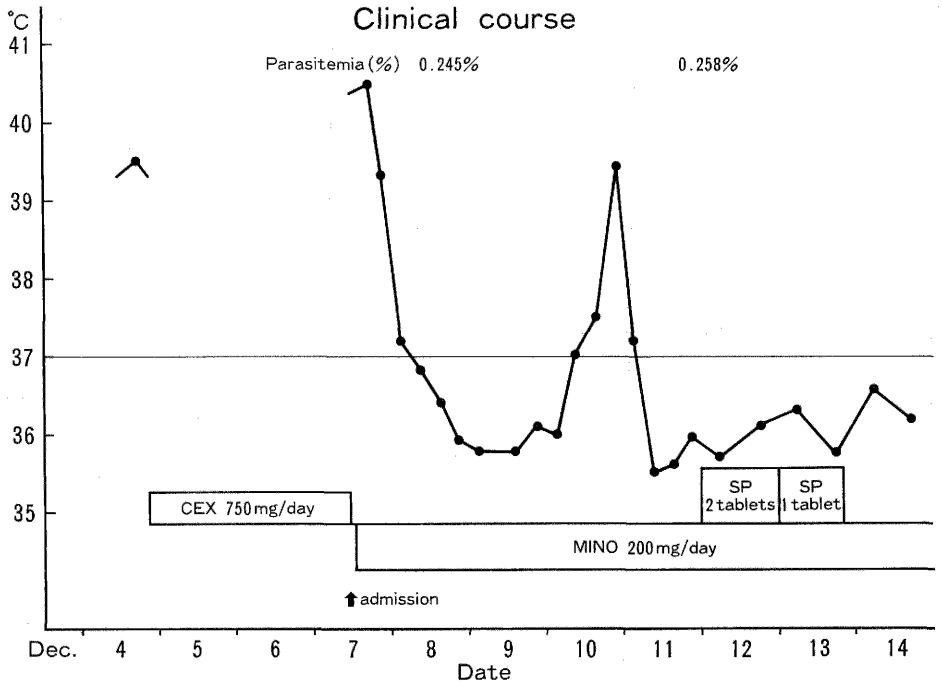

Figure 4 Clinical course of the patient.
CEX: cephalexin, MINO: minocycline,
SP: sulfadoxine-pyrimethamine

computed tomography (CT) scan of abdomen showed a markedly swelled liver and spleen (Fig. 3). Clinical course of this patient is summerized in Fig. 4. Typical intermittent fever of *P. malariae* malaria (three-day intervals) was observed.

The patient was orally treated with sulfadoxine-pyrimethamine (Fansidar®, pyrimethamine 25 mg; sulfadoxine 500 mg) 3 tablets (2 tablets on the first day and 1 on the second), and minocycline 200 mg per day for 7 days. All symptoms of *P. malariae* malaria abated.

Discussion

The risk of contracting malaria has recently been increasing because of the resurgence of the disease in many countries and areas of the world (Schultz, 1989). Nevertheless, specific diagnostic investigations for malaria can be sometimes difficult to prescribe in Japan, because Japanese physicians and medical technicians are not familiar with these infections. The clinical course in this patient was quite typical of *P. malariae* infection with intermittent fever (three-day interval), amenia and hepato-splenomegaly. And this patient informed us that he was suffering from malaria. Then we were able to make a diagnosis of *P. malariae* malaria easily.

Drug-resistant *P. falciparum* and *P. vivax* are documented to occur in the many malarial areas (WHO, 1992; Rieckman *et al.*, 1989; Whitey *et al.*, 1989; Collignon, 1991; Gravelli and Corti, 1992). A drug-resistant *P. malariae* case was not reported yet, anywhere the world. In this case, this patient had used chloroquine himself to treat malaria and experienced repeatedly recrudescences in Nigeria. Accodingly we did not select chloroquine, but sulfadoxine-pyrimethamine and minocycline, which were very effective against this *P. malariae* infection. The drug used for malaria must be chosen as the most effective one from several anti-malarial drugs which are available in Japan, such as chloroquine, sulfadoxine-pyrimethamine (Fansidar®), sulfamethoxazole-trimethoprim (Baktar®, Bactramin®), quinine, quinidine, mefloquine, minocycline *etc.* (Tanabe *et al.*, 1989; Yoshida *et al.*, 1987; Amano *et al.*, 1991, 1990). But we could not find a complete cure, because this patient left Japan when he did not have any signs of malaria.

Our Japanese physicians must recognize that malaria is increasing in Japan and drug-resistant *Plasmodium* infections are spreading throughout malarial areas.

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CONTENTS

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