日本熱帯医学会雑誌

第21巻 第4号 平成5年12月

内　　容

（裏面に続く）

JTM FOUNDED 1959

日熱医会誌　日本熱帯医学会

日本熱帯医学会雑誌
第21巻第4号
1993年12月15日　印刷
1993年12月15日　発行

発行所　日本熱帯医学会
編集者　板倉英音
印刷所　昭和堂印刷
諫早市長野町1007-2（〒854）
☎0957-22-6000

本雑誌の刊行にあたりその費用の一部を文部省科学研究費補助金
（研究成果公開促進費）によった。

Jpn. J. Trop. Med. Hyg., Vol. 21, No. 4, 1993, pp. 193-199

ADAPTIVE CHANGES IN PHYSIOLOGICAL RESPONSES OF MEN TO HEAT INDUCED BY HEAT ACCLIMATIZATION AND PHYSICAL TRAINING

SEIKI HORI[1] AND NOBUO TANAKA[2]

Received August 16 1993/Accepted October 7 1993

Abstract: Twelve healthy male nonathletic university students were selected as subjects. Anthropometric measurement and a work capacity test using a bicycle ergometer in a climatic chamber of 30°C and 60% R. H. were carried out in summer and winter. Observations of the physiological responses of the subjects were made while pedalling a bicycle ergometer at a constant work load of 50% $\dot{V}o_2max$ and at a cycling rate of 50 rpm for 30 min in a climatic chamber of 30°C and 60% R.H., in summer. The subjects took the same exercise for 13 successive days except the day after training, for 6 days in a climatic chamber of 30°C and 60% R.H., after the work capacity test was tested in winter. Body weight and skinfold thickness showed a tendency to decrease in summer. $\dot{V}o_2max$ per body weight was considerably greater in summer than in winter though this difference was statistically not significant. Na concentration in sweat and the increase in heart rate during exercise were significantly lesser in summer than in winter. Sweat volume induced by exercise increased and rise in rectal temperature during exercise showed a tendency to decrease in summer. Increase in heart rate during exercise was decreased significantly by physical training. Sweat volume during exercise tended to increase and rise in rectal temperature during exercise tended to decrease progressively during physical training. Decrease in heart rate during exercise induced by physical training was greater than that induced by climatic heat acclimatiztion, while decrease in Na concentration of sweat due to climatic heat acclimatization was greater than that observed during physical training. Indices representing the magnitude of strain including relative increase in heart rate, relative rise in core temperature and relative water loss are proposed for the assessment of work capacity in heat.

INTRODUCTION

It has been known that the physiological responses of unacclimatized individuals in heat are changed when they have been exposed repeatedly to a hot environment (Kuno, 1956; Shvartz *et al.*, 1979) or they have been adapted to work in heat (Robinson *et al.*, 1953; Wyndham *et al.*, 1964). In short-term heat acclimation, unacclimatized individuals sweat more readily, and more profusely, and the rise in their core temperature during exercise in heat is lessened due to greater heat dissipation accompanied by an increased amount of sweat. These adaptative changes to heat during short-term heat acclimation are considered to be favorable for individuals who must work in a hot environment (Ihzuka *et al.*, 1986). It is generally agreed that unacclimatized individuals show a marked rise in core temperature during exercise in heat due to a slower onset of sweating and a lower sweat rate (Robinson *et al.*, 1953; Kuno, 1956; Bass, 1963). After successive exposures to a combination of exercise and environmental heat, a lessening of cardiovascular strain has been observed as indicated by a lesser increase in heart rate due to physical training and a lower rise in core temperature due to an earlier onset of sweating and an increased sweat volume with a decreased salt concentration (Lind and Bass, 1963; Piwonka *et al.*, 1965; Araki *et al.*, 1981). However, the decrease in the salt concentration in sweat was much less and the decrease in the heart rate was much greater during successive exposure to a combination of exercise and environmental heat when compared with those observed in climatic heat acclimatization (Hori, 1977). It can be said, therefore, that there are different characteristics of heat adaptation, with regard

1 Department of Physiology, Hyogo College of Medicine, Nishinomiya, Hyogo 663, Japan
2 Department of Health and Physical Education, Kyoto Sangyo University, Kyoto 603, Japan

Table 1 Characteristics of subjects ($\bar{X}\pm SD$)

Season	Number	Age (Yr)	Height (cm)	Body weight (kg)	Area (m^2)	Area / Body weight (cm^2/g)	Mean skinfold thickness (mm)	Fat content (%)
Summer	12	20.1±1.4	170.1±5.3	58.9±3.8	1.67±0.10	0.287±0.010	10.9±3.1	12.4±3.2
Winter	12	20.6±1.4	170.1±5.3	59.8±4.2	1.70±0.12	0.283±0.010	12.9±4.2	14.4±3.5

to sweating reaction and cardiovascular reaction, during exercise in heat between climatic heat acclimatization and heat acclimation caused by physical training in a hot environment. Thus, an attempt was made to compare, in detail, changes in physiological responses of the same subjects during exercise in a hot environment between two types of heat adaptation-climatic heat acclimatization and heat acclimation induced by exercise in heat.

Materials and Methods

Twelve healthy young male nonathletic university students were selected as subjects after informed consent had been obtained. Anthropometric measurement and determination of the maximal oxygen uptake ($\dot{V}o_2$ max) using the bicycle ergometer in a climatic chamber of 30°C and 60% R.H. were performed in summer and winter. Experiments were performed at 15:00 h. Subjects were instructed to fast and remain at rest after lunch. The subjects, dressed in shorts only, rested on the saddle of the Monark bicycle ergometer for 5 min, then warmed up for one minute by pedalling at zero load. The pedal frequency was set at 50 rpm and the work load was incremented continuously by 15 watts/min until the subjects reached exhaustion. During exercise, a bipolar chest lead ECG was recorded and the volume of expired gas was recorded automatically. Expired gas samples were analyzed for oxygen and carbon dioxide. $\dot{V}o_2$max was determined by averaging the consecutive 15s oxygen uptake value before exhaustion. Each subject, dressed in shorts only, sat for 30 min, then pedalled the cycle ergometer at a constant work load of 50% $\dot{V}o_2$ max at a cycling rate of 50 rpm for 30 min in a climatic chamber of 30°C and 60% R.H., on the 7th day after the work capacity test, in summer.

Body weight was measured before and immediately after exercise using a platform balance with an accuracy of up to ±5g, and net body weight was obtained by subtracting the weight of the shorts. Rectal temperature was recorded continuously by a copper-constantan thermocouple. Heart rate was taken by a bipolar chest lead electrocardiogram. Sweat samples from the back were collected at 10 min intervals using filter paper method (Ohara, 1966). Na in sweat was eluted with distilled water from the filter paper and its concentration was determined by flame photometry. In winter, each subject took the same 30 minute exercise for 13 successive days except on the day after training, for 6 days. The subjects rested for one hour in the climatic chamber of 30°C and 60% R.H. on the day without training, to maintain the state of heat adaptation induced by exercise in a hot environment. Skinfold thickness was measured on the right side of the body. Skinfold sites and weighing factors used for calculating the mean skinfold thickness are as follows (Hori *et al.*, 1978).

Chest	Abdomen	Upper arm	Sobscapular	Waist	Thigh
0.143	0.139	0.141	0.143	0.139	0.295

The body fat (F %) was calculated from the mean skinfold thickness (X mm), body weight (W Kg) and body surface area (A m^2) by the following equation (Hori *et al.*, 1978)

$$F=28.9\frac{A\times X}{W}+3.67$$

Table 2 All out time, $\dot{V}o_2$max, $\dot{V}o_2$max per body weight and maximal heart rate ($\bar{X}\pm SD$)

Season	All out time (min)	$\dot{V}o_2$max (l/min)	$\dot{V}o_2$max / Weight (ml/kg/min)	Maximal heart rate (beats/min)
Summer	16.7±1.5	2.38±0.37	40.6±3.7	182±4.4
Winter	16.0±1.5	2.29±0.29	37.8±3.6	183±4.5

Table 3 Body weight loss, body weight loss per body weight, mean Na concentration in sweat, rise in rectal temperature, increase in heart rate, $\dot{V}o_2$ and $\dot{V}o_2$ per body weight ($\bar{X}\pm SD$)

Season	Training (week)	ΔW (kg)	$\frac{\Delta W}{W}$ (%)	C (mEq/l)	ΔTre (°C)	ΔH (beats/min)	$\dot{V}o_2$ (l/min)	$\dot{V}o_2$/W (ml/kg/min)
Summer	0	0.52±0.10	0.89±0.22	*S 41.1±8.3	0.74±0.17	*S 72.1±2.3	1.41±0.17	24.0±3.4
Winter	0	0.48±0.08	0.81±0.21	52.9±10.4	0.79±0.19	75.3±2.5	1.40±0.17	23.3±3.3
	1	0.53±0.11	0.89±0.22	51.0±10.9	0.74±0.18	*t 70.6±2.0	1.38±0.76	22.9±3.1
	2	0.54±0.12	0.91±0.20	50.3±11.0	0.72±0.19	*t 69.2±2.1	1.37±0.15	22.8±3.3

*S: Significant differences between summer and winter.
*t: Significant differences between before training and after training.
*: $P<0.01$
ΔW: Body weight loss, W: Body weight, C: Mean Na Concentration in sweat,
ΔTre: Rise in rectal temperature, ΔH: Increase in heart rate.

RESULTS

The physical characteristics of the subjects are given in Table 1. The mean values of body weight, body surface area, mean skinfold thickness and body fat percentage were smaller in summer than in winter while the mean value of the ratio of body surface area to body weight was greater in summer than in winter though these differences were statistically not significant. A greater value of the ratio of body surface area to body weight with smaller deposits of subcutaneous fat in summer indicates a body contour changed to a more slender body shape from winter to summer. The mean values and standard deviations of all out time, $\dot{V}o_2$max, ratio of $\dot{V}o_2$max to body weight and maximal heart rate in both seasons are represented in Table 2. The mean values of all out time, $\dot{V}o_2$max and ratio of $\dot{V}o_2$max to body weight were greater in summer than in winter. However, these differences were statistically not significant. Changes in body weight loss, ratio of body weight loss to body weight, mean Na concentration in sweat, rise in rectal temperature, increase in heart rate, oxygen uptake and oxygen uptake per body weight during exercise in a hot environment induced by climatic heat acclimatization and successive exposures to a combination of exercise and environmental heat are shown in Table 3. The mean values of body weight loss, ratio of body weight loss to body weight and oxygen uptake per body weight were greater in summer than in winter, while the mean values of mean Na concentration in sweat, rise in rectal temperature, and increase in heart rate were smaller in summer than in winter. Among these differences, there were significant differences in mean Na concentration in sweat and the increase in heart rate. The mean values of body weight loss and ratio of body weight loss to body weight increased considerably during the first week of training, and was followed by a more gradual increase during the last week of training. The mean values of rise in rectal temperature decreased during training. The mean values of increase in heart rate on the first week and the second week were significantly smaller than the mean value before training. The mean value of oxygen uptake and oxygen uptake per body weight decreased gradually during training. Mean Na concentration in sweat showed a tendency to be lower during training.

DISCUSSION

Acclimatization to heat appears to be not only a physical function concerning body temperature regulation but also a physical characteristics (Coon *et al.*, 1950). It is known that a rise in ambient temperature results in a decrease of subcutaneous fat due to a decrease in the caloric intake and the body shape accordingly becomes more slender by the decrease of subcutaneous fat (Hori *et al.*, 1982). Thus it is assumed that a thinner skinfold thickness and a greater value of the ratio of body surface area to body weight in summer might be caused by a hot climate. The thickness of subcutaneous fat prevents heat transfer from the body to the environment. Heat dissipation from the body to the environment is proportional to the body surface area and metabolic heat produced in the body is proportional

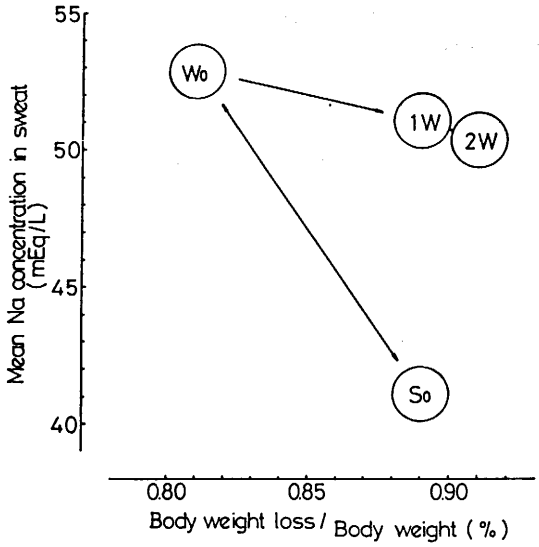


Figure 1 Changes in correlation between mean Na concentration in sweat and body weight loss per body weight induced by climatic heat acclimatization and physical training.
So: Summer, Wo: Winter, 1W,2W: Weeks after training
Circle: Drawn around the means with arbitrary radiuses.
⟷ Seasonal change
⟶ Training effect

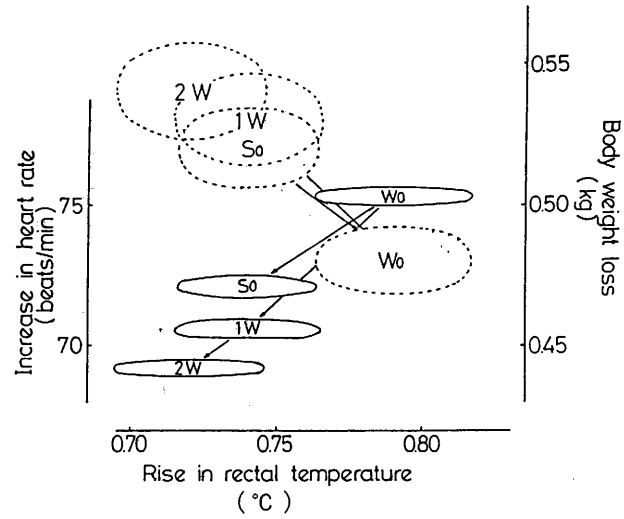


Figure 2 Correlation between increase in heart rate, body weight loss and rise in rectal temperature.
So: Summer, Wo: Winter, 1W,2W: Weeks after training
◯: Correlation between increase in heart rate and rise in rectal temperature.
◌: Correlation between body weight loss and rise in rectal temperature.
Circles: Drawn around the means with radiuses of half values of standard errors.
⟷: Seasonal change
⟶: Training effect

to the body weight during movement of the body, for example, in walking or running. Therefore, the lessening of subcutaneous fat and the greater ratio of body surface area to body weight caused by a hot climate are considered to be convenient for the regulation of body temperature in a hot environment. As shown in Table 3, the mean value of body weight loss in summer was greater than that in winter, while the mean value of the mean Na concentration in sweat in summer was significantly smaller than that in winter. These findings were in agreement with the results reported by many investigators (Ohara 1966; Ihzuka *et al.*, 1986). A decrease in salt concentration of sweat increases the difference in vapor pressure between the sweat on the skin and the surrounding air (Hori *et al.*, 1982). It may thus be considered that the smaller rise in rectal temperature during exercise in summer was caused by a greater heat dissipation due to profuse sweating with lower Na concentration as well as the physical characteristics favorable to heat dissipation. Lesser increase in heart rate during exercise in summer might be caused by a lower rise in rectal temperature and an increase in blood volume with an improvement of the skin circulation (Bass and Henschell, 1956; Senay 1972). As shown in Table 2, all out time, $\dot{V}o_2max$ and $\dot{V}o_2max$ per body weight were greater in summer than in winter though these differences were not significant. Greater values of all out time and $\dot{V}o_2max$ in summer might reflect a lesser increase in heart rate during exercise in heat, and greater values of $\dot{V}o_2max$ per body weight might be caused by a decrease in body weight and a lesser increase in the heart rate during exercise. As shown in Table 3, sweat volume increased progressively due to the imposition of internal and external heat stress during successive exposures to exercise in a hot environment (Ogawa *et al.*, 1982; Ogawa and Asayama 1986; Tsujita *et al.*, 1989). During the first week of physical training, sweat volume increased markedly and the increase in sweat volume was slight during the next week while the change in the Na concentration of the sweat was small. In Fig. 1. Seasonal change in correlation between Na concentration in sweat and body weight loss (sweat volume) was compared with the change that was induced by physical training in a hot environment. This figure indicates greater differences in the change of sweating reaction induced by two types of heat adaptation.

Since the concentration of sweat Na increases progressively as the rate of sweating increases (Kuno

1956), there can be no doubt about decrease in Na concentrations in sweat at a given sweat rate during physical training. However, the decrease in the concentration of the sweat rate from winter to summer was much greater than that induced by physical training in heat. According to Conn *et al.*, (1946), reabsorption of salt at the duct of the sweat gland from the precursor sweat secreted at the acinus of the sweat gland is enhanced by the increased secretion of mineralocorticoid in summer, and the Na concentration in sweat decreases in spite of the increase in the sweat rate in summer. It is presumed that two weeks were too short a time span for the effects of mineralocorticoid to appear on the reabsorption of salt at the duct of the sweat gland or that the increase in the secretion of aldosterone induced by physical training was too small to decrease the Na concentration in sweat. In heat adaptation, a reduction in the rise of rectal temperature is usually accompanied by lessening of the increase in the heart rate. Changes in the relationship between body weight loss, increase in heart rate and rise in rectal temperature during climatic heat acclimatization and physical training in heat were shown in Fig. 2. As shown in Fig. 2, changes in the correlation between increase in heart rate and rise in rectal temperature and the correlation between sweat volume and the rise in rectal temperature induced by climatic heat acclimatization were approximately the same as those induced by physical training in heat. Thus it seems certain that there was a discrepancy with respect to the changes in Na concentration in sweat and changes in other physiological

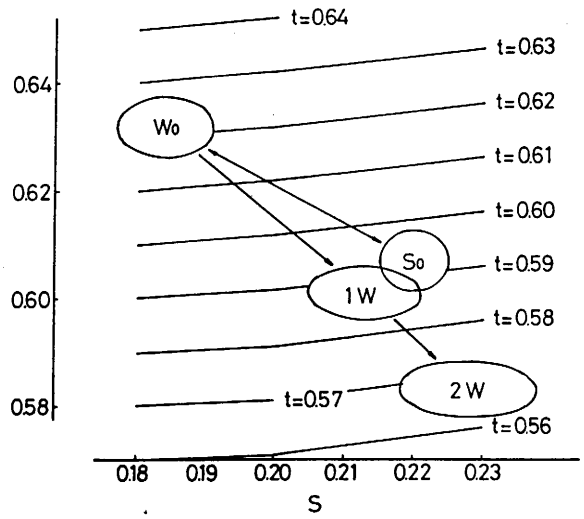


Figure 3 Changes in correlation between stress index and value of S induced by climatic heat acclimatization and training.
I,S,T: The same as in table 4.
So: Summer Wo: Winter 1W,2W: Weeks after training
Circles: Drawn around the means with radiuses of standard errors.
⟷: Seasonal change
⟶: Training effect

responses to heat exposure or exercise in a hot environment. To assess physiological strain induced in the body by exercise in heat, we expressed the magnitude of the strain by a combination of relative increase in heart rate, relative rise in rectal temperature, and relative water loss, using the critical values of these three fac-

Table 4 Stress index and its components ($\bar{X}\pm SD$)

Season	Training (week)	A	B	C	I	S	t
Summer	0	**S 0.556±0.018	0.213±0.049	0.126±0.022	**S 0.607±0.020	**S 0.220±0.019	**S 0.592±0.019
Winter	0	0.586±0.019	0.237±0.056	0.115±0.019	0.632±0.020	0.184±0.023	0.624±0.020
	1	**t 0.543±0.016	0.213±0.052	0.127±0.025	**t 0.601±0.017	*t 0.213±0.029	**t 0.583±0.017
	2	**t 0.531±0.016	0.201±0.053	0.130±0.029	**t 0.583±0.017	**t 0.228±0.034	**t 0.566±0.017

$A=\frac{\Delta H}{200-H}$, $B=\frac{\Delta Tre}{40.6-Tre}$, $C=\frac{\Delta W}{0.07W}$, $I=\sqrt{A^2+B^2+C^2}$, $S=\frac{C}{\sqrt{A^2+B^2}}$, $t=\sqrt{A^2+B^2}$

*S: Significant differences between summer and winter.
*t: Significant differences between before training and after training.
*: $P<0.05$, **: $P<0.01$
ΔH: Increase in heart rate (beats/min)
H: Heart rate before exercise (beats/min)
ΔTre: Rise in rectal temperature (C)
Tre: Rectal temperature before exercise (C)
ΔW: Body weight loss (kg)
W: Body weight before exercise (kg)

tors as those which cause all out (heart rate, 200 beats/min), heat stroke (rectal temperature, 40.6°C) and water depletion heat exhaustion (body weight loss, 7% of body weight) (Leithead and Lind 1964). The value of stress index I was calculated as follows. $I=\sqrt{A^2+B^2+C^2}$

$$A=\frac{\Delta H}{200-Ho} \qquad B=\frac{\Delta T}{40.6-To} \qquad C=\frac{\Delta W}{0.07W}$$

where: Ho=Heart rate before exercise (beats/min)
ΔH=Increase in heart rate at the end of the experiment (beats/min)
To=Rectal temperature before exercise (°C)
ΔT=Rise in rectal temperature at the end of the experiment (°C)
W=Body weight before exercise (Kg)
ΔW=Weight loss at the end of the experiment (Kg)

Since the value of I is defined as the magnitude of strain induced in the body, a smaller value of I during exercise in heat indicates a superior capacity for prolonged exercise in a hot environment and we can expect that heat acclimatization induced by a hot climate and successive exposures to a combination of exercise and a hot environment is accompanied by a reduction of the I value.

By calculation and transformation,

Equation $I=t\sqrt{1+S^2}$ can be derived as follows;

$$I=\sqrt{A^2+B^2}\sqrt{1+\frac{C^2}{A^2+B^2}}=t\sqrt{1+S^2}$$

where $t=\sqrt{A^2+B^2}$

$$S=\frac{C}{\sqrt{A^2+B^2}}$$

The decrease in the value of parameter "t" was much greater in physical training in heat than that in climatic heat acclimatization, while the increase in the value of parameter "S" was much greater in climatic heat acclimatization than in physical training in heat. Thus a change in the value of "t" represents the training effect and change in the value of "S" represents the effect of climatic heat acclimatization. The mean values and the standard deviation values of A, B, C, I, S and "t" calculated using the data obtained in the present experiment are given in Table 4. The mean values of A, I and "t" were significantly smaller in summer than in winter while the mean value of S was significantly greater in summer than in winter. The mean values of A, I and "t" decreased significantly and the mean value of S increased significantly during physical training in heat. The contribution of the relative increase in heart rate (A) to the value of I was greater than the relative rise in rectal temperature (B) and relative body weight loss (C). Reduction of the magnitude of strain as a whole (I) was brought about by climatic heat acclimatization and physical training at the expense of the increase in the magnitude of strain C. In Fig. 3, seasonal change in relation to the values of I and S was compared with the change in that induced by physical training in a hot environment. In this figure, iso-training lines are drawn by connecting the points of the same value of parameter "t". As shown in Fig. 3, a decrease in the value of "t" was usually accompanied by an increase in the value of S during both climatic heat acclimatization and physical training in heat. However, the decrease in the value of "t" was greater in physical training than in climatic heart acclimatization and the reduction of the values of I and "t" during physical training in heat for 1 week was greater than that induced by climatic heat acclimatization for 6 months. The increase in the value of S during physical training in heat for 2 weeks was greater than that caused by seasonal acclimatization to heat. Thus, it can be said that heat tolerance and the capacity for prolonged exercise in heat can be improved more rapidly by successive exposures to exercise and a hot environment as compared to climatic heat acclimatization.

REFERENCES

1) Araki, T., Matsushita, K., Inoue, Y. and Nakao, M. Effect of physical training on exercise-induced sweating in women. (1981): J. Appl. Physiol. 51, 1526-1532.
2) Bass, D.E. (1963): Thermoregulatory and circulatory adjustments during acclimation to heat in man. In: Temperature, Its Measurement and Control in Science and Industry, ed. by Herzfeld, C.M. Reinhold, New York, 3:299-305.
3) Bass, D.E. and Henschell, A. (1956): Responses of body fluid compartments to heat and cold. Physiol. Rev., 36: 128-144.
4) Coon, C.S., Garn, S.M. and Birdsell, J.B. (1950): A study of the problems of race formation in man. Charles C. Thomas, Springfield, Illinois.
5) Conn, J.W., Johnson, M.W. and Louis, L.H. (1946): Acclimatization to humid heat, A function of adrenal cortical activity. J. Clin. Invest., 25;912-913.
6) Hori, S. (1977): Changes in physiological reactions to a hot environment induced by climatic change and physical training. Jpn. J. Trop. Med. Hyg., 15(2):93-104.
7) Hori, S., Tsujita, J., Tanaka, N. and Mayuzumi, M. (1978): Studies on heat tolerance of subtropical natives after migration to a temperate zone. Int. J. Biometeor. 22(2):82-93.
8) Hori, S., Ihzuka, H. and Nakamura, M. (1982): Comparison of physiological responses to heat between subtropical and temperate natives. Jpn. J. Trop. Med. Hyg., 10 (3,4):229-237.

9) Ihzuka, H., Hori, H. and Akamatsu, T. (1986): Seasonal variations of physiological responses to heat of subtropical and temperate natives. Int. J. Biometeor., 30(2):107-113.
10) Kuno, Y. (1956): Human perspiration, Charles C. Thomas, Springfield, Illinois.
11) Leithead, C.S. and Lind, A.R. (1964): Heat stress and heat disorders. Davis, Philadelphia.
12) Lind, A.R. and Bass, D.E. (1963): The optimal exposure time for the development of accilmatization to heat. Federation Proc., 22:704-708.
13) Ohara K. (1966): Chloride concentration in sweat, its individual, regional, seasonal and some other variations, and interrelations between them. Jpn. J. Physiol., 16:274-290.
14) Ogawa, T. and Asayama, M. (1986): Quantitative analysis of the local effect of skin temperature on sweating. Jpn. J. Physiol., 36, 417-422.
15) Ogawa, T., Asayama, M. and Miyagawa, T. (1982): Effects of sweat gland training by repeated local heating. Jpn. J. Physiol., 32, 971-981.
16) Piwonka, R.W., Robinson, S., Gay, V.L. and Manald, R. S. (1965): Preacclimatization of men to heat by training. J. Appl. Physiol., 20:379-384.
17) Robinson, S., Turrell, E.S., Belding, H.S. and Horvath, S. M. (1953): Rapid acclimatization to work in hot climates. Am. J. Physiol., 140:168-176.
18) Senay, L.C., Jr. (1972): Changes in plasma volume and protein content during exposures of working men to various temperature before and after acclimatization to heat. Separation of the roles of cutaneous and skeletal muscle circulation. J. Physiol., 224:61-81.
19) Shvartz, E., Bhattacharta, A., Sperinde, S.J., Brock, P.J., Sciaraffa, D. and Beaumount, W.V. (1979): Sweating responses during heat acclimation and moderate conditioning. J. Appl. Physiol., 46, 675-680.
20) Tsujita, J., Hori, S., Mayuzumi, M., Meren, J. and Tanaka, N. (1989): Effect of ambient temperature on blood consitituents during prolonged exercise. Proceeding of the 11th ISB Congress. ed. Driscoll, D. and Box, E.O. 287-295.
21) Wyndham, C.H., Strydom, N.B., Morrison, J.F., Williams, C.G., Bredell, G.A.G., von Rahden, M.J.E., Holdsworth, L.D., van Graan, C.H., van Rensburg, A.J. and Munro, A. (1964): Heat reactions of Caucasians and Bantu in South Africa. J. Appl. Physiol., 19:598-606.

Jpn. J. Trop. Med. Hyg., Vol. 21, No. 4, 1993, pp. 201-206

BASIC STUDIES ON THE MONGOLIAN GERBIL AS A SUSCEPTIBLE HOST TO FILARIAL INFECTION: COMPARATIVE STUDIES ON SERUM BIOCHEMICAL VALUES BETWEEN THE WILD-COLORED GERBIL AND THE COAT COLOR MUTANTS

KAZUHIRO SHICHINOHE[1,2], MASUMI SHIMIZU[1,2], KATSUYA HAGA[2], TETSUO IEDOKORO[2] AND KOICHIRO FUJITA[2]

Received August 30 1993/Accepted October 7 1993

Abstract: We made a comparative study on serum biochemical values of mature Mongolian gerbils between the wild-colored (agouti) and the coat color mutants of both sexes by autoanalyzers. The coat colors of the mutants were white spotted-agouti, albino, black and white spotted-black. All of serum biochemical values we measured of different coat color gerbils were not significantly different each other. We did not find any lipemic sera and any hyperglycemia in all coat color gerbils. Compare to the other common laboratory rodents, patterns and values of serum protein fractions of the gerbils were different. Namely, A/G ratios and rates of γ-globulin fraction of gerbils were much higher than those of mice and rats and were the same as those of human beings.

INTRODUCTION

We made a comparative study on serum biochemical values of Mongolian gerbils between the wild-colored (called agouti) gerbils and the coat color mutants of both sexes in this report.

The Mongolian gerbil (*Meriones unguiculatus*) can be kept and handled so easily in laboratories and is so readily infected with some filarias (Ash and Riley, 1970a,b; Dalesandro and Klei, 1976; Matsuda *et al.*, 1976) that it has been used as an experimental host of filariasis. Biological characteristics of this animal were studied in 1960s when it was started to be used as a laboratory animal (Schwentker, 1963; Ruhren, 1965; Mays, 1969). However after that, it has been raised just only as a material, that means as a host only for providing parasites used for research, and it's own characteristics have not been studied systematically.

Genetic influences on host reactions against filarial infections could not be studied because those gerbils commonly used were only agouti type which was not controlled genetically at all. But we have raised and kept the closed colonies of the coat color mutants of Mongolian gerbils which have white-spotted agouti, albino, black or white-spotted black coat color and those coat color mutants are expected as a model to study influences of genetic background on response against filarial infections. We have studied to characterize the coat color mutants of Mongolian gerbils biologically and hematologically (Shimizu *et al.*, 1990, 1991) and we also need data on serum biochemical values.

MATERIALS AND METHODS

Mongolian gerbils were fed a commercial pellet for small rodents (MF; Oriental Yeast Inc., Tokyo, Japan) and water ad libitum under a conventional condition and housed 5 animals of the same sex in one cage. The room temperature and the humidity were maintained at 24± 2°C and 60±5 %, respectively. The coat color mutants of the gerbils maintained in our laboratory were agouti, white-spotted agouti, albino, black and white spotted -black and origins of these gerbils were described in our

1 Department of Laboratory Animal Science, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113, Japan
2 Department of Medical Zoology, Faculty of Medicine, Tokyo Medical and Dental University, 1-5-45 Yushina, Bunkyo-ku, Tokyo 113, Japan

Table 1 Serum biochemical values of the agouti type and the coat color mutants of Mongolian gerbils (1)

Coat color	Sex	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)
Agouti	Male	89±10	66±10	55± 3*
	Female	89±18	76± 9	37±10
White spoted-agouti	Male	77±14	77± 7	81±17**
	Female	90±18	91±12	45± 2
Albino	Male	91±18	74±15	41± 9
	Female	102±19	81±16	39±10
Black	Male	88±14	65± 8	57±18
	Female	87±16	76±23	44± 8
White spotted-black	Male	87±14	62±18	77±26
	Female	89±18	63± 9	70±18

The values are derived from 14 to 15-week-old gerbils and represent means ±standard deviations for 10 gerbils of each group. *, ** The difference between sexes is significant by Student's t-test (*$p<0.05$, **$p<0.01$). There are no significant coat color differences in those values.

Table 2 Serum biochemical values of the agouti type and the coat color mutants of Mongolian gerbils (2)

Coat color	Sex	GOT (IU/l)	GPT (IU/l)	LDH (IU/l)	ALP (IU/l)
Agouti	Male	388±152	50±16	950±452	164±47
	Female	363±188	61±23	728±476	172±79
White spotted-agouti	Male	355±152	41±22	910±393	99±21
	Female	408±120	29±16	887±433	88±22
Albino	Male	436± 97	43±22	1131±547	145±53
	Female	276±103	43±26	711±459	151±25
Black	Male	422± 55	54±30	1128±197	121±43
	Female	403±139	42±23	1012±559	129±29
White spotted-agouti	Male	342±143	37±14	900±205	82±20
	Female	389± 81	34±11	950±470	110±27

The values are derived from 14 to 15-week old gerbils and represent means ±standard deviations for 10 gerbils of each group. Glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, lactate dehydrogenase and alkaline phosphatase are abbreviated to GOT, GPT, LDH and ALP, respectively. There are no sex differences and no coat color differences in those values significantly by Student's t-test.

Table 3 Serum biochemical values of the agouti type and the coat color mutants of Mongolian gerbils (3)

Coat color	Sex	Uric acid (mg/dl)	Urea nitrogen (mg/dl)	Creatinine (mg/dl)	Sodium (mEq/l)	Potassium (mEq/l)	Chloride (mEq/l)	Calcium (mg/dl)
Agouti	Male	1.2±0.9	23± 8	0.4±0.1	157±2	7.3±1.5	121± 3	8.8±0.3
	Female	0.7±0.2	31± 5	0.5±0.1	157±6	7.0±0.9	123± 6	8.8±0.7
White spotted-agouti	Male	0.5±0.3	26± 6	0.5±0.1	156±2	6.5±2.1	122± 2	7.7±0.8
	Female	0.8±0.5	25± 2	0.5±0.1	155±2	7.8±1.1	121± 1	8.1±0.6
Albino	Male	0.9±0.3	31± 2	0.5±0.1	149±3	10.7±1.2	119± 3	9.0±0.8
	Female	1.2±1.1	21±13	0.5±0.1	147±9	9.4±2.3	113±10	9.4±0.6
Black	Male	0.8±0.2	18± 3	0.3±0.1	151±2	9.5±0.7	118± 1	9.0±0.7
	Female	1.4±0.8	20± 4	0.4±0.1	149±3	10.4±1.4	115± 3	9.2±0.4
White spotted-black	Male	0.7±0.4	18± 3	0.3±0.1	149±3	8.7±0.8	115± 4	9.1±0.9
	Female	1.0±0.7	25± 6	0.4±0.1	147±1	8.8±0.7	113± 2	9.4±0.7

The values are derived from 14 to 15-week old gerbils and represent means ±standard deviations for 10 gerbils of each group. There are no sex differences and no coat color differences in those values significantly by Student's t-test.

Table 4 Serum biochemical values of the agouti type and the coat color mutants of Mongolian gerbils (4)

Coat color	Sex	Total protein (g/dl)	Albumin (g/dl)	A/G ratio
Agouti	Male	5.98±0.30	3.61±0.16	1.56±0.29
	Female	6.24±0.34	3.68±0.22	1.44±0.09
White spotted-agouti	Male	5.92±0.15	3.61±0.13	1.57±0.18
	Female	6.32±0.25	3.65±0.18	1.39±0.25
Albino	Male	5.86±0.21	3.49±0.08	1.47±0.11
	Female	5.80±0.32	3.60±0.13	1.68±0.37
Black	Male	5.62±0.15	3.39±0.10	1.53±0.20
	Female	5.94±0.21	3.56±0.14	1.52±0.27
White spotted-black	Male	5.84±0.39	3.50±0.13	1.51±0.20
	Female	5.76±0.27	3.45±0.08	1.51±0.23

The values are derived from 14 to 15-week-old gerbils and represent means ±standard deviations for 10 gerbils of each group. There are no sex differences and no coat color differences in those values significantly by Student's t-test.

previous report (Shimizu *et al.*, 1990). Ten animals of each coat color of both sexes aged 14 to 15 weeks old were used in the experiment.

Samples of blood were collected from the hearts of animals anesthetized with ether in the morning after overnight fasting. They were centrifugalized and the sera were stored at -80°C until assays.

The autoanalyzer, Hitachi 736 (Hitachi co. Ltd. Japan) or Spotchem (Kyoto Daiichi Kagaku co. Ltd. Japan) was used to measure the followings: glucose (GOD-POD), glutamate oxaloacetate transaminase (GOT) (OAC-POP-POD), glutamate pyruvate transaminase (GPT) (POP-POD), lactate dehydrogenase (LDH) (lactate pyruvate method), alkaline phosphatase (ALP) (p-nitrophenylphosphate), cholesterol (CE-COD-POD), triglyceride (LPL-GYOD-POD), uric acid (uricase-POD), urea nitrogen (urease method), creatine (Jaffe reaction), sodium, potassium and chloride (electrode), calcium (OCPC), total protein (BCG). Protein fractions were measured by using electrophoresis. Those values were evaluated statistically by Student's t-test.

Results

Table 1 to 4 show serum biochemical values of Mongolian gerbils including the coat color mutants after overnight fasting. Means of some biochemical values were as follows: glucose 77-102 mg/dl, cholesterol 62-81 mg/dl, triglyceride 37-81 mg/dl (Table 1), GOT 276-436 IU/l, LDH 712-1131 IU/l (Table 2), uric acid 0.5-1.4 mg/dl in males and 0.7-1.4 mg/dl in females (no sex difference) (Table 3), total protein 5.62-6.32 g/dl, A/G ratio 1.39-1.68 (Table 4).

Figure 1 shows patterns of serum protein fractions of Mongolian gerbils. Mean values of all coat color gerbils of each fraction were as follows: Albumin 57.8-62.3 %, α_1-globulin 1.9-2.5 %, α_2-globulin 8.8-11.1 %, β-globulin 4.4-9.2 %, γ-globulin 18.1-23.6 %.

All the values we measured were not significantly different between the agouti gerbil and the coat color mutants.

Discussion

The present paper is the first report of a comparative study on serum biochemical values between the agouti type and the coat color mutants of Mongolian gerbils. In this experiment, significant differences between the agouti gerbil and the coat color mutants were not detected in serum biochemical values. But some values of the agouti gerbils were different from those reported by earlier workers.

Mays (1969) reported that uric acid value had male dominance in Mongolian gerbils, but we could not find such sex difference in that of each coat color gerbil (Table 3). Activity of γ-glutamyltranspeptidase of gerbils (data not shown) was under measurable level similar to that of the other small rodents (Tanimoto, 1988). GOT and LDH of gerbils were extremely higher than those of rats and mice (Table 2). As those samples were not hemolysis and those data were re-confirmed, we thought that is one of particular characteristics of gerbils. The more precise experiments in future are needed to determine it. All the sera from total 100 gerbils we tested were not lipemic at all, although earlier workers pointed out that 30 % of the sera were lipemic (Ruhren, 1965; Rich; 1968; Mays, 1969). This difference might be caused by that earlier workers did not establish the suitable way of feeding for gerbils

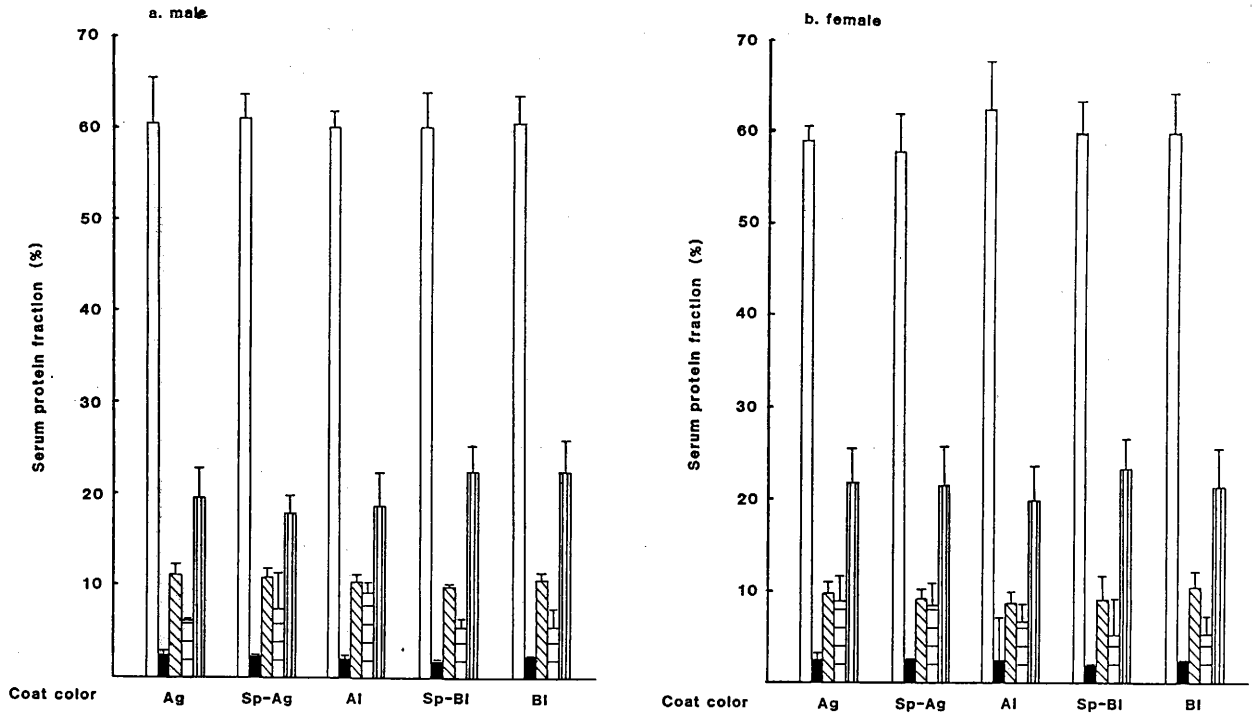


Figure 1 Serum protein fractions of the agouti type and the coat color mutants of male (a) and female (b) Mongolian gerbils. The values are derived from 14 to 15-week-old gerbils and represent means ± standard deviations for 10 gerbils of each group. The coat colors of agouti, white spotted-agouti, albino, black and white spotted-black are abbreviated to Ag, Sp-Ag, Al, Bl and Sp-Bl, respectively. Each bar represents one of protein fractions as follows; □ albumin, ■ α_1-globulin, ▨ α_2-globulin, ⊞ β-globulin and ▤ γ-globulin. There are no sex differences and no coat color differences in those values significantly by Student's t-test.

which were newly used as a laboratory animal in those days. Now they are raised in certain condition with nutritionally well-balanced commercial pellets. In fact, values of total cholesterol and triglyceride of gerbils were not so high and similar to those of the other common laboratory rodents (Wolford *et al.*, 1986). Nakama (1977) reported that the Mongolian gerbil was hereditary spontaneously diabetic animal. Bonquist (1972) said that there were a few animals which showed diabetes-like symptoms in a stock of old obese gerbils. But glucose values we tested were within a normal range like mice, rats (Wolford *et al.*, 1986) and humans (Ishii *et al.*, 1981) and glucosuria has not been observed at all either (data not shown). Moreover, food and water intake of gerbils has been stable (Shimizu *et al.*, 1990). These facts reveal that the Mongolian gerbil is not a hereditary spontaneously diabetic animal in general.

The patterns of serum protein fractions of gerbils were very different from those of rats and mice. Namely, A/G ratios (Table 4) and rates of γ-globulin fraction (Figure 1) of the Mongolian gerbils were much higher than those of rats and mice and were the same as those of human beings (Ishii *et al.*, 1981; Tanimoto, 1989). As globulin value changes according to environmental conditions, the breeding conditions of gerbils might bring the same level of globulin as human beings. Namely, gerbils in our laboratory have been kept under bacteriologically unsterilized condition called conventional condition and that condition was bacteriologically the same as where human beings live. We think that is one of advantages of the gerbil as an experimental model of human filariasis as well as their peculiar sensitivity because the breeding condition of animals would effect on sensitivity to parasites (Shichinohe *et al.*, 1990). The Mongolian gerbil has been known to be the most successful host for experimental filariasis such as *Brugia* spp. (Ash and Riley, 1970a,b), *Dipetalonema vitae* (Dalensandro and Klei, 1976) and *Litomosoides carinii* (Matsuda *et al.*, 1976). This character of the gerbil is really valuable because it is very expensive and difficult for studies of filariasis to use their own natural hosts in laboratories. For example, natural mammalian hosts of *Brugia pahangi* are monkeys, cats and dogs (Edeson, 1959; Edeson and Wilson, 1964) and numerous attempts to introduce this worm into commonly avail-

able laboratory rodents such as rats and mice have been failed (Laing *et al.*, 1961; Ahmed, 1967; Ash and Riley, 1970b; Sucharit and MacDonald, 1973). However, the reason why gerbils are readily infected with them has not been fully understood. The earlier studies on experimental filarial infections in Mongolian gerbils suggested that the sensitivity of gerbils to filarial worms were brought by less responsivility of cellular immunity against parasites (Lammie and Katz, 1983; Klei *et al.*, 1990). On humoral immunity of gerbils, they had IgG antibody (Tomisato *et al.*, 1983; Farrar *et al.*, 1991), IgE -like antibody in their intact sera, produced it against worm antigen derived from *Dirofilaria immitis* (Shichinohe *et al.*, 1992) and much higher γ-globulin level than mice and rats in the present experiment. These facts that gerbils had enough components of humoral immunity were thought to be interesting in contrast with their peculiar sensitivity to parasites. They might indirectly support the statement of earlier workers that their sensitivity was caused by less cellular immunoreactivity.

Mongolian gerbils commonly used were only agouti type and their genetic background have not been controlled at all in contrast with rats and mice which have been controlled genetically to establish many inbred strains. We have studied characteristics of the coat color mutants of gerbils as compared with the agouti gerbil and until now, we have not found any significant differences between the agouti gerbil and the coat color mutants in the serum biochemical values as well as in biological and hematological values (Shimizu *et al.*, 1990, 1991). Then, if the different coat color gerbils will respond differently each other against filarial infection, it is thought that genetic background of gerbils effects on response against filarial infection. Consequently, present study on serum biochemical values of the Mongolian gerbil would be useful as a basic data to analyze mechanisms of host-parasite relationship using gerbils and the coat color mutants of Mongolian gerbils would be expected an useful model to study an influence of genetic background on reactions against filarial infection.

REFERENCES

1) Ahmed, S.S. (1967): Studies on the laboratory transmission of sub-periodic *Brugia malayi* and *B. pahangi*. I. The resistance of guinea-pigs, rabbits and white mice to infection, Ann. Trop. Med. Parasit., 61, 93-100

2) Ash, L.R. and Riley, J.M. (1970a): Development of *Brugia pahangi* in the jird, *Meriones unguiculatus*, with notes on infections in other rodents, J. Parasitol., 56, 962-968

3) Ash, L.R. and Riley, J.M. (1970b): Development of subperiodic *Brugia malayi* in the jird, *Meriones unguiculatus*, with notes on infections in other rodents, J. Parasitol., 56, 969-973

4) Bonquist, L. (1972): Obesity and pancreatic islet hyperplasia in the Mongolian gerbil, Diabetologia, 8, 274-282

5) Dalesandro, D.A. and Klei, T.R. (1976): Evidence for immunodepression of syrian hamster and Mongolian jirds by *Dipetalonema vitae* infections, Trans. R. Soc. Trop. Med. Hyg., 70, 534-535

6) Edeson, J.F.B. (1959): Studies on filariasis in Malaya: the periodicity of the microfilariae of *Brugia malayi* and *B. pahangi* in animals, Ann. Trop. Med. Parasit., 53, 381-387

7) Edeson, J.F.B. and Wilson, T. (1964): The epidemiology of filariasis due to *Wuchereria bancrofti* and *Brugia malayi*, A. Rev. Ent., 9, 245-268

8) Farrar, R.G., Klei, T.R., McVay, C.S. and Coleman, S.U. (1991): Quantitative characterization of antibody responses to single and multiple *Brugia pahangi* infection in jirds, J. Parasitol., 77, 718-726

9) Ishii, A., Toyama, T. and Sakaguchi, T. (Traslated) (1981): Clinical biochemical and hematological reference values in normal experimental animals and normal humans, Mitruka, B.M. and Rawnsley, H.M. 2nd ed., 345pp., Seishi shoin Ltd., Tokyo

10) Klei, T.R., McVay, C.S., Dennis, V.A., Coleman, S.U., Enright, F.M. and Cassey, H.W. (1990): *Brugia pahangi*; Effects of duration of infection and parasite burden on lymphatic lesion severity, granulomatous hypersensitivity and immune responses in jirds (*Meriones unguiculatus*), Expl. Parasitol., 71, 393-405

11) Laing, A.B.G., Edeson, J.F.B. and Wharton, R.H. (1961): Studies on filariasis in Malaya: further experiments on the transmission of *Brugia malayi* and *Wuchereria bancrofti*, Ann. Trop. Med. Parasit., 55, 86-92

12) Lammie, P.J. and Katz, S. (1983): Immunoregulation in experimental filariasis II; Responses to parasite and non-parasite antigen in jirds with *Brugia pahangi*, J. Immun., 130, 1386-1389

13) Matsuda, H., Takaoka, M. and Tanaka, H. (1976): Effect of diethylcarbamazine on microfilaria of *Litomosoides carinii* in jird, *Meriones unguiculatus*, Jpn. J. Parasitol., 25, 94-98

14) Mays, A. Jr. (1969): Beseline hematological and biochemical parameters of the Mongolian gerbil (*Meriones unguiculatus*), Lab. Anim. Care, 19, 838-842

15) Nakama, K. (1977): Studies on diabetic syndrome and influences of long-term tolbutamide administration in Mongolian gerbils (*Meriones unguiculatus*), Endocrinol. Japon., 24, 421-433

16) Rich, S.T. (1968): The Mongolian gerbil (*Meriones unguiculatus*) in research, Lab. Anim. Care, 18, 235-243

17) Ruhren, R. (1965): Normal values for hemoglobin concentration and cellular elements in the blood of Mon-

golian gerbils, Lab. Anim. Care, 15, 313-320
18) Schwentker, V. (1963): The gerbil; A new laboratory animal, Illinois Vet., 6, 5-9
19) Shichinohe, K., Shimizu, M., Asakawa, M. and Ishizaki, M. (1990): Effect of rearing conditions on Encephalomyocarditis (EMC) virus-induced diabetes in mice, Exp. Anim., 39, 377-381
20) Shichinohe, K., Shimizu, M., Kato, K., Tamura, N., Tsukidate, S. and Fjita, K. (1992): Basic studies on the Mongolian gerbil as a susceptible host to filarial infection; IgE-like antibody formation against *Dirofilaria immitis* adult worm antigen, Jpn. J. Trop. Med. Hyg., 20, 271-281
21) Shimizu, M., Shichinohe, K., Tsukidate, S. and Fujita, K. (1990): Basic studies on the Mongolian gerbil as a susceptible host to filarial infection; Comparative studies on growth and reproduction among coat color mutants and genetic analysis of coat colors, Japan J. Trop. Med. Hyg., 18, 301-310
22) Shimizu, M., Shichinohe, K., Tsukidate, S. and Fujita, K. (1991): Basic studies on the Mongolian gerbil as a susceptible host to filarial infection; Comparative studies on hematological features between the wild -colored gerbil and the coat color mutants, Jpn. J. Trop. Med. Hyg., 19, 331-338
23) Sucharit, S. and MacDonald, W.W. (1973): *Brugia pahangi* in small laboratory animals: attempts to increase susceptibility of white rats to *Brugia pahangi* by host selections, S.E. Asian. J. Trop. Med. Publ. Hlth., 4, 71-77
24) Tanimoto, Y. (1988): Biochemistry of blood and urine in experimental animals, Laboratory Animal Series, 5, 189pp., Soft Science Inc., Tokyo
25) Tanimoto, Y. (Ed.) (1989): Serum biochemical finding, Tajima, Y. (Supervised), Biological reference data book on experimental animals, 151-201, Soft Science Inc., Tokyo
26) Tomisato, M., Nakajima, Y., Nariuchi, H. and Nogami, S. (1983): Detection of IgG antibodies in Mongolian jirds infected with *Brugia pahangi* by enzyme-linked immunosorbent assay, Jap. J. Parasit., 32, 43-54
27) Wolford, S.T., Schroer, R.A., Gohs, F.X., Gallo, P.P., Brodeck, M., Falk, H.B. and Ruhren, R. (1986): Reference range data base for serum chemistry and hematology values in laboratory animals, J. Toxicol. Environm. Health, 18, 161-188

Jpn. J. Trop. Med. Hyg., Vol. 21, No. 4, 1993, pp. 207-211

ANTIBODY FREQUENCY DISTRIBUTION CURVE FOR RISK ASSESSMENT OF A MALARIA EPIDEMIC IN THE SUDAN

SHIGEYUKI KANO[1], SALAH H. EL SAFI[2], FAKHR EL DIN M. OMER[2], PILARITA T. RIVERA[1], AHMED A. EL GADDAL[2] AND MAMORU SUZUKI[1]

Received September 6 1993/Accepted October 7 1993

Abstract: A seroepidemiological study of the prevalence of malaria, employing the frequency distribution curve of ELISA titers, was conducted. Comparison of the shape of the curves obtained in the two villages in the Sudan before and after the flood of the Blue Nile in 1988, clearly demonstrated the existence of certain malarial foci which might pose a potential risk for development of malaria epidemics in the area, although these malarial foci could not be detected by either slidepositivity rate or seropositivity rate. Thus, application of this method is of considerable value in identifying latent malarial foci where control measures should be strengthened.

INTRODUCTION

During the advanced stages of malaria control programmes, cases in which parasites in the peripheral blood are in such low numbers that they cannot be readily detected by microscopical examination become a majority (Sadun, 1972). On such an occasion, a more suitable method for epidemiological assessment is required, in order to detect latent malarial foci in apparently controlled areas. In the present study, follow up epidemiological surveys were conducted in two villages in the Sudan in 1987 and 1989. In 1987, the frequency distribution curve of antibody titers (Kagan, 1973) revealed a potential danger of future malaria epidemics in one of the villages. In 1988, the area was flooded by a heavy rainfall which increased the vector breeding sites and caused the occurrence of malaria epidemics even in the controlled area. In fact, our survey conducted in 1989 clearly confirmed an epidemic in a village where the potential risk had earlier been presumed. In the present study, the usefulness of the frequency distribution curve of antibody titers in the identification of latent malarial foci is discussed.

SUBJECTS, MATERIALS AND METHODS

Study area

The Gezira irrigated area, which covers a total of 2 million acres, lies between latitude 13°N and 15°N in the central part of the Sudan (Fig. 1). It has a population of about 2 million. The area is irrigated by gravity through a canal system from Sennar Dam on the Blue Nile. The area has a hot dry season from April to June with an average daily temperature of 32°C and relative humidity of 20%, and a cool dry season from December to March, with average daily temperature of 22°C, and relative humidity of 30%. Average annual rainfall is about 225 mm occurring between July and September (El Gaddal *et al.*, 1985). The Gezira area is covered by the Sudan Blue Nile Health Project which is responsible for control of water-borne diseases including malaria. In December 1987, two villages were selected for the present pilot study. Mobi, a well controlled area with low incidence of malaria cases. On the other hand, Sennar, located beside the dam, maintained many probable vector breeding sites, and was regarded as hyperendemic. A follow up survey was carried out in January 1989 in the same villages. In August 1988, between our two surveys, the heaviest rainfall in the last 40 years caused the flooding of the Nile.

1 Department of Parasitology, Gunma University School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371, Japan
2 Blue Nile Health Project, Ministry of Health, Wad Medani, Sudan

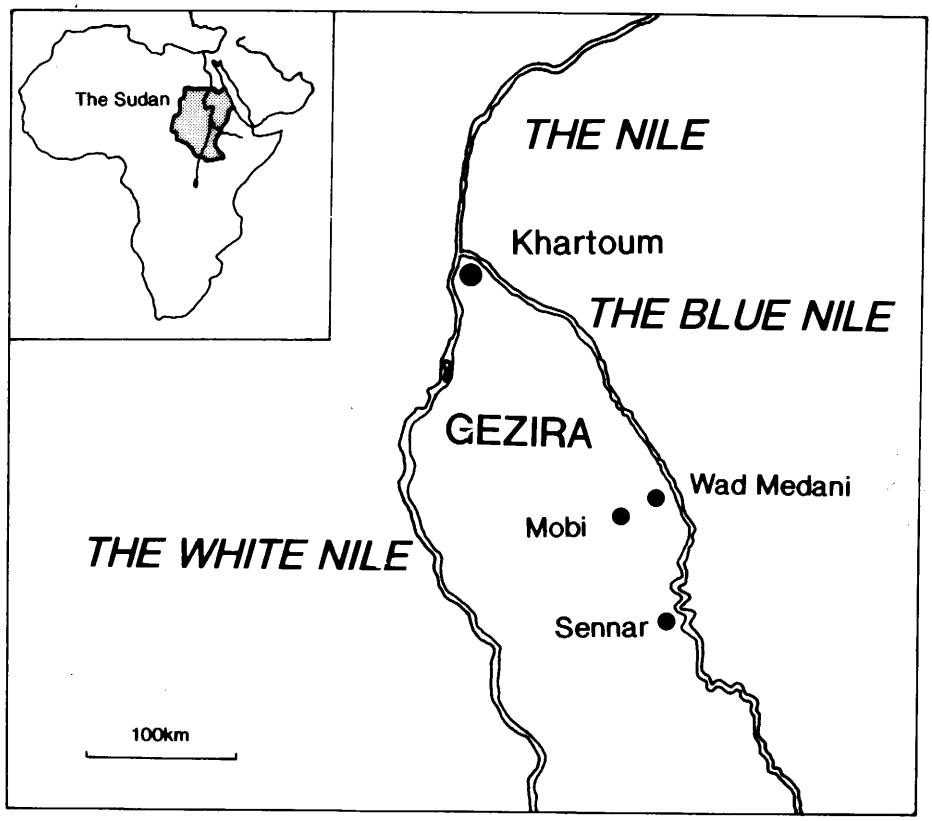


Figure 1 Map of the Sudan Gezira showing study area.

Blood sampling

Sera from the adult were collected by venipuncture. In children and infants to whom venipuncture was not acceptable, plasma specimens were obtained by a finger -prick method (Kano *et al.*, 1989). Thick smears were made simultaneously from all the donors and subjected to parasite detection by microscopic observation. In Sennar, most of the blood samples were taken from school children 7-14 years of age, 61 out of 72 of the children tested in 1989 had also been sampled in 1987. In Mobi, blood samples were obtained at the same health post in 1987 and 1989 where donors were gathered from the neighbourhood. Seventeen out of 46 in 1987 and 58 out of 90 in 1989, were school children 7-14 years of age.

ABC-ELISA

The measurement of antibodies by the ABC-ELISA was carried out according to the method described by Sato *et al.* (1990). This serological method was devised particularly for field use. Briefly, the antigen was obtained from an *in vitro* cultivation (Trager and Jensen 1976: Miyagami and Waki, 1985) of a *Plasmodium falciparum* (*P.f.*) strain (SGE1 strain from Gambie; donated by Dr. Ambroise-Thomas in 1979). The antigen was lyophilized and brought directly to the laboratory of Blue Nile Health Project at Wad Medani. On the day before the test was done, the lyophilized antigen was dissolved to a final protein concentration of 250μg/ml and distributed in each well of a 96-well U-bottom microtiter plate (Greiner, Germany). The plate was left overnight allowing the antigen to dry and coat the bottom of the wells. Sera or plasma at 2-fold dilutions, 1:32-1:1024, were applied. Enzyme reaction was performed by the Vectastain™ ABC kit, which consisted of biotinylated anti-human IgG goat serum and avidin biotin complex horseradish peroxidase (VECTOR Laboratories Inc., CA). Addition of the substrate, 4-chloro-1 -naphtol, generated a blue colour in the well with a positive serum, clearly readable with the naked eye. The final dilution of a serum sample which gave this colour was considered as the antibody titer. And the titer equal to or greater than 1:64 was considered as positive according to our previous report (Sato *et al.*, 1990).

Statistical analysis

The distribution of titers of each group were compared statistically by the Mann-Whitney Test (U -test). The seropositivity and slidepositivity rate of the groups were compared by the Chi square test.

Table 1 Distribution of malaria titers by the ABC-ELISA

		ABC-ELISA TITERS							
		<32	32	64	128	256	512	1024	Total
1987	Sennar	8	30(1)	26	2	4	8(4)	2	80(5)
	Mobi	13	17	12	2	2	0	0	46(0)
1989	Sennar	15(1)	3	7(4)	12(4)	8(5)	12(9)	15(11)	72(34)
	Mobi	63	1	1	7(3)	8(1)	3	7(3)	90(7)

( ) Number of people who showed parasitemia

RESULTS

1. *Comparison of the epidemiological features of malaria in Sennar and Mobi based on the results obtained in 1987.*

1-1) *Slide positivity rate*:

In Sennar, 5 out of 80 examined showed *P.f.* parasites in the thick smear, but none out of 46 in Mobi (Tab. 1). However, no significant difference was recognized by the Chi square test between the slide positivity rate of Sennar and that of Mobi ($P=0.123$).

1-2) *Antibody titration by the ABC-ELISA*:

Antibody titers obtained in Sennar and Mobi did not show normal distribution and the variables of the respective groups differed from each other. Therefore, instead of comparing the geometric mean of the reciprocal titers (GMRT) of each group by the t-test, statistical difference of the groups was tested by the U-test. Significant difference was not convincing between Sennar and Mobi in 1987 ($P=0.006$). Seropositivity rate was also compared by the Chi square test: 42 out of 80 (52.5%) manifested positive titers in Sennar; While in Mobi, 16 out of 46 (34.8%) were seropositive (Tab. 2). However significant difference was not recognized between the two groups ($P=0.055$).

1-3) *The frequency distribution curve of antibody titers*:

The frequency distribution curves of the ABC-ELISA titers are shown in Fig. 2. A bimodal distribution of titers was obtained for 1987 data in Sennar. It appeared that the peak at 1:512 was a reflection of small, recent past epidemics which had occurred in this village. While the antibody distribution profile in Mobi showed only a single peak at low titer of 1:32, which indicated no occurrence of recent epidemics among the inhabitants. Thus the frequency distribution curves showed some epidemiological difference between Sennar and Mobi. It was presumed that some potential foci which might be provoked by some elements into epidemics, existed in Sennar; whereas, in Mobi, the potential risk was minimal if any.

Table 2 Percentage positives by the ABC-ELISA and microscopy

	Sennar		Mobi	
	ABC-ELISA	microscopy	ABC-ELISA	microscopy
1987	52.5	6.3	34.8	0.0
1989	75.0	47.2	28.9	7.8

2. *Changes in the epidemiological features after the flood of 1988.*

2-1) *Sennar*:

In January 1989, when the second survey was conducted, every malariometric index dealt with in the present study showed a statistically significant rise compared to those in 1987. Parasite rate increased from 6.3% to 47.2% ($P<0.001$). Seropositivity rate in 1989 was 75.0% versus 52.5% in 1987 ($P<0.001$). And significant difference in GMRT was also recognized between 1987 ($1:2^{6.0}$) and 1989 ($1:2^{7.3}$), ($P<0.001$). Moreover the most remarkable change was observed in the pattern of frequency distribution curves of antibody titers. In 1989, the second peak became higher, at the highest titer of 1:1024, reflecting the epidemic occurring at that time.

2-2) *Mobi*:

Seven out of 90 (7.8%) were slidepositive and 26 (28.9%) were seropositive in 1989 (Tab. 1 and 2). Comparison with the slidepositivity rate and seropositivity rate obtained in 1987 showed no statistically significant differences ($P=0.052$ and 0.066 respectively). GMRT in 1989 ($1:2^{5.3}$) was also statistically compared to that in 1987 ($1:2^{5.2}$), but the difference was not significant ($P=0.114$). However, the antibody titer distribution curve showed a small second mode at 1:256 in 1989 (Fig. 2), indicating that small scale epidemics had occurred in this village. This finding could only be demonstrated by using the frequency distribution curve of antibody titers.

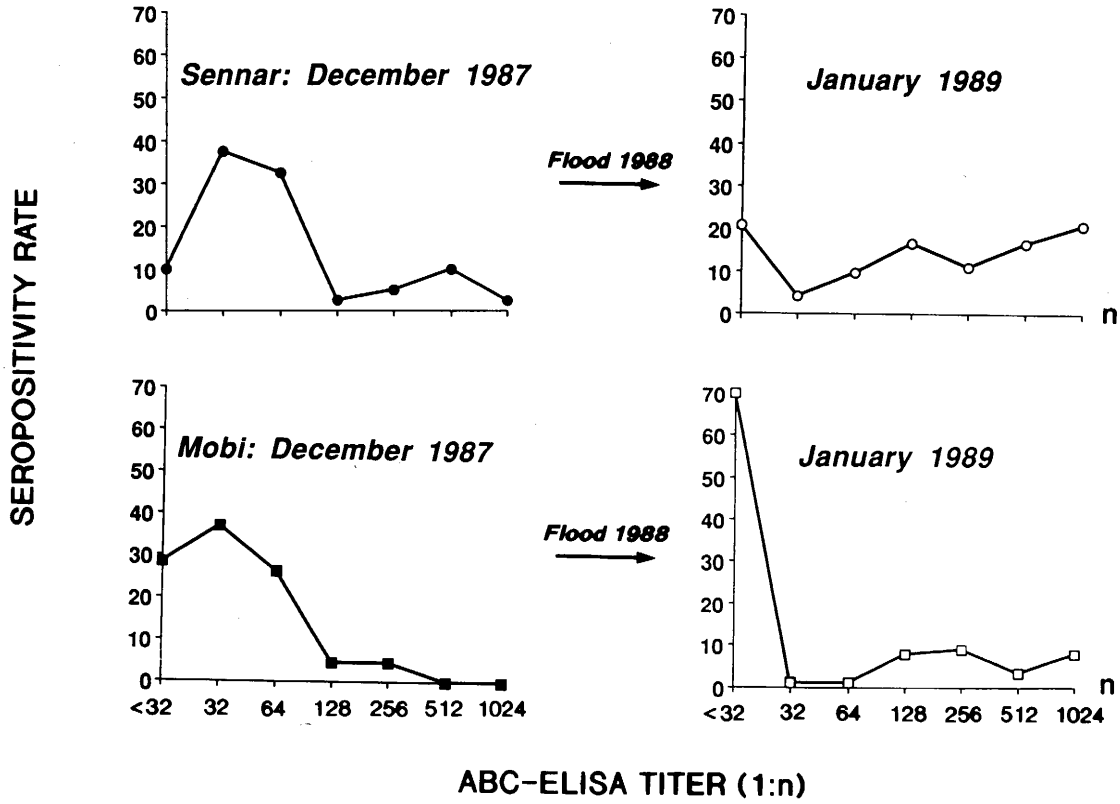


Figure 2 Comparison of the frequency distribution curves of the antibody titers.

DISCUSSION

Seroepidemiology is important, in that it provides more informative *period* prevalence data which cannot be obtained through microscopy (Draper *et al.*, 1972; Colins and Skinner, 1972). In particular seroepidemiological assessment is valuable when a malaria control programme reaches the more advanced stage and the endemicity of malaria becomes very low in the controlled areas (Sadun, 1972: Kagan, 1972). Usually the degree of transmission or prevalence of malaria is measured by either GMRT or percentage of the sample population that is serologically positive (Kagan *et al.*, 1969). However it was clearly shown in the present study that both parameters of assessing malaria prevalence were not adequate in providing detailed information particularly on the potential risk of an epidemic. In Table 2, statistical significant difference between the percentage positives of Sennar and Mobi in 1987, was not convincing by both serology and microscopy. However, the antibody titer distribution curve of Sennar in 1987 revealed a bimodal pattern showing a Second peak at titer 1:512 (Fig. 2). The subjects who manifested titer of 1:512 were those considered to have contracted malaria about the time when the survey was done, and they might constitute the probable *focus* of succeeding transmission in the community. Thus the second mode of the curve was regarded as the significant indicator of potential risk of malaria epidemics which might occur as a consequence of environmental or man-made changes. This assumption was proven to be true after the disastrous flood occurred in August 1988, which adversely affected the well implemented malaria control programme in the whole area including our study villages. In the study in 1989, increased malaria prevalence was actually observed by microscopy. And the frequency distribution curve of the ABC-ELISA titers obtained in Sennar changed into a hyperendemic pattern, showing the highest peak at the highest antibody titer 1:1024 (Fig. 2). This pattern was the reflection of the very recent past or current infection in the majority of the people at the time of the survey.

On the other hand in Mobi, the frequency distribution curve of the ABC-ELISA titer, characterized by a single low-titered peak in 1987, changed into a bimodal pattern suggestive of a small, recent past outbreak of malaria in the village in 1989. Comparison of seropositivity and slidepositivity rates did not present significant difference between the results before and after the flood. Indeed, seropositivity rate was lowered in

1989, in contradiction to what had actually happened.

It was thus affirmed in this study that the actual prevalence of malaria may be more precisely reflected in the shape of the frequency distribution curve of antibody titers. Application of this method will be of considerable value in identification of early or even presumptive malaria foci where control measures must be concentrated.

ACKNOWLEDGEMENTS

This study was supported in part by grants from Monbusho International Scientific Research Programme (NO. 62045009), and Japan-U.S. Medical Science programme. We thank Dr. Taiichi Sato, lecturer of the Department of Public Health, Gunma University School of Medicine, and Prof. Kumiko Sato of the College of Medical Care and Technology, Gunma University, for helpful discussion.

REFERENCES

1) Collins, W.E. and Skinner, J.C. (1972): The indirect flourescent antibody test for malaria, Am. J. Trop. Med. Hyg., 21, 690-695
2) Draper, C.C., Voller, A. and Carpenter, R.G. (1972): The epidemiologic interpretation of serologic data in malaria, Am. J. Trop. Med. Hyg., 21, 696-703
3) El Gaddal, A.A., Haridi, A.A.M., Hassan, F.T. and Hussein, H. (1985): Malaria control in the Gezira -Managil irrigated scheme of the Sudan, J. Trop. Med. Hyg., 88, 153-159
4) Kagan, I.G. (1972): Evaluation of the indirect hemagglutination test as an epidemiologic technique for malaria, Am. J. Trop. Med. Hyg., 21, 683-689
5) Kagan, I.G. (1973): Parasitic Diseases. *In*: Serological Epidemiology, 155-168, Academic Press, London
6) Kagan, I.G., Mathews, H.M., Rogers, W.A. and Fried, J. (1969): Seroepidemiological studies by indirect haemagglutination tests for malaria, Bull. World Health Organ., 41, 825-841
7) Kano, S., Takagi, T., El Gaddal, A.A. and Suzuki, M. (1989): A new method of plasma collection suitable for large scale seroepidemiological surveys of malaria in the tropics, Trans. Roy. Soc. Trop. Med. Hyg., 83, 304
8) Miyagami, T. and Waki, S. (1985): *In vitro* cultivation of *Plasmodium falciparum* under aerobic atmosphere in a CO_2 incubator, J. Parasitol., 71, 262-263
9) Sato, K., Kano, S., Yamaguchi, H., Omer, F.M., El Safi, S.H., El Gaddal, A.A. and Suzuki, M. (1990): An ABC -ELISA for malaria serology in the field, Am. J. Trop. Med. Hyg., 42, 24-27
10) Sadun, E.H. (1972): The research and development of serologic tests for malaria, Am. J. Trop. Med. Hyg., 2, 677-682
11) Trager, W. and Jensen, J.B. (1976): Human malaria parasites in continuous culture, Science, 193, 673-675

Jpn. J. Trop. Med. Hyg., Vol. 21, No. 4, 1993, pp. 213-217

MALARIA SEROLOGY AS AN ALTERNATIVE TO PARASITE DETECTION IN A HIGHLY ENDEMIC AMAZONIAN COMMUNITY

SHIGEYUKI KANO[1], YOSHIAKI UTSUNOMIYA[2], ALFRED TAKESHI HONDA[3], OSEAS SANTOS[4], WALDIR DE SOUZA MIRANDA[4], MILTON MASATO HIDA[5], KEIZO MATSUMOTO[2] AND MAMORU SUZUKI[1]

Received August 27 1993/Accepted October 22 1993

Abstract: The Indian colony under study is located in relatively inaccessible part of the Amazonian jungle, restricting frequent visits. Malaria survey in such a community requires methods which would yield reliable results even from a single visit. Malaria was regarded as a minor disease by the inhabitants who appeared to be free from malaria. The reported incidence was very low, and in fact, no parasitemia was detected in the blood smears of a group of inhabitants obtained through our short visit. But on the contrary, an avidin biotin peroxidase complexed enzyme-linked immunosorbent assay (ABC-ELISA) revealed a 100% prevalence of malaria antibodies in the population. Therefore, serological studies which can obtain the *period prevalence* are useful for the assessment of malaria situation in the highly endemic community which is not readily accessible, or whose populations seem to have acquired a certain degree of immunity depressing parasitemia to submicroscopic levels.

INTRODUCTION

Immunological methods are regarded as the most useful for determining malaria experience of populations especially in the advanced stages toward the eradication (Collins *et al.*, 1968; WHO, 1972). Ordinary slide observation cannot detect latent malaria foci in the controlled area because of the lowered possibility of encountering patent parasitemias (Pampana, 1969). However, we present another circumstance in which serological investigation in retrospective diagnosis of malaria is of high practical value.

We had an opportunity to visit an Amazonian Indian colony in the state of Pará, which per se had been isolated culturally from the surroundings. We could just stay there for a very short time, during which time we obtained the blood samples and detailed malarial histories through a questionnaire. Parasites were not detected in any individuals examined, and the declared malaria histories were rather incidental. However, the ABC-ELISA revealed a high antibody prevalence in the community. The serological profile was considered to give a truer picture of the endemicity of malaria at the place.

MATERIALS AND METHODS

Study area

The colony was located along the Xingu, about 700km to the south-west of Belém, the capital of the state of Pará (Figure 1). The inhabitants were of the Amazonian Indian tribe of Kaiapós with a population of 912 people then (Photo 1). They were basically a subsistent community, isolated from the rest of the people economically and culturally. However, the Brazilian Government started to preserve their way of life, helping the promotion of community health. A nurse was being sent who stayed with them and took care of their health and needs. The malaria situation of the state of Pará was one of the worst in Brazil. Annual report of 1988 of Fundação Nacional de Saúde (National Foundation of Health) described 125,628 positive cases out of a total population of 4,857,199 in Pará (Ministério da Saúde, 1988). In the nearest city

1 Department of Parasitology, Gunma University School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371, Japan
2 Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852, Japan
3 Hospital Amazônia, Beneficência Nipo-Brasileira da Amazônia, Belém, Pará, Brasil
4 Fundação Nacional de Saúde, Ministerio da Saúde, Belém, Pará, Brasil
5 Faculdade de Medicina de Botucatu, Universidade Estadual Paulista, Botucatu, São Paulo, Brasil

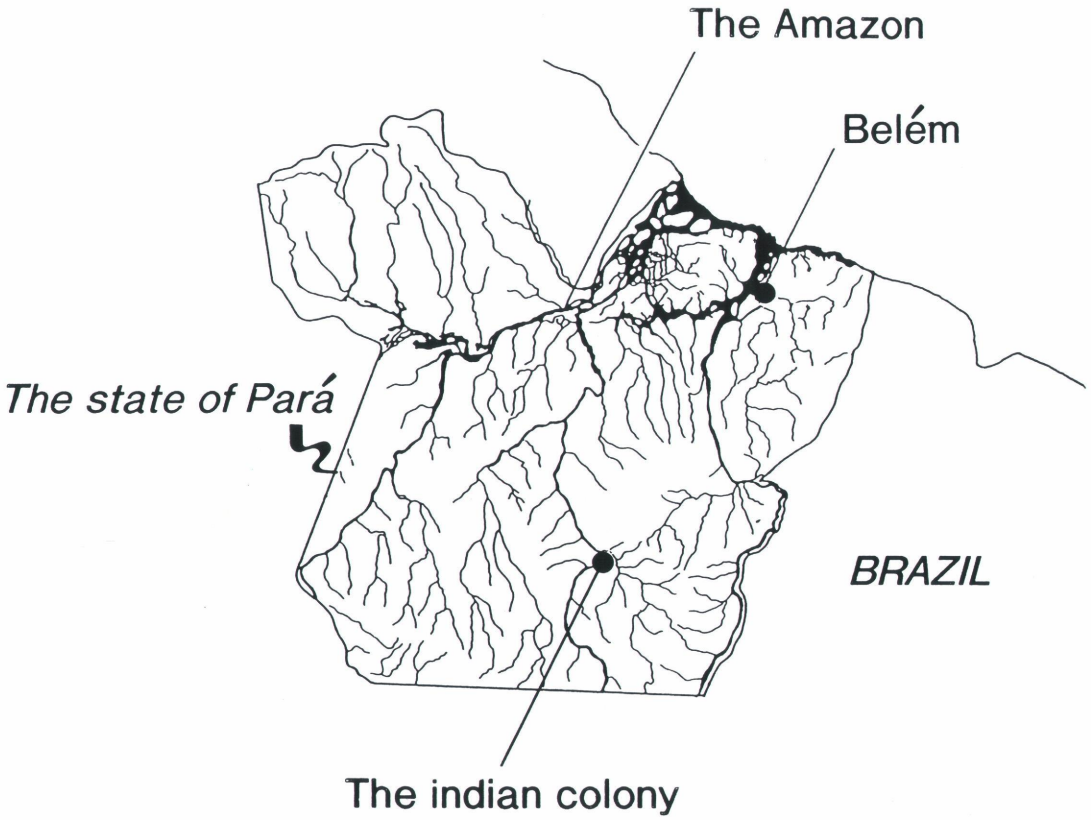


Figure 1 The study area.

Photo. 1 Beautifully painted and dressed indian children in the colony.
The photograph was taken with their permission.

(50km from the colony), Saõ Felix do Xingu, which was the center of deforestation and gold mining, 24,145 parasite positive cases out of 69,570 people were reported (Ministério da Saúde, 1993).

Subjects

Blood smears and sera were collected with informed consent from 20 donors, 8-75 years of age (September 5, 1990). They were asked their age, sex, and malaria episodes (Table 1). The samples were brought back to Gunma University School of Medicine and subjected to microscopical observation and the ABC-ELISA. The results were made known to them after the tests were completed.

ABC-ELISA

The measurement of antibodies by the ABC-ELISA was carried out according to the method described by Sato *et al.* Briefly, the antigen was obtained from an *in vitro* cultivation (Trager and Jensen, 1976; Miyagami and Waki, 1985) of *Plasmodium falciparum* (*P.f.*) strain (SGE1 strain; donated by Dr. Ambroise-Thomas in 1979). Twenty microliter of the antigen solution at a protein concentration of 250 μg/ml was placed in each well of a 96-well U-bottom microtiter plate (Greiner, Germany). The plate was left overnight allowing the antigen to dry and coat the bottom of the wells. Sera at 2-fold dilutions, 1:32-1:1024, were applied. Enzyme reaction was performed by the VECTASTAIN™ ABC Kit, which consisted of biotinylated, affinity-purified anti-human IgG made in goat, avidin DH and biotinylated horseradish peroxidase H (VECTOR Laboratories Inc., CA). Addition of the substrate, 4-chloro-1-naphtol, generated a blue color in the well with a positive serum, clearly readable with the naked eye. This test can be carried out without using major electrical equipment. The final dilution of a serum sample which gave this color was considered as the antibody titer.

RESULTS

Malarial incidence

No parasite was detected from microscopical observation of Giemsa-stained thick blood smears which were obtained from the 20 donors. Examination of local malarial records kept by Fundação Nacional de Saúde revealed 5 parasite-positives out of 222 feverish cases in the colony for the 2-week period prior to our visit. Inhabitants' response to the questionnaire indicated that malaria was considered as a minor disease and that no prophylactic measures were taken against the contraction of malaria.

The ABC-ELISA titers and declared malaria episodes

Table 1 is the individual records on age, sex, the ABC-ELISA titers and declared malaria episodes. Examinees numbered 3 and 9 declared the last malaria episode 5 days before the survey and showed consider-

Table 1 Individual records of a batch of 20 donors

No.	Declared age	Sex	ABC-ELISA titer	Declared malaria episode	
				No. of times	Last episode prior to survey
1	38	f	64	1	several years
2	26	f	128	2	7 months
3	32	f	1024	2	5 days
4	40	f	256	None	
5	73	m	512	1	1 year
6	44	f	64	1	several years
7	74	m	512	1	1 month
8	28	f	32	1	several years
9	8	m	256	3	5 days
10	73	f	128	1	several years
11	30	m	64	1	4 months
12	32	f	64	1	1 year
13	56	m	512	None	
14	28	f	64	None	
15	75	m	512	None	
16	25	f	64	2	5 months
17	47	f	512	None	
18	47	f	512	None	
19	24	f	64	1	1 year
20	50	f	128	1	1 month

able high ABC-ELISA titers at 1:1024 and 1:256 respectively. Six individuals declared that they had never contracted malaria, however, one of them showed the titer at 1:64, another at 1:256 and the rest 4 at 1:512. This discrepancy will be explained granting that these 6 people had acquired a certain degree of immunity against malaria. In fact, there is a correlation between the ABC-ELISA titers and ages of the donors (excluding No. 3 and 9) with a correlation coefficient at 0.73, which suggests that one is likely to manifest higher titer after the cumulative infection as one grows older in the community.

The frequency distribution curve of antibody titers

The frequency distribution curve of the ABC-ELISA titers is shown in Figure 2. A bimodal distribution of titers was obtained: the first peak which consists of the individuals showing the titer at 1:32 to 1:128 is suggestive of past malaria episodes from months to years before the survey, and the second one at 1:256 to 1:1024 may be reflecting the recent malaria episodes or cumulative malaria infection. This pattern of frequency distribution curve suggests that malaria transmission was actively taking place in the colony (Rivera, 1993).

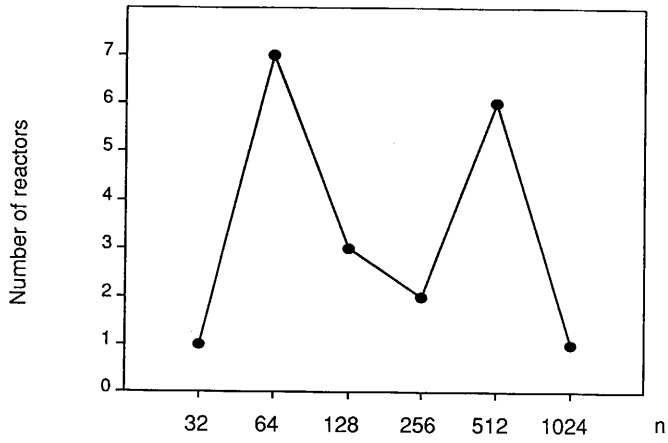


Figure 2 The frequency distribution curve of the ABC-ELISA titers.

DISCUSSION

Seroepidemiology provides more informative *period* prevalence data than can be obtained through microscopy (Collins and Skinner, 1972; Draper, 1972). It is particularly useful if, like in our present survey, the area where malaria epidemiological studies are to be conducted is not easily accessible, or one cannot stay there long enough to get standard malariometric indices of the population. In our present survey, *point* prevalence data obtained by microscopical observation of the blood smears showed no evidence of malaria transmission. But the serological method revealed a variety of positive antibody titers. Indeed, the frequency distribution curve of the ABC-ELISA titers with a peak of high antibody prevalence suggested potential risks of malaria epidemics in the population, or that active transmission of the disease were actually occurring in the area (Rivera, 1993). Thus, we could get a truer picture of the endemicity of malaria in the village by the serological method even though our visit to the place was short and temporary.

Generally, the absence of patent parasitemia can be misleading, since patency is influenced by the use of antimalaria drugs and often occurs only intermittently during malaria infections (Kagan, 1972). However this big discrepancy between parasite and antibody rates in the present study may probably be due to a certain degree of acquired immunity among the indians which depresses parasitemia down to submicroscopic levels (Draper, 1972). Repeated infection with malaria parasites maintaining continuous antigenic stimulation may have produced adequate antibody levels of the inhabitants which play, in part, a protective role against malaria (Kuvin and Voller, 1963).

Serological assessment has considerable value in the advanced stages of a malaria control program when the endemicity of malaria has become very low in the target area (Kagan, 1972; Voller and Draper, 1982). Despite this, we stress in this manuscript the importance of serological surveys of malaria for populations in a highly endemic area. The value and ease of application of a new serological method, the ABC-ELISA, which requires no major electrical equipment will also facilitate seroepidemiological studies in remote rural communities.

ACKNOWLEDGMENTS

This study was supported in part by grants from Japan International Cooperation Agency. We express sincere gratitude to Mr. Tsugio Shishido, secretary general, and other staffs of Beneficência Nipo-Brasileira da Amazônia, for providing us with local fascilities. We also thank Dr. B.D. Akanmori of Gunma University School of Medicine, and Professor Kumiko Sato of the College of Medical Care and Technology, Gunma University, for helpful discussion.

REFERENCES

1) Collins, W.E., Skinner, J.C. and Jeffery, G.M. (1968): Studies on the persistence of malarial antibody response, Am. J. Epidemiol., 87(3), 592-598
2) Collins, W.E. and Skinner J.C. (1972): The indirect fluorescent antibody test for malaria, Am. J. Trop. Med. Hyg., 21(5), 695-696
3) Draper, C.C., Voller, A. and Carpenter, R.G. (1972): The epidemiologic interpretation of serologic data in malaria, Am. J. Trop. Med. Hyg., 21(5), 696-703
4) Kagan, I.G. (1972): Evaluation of the indirect hemagglutination test as an epidemiologic technique for malaria, Am. J. Trop. Med. Hyg., 21(5), 683-689
5) Kuvin, S.F. and Voller, A. (1963): Malaria antibody titers of west Africans in Britain, Br. Med. J., 477-479
6) Ministério da Saûde, Superintendência de Campanhas de Saûde Pûblica, Diretoria Regional do Pará (1988): Relatório Técnico Administrativo, Belém, 29-117 (in Portugese)
7) Miyagami, T. and Waki, S. (1985): *In vitro* cultivation of *Plasmodium falciparum* under aerobic atmosphere in a CO_2 incubator, J. Parasitol., 71(2), 262-263
8) Pampana, E.J. (1969): A text book of malaria eradication, 2nd ed., Oxford University Press, London
9) Rivera, P.T., Kano, S., Miguel, E., Tongol, P. and Suzuki, M. (1993): Application of seroepidemiology in identification of local foci in a malrious community in Palawan, Philippines, Am. J. Trop. Med. Hyg., 49(5), 608-612
10) Sato, K., Kano, S., Yamaguchi, M., Omer, F.M., El Safi, S.H., El Gaddal, A.A. and Suzuki, M. (1990): An ABC-ELISA for malaria serology in the field, Am. J. Trop. Med. Hyg., 42(1), 24-27
11) Trager, W. and Jensen, J.G. (1976): Human malaria parasites in continuous culture, Science, 193, 673-675
12) Voller, A. and Draper, C.C. (1983): Immunodiagnosis and seroepidemiology of malaria, Br. Med. Bull., 38(2), 173-177
13) WHO (1972): Serological testing in malaria, Bull. Wld. Hlth. Org., 47, 527-532

Jpn. J. Trop. Med. Hyg., Vol. 21, No. 4, 1993, pp. 219-224

POSSIBLE APPLICATION OF SURGICAL TREATMENT OF CHAGASIC MEGACOLON AT SANTA CRUZ GENERAL HOSPITAL, BOLIVIA

EDWIN CRESPO MENDOSA[1], FREDDY UDALRICO GUTIERREZ VELARDE[1], SIMEON SENGOKU[1], CHIAKI MIYOSHI[2] AND TAMOTSU NAKASA[2]

Received September 6 1993/Accepted October 27 1993

Abstract: Chagas' disease, a chronic parasitic disease caused by the protozoan *Trypanosoma cruzi,* is one of the endemic diseases in Central and South America. In Bolivia its seroprevalence is very high and the most common digestive manifestation of chronic Chagas' disease is megacolon.

A new protocol for the surgical treatment of Chagasic megacolon has been started in Santa Cruz General Hospital. It consists of a peri-operative management, a choice of operative technique and a long term follow-up.

From July 1989 to August 1992, among 46 Chagasic seropositive megacolons, 37 definitive operations of colorectal resection and anastomosis were perfomed. Although five cases developed early postoperative complications, there were no deaths in the group which had definitive operations.

The purpose of this study is to establish a standard surgical management for Chagasic megacolon in Bolivia.

INTRODUCTION

Chagas' disease, a chronic parasitic disease caused by the protozoan *Trypanosoma cruzi,* is a disease endmic to all Central and South American countries. It is estimated that about 16 million people are infected (WHO, 1990; UNDP/WORLD BANK/WHO, 1991).

In Bolivia, Chagas' disease is a serious public health problem because its vector (*Triatominae*) is found in 83% of the national territory. The seroprevalence of Chagas' disease is very high (29.8%-70%) (Foianini, 1986).

There are three stages in Chagas' disease: acute, indeterminate and chronic (WHO Expert Commitee for Chagas Disease, 1991). After the short acute stage in which the symptoms can be very mild and atypical, a long asymptomatic phase may last several years. In this stage, up to 30% of people will suffer damage to their cardiac, digestive or neurological systems. In Bolivia, the most common digestive manifestation of the chronic Chagas' disease is megacolon.

Many authors have reported on the surgical treatment of Chagasic megacolon (Joffre and Moreira, 1988; Milton, 1985; Moreira *et al.,* 1988). However because there is a great difference as for economic situations and level of medical care among South American countries, it is difficult to directly apply these results to Bolivian cases.

Santa Cruz is the second largest city in Bolivia, located in the eastern tropical area where Chagas' disease is also endemic. Currently many people immigrate from the high Andian area to Santa Cruz that the prevalence of Chagas' disease is getting higher.

In Santa Cruz General Hospital (SCGH), a new protocol for the surgical treatment of Chagasic megacolon had started. It consists of a pre- and post-operative management, a choice of operative procedure and a long term (at least five years) follow-up.

The purpose of this program is to establish a standard surgical management for Chagasic megacolon in Bolivia.

This report is a preliminary evaluation of the new protocol.

MATERIALS AND METHODS

Figure 1 shows the algorithm for the management of Chagasic megacolon.

1 Department of Surgery, Santa Cruz General Hospital, Bolivia
2 Department of Surgery and Bureau of International Cooperation, International Medical Center of Japan, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162, Japan

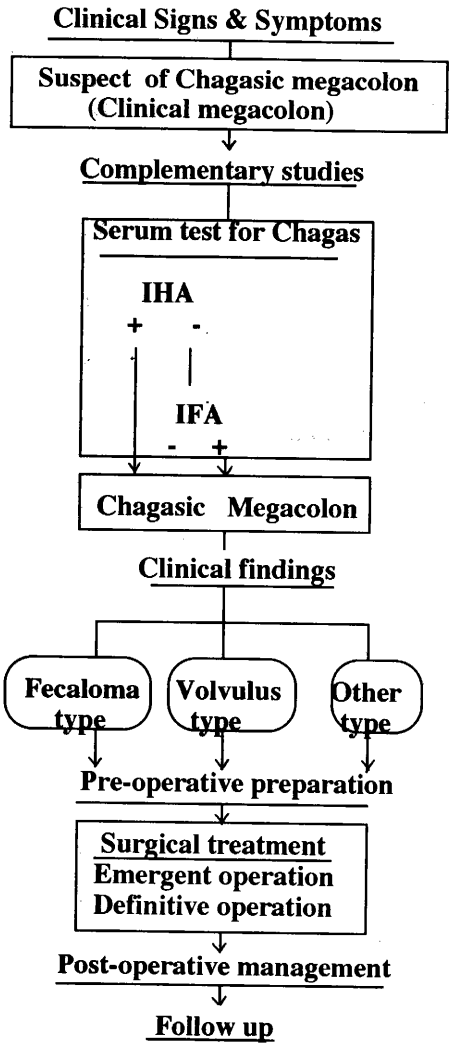


Figure 1 Algorithm for management of patients with Chagasic megacolon.

All cases diagnosed as megacolon in the Department of Surgery at SCGH were examined by serological Chagasic tests. The IHA (Indirect hemagglutination test) was performed first. When the IHA was negative, the IFA (Immunofluorecent assay) was examined. All seropositive cases with the IHA or the IFA were included as Chagasic megacolon cases in this protocol. These Chagasic megacolon cases were categorized into three types by clinical forms; Fecaloma type, Volvulus type and Other type. (Fecaloma is a fecal mass impacted by prolonged retension of feces.)

Diagnosis of fecaloma could be made by the history of a long period without defecation and palpable feces with rectal digital examination. Volvulus was characterized by a sudden onset of severe abdominal pain and usually found no palpable feces in rectum.

Each type has its own protocol consisting of pre

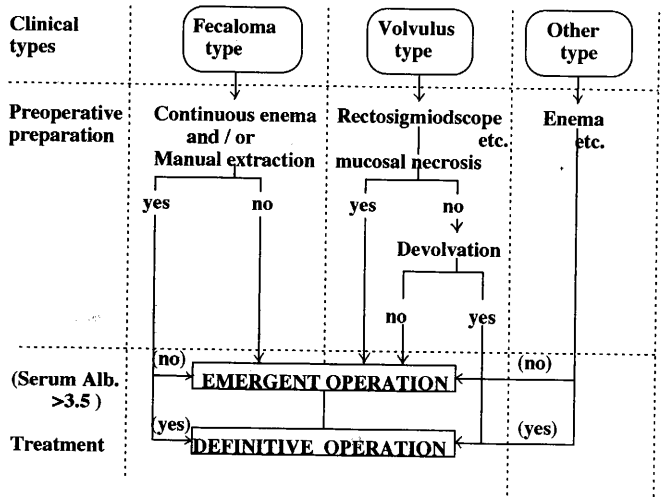


Figure 2 Peri-operative management of patients with Chagasic megacolon.

-operative preparation, surgical treatment and post -operative management (Figure 2).

For example, in the case of fecaloma, a continuous enema is the first choice, then a manual extraction. If the preparation is still not completed, first a colostomy (usually Hartmann's operation) is made. When the patient is suspected volvulus, the urgent rectosigmoidscopy has to be performed. If the necrosis of the colonal mucosa is found, an emergent Hartmann's operation is indicated. If its mucosa was intact, devolvulation with a rectosigmoidscope or a rectal tube has been the first choice.

All patients with hypoalbuminemia had their serum albumin levels corrected up to 3.5 mg/dl. Only the case with normal serum albumin levels (over 3.5 mg/dl) has to indicate the definitive operation of resection and anastomosis.

All operations listed above were perfomed by Bolivian surgeons. Colorectal anastomosis was done with manual sutures usually, while some of cases required mechanical suture devices (EEA stapler).

RESULTS

From July 1989 to August 1992, 61 clinical megacolon cases were admitted to the Department of Surgery of SCGH. 46 were seropositive for Chagasic test. These 46 cases included the following studies.

As it is shown Figure 3, the mean age of Chagasic megacolon patients was 52.7 years old and 31 were male. There were 25, 16 and 5 case of Fecaloma type, Volvulus type and Other type, respectively (Figure 4).

Associated problems were cardiomyopathy in 9 cases (24%), pulmonary tuberculosis in 2 cases (5%)

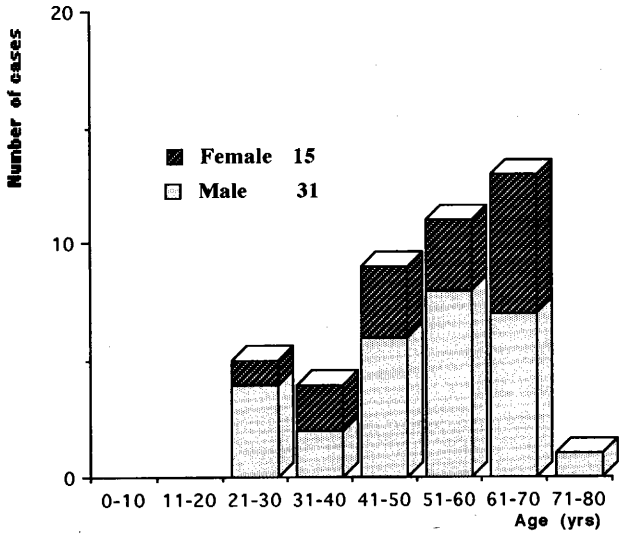


Figure 3 Distribution by age and sex of 46 patients with Chagasic megacolon.

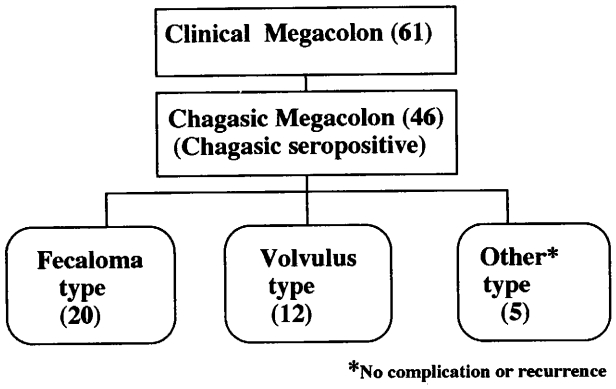


Figure 4 Clinical types of Chagasic megacolon.

etc. (Table 1).

Preperative serum albumin level is shown in Figure 5. Twenty four cases (65%) were under 3.5 mg/dl.

Among 46 Chagasic megacolon cases, seven received only non-surgical management. Although 6 of them were resolved non-surgically, they refused further definitive operation. The other case died of severe dehydration and acidosis before operation.

Surgical treatment was performed in 39 cases. Definitive operation of colorectal resection and anastomosis was done in 37 of them. In 2 cases only Hartmann's operation was performed due to refusal to the definitive operation.

Of the 37 definitive operations, one stage (direct) anastomosis was done in 28 cases and two stages (delayed) anastomosis was done in 9 other cases (Figure 6).

Table 1 Associated problems of 37 patients with definitive operation

Associated problem	No. of patients	%
Cardiomyopathy (including abnormal ECG)	9	24.3
Pulmonary tuberculosis	2	5.4
Rectal prolaps	1	2.7
Incisional hernia	1	2.7
Acute pancreatitis	1	2.7

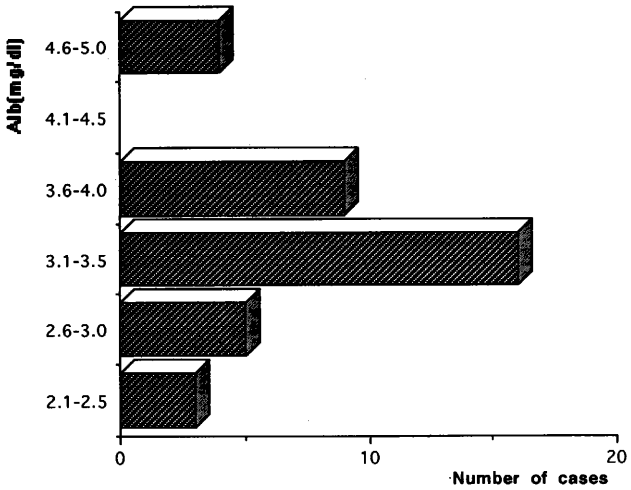


Figure 5 Pre-operative serum albumin level of 37 patients with definitive operation.

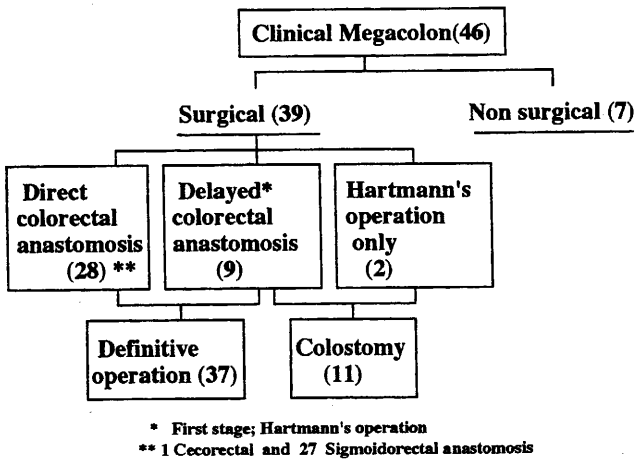


Figure 6 Surgical management of 46 patients with Chagasic megacolon.

The distance of the colorectal anastomosis from the anal verge is shown in Figure 7. The largest were between 5 to 10 cm. In three cases with very low anastomosis (less than 5 cm) mechanical suture devices (EEA stapler) were used.

Five cases had developed early post-operative com-

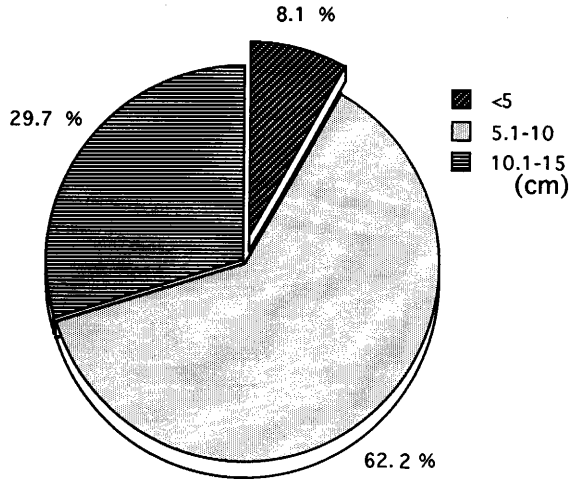


Figure 7 Location of colorectal anastomosis of 37 patients with definitive operation

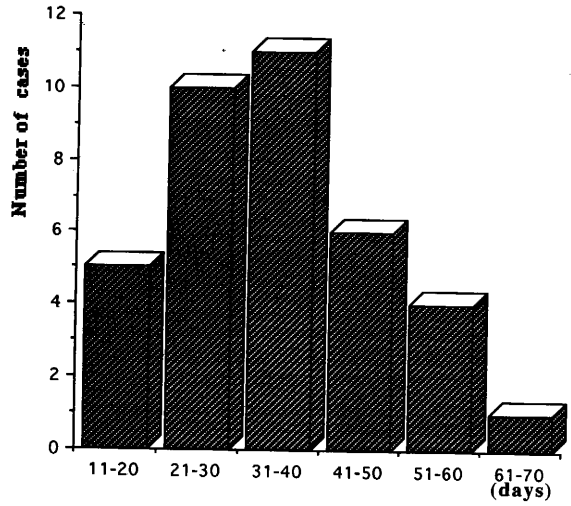


Figure 8 Length of hospitalization for 37 patients with definitive operation

Table 2 Early post-operative complication observe in 37 patients with difinitive operation

Complication	No. of patients	%
Stercoraceous fistula	3	8.1
Hemorrhage (intraperitoneal)	1	2.7
Abdominal wall abscess	1	2.7

Table 3 Patient mortality according to management of Chagasic megacolon

	Number	Complications	Death
Direct	28	4 (3.6)	0
Fecaloma type		3	
Volvulus type		1	
Delayed	9	1 (1.1)	0
Fecaloma type		0	
Volvulus type		1	

(%)

plications. Stercoraceous fistulas were developed in only 3 cases (8.1%) (Table 2).

The mean length of hospitalization was 34.5 days (Figure 8).

There were no deaths in the group which underwent definitive operation. Of the two cases which died: one was in the group of Hartmann's operation only and the other was in non surgical group (Table 3). The motality rate of total Chagasic megacolon was 4.3% (2/46), but that of the definitive operation group was 0% (0/37).

DISCUSSION

Because of the difference of the economic status and level of medical care among developing countries, the application of a surgical treatment established in one country may not be appropriate for another country.

Many studies about the surgical treatment of Chagasic megacolon have been reported, but there are very few from Bolivia (Saucedo, 1988). The prospective study of Chagasic megacolon was the first trial in Bolivia.

Usually the surgical treatment of Chagasic megacolon was only a colostomy for the fecaloma or a laparotomy for the devolvation of volvulus. In rural areas this is true even now where no specialist is available.

The seroprevalence of Chagas' disease is very high where some authors report it is over 70% in several areas (Foianini, 1986). Therefore, chronic Chagas' disease is becoming one of the most serious and common public health problems in Bolivia where the megacolon is more often encountered than the megaesophagus.

In such circumstance, the surgical management for Chagasic megacolon is indispensable for public health. The protocol, developed by Bolivian doctors, is intended to establish a standard surgical management for Chargasic megacolon in Bolivia.

Below are some points to be noted for information.

1) Operative technique

An operative technique should be determined by the physiopathology of the disease. Chagasic megacolon originates in the aganglionic portion of the intestine and a decreased number of ganglion cells are found throughout the colon (Earman, 1972; Todd *et al.*, 1969). From the point of the physiopathology, resection of the dilatat-

ed colon is inessential for the operation of the megacolon.

The enlarged intestine, usually rectosigmoid colon, is likely to be the most common locus of the fecaloma or volvulus. Therefore the resection of rectosigmoid is done for these complications (Earman, 1972; Todd *et al.*, 1969).

Some authors recommend Duhamel's operation (Milton, 1976; Joffre and Moreira, 1988). From this point of view, it is ideal for the relapse of complications should be minimal. However this technique reports the high rate of morbidity and mortality.

In developing countries, there are not many specialists in digestive surgery. Moreover very few patients can receive TPN (total parenteral nutrition). For these reasons, the operative technique should be as straight as possible.

Moreira *et al.* have operated 624 patients with Chagasic megacolon using Duhamel's technique. Mortality rate was 6.6% while early post-operative complications such as into-anal suppration (7.6%) and lower colon necrosis (2.4%) were developed.

On the other hand, the abdominal rectosigmoidectomy is easier and the rate of complication is not so high. Most general surgeons in Bolivia can perform it. This is the reason why the abdominal rectosigmoidectomy was chosen as a standard technique in this protocol.

2) Location of anastomosis

Another important point is the location of colorectal anastomosis from the anal verge. If it is lower, recurrence of fecaloma or volvulus is fewer, because more of the aganglionic portion of the rectum would be resected. However, the lower anastomosis makes it more difficult for the operative techniques and therefore is recognized higher rate of post-operative complications. Milton reported that the anastomosis should be performed within 2-4 cm from the pectinate line.

Mechanical suture devices are used for lower colorectal anastomosis. They can also be used also for cases of Chagasic megacolon (Cuitait, 1980), but in many cases the wall of the colon is too thick to be anastomosed with them.

Moreover it is so expensive that they can not be used for most patients in Bolivia.

In this study, most anastomoses were performed between 5 to 10 cm from the anal verge. Only 3 cases with lower anastomosis used mechanical suture devices.

3) Direct or delayed anastomosis

For decreasing complications, the delayed anastomosis (two or three stages operation) was recommended.

When the delayed anastomosis indicated, the patient generally went back home to the rural village with a colostomy. After that, most never came back to the hospital for the definitive operation unless any urgent problems appeared.

Therefore this protocol applied the direct anastomasis as first priority and the delayed anastomosis in the same hospitalization as second.

There were no deaths in either groups of the direct and delayed anastomosis. Morbidity was also low in both of them.

In this study, covering colostomy was performed for just a single case. Covering colostomy was worth considering but the result verifies it is not always necessary if the pre-operative preparation is completed.

One of the problems with this protocol was the lengthy hospital stay required when they received the definitive operation during the same hospitalization. SCGH and the Japanese project team supported some cases financially. Without such assistance, they would not have been able to continue further treatments. Ideally, the protocol will remain sustained by themselves, but financial condition of the hospital is quite inadquate.

Another problem is the difficulty of the follow-up of the patients. Most patients do not return to hospital unless they face severe problems. Alternative incentives, such as free charge of examination etc. should be considered.

This is a preliminary report and we regret it is too short to assess the study more in detail. However the early results of this are considered fairly satisfactory and the course should be continued.

ACKNOWLEDGEMENTS

This study was supported in part by Japan International Cooperation Agency (JICA).

We express our heartfelt thanks to all members of the Department of Surgery, Santa Cruz General Hospital and the Japanese technical cooperation team, all involved in Santa Cruz General Hospital project.

REFERENCES

1) Cutait, D.E. (1980): Anastmosis mecanica por grampeamento cirugia colorectal, Pren. med. argent., 67, 658-662

2) Earlam, R.J. (1972): Gastrointestinal aspect of Chagas' disease, Digestive Disease, 17, 559-571

3) Foianini, J.E. (1986): Enfermedad de Chagas—Revision Bibliografica, Santa Cruz (Bolivia)
4) Joffre M. de Rezende, and Moreira, H. (1988): Chagasic megaesophagus and megacolon. Historical view and present concepts, Arq. Gastroenterol. Sao Paulo, 25 (special issue), 32-43
5) Milton da Silveira, G. (1976): Chagas' disease of the colon, Br. J. Surg., 63, 831-835
6) Moreira, H., de Rezende, J.M., Sebba, F., de Azevedo, I. F., Leite, A.C.A. and Soares, E.P. (1985): Chagasic megacolon, colo-proctology, 5, 260-267
7) Saucedo, E. (1988): 15 anos de experiencia en el tratamiento de megacolon chagasico, Anurario del Ateneo de Medicina, 11-16, Santa Cruz de la Sierra
8) Todd, I.P., Porter, N.H., Morson, B.C., Smith, B., Friedmann, C.A. and Neal, R.A. (1969): Chagas disease of the colon and rectum, Gut, 10, 1009-1014
9) UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR) (1991): Tropical Diseases Progress in Research 1989 -1990, 69-77, WHO, Geneva
10) WHO (1990): Chagas Disease, Frequency and geographycal distribution, Weekly Epidemiological Record, 65, 257-264.
11) WHO Expert Committee on the Control of Chagas Disease (1991): WHO Technical Report Series 811, Control of Chagas Disease, WHO, Geneva

Jpn. J. Trop. Med. Hyg., Vol. 21, No. 4, 1993, pp. 225-229

AN OUTBREAK OF ASEPTIC MENINGITIS IN OKINAWA IN 1993

YOSHIHIRO MAKINO[1, 2], EIICHI TAMANAHA[3], ZENJI MIYAZATO[3], JUNKICHI SHIMABUKURO[3], CHIKARA MATSUMORA[3], CHORSON YAN[3], RYUJI ISHIHARA[3], TAKASHI MACHIDA[3], NAOTO YAMADA[3], SAKAE ARAKAKI[1], MASAYUKI TADANO[1] AND TOSHIHIKO FUKUNAGA[1, 2]

Received October 1 1993/Accepted November 5 1993

Abstract: In the summer of 1993, an epidemic of aseptic meningitis had broken out in Okinawa island, the southernmost area of Japan. It started in June (or probably in May), reached a peak early in July and subsided in August. A total of 216 specimens (cerebrospinal fluids, throat swabs and sera) was collected from the patients with aseptic meningitis and viruses were recovered from 83 specimens (38.4%). Five isolates randomly selected were examined for the identification of the virus. The virus was identified as echovirus type 30 (echo-30) by plaque reduction neutralization test using enterovirus antiserum pools and monospecific antiserum. Large-plaque and small-plaque phenotypes of echo-30 were isolated. The two were neutralized by anti-echo-30 sera. For the plaque assay of echo-30 in RD-18S cells, the use of tragacanth gum for the overlay medium could reduce the incubation period to 25-30 hr.

INTRODUCTION

Aseptic meningitis is a disease of central nervous system, mainly caused by viral infection. Most of the epidemics of aseptic meningitis in summertime are caused by enterovirus infection (7, 9). Echovirus type 30 (echo-30) was first isolated from a patient with aseptic meningitis in 1959 (3). In Japan, since the first report of echo-30 infection in 1978, a number of epidemics of aseptic meningitis were reported to be due to echo-30 infection (4, 10, 13). In Okinawa island, the southernmost area of Japan, we had experienced a big outbreak of aseptic meningitis in the summer of 1989. Many strains of echo-30 were recovered from cerebrospinal fluids (CSF) and throat swabs (TS) taken from the patients. In the summer of 1993, we encountered another outbreak of aseptic meningitis in Okinawa island. We collected specimens at a hospital and tried to isolate the etiological agent of the disease. In this paper we describe the results of the virological investigations of the outbreak of aseptic meningitis occurred in the summer of 1993.

MATERIALS AND METHODS

Specimens

The specimens were collected at a private hospital in Okinawa city, Okinawa Prefecture, during June through August 1993. Cerebrospinal fluid, serum and throat swab were collected from the children with the clinical symptoms of aseptic meningitis. They were stored at -70°C, then transferred to our laboratory.

Cells

A stable line of human rhabdomyosarcoma (RD -18S) (6) cells were kindly supplied by Dr. Hara, National Institute of Health, Japan. They were grown in Dulbecco's modification of Eagle's medium (DMEM) supplemented with 8% fetal calf serum (FCS) and antibiotics, and maintained in the same medium but FCS was reduced to 2% (MM).

Virus isolation

TS, obtained using cotton swab, was dissolved in 2 ml of DMEM containing antibiotics. The specimens (CSF, TS and serum) were centrifuged at 15,000 rpm for 5 min and the supernatant was used for the recovery of virus.

1. Department of Virology, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-01, Japan
2. Research Center of Comprehensive Medicine, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-01, Japan
3. Department of Pediatrics, Nakagami Hospital, 723, Chibana, Okinawa city, Japan.

Confluent monolayer of RD-18S cells was prepared in 25-cm² flask or 24-well plate. The culture medium was replaced with MM (1ml for flask or 0.5 ml per well for 24-well plate) and the monolayer was inoculated with 0.1 or 0.05 ml of the specimen. The cells were incubated at 37°C for 1.5 hr in a 5% CO_2 atmosphere. Then MM was added to the cultured cells (4 ml into flask or 0.5 ml per well to 24-well plate), and the cells were cultured at 37°C for one week and observed for cytopathic effect (CPE). When the cells showed maximum CPE, the cultured fluid was harvested. The centrifuge supernatant of the cultured fluid was inoculated into the new RD-18S cells (second passage). The virus stock was prepared at the second passage and was used for the identification of the virus. All the virus preparations were stored at -70°C until used.

Plaque assay

Confluent monolayer of RD-18S cells in 6-well plate was prepared. General procedures were described elsewhere (15). For the overlay medium, 1% ethanol -washed tragacanth gum (Wako Pure Chemical, Japan) in Eagle's medium (5, 11) containing 100 μg/ml of DEAE-dextran and antibiotics was used. The infected cell culture was incubated at 37°C for 25-30 hr. After the incubation period, the overlay medium was decanted and the cells were washed 3 times with phosphate-buffed saline (PBS), and fixed with methanol for 5 min. The cells were washed 3 times with PBS and stained with 0.2% crystal violet solution for 5 min. After washing the cells 3 times with deionized water, the plaques were counted.

Identification of recovered viruses

The recovered viruses were identified by 50% plaque reduction neutralization test (NT) on RD-18S cells using pooled antisera against enteroviruses (Denka Seiken Co. group E-O). The serotype of the virus was further confirmed by NT using monospecific antiserum. Three different preparations of anti-echo 30 serum were used : anti-Bastianni serum was purchased from Denka Seiken Co.: anti-echo-30 (5353) was a kind gift from Kagawa Prefectural Institute of Public Health; and anti -8914 serum was prepared by inoculating mice with 8914 strain of echo-30 virus, which was isolated in Okinawa in 1989.

RESULTS

Number of specimens brought into laboratory by week

A total of 216 specimens of CSF, sera and TS was taken from 123 children with the clinical symptoms of meningitis and brought into our laboratory. The number of meningitis patients from whom the specimens were taken are summed up by week and shown in Fig. 1. The epidemic of meningitis appeared to have occurred since June, reached a peak early in July, and subsided in August 1993.

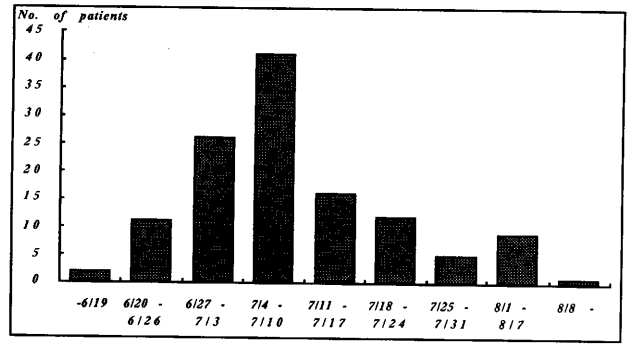


Figure 1 Number of patients specimens were taken by week. The specimens (CSF, TS and Serum) were collected at a hospital in Okinawa city, Okinawa, during June through August 1993.

Virus isolation

A total of 101 CSF specimens was inoculated into RD-18S cells in 25-cm² flasks or 24-well plates. In case of 24-wells, duplicate wells were used for one specimen. Characteristic CPE (rounding up, shrinking and nuclear pyknosis) was usually observed in 4-5 days after inoculation. The viruses were recovered from 29 specimens (isolation rate : 28.7%) (Table 1). The isolated viruses were further inoculated into new RD-18S cells and confirmed the appearance of the same CPE.

Table 1 Results of virus isolation

Specimens	Virus isolation		Total
	Positive	Negative	
Cerebrospinal fluid	29 (28.7%)	72 (71.3%)	101
Serum	0 (0%)	37 (100%)	37
Throat swab	54 (69.2%)	24 (30.8%)	78
Total	83 (38.4%)	133 (61.6%)	216

A total of 78 TS specimens was examined for virus isolation as described above. Same type of CPE as above was appeared in 2-4 days after inoculation. Viruses were recovered from 54 TS specimens (isolation rate : 69.2%). Thirty-seven serum specimens were inoculated into RD-18S cells. No virus was recovered.

Plaque assay described below was performed on the 12 clinical specimens to know the infectivity titers of the specimens. The titers of these specimens were varied

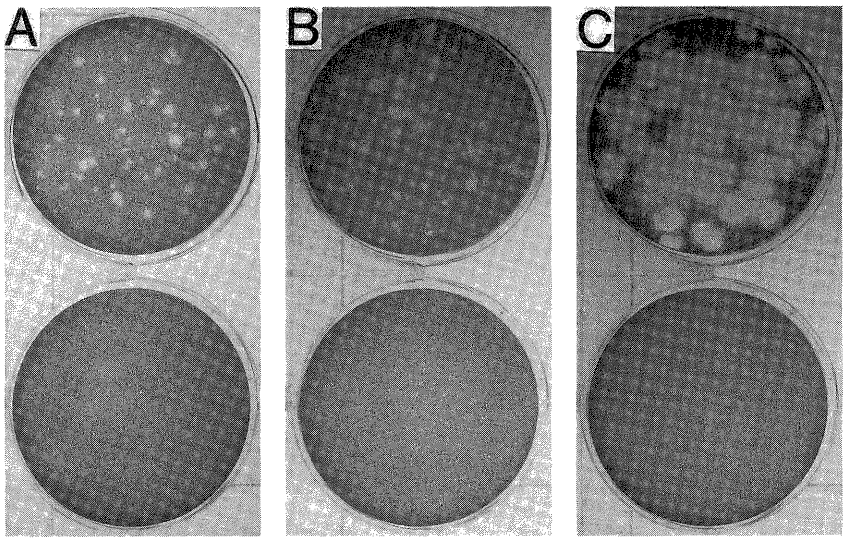


Figure 2 Plaques of Okinawan strain of echo-30. Confluent monolayers of RD-18 cells were infected with Okinawan strain of echo-30 (upper column) or mock-infected (down column) and incubated for 25 (A), 30 (B), and 45 hr (C), under tragacanth gum overlay medium. After the incubation period, the overlay medium was decanted and the cells were stained as described in the text.

between 0-250 plaque forming unit (PFU) per ml for CSF and $4.0\times10^3-5.4\times10^4$ PFU per swab for TS.

Identification of the recovered virus

The viruses recovered from CSF were identified by plaque reduction NT. For the infectivity assay of the virus, plaque titration in RD-18S cells was performed. We used 1% tragacanth gum containing DEAE-dextran for the overlay medium. The incubation period of the infected cells appeared to be enough for 25-30 hr (Fig. 2). In the beginning, 11 sets of antiserum pools were used. These sets of antiserum pools contained the antibodies to Coxackie A9, Coxackie B1-6, and Echo 1 -30 viruses. Five isolates were randomly selected and examined. These isolates were neutralized effectively by the antiserum pools that contained anti-echo-30 serum (antiserum pool -N and O). In order to confirm the serotype of the isolates, NT was performed using three different preparations of monospecific antisera (anti-echo-30). All of the isolates tested were neutralized by the anti-echo-30 (5353) and anti-8914 sera (Table 2). The 8914 strain of echo-30 was isolated in Okinawa in 1989. Since all the strains were neutralized by antiserum to 8914 strain, the etiological agent of the meningitis appeared to be quite similar strain as that of four years ago. It should be noted that prototype echo -30 (Bastianni strain) was neutralized effectively by 10 -20 units of anti-Bastianni serum, while the Okinawan isolates were partially neutralized (less than 50%) (Table 2) by this antibody concentration, indicating that the isolates were antigenically slightly different from the prototype echo-30 virus.

Table 2 Neutralizing activities of various antisera on the echo-30 strains

	Echovirus type 30					
	#93090	#93110	#93117	#93001	#93002	Bastianni
Anti-Bastianni	48[a](40.7)	36 (37.9)	35 (39.7)	22 (47.6)	51 (38.6)	1 (>99)
Anti-5353	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	ND
Anti-8914	0 (100)	0 (100)	0 (100)	1 (97.6)	0 (100)	ND
Pooled sera-O	3 (96.3)	18 (69.0)	13 (77.6)	2 (95.2)	23 (72.3)	ND
Diluent	81	58	58	42	83	>100

a) No. of plaques. Numbers in parentheses represent the reduction rates in per cent.

Plaque morphology of the isolates

The plaques of the isolates showed varieties in size (Fig. 2). Direct plaquing of CSF specimens also showed various plaque sizes. Plaque purification of the isolates was performed and the large-plaque and small-plaque phenotypes were isolated. The two phenotypes were neutralized by antisera to echo-30 (5353), anti-8914 and antiserum pool -O(data not shown).

DISCUSSION

The epidemic of aseptic meningitis had broken out since June (or probably May), reached a peak early in July and subsided in August, 1993. During this period, not only children but also adults were hospitalized with aseptic meningitis. Viruses were recovered more frequently from TS (69.2%) than from CSF (28.7%). No virus was recovered from sera. Five isolates, randomly selected, were examined by antiserum pools and all turned out to be echo-30 viruses. In Okinawa island, there had a big outbreak of aseptic meningitis in the summer of 1989. We recovered many strains of echo-30 from CSF and TS. Since the first report of echo-30 infection in Japan in 1978, a number of similar outbreaks were observed in several areas in Japan in 1983, 1989 and 1991 (4, 10, 13). High communicability of enteroviruses, including echo-30, has been documented (9). It has been reported that preexisting serum antibody did not protect against the same serotype of echovirus infection (14). These natures of the echoviruses may cause repeated outbreaks in the same area.

The isolates were neutralized by anti-echo-30 serum. They were fully neutralized by the antiserum to the Okinawan strain, isolated in 1989, while they were partially neutralized by the 10-20 units of antiserum to the prototype echo-30 (Bastianni strain), indicating that the isolates might be antigenically slightly different from the prototype strain (prime strain). We isolated large-plaque and small-plaque phenotypes. The two were neutralized by anti-echo-30. It has been reported that a single mutation in 5' non-cording region of poliovirus genome resulted in the production of small -plaque phenotype (12). Characterization of these phenotypes are being under way.

For the plaque assay of echo-30 virus in RD-18S cells, we found 1% tragacanth gum containing DEAE -dextran to be a good overlay medium. After an incubation period of 25-30 hr in the stationary culture, the cells infected with the virus formed visible foci, which were fixed and stained. When agar overlay medium was used, it took 5 days to form foci. For the neutralization test of echo-30, inhibition of CPE in microplate culture has been used (9). It takes longer observation time and frequently encountered break-through phenomenon, which made the results complicated. The plaque method we used was more simple and the results were obtained in a short incubation period. The cells infected with CSF usually showed maximum CPE in 4 days, while it usually took 2 days for those with TS, indicating that more infective viruses were present in TS than in CSF. In order to know the infectivity titers of the clinical specimens, direct plaque assay was performed on the virus-positive specimens. The geometric mean titers of the randomly selected specimens were 42.9 PFU/ml for CSF and over 2.8×10^4 PFU/swab for TS. In the study of echo-12, 50% human infectious dose (for adult volunteers) was 919 PFU (14). High infectivity titer of TS observed in this experiment may indicate that the droplets and aerosols from coughing or sneezing can be the important sources of infection together with the fecal contamination.

In this experiment, we could not recover any virus from serum specimens. Neutralization test was performed on the sera, whose CSF and/or TS were positive for echo-30. All the sera were taken during the acute phase of the illness. Neutralizing antibody (titer >1:5) was detected in 25 of 26 sera (96.2%). The viruses in the serum might be inactivated by the antibody, although viruses were recovered from CSF and/or TS. Protective role of serum antibody against poliovirus illness has been reported (8). While, the presence or absence of serum antibody to echo-12 caused no significant infection rate among volunteers infected with the same virus (14). The presence of serum antibody to Norwalk virus, another enteric virus, failed to protect against the challenge with the same virus (1). High titer of serum antibody to rotavirus (>1:128) was protective against homotypic reinfection, while titers less than that failed to protect (2). Echo-30 causes high incidence of aseptic meningitis (10) and sometimes fatal infection to immunodeficient person (16). It may be important to know how circulating serum antibody affects the appearance of illness. At the same time, seroepidemiological study of this virus will be necessary to cope with the possible outbreak in the future.

REFERENCES

1) Blacklow, N.R., Cukor, G., Bedigian M.K., Echeverria, P, Greenberg, H.B., Schreiber, D.S., and Trier, J.S. (1979): Immune response and prevalence of antibody to Norwalk enteritisw virus as determined by radioim-

munoassay, J. Clin. Microbiol., 10, 903-909
2) Chiba, S., Yokoyama, T., Nakata, S. (1986): Protective effect of naturally acquired homotypic and heterotypic rotavirus antibodies, Lancet, 2, 417-421
3) Ducan, I.B.R. (1960): Aseptic meningitis associated with a previously unrecognized virus, Lancet, 2, 470-471
4) Fujita, N., Sasaki, N., Ookita, K., Takeda, M., and Takao, N. (1991): Receent outbreak of echovirus 30 aseptic meningitis in Onomichi area in 1989, Jap. J. Pediatr., 44, 484-490
5) Kedmi, S., and Katzenelson, J. (1978): A rapid quantative fluoresces antibody assay of poliovirus sing tragacanth gum, Arch. Virol., 56: 337-340
6) McAllister, RM, Melnick, J.L., Finkelstein J.Z., Adams E.C. Jr., and Gardner M.B. (1969): Cultivation *in vitro* of cells derived from a human habdomyosarcoma, Cancer, 24, 520-526
7) Melnick, J.L. (1965): Echoviruses, 513-545, *In* Horsfall F.L., J., and Tamm, I. (eds), Viral and rickettsial infections of man, 4th ed, Lippncott Co., Philadelphia
8) Melnick, J.L. (1978): Advantages and disadvantages of killed and live poliomyelitis vaccines, Bull. WHO., 56, 21-38
9) Melnick, J.L., Wenner, H.A. and Phillips, C.A. (1979): Enteroviruses, 471-534, *In* Lennette, E.H. and Schmidt, N.J. (eds), Diagnostic Procedures for Viral, Rdckettsial and Chlamydial Infections, American Public Health Association, Inc., Washington, D.C.
10) Miki, K., Fujii, K., Ikejiri, K., Yamanishi, S., and Okade, T. (1993): Epidemic of aseptic meningitis due to echo virus 30 and its nosocomial infection in neonates, Clin. Virol., 21, 22-27
11) Okuno, Y., fukunaga, T., Tadano, M., Okamoto, Y., Ohnishi, T., and Takagi, M. (1985): Rapid focus reduction neutralization test of Japanese encephalitis virus in microtiter system, Arch. Virol., 86, 129-135
12) Pelletier, J., Flynn, E.E., Kaplan, G., Racaniello, V., and Sonenberg, N. (1988): Mutational analysis of upstream AUG codons of poliovirus RNA, J. Virol., 62, 4486-4492
13) Sato, H., Saito H., and Morita, M. (1993): An outbreak of dcho virus 30 and it's seroepidemiology, Clin. Virol., 21, 13-16.
14) Schiff, G.M., Stefanovic, G.M., Young, E.C., Sander, D.S., Pennekamp, J.K., and Ward, R.L. (1984): Studies of echovirus-12 in volunteers: Determination of Minimal infectious dose and the effect of previous infection on infectious dose, J. Inf. Dis., 150, 858-866
15) Schmidt, N.J. (1979): Cell culture techniques for diagnostic procedures for viral, rickettsial and chlamydial infections, 5th ed, Americal Public Health association, washington, D.C.
16) Ziegler, J.B., and Penny, R. (1975): Fatal echo 30 virus infection and amyloidosis in X-linked hypogammaglobulinemia, Clin. Immunol. Immunopathol., 3, 347-352

Jpn. J. Trop. Med. Hyg., Vol. 21, No. 4, 1993, pp. 231-237

STUDIES ON EPIDEMIOLOGY OF *DIROFILARIA IMMITIS* IN HOUSE DOGS IN NAGASAKI CITY, JAPAN, WITH CONSIDERATIONS ON YEARLY CHANGES IN MICROFILARIAL PREVALENCE

TSUTOMU ODA[1], OSAMU SUENAGA[2], AKIO MORI[3], KOICHIRO FUJITA[4], MAKOTO ZAITSU[5], KENJI KUROKAWA[5], TAKESHI NISHIOKA[7], TATSUYA ITOH[7] AND MARIKO MINE[8]

Received October 6 1993/Accepted November 8 1993

Abstract: The positive rate of *Dirofilaria immitis* infection was studied in the eastern, western, southern and northern parts of Nagasaki City 3 times between 1968 and 1983. Chronological changes in the positive rate of dogs for microfilariae in these 4 parts and the roles of epidemic factors in the changes of the positive rate were evaluated. The positive rate decreased in the northern part, where houses and buildings increased annually, whereas the number of dog owners and the density of dogs decreased. Improvements in the drainage such as side ditches, which are primary breeding sites of vector mosquitoes (*Culex p. pallens*), are assumed to have led to a decrease in the mosquito density, resulting in a decrease in the positive rate of dogs. However, the positive rate increased in the eastern, western, and southern parts. The density of dogs decreased also in these parts, but it remained higher than in the northern part as the number of houses was smaller, and more people kept dogs. Also, as the sewage system did not seem to be so well developed as in the northern part, more mosquitoes might be present. The high positive rates in these parts may be ascribed to these conditions.

INTRODUCTION

The adult worm of *Dirofilaria immitis* transmitted by mosquitoes usually lives in the heart of dogs. Human infections are also known in Japan (e.g., Yoshimura 1989; Suzumiya and Nawa 1990). In Nagasaki City, house dogs were infected with this worm in high rates and the main vector was *Culex pipiens pallens* (Suenaga *et al.*, 1971; Suenaga and Itoh, 1973; Suenaga *et al.*, 1980). However, the relative importance of the factors actually related to the prevalence remains unknown. Therefore we conducted an epidemiological study of *D. immitis*. Herein, the changes in microfilarial prevalence from 1968 to 1983 are reported with a consideration of the factors responsible for these changes.

PLACES AND METHODS

As Suenaga *et al.* (1971) reported, Nagasaki City is divided into 44 elementary school districts, or into 4 parts, eastern, western, southern and northern. To examine the microfilariae of *D. immitis* in each division, we sampled blood from an earlobe of a dog in April and May, 1983 when the City Health Center was conducting rabies vaccination to registered dogs. A drop of blood was taken, smeared on a slide glass, and stained with 10% Giemsa's solution. The number of microfilariae in the blood specimen was counted with a stereomicroscope. The data on sex and age of examined dogs were taken from the record of Nagasaki City Health Center.

Cx. p. pallens, the main mosquito vector, were collected by a light trap with black light (20 watts) from

1. Department of General Education, the School of Allied Medical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki 852, Japan
2. Reference Center, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852, Japan
3. Department of Medical Zoology, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852, Japan
4. Department of Medical Zoology, Faculty of Medicine, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku 113, Japan
5. Sasebo City Health Center, 1-10 Yahata-machi, Sasebo 857, Japan
6. Department of Bacteriology, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852, Japan
7. Nagasaki City International Culture Hall, 7-8 Hirano-machi, Nagasaki 852, Japan
8. Scientific Data Center of Atomic Bomb Disaster, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852, Japan

Table 1 Positive rates for microfilariae of *D. immitis* in dogs with the number of registered dogs per house in the districts of Nagasaki City in 1983

Part	District		No. of dogs			No. of houses	No. of dogs registered	No. of dogs per house
	No.	Name	examined	positive	%	(A)	(B)	(B/A)
Eastern	1	Irabayashi	3	0	0.0	9072	418	0.0461
	2	Togiya	1	0	0.0	2524	69	0.0273
	3	Katsuyama	3	3	100.0	2993	108	0.0361
	4	Shinkozen	1	0	0.0	1938	27	0.0139
	5	Kaminagasaki	4	2	50.0	4939	250	0.0506
	6	Koshima	0	—	—	6528	352	0.0539
	7	Nishizaka	49	16	32.7	2459	74	0.0301
	8	Himi	0	—	—	3280	163	0.0497
	9	Yagami	80	28	35.0	3448	122	0.0354
	10	Koga	68	28	41.2	1849	103	0.0557
	11	Toishi	56	22	39.3	1113	72	0.0647
		Total	265	99	37.4*	40143	1758	0.0438
Western	12	Inasa	69	20	29.0	5715	216	0.0378
	13	Asahi	56	13	23.2	2571	96	0.0373
	14	Akunoura	89	24	27.0	2787	116	0.0416
	15	Tategami	0	—	—	989	66	0.0667
	16	Kosakaki	0	—	—	1381	58	0.0420
	17	Fukuda	74	29	39.2	2489	101	0.0406
	18	Teguma	4	0	0.0	2063	111	0.0538
	19	Shikimi	1	0	0.0	1482	66	0.0445
		Total	293	86	29.4	19477	830	0.0426
Southern	20	Sako	19	1	5.3	3139	82	0.0261
	21	Nita	11	2	18.2	2098	84	0.0400
	22	Kitaohura	6	2	33.3	4668	248	0.0531
	23	Minamiohura	17	8	47.1	2491	127	0.0510
	24	Naminohira	24	3	12.5	1463	71	0.0485
	25	Tomachi	149	57	38.3	5044	232	0.0460
	26	Kogakura	46	22	47.8	1779	64	0.0360
	27	Doinokubi	49	29	59.2	4574	221	0.0483
	28	Fukahori	64	27	42.2	3545	104	0.0293
	29	Minami	13	5	38.5	244	16	0.0656
	30	Mogi	57	12	21.1	2049	96	0.0469
	31	Hayasaka	0	—	—	1955	131	0.0670
	32	Hiyoshi	0	—	—	343	18	0.0525
		Total	455	168	36.9*	33392	1494	0.0447
Northern	33	Zenza	53	11	20.8	2563	75	0.0293
	34	Sakamoto	32	5	15.6	4051	79	0.0195
	35	Takao	134	34	25.4	6628	227	0.0342
	36	Yamazato	30	6	20.0	5079	110	0.0217
	37	Nishishiroyama	7	1	14.3	4387	186	0.0424
	38	Shiroyama	9	2	22.2	2232	86	0.0385
	39	Nishiurakami	207	56	27.1	8692	387	0.0445
	40	Nameshi	12	4	33.3	9255	420	0.0454
	41	Nishikita	42	14	33.3	4273	166	0.0388
	42	Nishimachi	22	7	31.8	6476	299	0.0462
	43	Kawabira	7	4	57.1	2573	115	0.0447
	44	Ohzono	0	—	—	2950	61	0.0207
		Total	555	144	25.9*	59159	2211	0.0374
Sum total			1568	497	31.7	152171	6293	0.0421

*Significant ($P<0.01$)

1972 to 1983 in 4 points in residential area: Irabayashi (Eastern part), Inasa (Western part), Tomachi (Southern part) and Sakamoto (Northern part). In addition, the collection was made in Sakamoto-machi also from 1967 to 1971. The light trap was regularly operated from 6 PM to 7 AM (one night), once a week from May to October.

RESULTS

1. Microfilarial prevalence in house dogs in Nagasaki City

Table 1 shows the results of the blood examination of dogs in Nagasaki City in 1983. The positive rate for microfilariae was 31.7% for the whole city. The positive rate in the eastern or southern part was significantly higher than that in the northern part (x^2-test, $p<0.01$). We thought that the difference in the positive rate might be related with the dog density (the number of dogs per house). To make clear this hypothesis, the numbers of dogs per house were also examined in these parts (Table 1). As explained later, the positive rate is correlated with the dog density, which was lowest in the northern part with the lowest positive rate among the 4 parts.

2. Comparison of positive rates in dogs by sex and age in the 4 parts of the city

The positive rates were compared according to sex and age in the 4 parts in Table 2. The rates were calculated only for dogs with an exact record of age. The positive rate is generally lower at any age for either sex in the northern part of the city than in the other 3 parts. This means that the lower prevalence in the northern part was not due to different age distribution nor sex ratio in the dogs examined.

3. Correlation between positive rate and dog density

We examined the correlation between positive rate and dog density in districts in Figure 1, where points were plotted only when the number of dogs examined was over 10. The microfilaria positive rate was positively correlated with dog density in the city (correlation coefficient 0.4163, $0.01<p<0.05$).

4. Yearly changes in positive rates of the dogs in the 4 parts of the city

The yearly changes in positive rates in the 4 parts were examined by comparing the results reported by Suenaga *et al.* (1971; 1980) with the present data by the linear trend test of Armitage (1955) (Table 3). In the northern part, the total positive rate of 40.5% in 1968 clearly decreased to 25.9% in 1983, while the rate increased in the eastern, western and southern parts.

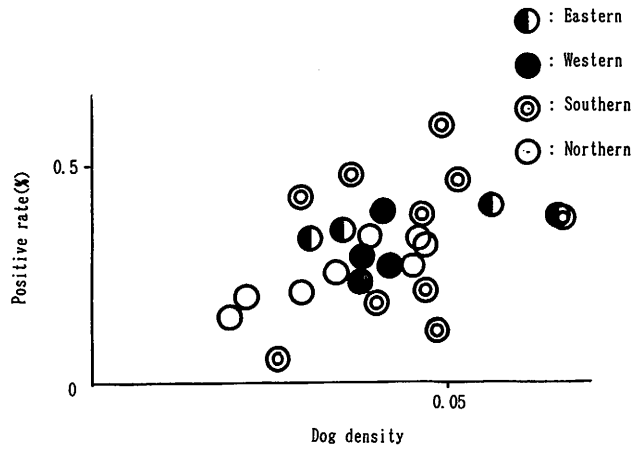


Figure 1 Relation between dog density and positive rate of dogs in districts in Nagasaki City in 1983.
* Points were plotted only when the number of dogs examined was over 10.

In 1977 and 1983, the results of positive rates in the 4 parts were obtained in spring, but in 1968 the results were composed of data taken in spring and autumn. The positive rate calculated only for spring was 34.6%, 19.5%, 21.6% and 41.8% in the eastern, western, southern and northern parts, respectively, there being not much difference between the results obtained only in spring and those obtained in spring and autumn combined (Table 1).

5. Yearly changes in the number of the registered dogs in Nagasaki City

Figure 2 shows the yearly change in the total number of dogs registered in whole Nagasaki City. The number of these dogs decreased gradually from 1968 to 1983, with some variations. Changes in numbers of dogs and houses in each of 4 parts of Nagasaki City are shown in Table 4. In each part, the decrease in the number of dogs was apparent, while the number of houses increased.

6. Yearly changes of the relation between positive rate and dog density in the 4 parts

As the numbers of houses and registered dogs in each district were available for 1968 and 1983, the dog density (number of dogs per house) was calculated. The relation between the microfilaria positive rate in dogs and the dog density in each district in 1968 was compared with that in 1983 in Figure 3. The positive rate

Table 2 Number and percentage of dogs positive for *D. immitis* microfilariae by age and sex in 4 parts in Nagasaki City, 1983

Part	Sex	No. and %	Age							Total
			0	1-2	3-4	5-6	7-8	9-10	11≦	
Eastern	Male	Dogs examined	0	65	40	28	18	9	7	167
		Dogs positive	0	17	12	11	10	6	6	62
		% positive	0	26.2	30.0	39.3	55.6	66.7	85.7	37.1
	Female	Dogs examined	0	31	19	16	10	7	12	95
		Dogs positive	0	7	7	8	4	4	7	37
		% positive	0	22.6	36.8	50.0	40.0	57.1	58.5	38.9
Western	Male	Dogs examined	1	51	43	35	13	15	16	174
		Dogs positive	0	8	21	15	7	4	6	61
		% positive	0	15.7	48.8	42.9	53.8	26.7	37.5	35.1
	Female	Dogs examined	0	37	33	15	12	11	8	116
		Dogs positive	0	7	6	5	1	4	2	25
		% positive	0	18.9	18.2	33.3	8.3	36.4	25.0	21.6
Southern	Male	Dogs examined	3	99	72	43	30	11	14	272
		Dogs positive	0	25	36	18	16	7	9	111
		% positive	0	25.3	50.0	41.9	53.3	63.6	64.3	40.8
	Female	Dogs examined	1	54	42	32	15	19	8	171
		Dogs positive	0	10	16	14	3	7	3	53
		% positive	0	18.5	38.1	43.8	20.0	36.8	37.5	31.0
Northern	Male	Dogs examined	3	95	72	65	42	29	29	335
		Dogs positive	1	15	22	25	17	10	9	99
		% positive	33.3	15.8	30.6	38.5	40.5	34.5	31.0	29.6
	Female	Dogs examined	1	76	41	41	24	20	16	217
		Dogs positive	0	5	6	13	6	6	7	43
		% positive	0	6.6	14.6	31.7	25.0	30.0	43.8	19.8

Table 3 Yearly changes in *D. immitis* positive rates of dogs in 4 parts in Nagasaki City from 1968 to 1983

Part	1968*			1977*			1983			Increase (+) or decrease (−) in prevalence 1968 to 1983
	No. of dogs examined	positive	Positive rates (%)	No. of dogs examined	positive	Positive rates (%)	No. of dogs examined	positive	Positive rates (%)	
Eastern	675	194	28.7	328	113	34.5	265	99	37.4	+**
Western	337	68	20.2	334	98	29.3	293	86	29.4	+**
Southern	639	137	21.4	504	178	35.3	455	166	36.9	+**
Northern	719	291	40.5	555	174	31.4	555	153	25.9	−**

* : Cited from Suenaga *et al.* (1971, 1980).
** : Significant $P<0.001$

decreased in parallel with the decrease of the dog density in the northern part, but in the other parts the opposite tendency was generally observed.

7. Yearly changes in number of *Cx. p. pallens* females at 4 points

In Figure 4, the mean number of *Cx. p. pallens* females per night was shown as an annual population index. The mosquito population was not large from

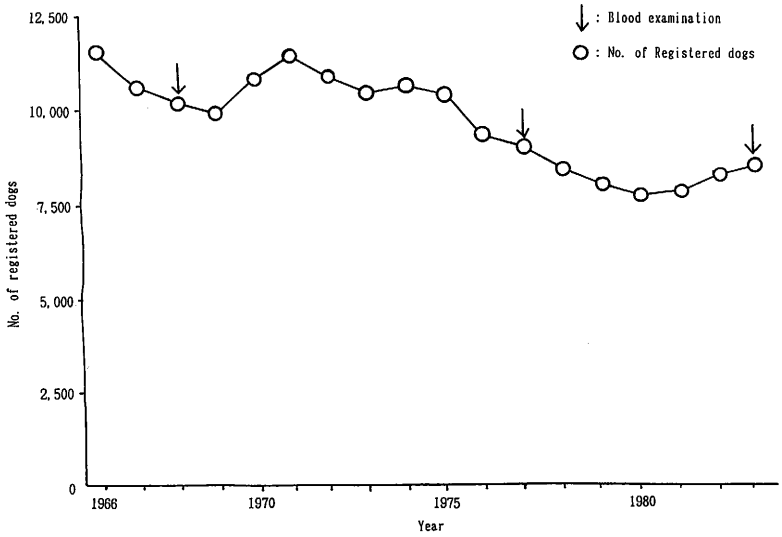


Figure 2 Yearly changes in the total number of registered dogs in Nagasaki City.

1972 to 1983, in the eastern, western and southern parts, though population varied with year. In Sakamoto (northern part) where the mosquitoes were collected from 1967 to 1983, the density was also low since 1971.

DISCUSSION

We studied the infections of *D. immitis* in registered dogs in the eastern, western, southern and northern parts of Nagasaki City in 1968, 1977 and 1983, and found a significant difference between the northern part and the other 3 parts. In the northern part, the positive rate had decreased, whereas in the other 3 parts, it had increased markedly. As one of the factors causing this phenomenon, introduction of Ivermectin, an effective

Table 4 Changes in numbers of dogs and houses in 4 parts between 1968 and 1983

Part	Dog and House	1968*	1983
Eastern	No. registered dogs (A)	3,666	1,758
	Dog density (A/B)	0.1212	0.0438
	No. houses (B)	30,243	40,143
Western	No. registered dogs (A)	1,428	830
	Dog density (A/B)	0.0932	0.0426
	No. houses (B)	15,316	19,477
Southern	No. registered dogs (A)	2,491	1,494
	Dog density (A/B)	0.1034	0.0447
	No. houses (B)	24,085	33,392
Northern	No. registered dogs (A)	3,733	2,211
	Dog density (A/B)	0.1050	0.0374
	No. houses (B)	35,566	59,159

*Cited from Suenaga *et al.* (1971, 1980)

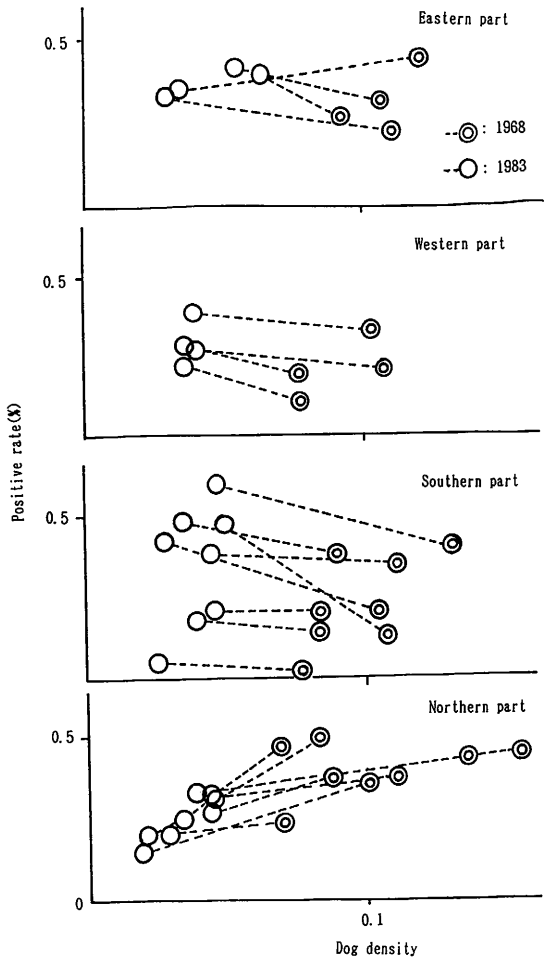


Figure 3 Relation between the dog density and the positive rate for *D. immitis* in the districts of Nagasaki City in 1968 and 1983.

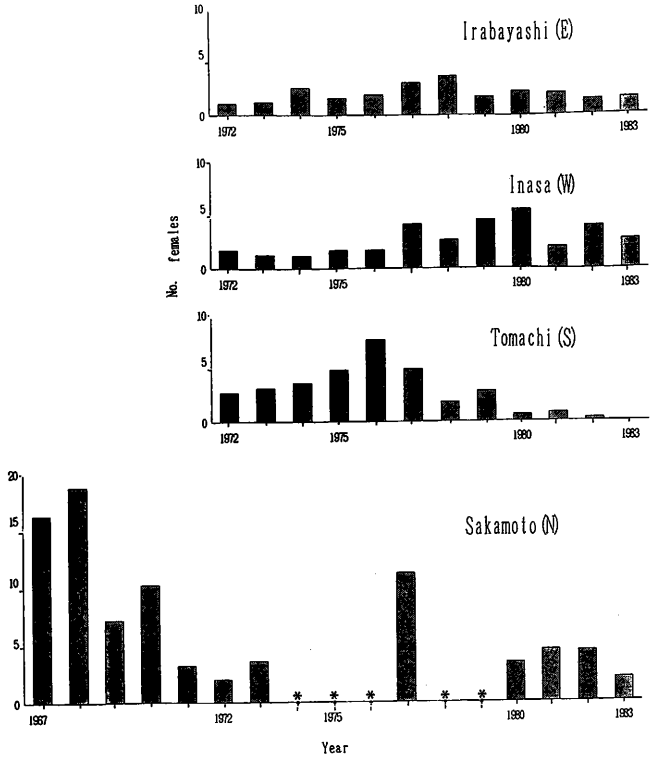


Figure 4 Yearly changes in the number of *Cx. p. pallens* females per night in Nagasaki City. E, W, S and N show eastern, western, southern and northern part respectively.
* The collection by light trap was not made.

preventive drug for *D. immitis*, was considered, but it can not be a reason for the decrease in the positive rate in the northern part since 1968 because its introduction was 1988. The present result showed that mosquito density was scarcely different in all the parts after 1972. But the density decreased in the northern part from 1968 to 1972, and thus, this decrease in mosquito density may be related to the decrease of the rate.

Dog density, which is an important factor responsible for infection (Wada *et al.*, 1989), decreased apparently in all 4 parts, but the positive rate decreased in the northern part and increased in the other 3 parts. As shown in Table 4, the number of houses in the northern part, which was the largest among the parts, had increased. This implies that many new residential areas had developed in this part, where it is assumed that the space to keep dogs outside was limited and there was some environmental complaint against dogs. Therefore, many dogs may have been kept within the house, and prevented from attack of the vector mosquito, *Cx. p. pallens*, which occurs in the breeding sites such as side ditches. Furthermore, when we took dog's blood, we had an impression that in the northern part more dog owners became to take measures to prevent exposure of their dogs to mosquitoes by, for example, letting them within the house at night and providing mosquito repellents. In the northern part, the increase in houses and office buildings was associated with the development of the sewage system thus decreasing drainages such as side ditches, according to the Department of Sewage System in Nagasaki City. Such improvements in the sanitary environment are considered to have been a factor in the decrease of the *Cx. p. pallens*. These conditions are assumed to relate to the decrease in the positive rate in the northern part.

On the other hand, the positive rate increased in eastern, western and southern parts. The density of dogs increased also in these parts, but it remained higher than in northern part as the number of houses was smaller, and more people kept dogs. In addition, it seems that the sewage system was poorly developed in these parts, because the number of houses was smaller than in northern part. From this we assumed that as the drainage has not been so well developed as in the northern part, more mosquitoes would be present in the 3 parts, though this result was not clearly shown by the data of light trap. According to dog owners, more dogs seem to be bred outdoors. Therefore it is suspected that dogs had more chance to be attacked by mosquitoes even if mosquito density was low in these parts.

As for the role of other mosquitoes in the transmission of *D. immitis*, Suenaga and Itoh (1973) reported that *Aedes albopictus* may be the secondary important vector, and *Cx. tritaeniorhynchus* may also be some bearing on the transmission in Nagasaki City. However, there is no evidence that these mosquitoes increased in the 3 parts in particular, and they are not considered to have contributed much to the increase of positive rate. The number of stray dogs may be another factor influencing the transmission. Their number, as given by the number of arrested dogs (unpublished data), decreased with years, therefore stray dogs are not an important factor for the increase of positive rate.

ACKNOWLEDGMENTS

Professor Y. Wada, Department of Medical Entomology, Institute of Tropical Medicine, Nagasaki University kindly read this manuscript and provided detailed suggestions. Dr. T. Fukuda, President of the Society of Veterinarian, in Nagasaki City and their member, veterinarians really cooperated with our research. We wish to express our sincere gratitude to these persons.

REFERENCES

1) Armitage, P. (1955): Test for linear trends in proportions and frequencies. Biometrics 11, 375-386.
2) Suenaga, O., Itoh, T. and Nishioka, T. (1971): Studies on the filarial prevalence among house dogs and the mosquito vectors in Nagasaki City, Western Japan. 1. On the microfilarial prevalence among house dogs in Nagasaki City. Trop. Med., 12, 169-178. (in Japanese with English summary).
3) Suenaga, O., Itoh, T. and Nishioka, T. (1973): Studies on the filarial prevalence among dogs and the mosquito vectors in Nagasaki City, Western Japan. 5. On the vector mosquitoes of *Dirofilaria immitis* in Nagasaki City. Trop. Med., 15, 131-140. (in Japanese with English summary).
4) Suenaga, O., Nishioka, T. and Itoh, T. (1980): On the transition of canine heartworm prevalence among dogs in Nagasaki City, Western Japan. 1. The filarial prevalence among dogs in the city. Trop. Med., 22, 35-45.
5) Suzumiya, J. and Nawa, Y. (1990): Three cases of pulmonary Dirofilariasis found in Miyazaki prefecture. Jpn. J. Parasit., 39, 67-71.
6) Wada, Y., Tsuda, Y. and Suenaga, O. (1984): Transmission dynamics of *Dirofilaria immitis* in a Southwestern part of Japan. Trop. Med., 31, 35-47.

Jpn. J. Trop. Med. Hyg., Vol. 21, No. 4, 1993, pp. 239-243

AN ULTRASTRUCTURAL STUDY OF AFRICAN ENDEMIC KAPOSI'S SARCOMA

HIDEAKI ETO, KAN TORIYAMA and HIDEYO ITAKURA

Received May 12 1993/Accepted November 9 1993

Abstract: An ultrastructural study of African endemic Kaposi's sarcoma (KS) of the skin and lymph nodes revealed an irregularity of blood vascular tissues and prominent diapiresis of erythrocytes in the early stage of the disease. In the advanced stage, incomplete blood capillary structures covered with primitive mesenchymal cells were observed. Erythrophagia was frequently seen. These results suggest that KS cells are derived from primitive mesenchymal cells which may differentiate to immature endothelial cells.

INTRODUCTION

KS is now thought to be a reversible hyperplasia at least at its inception and gradually progresses towards a true neoplasm at an advanced stage (Brooks, 1986; Itakura *et al.*, 1986). KS cells are characterized by spindle-shaped cells forming slit-like structures. On the histogenesis of KS cells, several hypotheses about original cells have been suggested by ultrastructural studies; vascular endothelial cell (Hashimoto and Lever, 1964; Ramos *et al.*, 1975), lymphatic endothelial cell (McNutt, *et al.*, 1983), Schwann cell (Pepler and Theron, 1962) and mesenchymal cell (Harrison and Kahn, 1978; Leu and Odermatt, 1985; Weich *et al.*, 1991; Kostianovsky *et al.*, 1992). However, the histogenesis of KS cells still remains obscure.

We performed an ultrastructural study of African endemic KS and discussed the histogenesis of KS cells.

MATERIALS AND METHODS

Materials:
Four cases of KS were obtained at Rift Valley Provincial General Hospital in Nakuru and Nyanza Provincial General Hospital in Kisumu, in Republic of Kenya. Clinical data and relevant information were recorded as accurately as possible.

Light microscopy:
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 and cellularity were determined.

Ultrastructural studies:
Small pieces of formalin-fixed, paraffin embedded specimens were refixed in 2.5% glutaraldehyde, post-fixed in 2% osmium tetroxide, dehydrated in graded ethanol solutions, and embedded in Epon 812. Sections were observed with a JEM-100CX transmission electron microscope.

RESULTS

Light microscopy:
The clinical aspects and histological features of the four cases are shown in Table 1. According to the predominant histological features, two types of growth patterns; a granulation tissue type and a spindel cell/fibrosarcoma type were recognized. The former was characterized by an angioproliferative process with edema and an inflammatory cell infiltration, but less cellular (Figure 1). This type was seen in Case 1, at an early stage of the disease. The latter (in Case 2-4, at an advanced stage) was classified as a spindle cell/fibrosarcoma type. This type showed a high cellularity, but its cellular atypism and mitotic figures were not prominent (Figure 2).

Electron microscopy:
In the case of granulation tissue type, endothelial cells were arranged with no continuity and basal lamina were fragmented. Diapedeses of erythrocytes were prominent. Fibroblastic cells appeared between these capil-

Department of Pathology, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852, Japan

Table 1 Clinicopathological findings of African Endemic Kaposi's sarcoma

Case	Age(yr)	Sex	Site of Location	Histological type	Cellularity
1	38	M	skin of leg	G	+
2	53	M	skin of leg	S	+++
3	10	M	scrotum	S	++
4	4	M	axillary lymph nodes	S	+++

G: granulation tissue-like type
S: spindle cell/fibrosarcoma-like type

laries (Figure 3). In the case of spindle cell/fibrosarcoma type, incomplete blood capillary structures were covered with the enlarged and irregular-shaped cells. The nuclei of the cells had irregular membrane with fungating (powdery) chromatin and small nucleoli. These cells had abundant cytoplasm with few organelles and no Weibel-Palade bodies. Diapedeses of erythrocytes and erythrophagia were also observed (Figure 4).

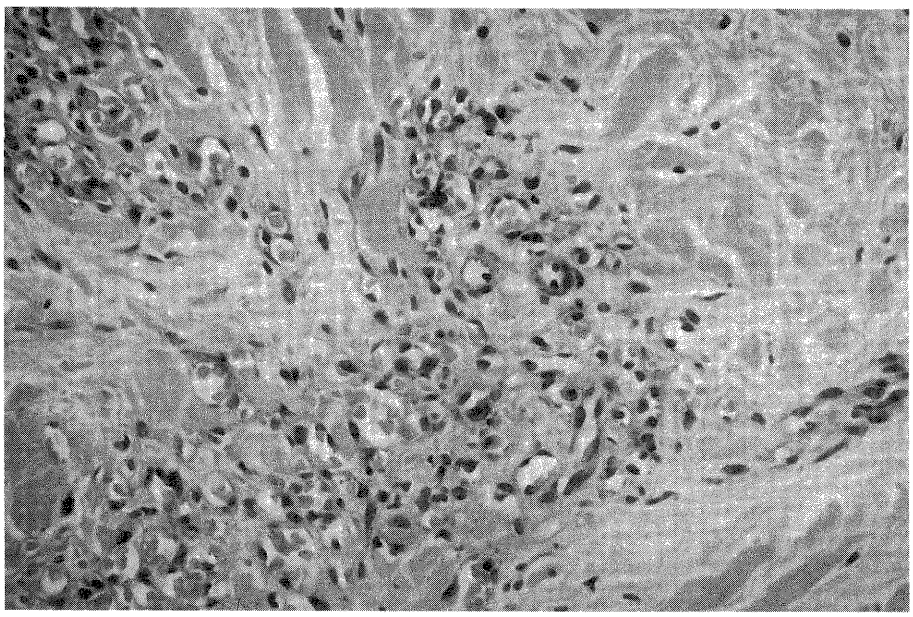


Figure 1 Case 1. Granulation tissue type of KS. The lesion is characterized by an angioproliferative process with an inflammatory cell infiltration. (H.E. ×100)

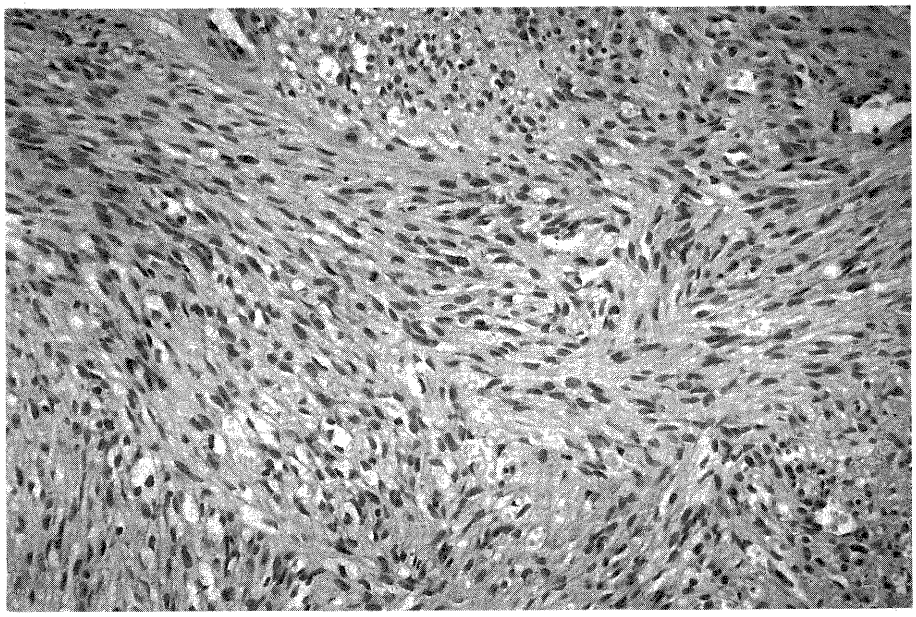


Figure 2 Case 2. Spindle cell/fibrosarcoma type of KS. The lesion is formed by interlacing bundles of spindle-shaped cells. (H.E. ×100)

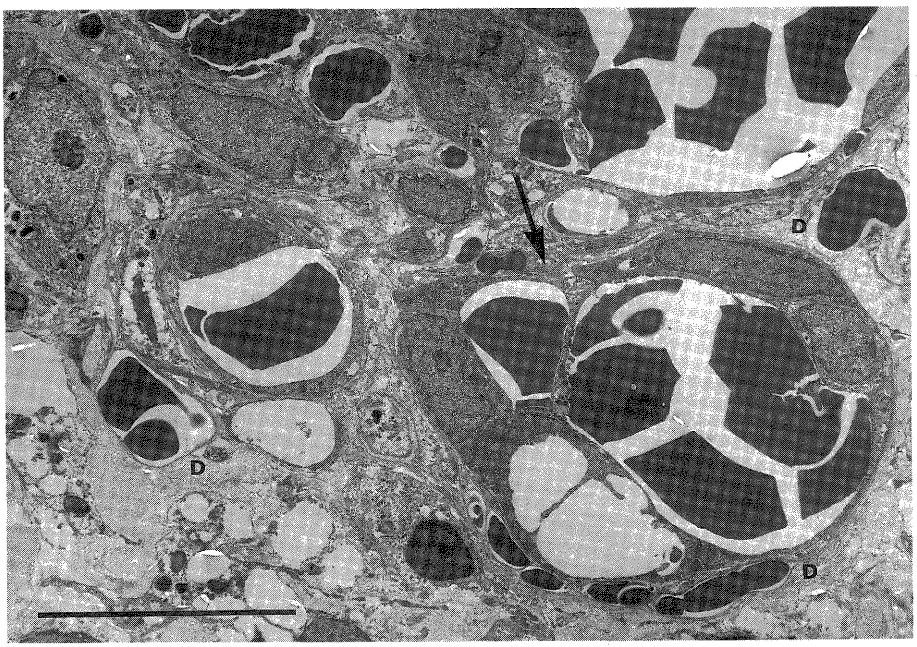


Figure 3 Case 1. Granulation tissue type of KS. Endothelial cells are arranged with no continuity (arrow). Diapedeses of erythrocytes are prominent (D). (×3800, Bar: 10μm)

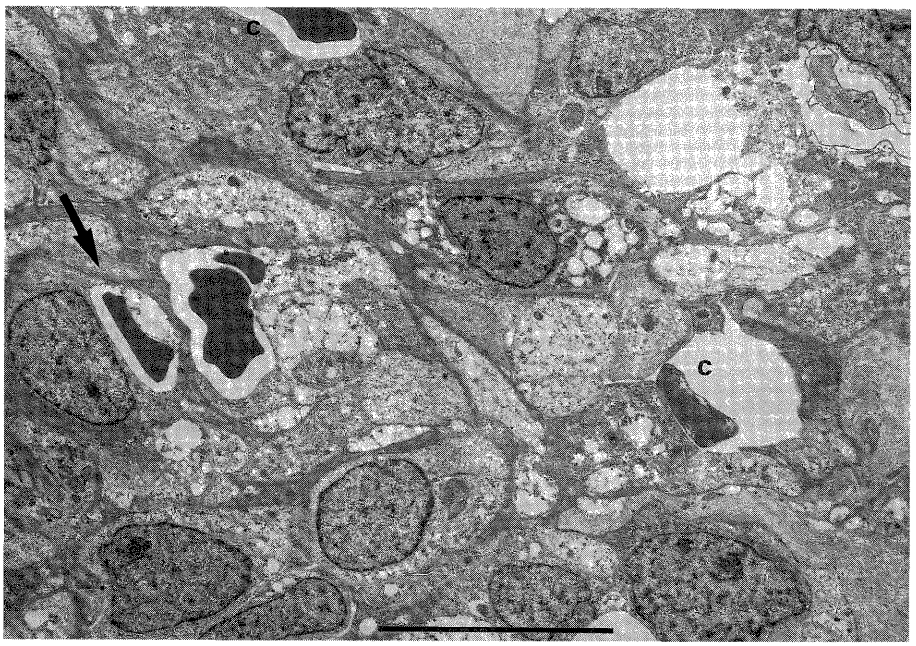


Figure 4 Case 2. Spindle cell/fibrosarcoma type of KS. Incomplete capillary structures (C) covered with enlarged and irregular-shaped cells. Erythrophagia (arrow) is characteristic. (×4000, Bar: 10μm)

DISCUSSION

KS shows a wide spectrum of histological changes in the course of the disease. At an early stage, similar lesion to non-specific granulation tissue, with cutaneous edema and perivascular inflammatory cell infiltration, are characteristic. Then spindle-shaped cells appears gradually in the course of the disease. At an advanced stage, slit-like structures with characteristic spindle -shaped cells, similar structure to angiosarcoma and fibrosarcoma, are observed (Itakura *et al.*, 1986; Enzinger and Weiss, 1988). According to McNutt *et al.* (1983), ultrastructural findings of KS at an early stage revealed more prominent irregularity of vascular structure than other vascular proliferative diseases. In this study, the capillaries of KS showed fragmented basal lamina and no continuity of the endothelial arrangement. Diapedeses of erythrocytes were also prominent at an early stage. Some immune factors related to angiogenic activity, such as thymosin, interferon, lymphokines and prostaglandin, are thought to be crucial in the development of KS (Levy and Ziegler, 1983). The lesions mentioned above may be caused by some immune factors. At an advanced stage, incomplete blood capillary structures covered with enlarged and irregular-shaped cells were observed. These cells had abundant cytoplasm with few organelles and no Weibel -Palade bodies, as ocassionally seen in primitive mesenchymal cells. Erythrophagia was frequently seen. Using immunohistochemical methods we have observed that KS cells show a positive reaction for vimentin, which is a marker for mesenchymal cells, and that KS cells are partially positive for factor Ⅷ-related antigen, which is a marker for vascular endothelial cells (Komuro and Toriyama, 1991). These results suggest that KS cells are derived from primitive mesenchymal cells which may differentiate to immature endothelial cells.

ACKNOWLEDGMENTS

The authors are grateful to Dr. A. Makatta for helpful comments and advice on the manuscript in English, and are also indebted to Mr. A. Ichinose for his help of electron microscope.

REFERENCES

1) Brooks, J.J. (1986): Kaposi's sarcoma: A reversible hyperplasia, Lancet, 2, 1309-1310
2) Enzinger, F.M. and Weiss, S.W. (eds). (1988): Soft tissue tumors. 2nd. ed. pp. 533-544. C.V. Mosby Co. Washington
3) Harrison, A.C., and Kahn, L.B. (1978): Myogenic cells in Kaposi's sarcoma: An ultrastructural study, J. Path-

ol., 124, 157-171
4) Hashimoto, K. and Lever, W.F. (1964): Kaposi's sarcoma: Histochemical and electron microscopic studies, J. Invest. Dermatol., 43, 539-549
5) Itakura, H., Toriyama, K. and Uzuta, F. (1986): Kaposi's sarcoma: Pathology and local epidemiology in Kenya, Trop. Med., 28 (suppl.), 3-8
6) Komuro, S. and Toriyama, K. (1991): Geopathology of endemic pediatric lymph node Kaposi's sarcoma in western Kenya, Jpn. J. Trop. Med. Hyg., 19, 251-263
7) Kostianovsky, M., Lamy, MAJ. Y. and Greco, M.A. (1992): Immunohistochemical and electron microscopic profile of cutaneous Kaposi's sarcoma and bacillary angiomatosis, Ultrastruct. Pathol., 16, 629-640
8) Leu, H.J. and Odermatt, B. (1985): Multicentric angiosarcoma (Kaposi's sarcoma): Light and electron microscopic and immunohistological findings of idiopathic cases in Europe and Africa and of cases associated with AIDS, Virchows. Archiv. A., 408, 29-41
9) Levy, J.A. and Ziegler, J.L. (1983): Acquired immunodeficiency syndrome is an opportunistic infection and Kaposi's sarcoma results from secondary immune stimulation, Lancet., 1, 78-80
10) McNutt, N.S., Fletcher, V. and Conant, M.A. (1983): Early lesion of Kaposi's sarcoma in homosexal men: An ultrastructural comparison with other vascular proliferations in skin, Am. J. Pathol., 111, 62-77
11) Pepler, W.J. and Theron, J.J. (1962): An electron microscope study of Kaposi's haemangiosarcoma, J. Path. Bact., 83, 521-525
12) Ramos, C.V., Taylor, H.B., Hernandez, B.A. and Tucker, E.F. (1975): Primary Kaposi's sarcoma of lymph nodes, Am. J. Clin. Pathol., 66, 998-1003
13) Weich, H.A., Salahuddin, S.Z., Gill, P., Nakamura, S., Gallo, R.C. and Folkmann, J. (1991): AIDS-associated Kaposi's sarcoma-derived cells in long-term culture express and synthesize smooth muscle alpha-actin, Am. J. Pathol., 139, 1251-1258

Jpn. J. Trop. Med. Hyg., Vol. 21, No. 4, 1993, pp. 245-249

Case Report

FOUR IMPORTED CASES OF FALCIPARUM MALARIA SUCCESSFULLY TREATED WITH HALOFANTRINE

SHIGEYUKI KANO[1], GOHTA MASUDA[2], YUZURU KUBO[3], MASAYUKI SAITO[3], TSUYOSHI YAMAGUCHI[2] AND MAMORU SUZUKI[1]

Received September 29 1993/Accepted October 15 1993

Abstract: Halofantrine, a phenanthrene derivative, has been used for the treatment of human malaria since 1984, and reported to be effective against all parasite species without any major side effects. The chemical structure of the drug is unique and not closely related to any other antimalarials, therefore, it is particularly effective for the treatment of the wide-spread drug resistant malaria. Four cases of imported falciparum malaria, including chloroquine and pyrimethamine resistant malaria, were treated with halofantrine and reported in this paper. Halofantrine cleared parasites and fever of all the cases rapidly and produced salutary symptomatic improvement of the patients. No adverse reactions were recognized after the administration of the drug, nor was there recrudescence. Fansidar is the only antimalarial drug commercially available in Japan, and imported drug resistant malaria not only against chloroquine but also Fansidar has been on the increase. The general use of halofantrine is thus expected in Japan.

INTRODUCTION

In 1992, the total number of Japanese who went abroad amounted to as many as 11,790,699, and of foreigners who entered Japan to 3,251,753 (Annual record of the office of statistics and information, Division of Immigration Service, Ministry of Justice). Consequently the reported annual number of imported malaria cases increased to not less than 100 (Ohtomo *et al.*, 1993), and therefore, malaria is no longer recognized as a very rare disease in Japan. Nevertheless effective drugs against malaria are not easily available and some severe cases have been reported because of the delay in the onset of treatment (Kano *et al.*, 1998; Hosaka *et al.*, 1992). Particular attention has to be focused on quick and proper treatment with safe and effective antimalarials. Four cases of falciparum malaria which were successfully treated with Halfan™ (halofantrine hydrochloride, 233mg base/tablet, SmithKline Beecham Pharmaceuticals) are reported in this paper.

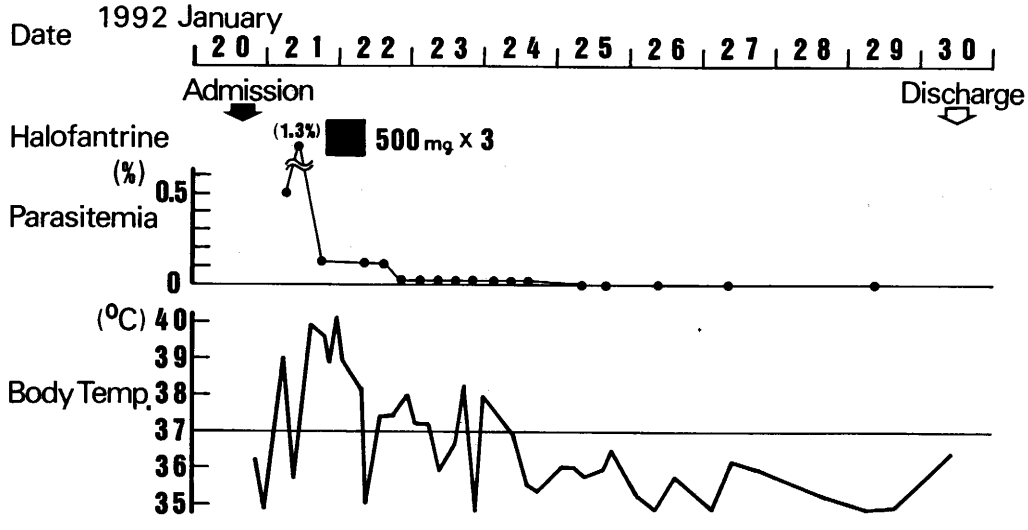
CASE 1

The patient was a Japanese T.V. cameraman, 39 years old, who stayed in Uganda from December 24, 1991 to January 15, 1992. He was taking chloroquine once a week during this period. In spite of the prophylaxis, on his way back to Japan (Jan. 16), he showed fever of over 40°C. On admission to Isesaki-Sawa Medical Association Hospital on Jan. 20, he manifested chills, a headache, slight splenomegaly, and thrombocytopenia, but anemia. The following morning, *Plasmodium falciparum* (*P.f.*) parasites were detected from a thin blood smear at the density of 0.5% of red blood cells, but antibody titer against malaria was negative by the indirect fluorescent antibody test (IFAT). Two tablets each of Halfan™ were administered orally at 19:30 on Jan. 21, 2:30 and 8:30 on Jan. 22. Body temperature fell to normal at 10:00 on Jan. 24 (36.9°C) and parasites were cleared at 9:00 on Jan. 25. No adverse reaction was recognized after the administration of the drug, nor was there recrudescence of *P.f.* malaria. (Fig. 1).

CASE 2

The patient was a Japanese male, 37 years old, who stayed at Vangunu in Solomon Islands during April 11-25, 1992. He was on regular weekly chloroquine pro-

1. Department of Parasitology, Gunma University School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371, Japan
2. Department of Infections Diseases, Tokyo Metropolitan Komagome Hospital, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113, Japan
3. Isesaki-Sawa Medical Association Hospital, 481 Shimoueki, Isesaki, Gunma 372, Japan



WBC (/mm³)	3600	3200	4100	5800	5500	5000	5200	7200	7300
RBC (x10⁴/mm³)	518	517	497	467	465	497	466	450	458
Hb (g/dl)	15.9	16.3	14.9	13.6	14.0	14.9	14.1	13.6	14.0
Ht (%)	45	46	43	41	41	44	41	41	42
Plts (x10⁴/mm³)	3.3	3.2	3.5	4.8	7.8	11.3	18.1	22.8	33.4

Figure 1 Clinical course of Case 1. 39yr. Male.

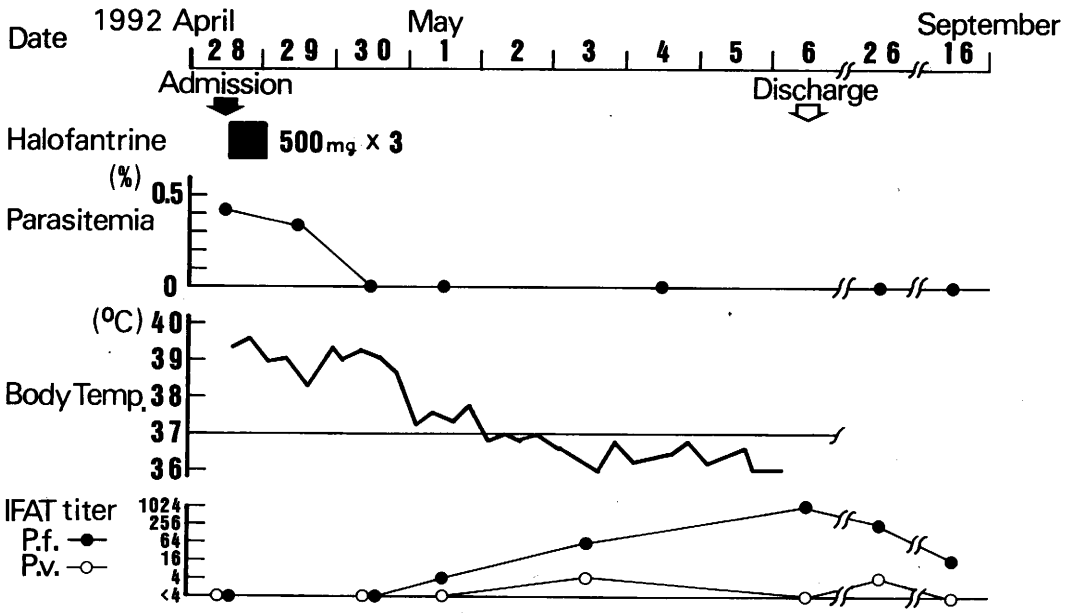
phylaxis. He was admitted to Tokyo Metropolitan Komagome Hospital on April 28, complaining of fever of 39.3°C, a headache, and general fatigue. On examination, there was hepatomegaly, 2 fingers in width, but the spleen was not palpable. Microscopic observation of a thin blood smear revealed *P.f.* parasites at 0.4% of red blood cells. He was treated with a total of 6 tablets of Halfan™, because he was clinically suspected of suffering from chloroquine resistant *P.f.* malaria. However, *in vitro* drug susceptibility tests indicated that the parasites were susceptible to chloroquine (IC_{50}=84.5 nM), and highly resistant to pyrimethamine (IC_{50}>1000 nM). His clinical course after the drug administration is illustrated in Figure 2: parasitemia was cleared by April 30 and body temperature fell to normal on May 2. Antibody levels of *P.f.* and *P.v.* titers are also shown in the figure. The case was successfully treated with Halfan™ and showed no adverse reaction nor recrudescence.

CASE 3

The patient was a Japanese male student, 23 years old, who visited China, Pakistan, India, some African countries (Kenya, Tanzania, Zaire, Zambia, Uganda and Malawi), and Thailand, between April 1991 and April 24, 1992. He was taking chloroquine irregularly during that period. He manifested fever in March, 1992 and was diagnosed as having malaria by a local doctor, and treated with Fansidar. Despite the treatment, he showed fever on April 25 and May 13 (38.6 °C), and was then seen at Tokyo Metropolitan Komagome Hospital on May 15. Peripheral blood smear showed *P.f.* parasitemia at 0.08% of RBCs and he was hospitalized on the following day. Treatment with 1500mg of Halfan™ cleared the parasites in 72 hours and fever in 63 hours. On admission, the IFAT titer was 1:64 and increased to 1:1024 on May 18 (Fig. 3). No adverse reaction nor recrudescence of the parasites was recognized after the drug administration. Besides malaria, examination of stool on admission revealed *Shigella flexneri* infection. The patient was also successfully treated with a total of 2000mg (400mg×5days) of lomefloxacin (LFLX), a quinolone derivative, and discharged from the hospital on June 3.

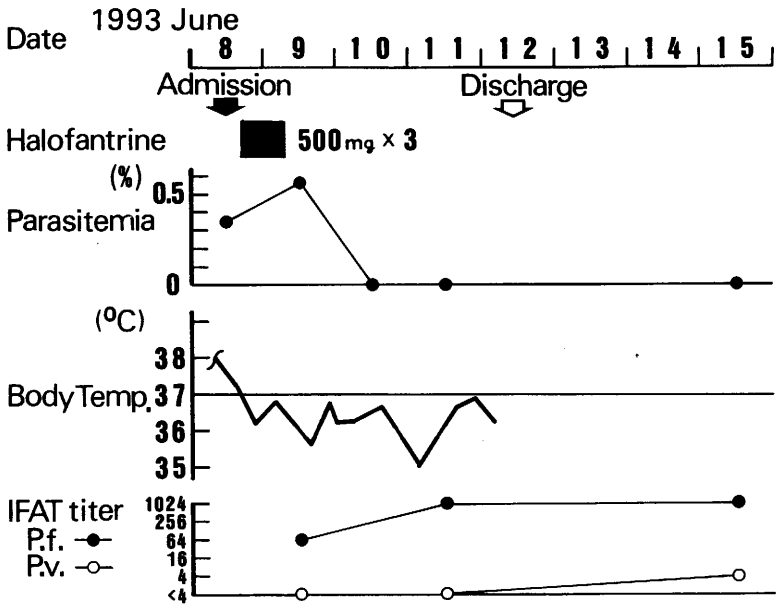
CASE 4

The patient was a Japanese female and a dentist, 53 years old, who stayed in Mali from September 1989 to May 3, 1993. She suffered from several episodes of malaria in September-November 1991 and was treated with chloroquine, Fansidar, or quinine. She was not taking any prophylactic measures against malaria dur-



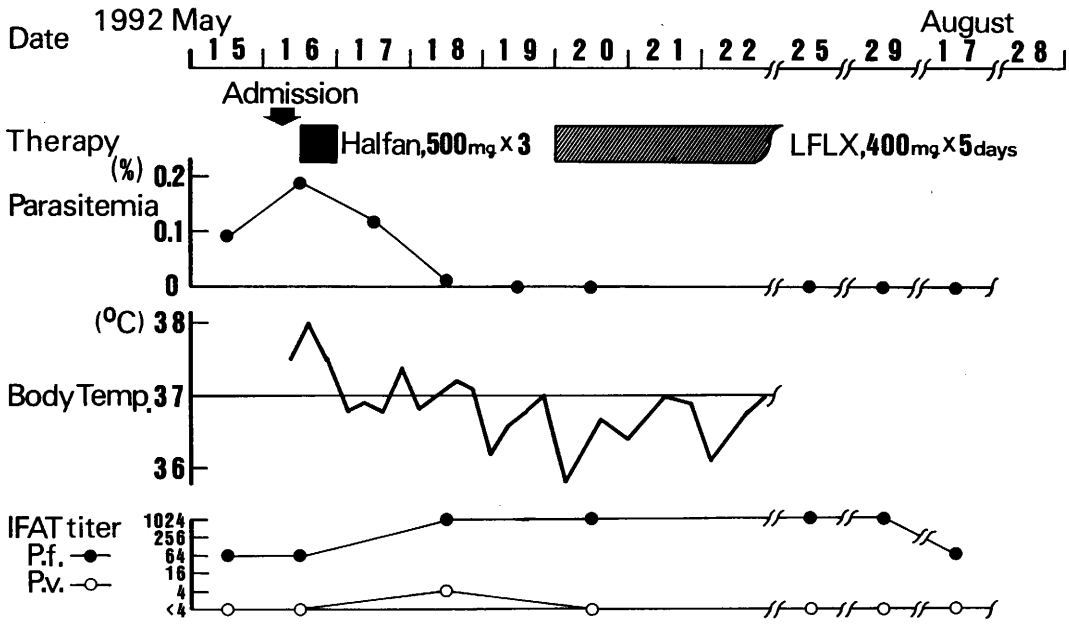
WBC(/mm³)	6800	5800	5000	4400	5200	6400	6500	7100	7300
RBC(x10⁴/mm³)	582	593	613	630	585	527	534	566	568
Hb (g/dl)	17.1	17.3	17.3	18.2	16.6	15.0	15.5	16.2	16.2
Ht (%)	49.7	51.2	53.3	53.7	49.7	44.5	45.9	49.5	48.8
Plts (x10⁴/mm³)	19.0	14.8	8.0	4.4	4.9	8.2	21.1	19.9	17.3

Figure 2 Clinical course of Case 2. 37yr. Male.



WBC (/mm³)	4900	4500	3900	4200	4300
RBC (x10⁴/mm³)	373	369	347	357	379
Hb (g/dl)	11.2	11.1	10.4	10.6	11.3
Ht (%)	32.8	33.4	31.0	31.9	33.6
Plts (x10⁴/mm³)	8.5	11.5	11.4	14.0	20.9

Figure 3 Clinical course of Case 3. 23yr. Male.



WBC (/mm³)	3100	3900	4300	4200	4000	4800	4500	5000	5400
RBC (×10⁴/mm³)	319	340	318	346	328	332	365	410	451
Hb (g/dl)	10.0	10.6	9.8	10.7	10.3	10.5	11.6	12.8	13.5
Ht (%)	29.6	31.5	29.6	32.3	30.8	31.2	34.4	37.9	40.4
Plts (×10⁴/mm³)	13.2	14.7	13.3	16.1	17.4	22.6	27.3	26.3	35.1

Figure 4 Clinical course of Case 4. 53yr. Female.

ing her stay in Mali. After she came back to Japan, she showed fever of 39.2°C on May 16, 1993, and had been feverish since then. On June 8, she was admitted to Tokyo Metropolitan Komagome Hospital complaining of fever, chills, a headache and nausea. However, her general condition looked apparently milder than that of other Japanese patients with malaria. On admission, she had mild anemia and hepatomegaly but no splenomegaly. Falciparum malaria parasites were detected from a thin blood smear at the density of 0.34% of RBCs. Antibody titer against *P.f.* antigen was 1:64 by the IFAT (Fig. 4). Two tablets of Halfan™ were administered orally three times at 6 hourly intervals, which cleared parasites by June 10. No adverse reaction was recognized after the drug administration.

Discussion

Halfan has been used for the treatment of human malaria since 1984. To date, clinical studies have involved more than 2500 patients in 30 countries (Smith-Kline Beecham Pharmaceuticals (SB), 1992), but in Japan, few cases have been reported (Kano *et al.*, 1992; Masuda *et al.*, 1992; Yodonawa *et al.*, 1992). This is practically the first report of imported falciparum malaria cases which were successfully treated with halofantrine. Halofantrine is an antimalarial drug, unrelated to existing antimalarials, effective against all species, effective in drug resistance, very well tolerated, and with a simple dosage regimen. Treatment with halofantrine cleared parasitemia within 7 days in more than 99% of 1315 patients with falciparum malaria, and recrudescences occurred in only 78 patients (6%). Mean parasite clearance time was 57.9 hours in *P.f.* malaria patients, and mean fever clearance time was 50.2 hours (SB, 1992). Parasite clearance times of the reported cases 1, 2, 3, and 4 in this paper were 85.5h, 48h, 63h, and 48h respectively, and fever clearance times of Cases 1, 2, and 3 were 62.5h, 87h, 72h, which are well within the ranges seen for the drug. Case 4 was a patient who suffered from malaria several times before, and consequently, she was clinically semi-immune; her symptoms were rather mild, parasites were cleared more rapidly, and her body temperature was only slightly elevated. Therefore, changes in her body temperature following the administration of halofantrine may not in itself be indicative of the effectiveness of the drug.

Halofantrine is also reported to produce rapid symptomatic improvement, and is very well tolerated without showing major side effects. One of the typical

findings in the 4 cases in this paper was thrombocytopenia, which is commonly seen in acute malaria. Halfan product data (1992) shows that mean platelet counts steadily improved from day 3 and reached a plateau between days 7 and 14 after onset of treatment. Platelets count of the 4 cases in this paper increased to normal in 4-7 days after the drug administration (Fig. 1-4). No apparent side effect was recognized after the treatment with halofantrine in any of the 4 cases.

The IFAT for the detection of the malaria antibodies is useful for retrospective diagnosis of malaria. We can also distinguish the species of the parasites which a patient has contracted (Kano *et al.*, 1990). Cases 1 and 2 represent patients who suffered from malaria for the first time, who were thus seronegative on admission although showing parasitemia. However, Cases 3 and 4 had earlier malaria episodes and showed considerable levels of antibody titer on admission to the hospital. Both manifested *P.f.* titers as high as 1:1024 in a very short time. In Cases 2, 3, and 4, the IFAT titers against *P.f.* antigen were always higher than those against *P.v.* antigen throughout the clinical courses of the cases. Although the IFAT is not always useful for the diagnosis of malaria at the early stages of the infection, we can picture the episodes and course of the infection retrospectively through the changes in the titers.

Halofantrine belongs to a class of compounds—the phenanthrene-methanols—, which does not share chemical structure with any other animalarials, and is therefore particularly effective in the treatment of drug resistant malaria (SB, 1992). Elimination half-life of halofantrine is 1.3-6.6 days (SB, 1992) which is longer than that of quinine but considerably shorter than that for mefloquine or chloroquine, and the therapeutic course of treatment is completed within 12 hours. Because of these properties the parasite is not exposed to sub-optimal levels of the drug for an unnecessarily long time, thus the risk of *P.f.* strains emerging which are resistant to halofantrine is thought to be minimized. WHO data for 1993 shows that there is a widespread distribution of chloroquine-resistant *P.f.*, while resistance to Fansidar is also on the increase (WHO, 1992). However, Fansidar is the only antimalarial drug commercially available in Japan so far despite the fact that the number of imported malaria cases is increasing, including drug resistant malaria. In fact, cases 1 and 2 in this paper were considered as chloroquine resistant clinically, and case 2 as *in vitro* pyrimethamine resistance. The general use of halofantrine in Japan is thus expected.

We obtained the informed consent from every patient reported in this paper prior to the treatment with halofantrine.

ACKNOWLEDGEMENTS

The authors are grateful to Mrs. Rumiko Kosugi for her excellent technical assistance. We also thank Dr. B. D. Akanmori for helpful discussion.

REFERENCES

1. Kano, S., Tsuji, M., Hosoya, J. and Suzuki, M. (1988): A case of severe malaria treated with qinghaosu. Jpn. J. Trop. Med. Hyg., 16(4), 301-307
2. Kano, S., Waki, S., Igarashi, I., Nakazawa, S., Masuda, G. and Suzuki, M. (1990): Retrospective malaria diagnosis by indirect fluorescent antibody titration on Japanese patients. Jpn. J. Parasitol., 39(5), 475-481
3. Kano, S., Yodonawa, S. and Suzuki, M. (1992): Two cases of vivax malaria treated with halofantrine. Jpn. J. Trop. Med. Hyg., 20, 72
4. Hosaka, S., Ishikawa, A., Hosono, T., Iikuni, Y., Kondo, H., Kobayashi, Y., Nakamura, T., Ito, Y., Ohtomo, H., Kano, S. and Suzuki, M. (1992): A case of severe mixed *Plasmodium falciparum* and *P. vivax* infection successfully treated with artemether. Jpn. J. Trop. Med. Hyg., 20(4), 305-312
5. Masuda, G., Ajisawa, A., Negishi, M., Yamaguchi, T., Kano, S. and Suzuki, M. (1992): A case of mix infection with *Plasmodium falciparum* and *P. malariae* treated with halofantrine. Clin. Parasitol., 3, 38-40
6. Ohtomo, H., Takeuchi, T., Masuda, G., Akao, M., Takada, S., Araki, T., Tsuji, M. and Tanaka, H. (1993): Malaria incidence in Japan for past 5 years. Jpn. J. Parasitol., 42, (suppl.), 137
7. SmithKline Beecham Pharmaceuticals (1992): Halfan product data, SB House, Middlesex, UK
8. WHO (1993): Weekly Epidemiological Record, 68(34), 245-252, and 68(35), 253-260
9. WHO expert committee on malaria (1992): Nineteenth Report, 8
10. Yodonawa, S., Kano, S., Saito, T., Yokoyama, Y., Kawai, S. and Suzuki, M. (1992): An imported case of vivax malaria treated with halofantrine. Jpn. J. Trop. Med. Hyg., 20(4), 299-303

Jpn. J. Trop. Med. Hyg., Vol. 21, No. 4, 1993, pp. 251-254

Research Note

SEXUAL MOSAICS IN THREE BLACKFLY SPECIES (DIPTERA: SIMULIIDAE) IN JAPAN

UPIK KESUMAWATI HADI AND HIROYUKI TAKAOKA

Received October 7 1993/Accepted October 29 1993

Abstract: Sexual mosaics were found in eight adult Japanese blackflies, i.e., two *Simulium arakawae* Matsumura, 1921, four *S. bidentatum* (Shiraki, 1935), all collected from cattle sheds while biting, and two *S. aokii* (Takahasi, 1941) which emerged from pupae. They were distinguished into three types, i.e., bilateral, antero-posterior and other types of gynandromorph.

Sexual mosaicism, the appearance of morphological (phenotypic) features of both sexes in the same individuals, is known to occur in insects. Such sexually mosaic individuals can be classified either as gynandromorphs (a mixture of genetically male and female cells) or intersexes (only cells of a single genotype) (Brust, 1966).

In family Simuliidae (Diptera), sexual mosaicism has been reported to occur in several different species, i.e., *Simulium grisescens* var. *palmatum* Puri, 1932 in India (Puri, 1933), *S. venustum* Say, 1823 in Canada (Wolfe and Peterson, 1959), *S. arcticum* Malloch, 1914 in Canada (Fredeen, 1970), *S. soubrense* Vajime and Dunbar, 1975 in Ivory Coast (Dang and Peterson, 1979), *S. damnosum* Theobald, 1903 (complex) in West Africa (Cheke and Garms, 1985) and *S. aokii* (Takahasi, 1941), *S. bidentatum* (Shiraki, 1935), *S. takahasii* (Rubtsov, 1962), and *S. iwatense* (Shiraki, 1935) in Japan (Saito and Kanayama, 1986). We report here eight cases of sexual mosaics found in three Japanese blackfly species collected from Oita in Kyushu island, southwest Japan.

MATERIALS AND METHODS

Fly numbers 1-6 were collected at cattle sheds in Oita City, and the remaining two (fly numbers 7 and 8) were found among those that emerged from pupae collected from a stream in Yufuin, Oita. All specimens were identified according to descriptions given by Takaoka (1976, 1977). As in most other blackfly species, sexually dimorphic characters in the three blackfly species mentioned below were evident, being chiefly recognized in the eye facets, frons, antennae, mandibles and maxillary lacinia on the head; in the scutal pattern of the thorax; in the subcosta of the wings; in the claws of the legs; in the tergites and sternites of the abdomen; and in the genitalia. Wing length represents the distance from arculus to the wing tip.

DESCRIPTION OF THE SEXUAL MOSAICS

Table 1 shows the descriptions of the eight sexually mosaic individuals.

No. 1 *Simulium arakawae* Matsumura, 1921

The body length was about 3.2 mm and wing length was about 2.5 mm. Collected on May 5, 1989 from Kokubu cattle shed in Oita.

This appeared to be an almost complete bilateral gynandromorph, showing a male phenotype on the left side and a female phenotype on the right side (Figs. 1, 2, and 3a). With an exception, left mandible was serrated like the right mandible. The epipharynx and cibarium were of female phenotype. The genitalia was of female phenotype on the right side, although the paraproct (Fig. 2e) was incompletely formed and the cercus (Fig. 2f) was small like that of a male (Fig. 2g). The genital fork (Fig. 2b) was incomplete, lacking a left arm. The ventral plate (Fig. 2j) of the male genitalia was shaped only on the left side. The median sclerite (Fig. 2k) was present but looked narrower than a normal one.

Division of Medical Zoology, Oita Medical University, Hasama, Oita 879-55, Japan

Table 1 Descriptions of eight sexual mosaics of blackflies collected from Oita, Japan

No.	Species	Head		Thorax		Leg		Wing		Abdomen		Genitalia	
		L	R	L	R	L	R	L	R	L	R	L	R
1.	*S. arakawae*	M	F	M	F	M	F	M	F	M	F	M	F
2.	*S. arakawae*	F	X	F	M	F	M	F	M	X	X	M	M
3.	*S. bidentatum*	F	F	F	M	X	X	F	F	X	X	M	M
4.	*S. bidentatum*	F	F	X	X	X	X	F	F	X	X	M	M
5.	*S. bidentatum*	F	X	F	F	M	M	F	F	F	F	F	F
6.	*S. bidentatum*	F	F	F	X	X	F	M	F	M	M	M	M
7.	*S. aokii*	M	M	M	X	X	F	M	F	F	X	F	F
8.	*S. aokii*	F	F	F	M	F	M	F	M	M	M	M	M

*L, left; R, right; M, male; F, female; X, mixed phenotype (see detailed descriptions in the text).

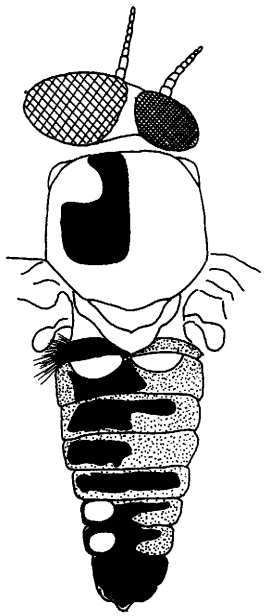


Figure 1 Head, thorax and abdomen (dorsal view) of *S. arakawae* No 1, showing a bilateral gynandromorphism, with a female character on the right and a male one on the left side.

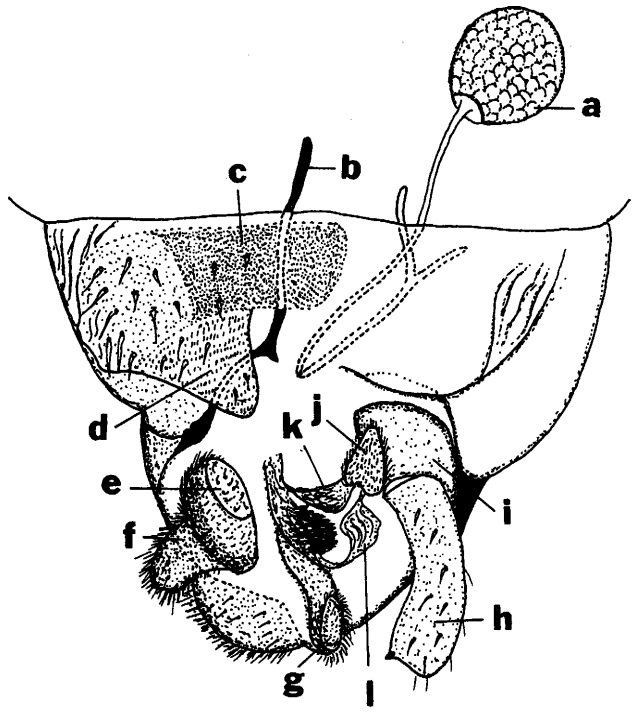


Figure 2 Terminalia (ventral view) of *S. arakawae* No 1. a, spermatheca; b, genital fork; c, 8th sternite; d, anterior gonapophysis; e, paraproct; f, cercus (female); g, cercus (male); h, style; i, coxite; j, ventral plate; k, median sclerite; l, paramere.

No. 2 *Simulium arakawae*

The body length was about 2.7 mm and wing length was about 2.3 mm. Collected on September 10, 1989 from Imanaga cattle shed in Oita.

This fly possessed a female character on the head except for the eye facets near the right front-occular area which were somewhat enlarged. The thorax (Fig. 3b), legs and wings were bilaterally divided, with female phenotype on the left side and male one on the right. Abdominal segments 2 to 5 showed a female phenotype whereas segments 8 and 9, and genitalia were of a normal male. Abdominal segments 6 and 7 were mosaic, each with a female phenotype on the left and a male phenotype on the right side.

No. 3 *Simulium bidentatum*

The body length was about 3.2 mm and wing length was 2.4 mm. Collected on October 29, 1989 from Imanaga cattle shed.

This fly possessed a female head and male genitalia. The scutal pattern on the thorax showed a female phenotype on the left and a male one on the right as in No. 2. The fore legs showed a mixed phenotype, i.e., a male character on the left side, and a female one on the right, while mid and hind legs were of female and male characters, respectively. The wings were of female

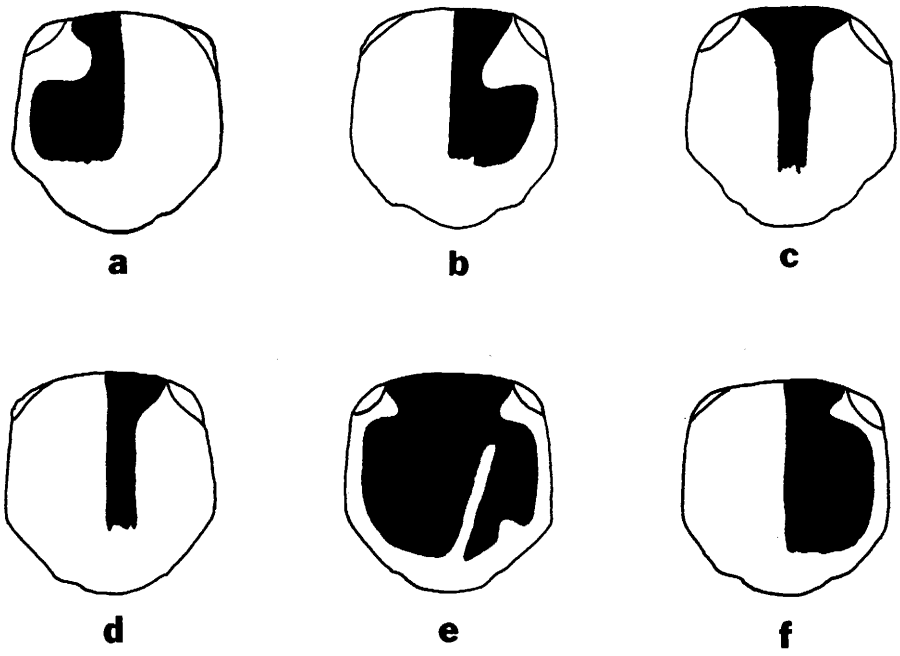


Figure 3 Scutal patterns of sexually mosaic blackflies. a, *S. arakawae* No 1; b, *S. arakawae* No 2; c, *S. bidentatum* No 4; d, *S. bidentatum* No 6; e, *S. aokii* No 7; and f, *S. aokii* No 8.

phenotype on both sides. The ventral surface of the abdomen showed a male phenotype. However, dorsal surface of the abdomen was mosaic as in No. 2.

No. 4 *Simulium bidentatum*

The body length was about 3.9 mm and wing length was about 3.0 mm. Collected on April 26, 1990 from Imanaga cattle shed.

This specimen possessed a female head and male genitalia as in No. 3. The thorax showed an irregular scutal pattern having a dark and wide longitudinal line medially (Fig. 3c). The hind legs were of female phenotype on both sides whereas fore and mid legs showed a mixed phenotype with a male character on the left side and a female character on the right side. The wings were of female phenotype on both sides. The ventral surface of the abdominal segments 2 to 4 showed a mixed phenotype with a female phenotype on the right and a male one on the left side, whereas that of the remaining segments was of normal male. The dorsal surface of the abdomen showed a male phenotype. This fly contained a blood meal in the midgut.

No. 5 *Simulium bidentatum*

The body length was about 4.2 mm and wing length was about 3.0 mm. Collected on March 10, 1990 from Imanaga cattle shed.

This fly was, for the most part, of female phenotype, except for all the legs and the small area of the right eye which were of male character. This fly was infected by mermithid.

No. 6 *Simulium bidentatum*

The body length was about 3.4 mm and wing length was about 2.4 mm. Collected on April 10, 1990 from Imanaga cattle shed.

This fly had a female head and a male abdomen including the genitalia. The scutum showed an unusual phenotype on the right side, but had a normal female pattern on the left side (Fig. 3d). The legs also had a female phenotype except the left midleg being of male phenotype. The wings were bilaterally divided, a male phenotype on the left side and a female phenotype on the right side.

No. 7 *Simulium aokii*

The body length was about 3.8 mm and wing length was about 2.9 mm. Collected as a pupa on March 21, 1989 in Yufuin and emerged in the laboratory.

This specimen possessed a male head and female genitalia. The thorax showed a normal male scutal pattern on the left side, but had a slightly aberrant pattern on the right side (Fig. 3e). The legs were of female character, except for the fore and mid legs on the left side being of male character. The wings were as in No. 6. The abdomen was of female phenotype except

for the basal scale and segment 2 on the right side, which were of male character.

No. 8 *Simulium aokii*

The body length was about 3.0 mm and wing length was about 2.8 mm. Collected as pupa on April 7, 1990 and emerged in the laboratory.

This specimen exhibited a female head and a male abdomen including the genitalia as in No. 6. The scutal pattern on the thorax (Fig. 3f), legs and wings were divided bilaterally, bearing a male phenotype on the right side and a female phenotype on the left side.

REMARKS. As previously reported in other blackfly species (Wolfe and Peterson, 1959; Dang and Peterson, 1979 and Puri, 1933), the eight sexually mosaic specimens reported here are distinguished into three types of gynandromorph: (1) bilateral type, as found in one *S. arakawae* (No. 1); (2) antero-posterior type, as found in six specimens i.e., one *S. arakawae* (No. 2), three *S. bidentatum* (No. 3, 4, and 6), and one *S. aokii* (No. 8), all having a female phenotype on the anterior part and a male phenotype on the posterior part, while other body areas showed a mixed phenotype; and one more specimen, *S. aokii* (No. 7), with reverse character; (3) another type, as found in one *S. bidentatum* (No. 5), mainly having a female phenotype and sexually mosaic areas of the body being distributed in an irregular pattern. None of these flies was interpreted as intersexes.

REFERENCES

1) Brust, R.A. (1966): Gynandromorphs and intersexes in mosquitoes (Diptera: Culicidae), Can. J. Zool., 44, 911-921.
2) Cheke, R.A. and Garms, R. (1985): Sexual mosaics in *Simulium damnosum* species complex (Dipt, Simuliidae) in West Africa, Entomologist's Monthly Magazine, 121, 137-141.
3) Dang, P.T. and Peterson, B.V. (1979): A case of bilateral gynandromorphism in *Simulium soubrense* Vajime and Dunbar (Diptera: Simuliidae), Tropenmedizin und Parasitologie, 30, 548-550.
4) Fredeen, F.J.H. (1970): Sexual mosaics in the black fly *Simulium arcticum* (Diptera: Simuliidae), Canadian Entomologist, 102, 1585-1592.
5) Puri, I.M. (1933): A case of gynandromorphism in *Simulium,* Indian J. Med. Res., 20, 801-803.
6) Saito, I and Kanayama, A. (1986): Gynandromorphism of Japanese blackflies, Jpn. J. Sanit. Zool., 37, (3), 292 (In Japanese).
7) Takaoka, H. (1976): Studies on black flies of the Nansei Islands, Japan (Simuliidae; Diptera). II. On six species of the subgenera, *Gomphostilbia* Enderlein, *Morops* Enderlein, *Odagmia* Enderlein and *Gnus* Rubzov, with a description of *Simulium* (*Gomphostilbia*) *okinawense* sp. nov., Jpn. J. Sanit. Zool., 27, (4), 385-398.
8) Takaoka, H. (1977): Studies on black flies of the Nansei Islands, Japan (Simuliidae; Diptera). III. On six species of the subgenus *Simulium* Latreille, Jpn. J. Sanit. Zool., 28, (2), 193-217.
9) Wolfe, L.S. and Peterson, D.G. (1959): Blackflies (Diptera: Simuliidae) of the forests of Quebec, Can. J. Zool., 37, 137-159.

Jpn. J. Trop. Med. Hyg., Vol. 21, No. 4, 1993, pp. 255-258

Short Communication

SERO-EPIDEMIOLOGICAL STUDY ON HUMAN TOXOCARIASIS IN THE RURAL SECTOR AROUND RECIFE, NORTHEAST BRAZIL

YUZURU IWANAGA[1,2], JOSE FELIPE GONCALVES[3], MASANOBU TANABE[2,4], SEIKI TATENO[2,4], MORIYASU TSUJI[5] AND TSUTOMU TAKEUCHI[4]

Received August 23 1993/Accepted October 7 1993

Until 1981, over 1,900 human cases with toxocariasis had been reported in the world (Glickman and Shantz, 1981). Prevalence and epidemiology of human toxocariasis have been extensively studied in many countries (Brunello *et al.*, 1986; Salinas *et al.*, 1987; Lynch *et al.*, 1988). In Brazil, two cases of infant toxocariasis (Kawakami *et al.*, 1984) and prevalence of human toxocariasis (Chieffi *et al.*, 1988) have been reportod in São Paulo. Recently, two human toxocariasis of inpatient and outpatient have been reported in two hospitals located in the urban area of Recife, northeast Brazil (Virginia *et al.*, 1991). However, to our knowledge, there is no reliable datum on the incidence, prevalence and epidemiology of human toxocariasis in the rural sector around Recife. According to parasitological examinations in São Lourenco da Mata, northeast Brazil (Goncalves *et al.*, 1990), four out of 485 inhabitants were positive for unembryonated eggs of *Toxocara canis* in stools. Therefore, a sero-epidemiological study was conducted to obtain preliminary data on the prevalence of human toxocariasis in São Lourenco da Mata.

Serum samples were collected from 288 inhabitants in four villages (Ceramica Bicopeba, Engenho Pitangueira, Engenho Camurim and Engenho General) in Sao Lourenco da Mata during July to December, 1989. Prevalence of intestinal parasitic infections of these subjects has been reported previously (Goncalves *et al.*, 1990).

The serological examinations using Ouchterlony and immunoelectrophoresis were performed according to the methods of Tsuji (1974, 1975). Crude extracted antigens were prepared according to the previous method (Tsuji, 1974) from the adult worms of *T. canis, Ascaria suum, Dirofilaria immitis Schistosoma mansoni* and *Taenia saginata,* and from the larvae of *Anisakis* sp., Two hundreds and eighty-eight sera were primarily examined by Ouchterlony using *T. canis* antigen. Seven sera (2.4%) showed positive reaction with *T. canis* antigen as shown in Table 1. However, these seven sera also revealed positive reaction with other helminthous antigens: all sera were positive for *S. mansoni* and *D. immitis* antigens, and one reacted with both *A. suum* and *Anisakis* sp. antigens. Stool examination of all of these seven cases showed positive for *S. mansoni* eggs. Six subjects were also positive for hookworm eggs, two were positive for *T. canis* eggs, and one was positive for *Ascaris lumbricoides* eggs (Table 1).

To evaluate the specificity of precipitation reaction on Ouchterlony test, these seven sera were further analyzed by immunoelectrophoresis. All sera did not show any precipitin band with the antigens prepared from *A. suum, T. saginata* and *Anisakis* sp. But all sera revealed positive reaction with *S. mansoni* antigen, and one reacted with *D. immitis* antigen. On the other hand, all of those specimens showed plural precipitation lines with *T. canis* as demonstrated in Figs. 1 and 2, and the species-specific precipitin band for *T. canis* (Tsuji, 1975) were detected in four serum samples.

Although definitive diagnosis of the toxocariasis is

1. Department of Parasitology, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima City, Hiroshima 734, Japan
2. Laboratório de Imunopatologia Keizo Asami, Universidade Federal de Pernambuco, Cidade Universitaria, Recife, Pernambuco CEP=50,730, Brazil
3. Centro de Pesquisas Aggeu Magalhaes, Cidade Universitaria, Recife, Pernambuco CEP=50,730, Brazil
4. Department of Tropical Medicine and Parasitology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan
5. Department of Parasitology, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka City, Tokyo 181, Japan

Table 1 The results of Ouchterlony test and stool examination for inhabitants in São Lourenco da Mata, Brazil

Subjects			Ouchterlony test					Stool examination			
No.	Sex	Age	T.c	S.m	D.i	A.s	Ani	S.m	H.w	A.l	T.c
1019	F	7	+	+	+	+	+	+	+	+	
1028	F	32	+	+	+			+	+		
3009	M	17	+	+	+			+	+		+
3053	F	36	+	+	+			+			
3075	F	54	+	+	+			+	+		
4008	F	8	+	+	+			+	+		+
4185	F	19	+	+	+			+	+		

T.c: *Toxocara canis*
S.m: *Schistosoma mansoni*
D.i: *Dirofilaria immitis*
A.s: *Ascaris suum*
Ani: *Anisakis* larvae
H.w: Hook worm
A.l: *Ascaris lumbricoides*

based upon the larvae in biopsy specimens, demonstration of larvae appears to be difficult. Therefore, the diagnosis of human toxocariasis has been usually performed by various serological tests using different types of antigens (Cypess *et al.*, 1977; Savigny and Tizard, 1977; Kawamura, 1983; Clemett *et al.*, 1985; Inoue and Tsuji, 1989). However, in the case of toxocariasis as well as other nematoda infections, there exist many cross-reactions with other parasitic antigens on any kind of serological methods. In contrast, Tsuji (1975) has demonstrated that toxocariasis can be diagnosed by the immunoelectrophoresis according to the presence of species-specific precipitin band. In the present study, such a species-specific precipitin band was identified in four serum samples by immunoelectrophoresis. It is, therefore, likely that at least four cases may considered to be positive cases of human toxocariasis serologically. Goncalves *et al.* (1990) suggest that the circumferential environments around their housings may be heavily contaminated with *T. canis* eggs. So, a high incidence of toxocariasis is expected in this area. But, in the present study, toxocariasis were a few cases. This finding suggested that some of ingested *T. canis* eggs potentially developed in humans. As mentioned above, no reliable data on human toxocariasis in the rural sector around Recife, northeast Brazil have been reported before so that the present communication may be first report on the occurrence defined cases of human toxocariasis in this area by serological approach.

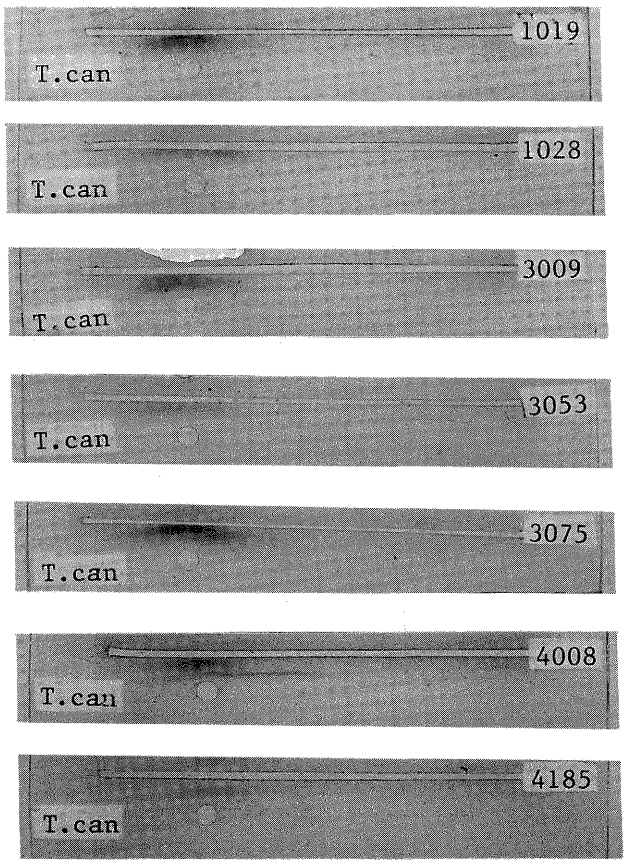


Fig. 1 Immunoelectrophoresis against *Toxocara canis* antigen for inhabitants in São Lourenco da Mata, Brazil
T. can: *Toxocara canis* adult worm.

Acknowledgement

The authors are indebted to Dr. Luis Carvalho, Universidade Federal de Pernambuco, Brazil, for his suggestion.

This study was supported by Research Grants from the Japan International Cooperation Agency (JICA).

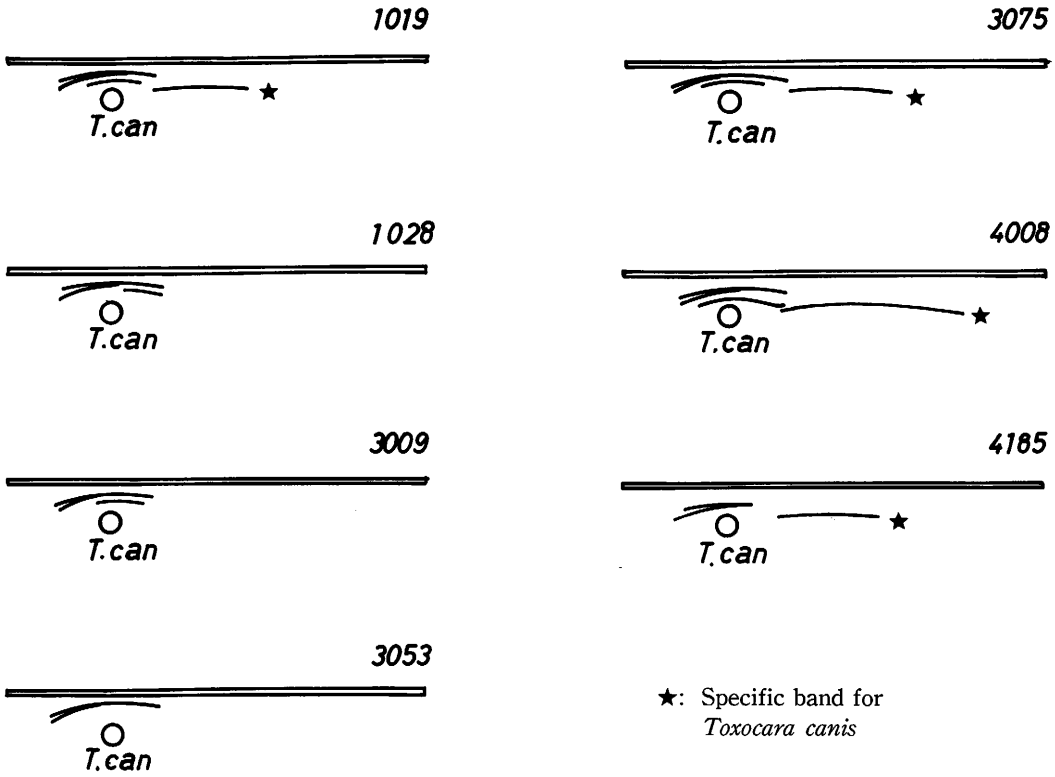


Fig. 2 Immunoelectrophregrams against *Toxocara canis* antigen for inhabitants in São Lourenco da Mata, Brazil

REFERENCES

1) Brunello, F., Falagiani, P. and Genchi, C. (1986): Enzyme linked immunosorbent assay (ELISA) for the detection of specific IgG antibodies to *Toxocara canis* ES antigens, Boll. Ist. Sieroter Milam., 65, 54-60
2) Chieffi, P.P., Ueda, M., Camargo, E.D., Souza, A.M.C., Silva, C.L., Nova, A.V. e Guedes, M.L.S. (1988): Contacto domiciliar e profissional com cães como fatores de risco para infeccão humana por larvas de *Toxocara,* Rev. Inst. Med. Trop. São Paulo., 30, 379-382
3) Clemett, R.S., Hidajat, R.R., Allardyce, R.A. and Stewart, A.C. (1985): Toxocaral infection in hydatid control officers; diagnosis by enzyme immunoassay, New Zealand Med. J., 98, 737-739
4) Cypess, R.H., Karol, M.N., Zidian, J.L., Glickman, L.T., Gitlin, D. (1977): Larva-specific antibodies in patients with visceral larva migrans, J. Infect. Dis., 135, 633-640
5) Glickman, L.T. and Shantz, P.M. (1981): Epidemiology and pathogenesis of zoonotic toxocariasis, Epidemiol. Rev., 230-250
6) Goncalves, J.F., Tanabe, M., Melo, F.P.M., Goncalves, F. J., Aca, I.S., Motta, S.R.N., Tateno, S. and Takeuchi, T. (1990): Parasitological and serological studies on amoebiasis and other intestinal parasitic infections in the runal sector around Recife, northwest, Brazil, Rev. Inst. Med. Trop. São Paulo., 32, 428-435
7) Inoue, H. and Tsuji, M. (1989): Studies on visceral larva migrans: Detection of anti-*Toxocara* canis IgG antibodies by ELISA in human and rat sera, Jap. J. Parasitol., 38, 68-76
8) Kawakami, E., Fagundes, N.U., Wehba, J. and Patricio, F.R. (1984): Visceral larva migrans in infancy; report of 2 cases, Arg. Gastroent., 21, 83-87
9) Kawamura, H. (1983): Detection of circulating antigen and antibody of *Toxocara canis* by radioimmunoassay and antigen-binding assay with a purified *Toxocara canis* antigen, Hiroshima Med. J., 31, 265-274
10) Lynch, N.R., Wilkes, L.K., Hodgen, A.N. and Turner, K. J. (1988): Specificity of *Toxocara* ELISA in tropical populations, Parasit. Immun. 10, 323-337
11) Salinas, P., Reyes, L., Sotomayor, M.T. e Letonja, T. (1987): Prevalencia de huevos de *Toxocara* sp. en algunas pazas publicas de la region metropolitana de Santiago, Chili Boletin Chileno de Parasitol., 42, 33-36
12) Savigny, D.H. and Tizard, I.R. (1977): Toxocaral larva migrans: The use of larval secretory antigens in haemagglutination and soluble antigen fluorescent antibody tests, Trans. R. Soc. Med. Hyg., 71, 501-507
13) Tsuji, M. (1974): On the immunoelectrophoresis for helminthological researches, Jap. J. Parasitol., 23, 335-345
14) Tsuji, M. (1975): Comparative studies on the antigenic structures of several helminths by immunoelectrophor-

esis, Jap. J. Parasitol., 24, 227-236
15) Virginia, P., Nagakura, K., Ferreira, O. and Tatemo, S. (1991): Serologic evidence of Toxocariasis in northeast Brazil, Jpn. J. Med. Sci. Biol., 44, 1-6

ISSN 0304-2146

JAPANESE JOURNAL OF TROPICAL MEDICINE AND HYGIENE

VOL. 21 NO. 4 DECEMBER 1993

CONTENTS

(Contents continued on reverse of this cover)

JTM FOUNDED 1959

Jpn. J. Trop. Med. Hyg.

JAPANESE SOCIETY OF TROPICAL MEDICINE