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VIROLOGIC-EPIDEMIOLOGICAL STUDIES ON INDONESIA*

Survey of Anti-Arboviral Antibodies in Sera from Residents of Lombok, Sumatera and Djawa

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Susumu HOTTA, Hideo AOKI, Tazuko YASUI and Susumu SAMOTO. *Virologic-Epidemiological Studies on Indonesia: Survey of Anti-Arboviral Antibodies in Sera from Residents of Lombok, Sumatera ana Djawa.* Kobe J. Med. Sci. **13**, 221-234, September 1967——Sera were collected from residents of Indonesia, i. e., Lombok Island in 1964, Lampung State, South Sumatera, in 1965, and Madjalengka State, West Djawa, in 1966.

HI antibodies against dengue types 1 and 2 (D1 and D2), Japanese encephalitis (JE) and yellow fever (YF) viruses were measured using a microtiter technique (Takatsy-Sever).

Results obtained indicate :

(1) Antibodies against D1, D2 and JE are found.

(2) Quantitative distributions of the antibodies are different among the areas examined ; for instance, JE-antibodies are predominant in the 1964 Lombok sera, D1 in the 1965 Lampung sera, and D2 in the 1966 Madjalengka sera, respectively. It is presumed that each particular virus is predominantly distributed in the respective area.

(3) Anti-YF antibodies are also detected in sera from all the areas surveyed. Because of their comparatively low titers, however, the YF-antibodies are presumably present due to crossing reactions, especially by dengue infections.

On schedule are further studies, employing more specimens from different parts of Indonesia and other viral antigens such as those of other types of dengue and chikungunya.

INTRODUCTION

It is of importance and interest from medical and public health points of view to know what kinds of viruses are distributed in human and/or animal populations of a particular area. Results of such investigations of Indonesia, however, have so far been scanty.

The Kobe University School of Medicine has made a long-term project of medical and public health investigations of Indonesia, and the First (1964), Second (1965) and Third (1966) Medical Missions were sent as scheduled. Each team opened ambulatory clinics at selected places in Indonesia : Lombok in 1964, Sumatera in 1965, and Djawa in 1966. Members of the teams and general outlines of their activities

* Preliminary accounts for part of the work were published in Japanese (Hotta et al, 1966)[7]

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were previously described (Reports; Shirai, 1966 a[17], b[18], Miyasaki, 1966[12]). The present authors participated in the investigations, carrying out microbiologic and serologic researches.

Since the geography and climate of Indonesia are typically tropical, our primary attention has been paid to the study of tropical diseases, especially arboviral infections. This paper will report results of the works dealing particularly with types 1 and 2 dengue (D1 and D2), Japanese encephalitis (JE), and yellow fever (YF), all of which are of important significance not only for Indonesia but also for countries including Japan. Although the study is still in progress, the results obtained up to the present time will be described here to meet the current need.

MATERIALS AND METHODS

Serum:

Specimens[a] were collected from residents of Lendang-Nangka Village, Lombok Island (in 1964), Lampung State, Sumatera Island (in 1965), and Madjalengka State, Djawa Island (in 1966).

While the sera used were taken from individuals complaining of subjective and/or objective disorders, sera from cases diagnosed as acute infectious diseases were excluded from the experiment.

Sera were separated, under sterile precaution, from the blood taken from the cubital vein of individuals who visited our teams' ambulatory clinics for consultation. The sera were stored in a cold and dark place or in a refrigerator at about 4°C as often as possible until their arrival at Kobe; however, they were unavoidably left at room temperatures of the tropics for a few weeks. To prevent bacterial or fungal contamination, phenol mercuric borate ($(C_6H_5Hg)_2B_4C_7$) was added at a concentration of approximately 1 : 200,000. After arrival at Kobe, they were kept frozen in an electric refrigerator.

Just prior to test, the sera were inactivated by heating at 56°C for 30 minutes, and received the following treatments in order to remove serologic inhibitor(s). Two-tenths ml of each serum specimen was mixed with 4 ml of cold acetone, and the mixture was shaken vigorously for 5 to 10 minutes. After centrifugation at 1,500 g for 5 minutes, the sediment was dried in a desiccator by continuous pressure reduction. The dried powder was resuspended in 2 ml of borate buffer saline (BBS; pH 9.0) and held at 4°C overnight. This was designated as 1 : 10 acetone-treated serum. After that, the material was subjected to "adsorption" with 0.05 ml of packed goose erythrocytes in ice water for 15 minutes. Following centrifugation

a) Collection and transportation of the specimens were done by all participants of the teams : Dr. Toda, Dr. Iwasaki, Dr. Fujimoto, Dr. Mori, Mr. Tozawa, Mr. Shimizu, Mr. Matsumoto and Mr. Shirane, of the 1964 Mission ; Dr. Hotta, Dr. Shirai, Dr. Ohyama, Dr. Noda, Dr. Takao, Dr. Matsuo, Mr. Tomioka, Mr. Yasue, and Mr. Morita of the 1965 Mission ; and Dr. Hotta, Dr. Miyasaki, Dr. Kayama, Dr. Nakanoin, Dr. Matsuo, Dr. Samoto, Dr. Kamidono, Mr. Matsunaga, and Mr. Okamoto, of the 1966 Mission.

at 3,000 g for 7 minutes, the supernatant was taken and used for hemagglutination inhibition (HI) tests.

Virus antigen:

Dengue type 1 (D1, Mochizuki strain), type 2 (D2, Trinidad-1751 strain), Japanese encephalitis (JE, JaGAr #01 strain), and yellow fever (YF, 17D strain) were employed. All the strains were mouse-passaged. The antigens of D1, D2 and YF were partially purified in our laboratory by the following method[b] and the JE antigen was a commercial product[c].

(1) Infected mouse brains were emulsified by being added with 10 times their volume of Tris-cystine saline (TCS) (including 0.02M tris, 0.14M NaCl and 100 μg/ml cystine; pH 7.8). (2) The supernatant obtained by a centrifugation at 8,500 g for 15 minutes was added with protamine sulfate (final concentration, 1 mg/ml) and held at 4°C for 30 to 60 minutes with occasional shaking. (3) After having been centrifuged at 4,000 g for 15 minutes, the supernatant was added with pre-cooled ethanol (final concentration, 25%), and was held at 4°C for 60 minutes with occasional agitation. (4) The precipitate obtained by centrifugation at 4,000 g for 15 minutes was re-suspended in one-tenth its volume of TCS. (5) After low speed centrifugation, the supernatant was taken and designated as the final purified material.

JE antigen, a powdered product, was dissolved in sterile distilled water and kept at 4°C for one hour prior to use.

Hemagglutination (HA) and hemagglutination-inhibition (HI)

The microtitration methods described by Takatsy (1955) and by Sever (1962) were used. Their general outlines are as follows:

(a) HA:

1. One drop (0.025 ml) of BBS (pH 9.0) was put into each well of a Microtiter ® V-plate[d], using a microdropper[d].
2. Antigen was taken by a dilution loop and transferred into the first well. After thorough mixing by rotating the loop with fingers, the loop was moved to the next well.
3. By the same procedures, two-fold dilutions of antigen were carried on successively.
4. One drop of BBS was added to each of the wells.
5. Five-hundredths ml (2 drops of the microdropper) of 0.33% goose erythrocyte suspension (1.88×10^7 cells/ml, in phosphate buffer solution[e,f]) was

b) A modification of the procedures originally proposed by Nakamura *et al* (1963)[13] and Fukai (personal communication)[5] (Refer to Matsumura, 1965[10]).

c) Purchased from the Takeda Pharmaceutical Co., Ltd., Osaka, Japan.

d) Procured from the Cooke Engineering Company, San Mateo, California, USA.

e) So-called virus adjusting diluent (VAD), pH's of which should be 6.2 for D1 and D2, 6.4 for JE, and 6.0 for YF.

f) The whole volume of fluid contained in each well was 0.1 ml after completion of the procedures.

added to the wells and mixed thoroughly by agitation. The plate was sealed with cellophane tape to prevent evaporation and was kept in an incubator at 37°C for 30 to 60 minutes.

6. The highest dilution of antigen showing the complete agglutination was regarded as representing one HA unit.

(b) HI:

1. Serum (originally 1 : 10 diluted) was diluted two-fold with BBS, according to the procedures described above for HA tests.
2. One drop (0.025 ml) of antigen adjusted to possess 4 to 8 HA units was added to each well, and the constituents were mixed thoroughly. One separate drop of antigen was put into another well containing one drop of BBS, in order to confirm the titer of antigen on the following day (Refer to Procedure (5) described below).
3. The plate was sealed with cellophane tape, which then was held in a refrigerator at 4°C overnight.
4. The next day two drops (0.05 ml) of the goose erythrocyte suspension were added to each well and mixed thoroughly. Sealed with cellophane tape, the plates were kept in an incubator at 37°C for 30 to 60 minutes. The highest dilution of serum displaying the complete inhibition of HA was regarded as the HI titer. Since the dilution of serum in the first well was 1 : 20, this was the minimum positive reaction so far in the study.
5. The antigen was re-tested to ascertain its titer.

RESULTS

Table 1 indicates districts surveyed and the number of specimens collected.

Table 1. Districts surveyed and number of serum specimens collected.

Lombok Island (1964)							
Village	Lendang-Nangka						Total
No. of specimen	129						129
Lampung State, Sumatera (1965)							
Village	Metro	Sritedjo-kentjono	Batang-hari	Pekalon-gan	Simbar-waringin	Josodady	Total
No. of specimen	63	51	68	57	37	24	300
Madjalengka State, Djawa (1966)							
Village	Nunuk	Tibodas	Argalinga	Madjalengka	Tikijin		Total
No. of specimen	100	30	101	31	36		298

Lombok is an island, Lampung is an agricultural zone in Southern Sumatera, and Madjalengka is a mountain region about 300 to 1,500 m above sea level in Western Djawa (See a map illustrated in Fig. 1).

The feeling is that communication is rather easy between Sumatera Island and Djawa Island, but more difficult between Lombok Island and Djawa Island. On each island the inhabitants of the different villages can communicate with one another rather easily, but there is a strong tendency for such inhabitant to reside permanently on his own island.

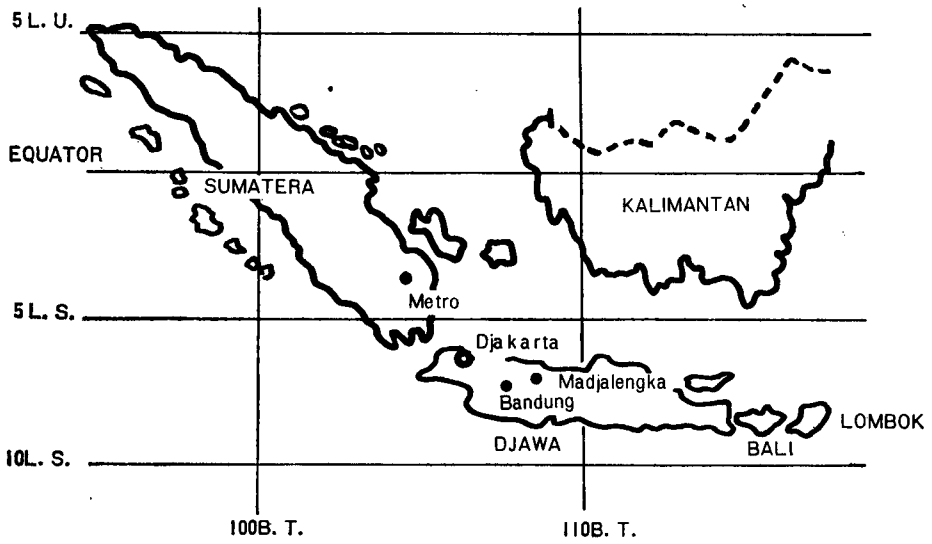

Fig. 1. Map of Indonesia

Figs. 2, 3 and 4 illustrate distributions of HI antibodies against the four kinds of antigens tested.

Relatively predominant are: anti-JE antibodies in 1964 Lombok sera, anti-D1 antibodies in 1965 Lampung sera, and anti-D2 antibodies in 1966 Madjalengka sera, respectively.

Numbers of specimens showing positive reaction to any (one or more) of the four antigens used are summarized in Table 2.

Since there is a considerable overlapping in the serologic reactions of arboviruses, especially among members belonging to the same groups, the over-all results should be analyzed from various angles.

Distributions of apparently positive or negative reactions among the specimens tested are summarized in Table 3 (for 1964), Table 4 (for 1965) and Table 5 (for 1966).

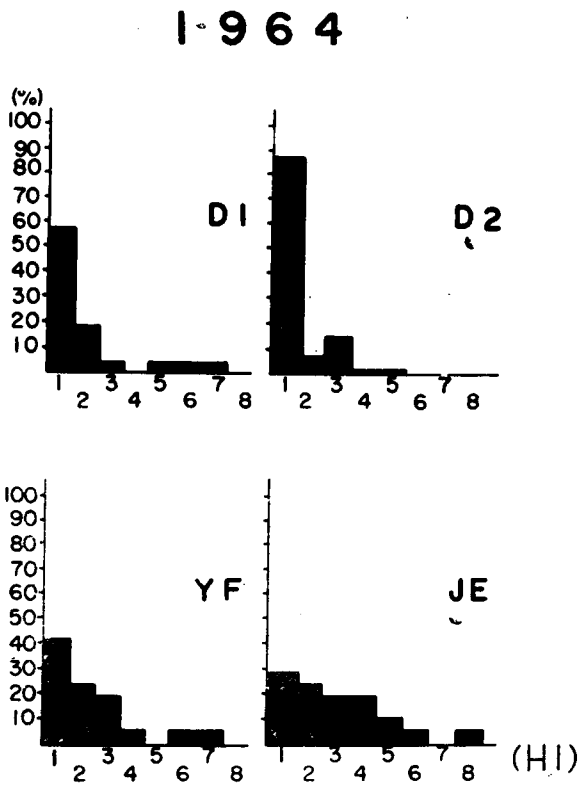

Fig. 2. HI antibodies among residents of Lendang-Nangka, Lombok Island, in 1964.
Ordinate indicates percentage of cases to the total specimens tested.
Abscissa indicates HI titers:
1<20; 2=20; 3=40; 4=80; 5=160; 6=320; 7=640; 8=1280 or more. These legends will be applied also in Figs. 3 and 4.

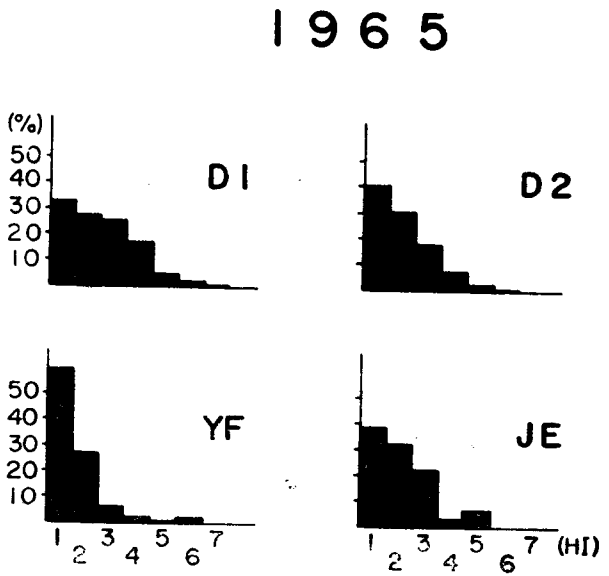

Fig. 3. HI antibodies among residents of Lampung State, Sumatera, in 1965.
Legends are the same as those in Fig. 2.

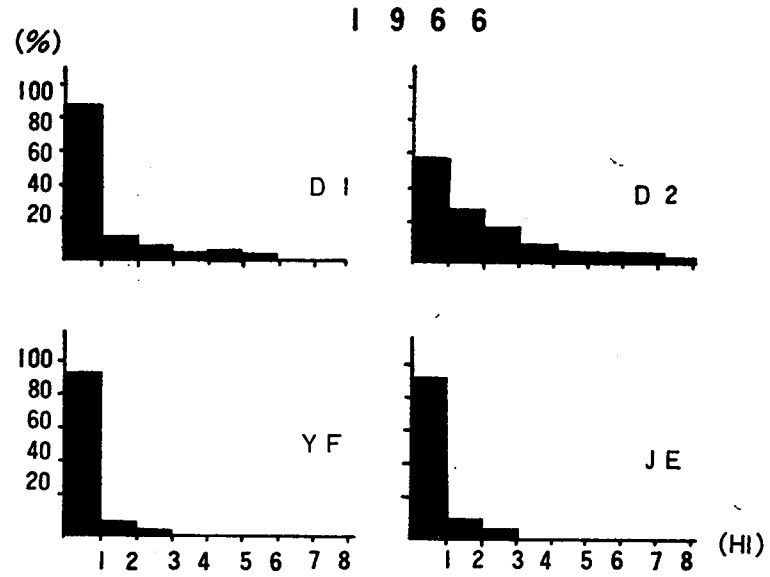

Fig. 4. HI antibodies among residents of Madjalengka State, Djawa, in 1966.
Legends are the same as those in Fig. 2.

Table 2. No. of specimens showing positive reaction to any (one or more) of the antigens used.

Year	Place	No. of specimen tested	Positive reaction	
			No.	%
1964	Lombok	129	50	38.8
1965	Sumatera	300	211	70.7
1966	Djawa	298	165	55.4

Table 3. Distribution of apparently positive or negative HI reaction among sera tested. (1964)

	Antigen					
Group	D1	D2	J E	Y F	No.	%
1	+	+	+	+	4	3.1
2	+	+	+	−	1	0.7
3	+	+	−	−	1	0.7
4	+	−	−	−	4	3.1
5	−	+	+	+	1	0.7
6	−	+	+	−	3	2.3
7	−	+	−	−	4	3.1
8	+	−	+	+	6	4.7
9	−	−	+	+	2	1.6
10	+	−	+	−	2	1.6
11	−	−	+	−	19	14.7
12	+	+	−	+	2	1.6
13	+	−	−	+	1	0.7
14	−	+	−	+	0	0.0
15	−	−	−	+	0	0.0
16	−	−	−	−	79	61.2
No. of positive	21	16	38	16	129	
%	16.3	12.4	29.3	12.4		

Table 4. Distribution of apparently positive or negative HI reaction among sera tested. (1965)

	Antigen					
Group	D1	D2	J E	Y F	No.	%
1	+	+	+	+	62	20.7
2	+	+	+	−	27	9.0
3	+	+	−	−	19	6.3
4	+	−	−	−	26	8.7
5	−	+	+	+	4	1.3
6	−	+	+	−	7	2.3
7	−	+	−	−	6	2.0
8	+	−	+	+	5	1.7
9	−	−	+	+	0	0.0
10	+	−	+	−	20	6.7
11	−	−	+	−	9	3.0
12	+	+	−	+	16	5.7
13	+	−	−	+	6	2.0
14	−	+	−	+	2	0.7
15	−	−	−	+	2	0.7
16	−	−	−	−	89	29.7
No. of positive	178	143	131	97	300	
%	59.3	47.7	43.7	32.3		

Table 5. Distribution of apparently positive or negative HI reaction among sera tested. (1966)

	Antigen					
Group	D1	D2	J E	Y F	No.	%
1	+	+	+	+	4	1.3
2	+	+	+	−	3	1.0
3	+	+	−	−	11	3.7
4	+	−	−	−	7	2.3
5	−	+	+	+	6	2.0
6	−	+	+	−	11	3.7
7	−	+	−	−	68	22.8
8	+	−	+	+	0	0.0
9	−	−	+	+	0	0.0
10	+	−	+	−	0	0.0
11	−	−	+	−	1	0.3
12	+	+	−	+	10	8.9
13	+	−	−	+	0	0.0
14	−	+	−	+	25	8.4
15	−	−	−	+	19	6.4
16	−	−	−	−	133	44.6
No. of positive	35	138	25	64	298	
%	11.8	46.3	8.4	21.5		

Specimens showing positive reaction to a single antigen are tabulated in Table 6.

Table 6. No. of specimens showing positive reaction to a single antigen (1964, 1965 and 1966).

Year	1964				1965				1966			
Antigen	D1	D2	J E	Y F	D1	D2	J E	Y F	D1	D2	J E	Y F
HI 20	3	4	8	0	16	5	5	2	2	38	0	16
40	1	0	6	0	8	1	4	0	0	19	1	3
80	0	0	4	0	2	0	0	0	0	9	0	0
160	0	0	1	0	0	0	0	0	0	1	0	0
320	0	0	0	0	0	0	0	0	1	1	0	0
640	0	0	0	0	0	0	0	0	0	0	0	0
No. of positive	4	4	19	0	26	6	9	2	3	68	1	19
%	3.1	3.1	14.7	0	8.3	2.0	3.0	0.7	1.0	22.8	0.3	6.4

Specimens showing titer to a particular antigen at least four times more than the others are summarized in Table 7.

Table 7. Specimens showing HI titers to a particular antigen at least four times more than the others.

Year	Antigen			
	D1	D2	J E	Y F
1964	2 (1.6)*	0 (0)	15 (11.6)	0 (0)
1965	18 (6.8)	0 (0)	6 (2.0)	0 (0)
1966	2 (0.7)	14 (4.7)	0 (0)	5 (1.7)

* Figure in parenthesis indicates percentage of cases to the total positive cases.

Tables 3 to 7 inclusive show that JE is predominant in the 1964 Lombok specimens, D1 in the 1965 Lumpung specimens, and D2 in the 1966 Madjalengka specimens, respectively.

Cases showing the highest or nearly highest HI titers to particular antigens are excerpted and are tabulated in Table 8.

Table 8. Cases showing the highest or nearly highest HI titers to particular antigens.

Year	Case	Antigen			
		D1	D2	J E	Y F
1964	No. 1	640	160	2560	640
	No. 2	1280	80	160	1280
1965	No. 3	2560	80	40	320
	No. 4	1280	160	80	80
	No. 5	320	40	1280	20
1966	No. 6	160	20	20	20
	No. 7	160	80	20	20
	No. 8	20	20	40	40
	No. 9	20	640	20	20

Probably, Nos. 1 and 5 are JE infection cases, Nos. 3, 4 and 6 are D1 infection cases, and No. 9 is a D2 infection case. At least within the scope of the present surveys, there was no case regarded as single YF infection.

DISCUSSION

Surveys for anti-arboviral antibodies in sera collected from various parts of Southeast Asia and Western Pacific regions have been carried out by a number of workers (Smithburn, 1954[20]; Hammon, *et al.*, 1958[6]; Smith, 1958[19]; Rosen, 1958[14]; Doherty and Carley, 1960[2]; Doherty, *et al.*, 1964[3]; Doherty, 1964[4]; Miles, *et al.*, 1964[11]; Wisseman, *et al.*, 1964[22]; Basaca-Scilla and Halstead, 1966[1]; etc. Also refer to review articles by Wisseman and Sweet, 1961[23], Wisseman, 1966[24]). These are useful to look into the distibution of viruses in the particular areas. However, investigations on Indonesia from the same purpose have so far been few, excepting reports by Lo *et al.* (1964[8], 1965[9]); they examined HI antibodies in sera collected in Bandung and its vicinities, Djawa Island, employing the method by Zhdanov (1961[25]), and stated that they found anti-D1 and D2 antibodies but not anti-JE antibodies.

The present paper is concerned with the surveys of anti-arboviral HI antibodies in Indonesian residents. A microtechnique developed by Takatsy (1955[21]) and by Sever (1962[16]) was used for the antibody titration. The applicability of this method has been reported by previous investigators (Rosenbaum, *et al*, 1963[15]), and it was confirmed in our study in which as much as possible antigens must be tested using limited amounts of sera.

The results indicate that group B arboviral infections are widely distributed among inhabitants of Indonesia. While this is not surprising, one point to be emphasized is that centain viruses are apparently predominant in each of the areas examined. For instance, it appears that (i) JE is more prevalent on Lombok Island; (ii) D1 is more widely distributed in the Lumpung district; and (iii) Madjalengka district has more cases of D2. The differences are probably attributed to environmental conditions of the respective areas, such as weather, altitude above sea level, flora and fauna, *etc*, which uniquely influence mosquito populations.

It is well known that no YF case has yet been noticed in Asian areas, in spite of the abundance of the specific vector mosquitoes. One possible explanation for this fact is the existence of immunologic overlapping between group B arboviruses. Our data are compatible with this concept. Further studies employing more specimens collected from different places are being planned to confirm the present hypothesis. In addition to this, other sorts of viral antigens should be tested, for instance, those of other types of dengue or chikungunya, which are intimately correlated with hemorrhagic fever now seriously prevalent in Southeast Asia. Such data will be useful in predicting the possible occurrence of these diseases and in giving a guiding formula for preventing the epidemics.

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