ON THE GEOGRAPHICAL DISTRIBUTION OF JAPANESE B ENCEPHALITIS IN THE FAR EAST ASIA

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(Received for Publication, June 30, 1950)

Japanese B encephalitis (JBE) seemed to be known in the another name of disease in Japan about one thousand and two hundreds years ago (Kakinuma¹⁾ and Mitamura²⁾), however, attention was paid first by the Japanese workers, who published their views on clinical and experimental findings of it, since Economo had described an encephalitis lethargica in Europe in 1917.

In 1933 the Third Sub-Committee of Encephalitis was organized in the Japanese Association for Promoting Science and Prof. Dr. R. Inada was appointed a chief of the Sub-Committee and the systematical study on a large scale has started. All the members of the Sub-Committee and the others who did not belong to the Sub-Committee have done their utmost to remove the misterious veil covering JBE. In the summer of 1933 an epidemic of JBE occurred in Japan. Hayashi,³⁾ Okayama Medical College, was successful experimentally to transfer the disease to monkey in the same year. On the occasion of the next epidemic which took place in 1935, many workers were able to isolate the JBE virus in Fukuoka, Okayama, Hyogo, Osaka, Kyoto, Niigata and Tokyo by using mice in the same manner of Webster in St. Louis encephalitis (SLE) in 1933. Kawamura⁴⁾, Mitamura⁵⁾ and Takagi⁶⁾ have accordingly made clear the fact that the disease was immunologically different from the other known encephalitiden. Mitamura²⁾ pointed out that those 30 newly isolated virus strains were almost the same though some minor variant might be found in their antigenic structure among them, and that there was one kind of JBE virus in Japan.

The studies on the intensity and the frequency of distribution of the virus in various districts in the Far East are, not only of scientific significance but also of importance for the preventive medicine. The indicators as to presume the geographical distribution of the disease are, first by the isolation of the causative agent from the host in areas, second by the positivity rate of neutralizing antibody against the virus in human and animal sera collected in such areas where the disease is reported or not and third by the report of the cases in such areas of which the clinical findings are similar to those of JBE.

The positive isolation of the virus indicates an actual existence of the disease, however, it is not always successful because of troubles in the technic and the material inspite of a case confirmed serologically while the appovement of the neutralizing antibody gives a reliable suggestion to presume the geographical distribution of the virus because the technique is simple and the reaction in general specific even if it has been known that there is some nonspecific virulicidal substance in some sera. The case report is

not always a liable indicator if it is confirmed by neither serological tests nor autopsy findings.

Takenouchi⁷⁾, Takagi⁹⁾ and Mitamura⁹⁾ have presumed, through the results observed in the neutralization test, that the disease is spread but very rarely, if not at all, in the district of Hokkaido till 1938. But a few cases of JBE virus was isolated by Hayashi, Mitamura and others¹⁰⁾ from the brain of a human case. As for other areas in the Far East Asia the virus was also isolated by Kobayashi¹¹⁾ in Formosa in 1938, by Smorodintseff¹²⁾ in the Maritime Province in 1938-39 where the Autumn encephalitis which is called by Russian workers and is known to be immunologically similar to Japanese B encephalitis occurred in the same years, by Yen¹³⁾ in North China in 1941, by Sabin¹⁴⁾ in Okinawa in 1945 and by the same author and his colleagues in Korea¹⁵⁾ in the following year, 1946.

As regards to isolation of the virus in Manchuria no report has been published, however, it is said that the virus was surely isolated by the Japanese workers in the years after the war according to the personal communication with Dr. Hiroki, ex-Manchuria Medical College, and Dr. Takemori, ex-Dairen Hygienic Institute. Both workers repatriated in 1948 and Takemori is now a assistant of the Kitaoka's laboratory. Hiroki failed to isolate the virus from the patient owing to the lack of mice while the disease was apparently prevailing among the South Manchurian natives during the years 1945, 46 and 47. Kanazawa, a friend of Takemori had succeeded to isolate a strain by using mice from a brain of a dead case among 13 Japanese who developed the typical encephalitis symptoms in Dairen in 1944. The newly isolated virus was identified by Kanazawa to the well known JBE virus "Kalinina" strain.

It was said that cases broke out also in 1945, and many cases were reported among the Soviet troops in the summer of 1946. Out of 15 autopsy materials supplied by the Soviet sanitary authorities 8 virus strains were isolated in mice at Dairen Hygienic Institute and were identified to JBE virus. It was also said that an epidemic has broken out in this district during the year of 1947.

All such informations realize that the disease has, as Mitamura pointed out repeatedly before from his results of the neutralization test with sera collected from habitants in various areas, been widely spread, not only within Japan but also in all the surrounding areas.

There are many clinical reports on JBE in various parts of Japan though not all of them had been confirmed serologically or through the isolation of virus, and also many reports on outbreaks of the disease have been published in various districts in the Far East, namely, in Okinawa, in Korea reported by Kin¹⁶⁾ in 1939 and by Hironaka¹⁷⁾ in 1940, and in the South Manchuria by Mochizuki¹⁸⁾ in 1939, by Fushiki¹⁹⁾ in 1940, by Nagazumi²⁰⁾ in 1940 and by Fujiki²¹⁾ in 1941 where are considered as an endemic area by the virus isolation later respectively. Ishii²²⁾ reported the JBE cases in Fukien Province in South China in 1930 and Kitamura²³⁾ has announced of an outbreak of 37 cases in Saghali during five years 1926-30 and 5 cases in 1939.

The geographical distribution of JBE represented was computed by Takenouchi²⁴⁾

from the results of the neutralizing antibody against JBE virus in sera collected from the natives in Formosa, Manila, Korea and Manchuria. They were positive in 33/50 or 66%, 0/40 or 0%, 5/38 or 13% and 13/59 or 22% respectively. These positivity rates were a bit lower than these in the original report because they were recalculated by us according to the American method, and the other positivity rates also were recalculated by us except the case specially noted. Anyway the geographical distribution of JBE virus in the Far East was computed from the results obtained in the neutralization test. Takagi⁸⁾ proved it in South Manchuria (38 samples) and in Formosa (25 samples) to be 33% and 80% respectively (original figures) and Mitamura²⁵⁾ found 20–80% positivity in swine (20/38), goats (5/10), buffalos (*Bubalus babalis*, Lin.) (8/10) and the yellow cattle (*Bos chinensis*, Lin.) (1/5) in Formosa.

METHOD

The neutralization test was done and the result was calculated according to the American method. The results which had been already published by Kii²⁸⁾ and us were summarized together with ours. The positivity rate in our previous reports was judged a bit higher than that calculated after the American method. For example the former signs $\equiv$, $\equiv$, $+$, $+$, $\pm$ and $-$ in the neutralization test indicate the differences 10^1 , 10^3 , 10^3 , $10^{1.5}$ and 0 respectively between the neutralizing power of the test serum and that of the negative control one. Now the neutralizing power of serum is indicated with LD_{50} calculated after the Reed-Muench's method and the signs $\equiv$, $+$, $+$, $\pm$ and $-$ are correspondent to the difference 10^3 , 10^3 , $10^{1.7}$, 10 and less than 10 LD_{50} between LD_{50} of the test serum and that of the negative control serum. The JBE virus was always employed for the neutralization test and the other encephalitis viruses, for example, St. Louis encephalitis (SLE), louping-ill, eastern equine encephalomyelitis (EEE) and western equine encephalomyelitis (WEE) viruses were employed on one hand to confirm the specificity of the neutralizing antibody in the test serum and on the other hand to presume whether or not such viruses were spread in the Far East.

RESULTS

Positivity rates of the neutralizing antibody in horse and bullock sera collected in various places in the Far East against JBE, SLE, EEE and WEE viruses are shown in Table 1. These original works observed by Kii, Kitaoka and others²⁸⁾ are summarized as the reference together with ours (Table 2 and 3) of which the abstracts have been previously reported by us (2, 29, 30) on the geographical distribution of JBE in Java, French Indo-China, Hainan Island, Central China, Manchuria and Saghalien.

Findings in Saghalien: Sera collected from 19 nurses through the kindness of the Director of the Toyohara Hospital, Saghalien, in May 1937, were tested for the neutralizing antibody against JBE and SLE viruses, however, all the results were negative as shown in Table 1.

Findings in Manchuria: Sera from 13 white Russian in Harbin, were supplied during the period of April to May, 1942 through the kindness of Dr. Chiba, the Central

Table 1. Positivity rate of neutralizing antibody against JBE, SLE, EEE and WEE viruses in sera from horses and cattle in various area in the Far East Asia.

		*1		JBE					SLE				EEE	WEE	Date of bleeding		
				##	+	+	+	-	positive rate	+	+	+	-	posit.rate		posit.rate	posit.rate
Horse	Japan (Tokyo)		101	61	35	4	1	$\frac{99}{101}=98\%$								1942.6**	
	South Korea		15	8	5	2		$\frac{15}{15}=100\%$	7	1	7	$\frac{7}{15}=47\%$	$\frac{0}{15}=0\%$	$\frac{0}{10}=0\%$		1941.2	
	For- mosa		32	3	8	7	8	$\frac{18}{32}=56\%$	1	3	25	$\frac{1}{29}=3\%$	$\frac{0}{32}=0$	$\frac{0}{32}=0$		1943. 5~ 6	
	South China	7~16	35		2	7	15	$\frac{9}{35}=26\%$	18	17		$\frac{0}{35}=0$	$\frac{0}{35}=0$	$\frac{0}{35}=0$		1942.11~12	
	Burma	4~15	25	2	5	6	6	$\frac{13}{25}=52\%$	1	2	22	$\frac{1}{25}=4\%$	$\frac{0}{25}=0$	$\frac{0}{25}=0$		1943. 5~ 7	
	Philipi- pine		44	3	7	15	12	$\frac{25}{44}=57\%$	2	2	40	$\frac{2}{44}=5\%$	$\frac{0}{44}=0$	$\frac{0}{44}=0$		1943. 5~ 7	
	Malay	6~ 9	41	3	8	19	5	$\frac{30}{41}=73\%$	4	17	20	$\frac{4}{41}=10\%$	$\frac{0}{41}=0$	$\frac{0}{41}=0$		1942.11~12	
	Suma- tra	6~12	25		1	7	7	$\frac{8}{25}=32\%$		4	21	$\frac{0}{25}=0$	$\frac{0}{25}=0$	$\frac{0}{25}=0$		1943. 5~ 6	
	Java	4~14	167		5	26	74	$\frac{31}{167}=19\%$	11	27	125	$\frac{11}{163}=7\%$	$\frac{0}{166}=0$	$\frac{0}{163}=0$		1942.11~12 1943. 5~ 6	
Cattle	For- mosa		44	1	5	11	13	$\frac{17}{44}=39\%$	2	11	31	$\frac{2}{44}=5\%$	$\frac{0}{44}=0$	$\frac{0}{44}=0$		1942.11~12	
	Burma	1~ 5	7			1	2	$\frac{1}{7}=14\%$			7	$\frac{0}{7}=0$	$\frac{0}{7}=0$	$\frac{0}{7}=0$		1943. 5~ 7	
	Philipi- pine		38			4	6	$\frac{4}{38}=11\%$		3	35	$\frac{0}{38}=0$	$\frac{0}{38}=0$	$\frac{0}{38}=0$		1943. 5~ 7	
	Thailand & Malay	3~12	26		1	3	9	$\frac{4}{26}=15\%$	3	3	23	$\frac{3}{29}=10\%$	$\frac{0}{26}=0$	$\frac{0}{26}=0$		1943. 5~ 7 1942.11~12	
	Java	2~ 6	48			2	4	$\frac{2}{48}=4\%$		3	45	$\frac{0}{48}=0$	$\frac{0}{48}=0$	$\frac{0}{48}=0$		1942.11~12 1943. 5~ 6	

*1=virus 2=grade of neutralization 3=No of test sample 4=age 5=area 6=animal **6=June

Hospital, Harbin and have undergone the neutralization test against JBE virus and the results were all in negative.

Out of another bunch of 20 sera collected from Chang Chun and Kirin natives through the kindness of Dr. Abe during the month of January, 1936, six (or 30%) were found positive against JBE virus in the neutralization test, and none against SLE virus.

Finding in Central China: One hundred four sera in total collected from natives in Central China, namely, 26 in Hangkow, 26 in Kiewkiang and 52 in Nankin in August, 1941, have proved positive in the neutralization test against JBE virus in 17 sera (65%), 17 sera (65%) and 30 sera (58%) respectively while all negative against SLE virus.

Findings in Hainan Island: Sera collected from 8 native adults in San-Ah township.

Table 2.
Positivity rate of neutralizing antibody against JBE and SLE viruses
in sera from human being in the Far East Asia.

4)	3)	JBE				SEL				Date of bleeding	Remarks
		++	+	±	—	posit. rate	++	±	—	posit. rate	
Saghalien	19			1	18	$\frac{0}{19} = 0\%$			19	$\frac{0}{19} = 0\%$	nurses of the Toyohara Hospital
Harbin	13			1	12	$\frac{0}{13} = 0$				1942, 4, 5	including 9 white Russians
Kirin	20	3	3	4	10	$\frac{6}{20} = 30$	1	19		$\frac{0}{20} = 0$	1936, 1 including 9 natives
Hankow	26	3	14	5	4	$\frac{17}{26} = 65$	1	25		$\frac{0}{26} = 0$	1941, 8 chineses
Kiewkiang	26	2	8	7	5	$\frac{17}{26} = 65$	6	20		$\frac{0}{26} = 0$	" , " ditto
Nankin	52	1	13	16	12	$\frac{30}{52} = 58$	2	50		$\frac{0}{52} = 0$	" , " ditto
Hainan	8			4	4	$\frac{0}{8} = 0$		8		$\frac{0}{8} = 0$	" , 2 natives
Fsench Indo-China	69	2	8	20	25	$\frac{30}{69} = 44$	2	13	50	$\frac{2}{65} = 3$	, 1 natives near Hanoi
Java	91	1		1	6	$\frac{2}{91} = 2$		91		$\frac{0}{91} = 0$	1939, 4 published
Manila	96			1	8	$\frac{1}{96} = 1$	1	17	77	$\frac{1}{95} = 1$	1938, 5 ditto

*1) = virus 2) = grade of neutralization 3) = No of test sample 4) = area **5 = May

Table 3.
Positivity rate of neutralizing antibody against JBE, SLE, and louping-ill
viruses in sera from animals in Hokkaido and Saghalien.

5)	3)	4)	JBE				SLE				Louping-ill				Date of bleeding	Remarks
			++	±	—	posit rate	++	±	—	posit rate	++	±	—	posit rate		
Hokkaido pig		60			60	$\frac{0}{60} = 0\%$			60	$\frac{0}{60} = 0$			60	$\frac{0}{60} = 0$	1937, 3	
ditto cow	1~3	17	1	16		$\frac{1}{17} = 6$			17	$\frac{0}{17} = 0$			17	$\frac{0}{17} = 0$	"	Blood drawn in Tokyo
Saghalien Sheep	1~12	25			25	$\frac{0}{25} = 0$			25	$\frac{0}{25} = 0$			25	$\frac{0}{25} = 0$	"	
ditto fox		19			19	$\frac{0}{19} = 0$			19	$\frac{0}{19} = 0$			19	$\frac{0}{19} = 0$	"	
ditto fox		31			2	$\frac{1}{3} = 33$										over-summering in Tokyo

*1) = virus 2) = grade of neutralization 3) = No of test animals 4) = age
5) = area and name of animals

in Hainan, through the kindness of Dr. Miyao in January, 1941 were found all resulting in negative in the neutralization test against both JBE and SLE viruses.

Findings in French Indo-China: Sera collected from 69 natives in Hanoi in January 1941 have proved positive in the neutralization test in 30 (44%) against JBE virus and in 2 (3%) against SLE virus.

DISCUSSION

If any conclusion is desirous from the results of neutralization test it should be kept in mind that the serum to be tested is to be drawn from the natives born, grown and lived in their home land limitedly and that they have never travelled out of the native land to avoid the infection in the other place. Collection of test serum should essentially be made not during the summer season when the disease usually occurs as Mitamura, Kii and others already pointed out, because the development of the neutralization antibody in serum following the invasion of virus into body which mainly takes place in the summer season depending on the intensity of epidemic.

We do not say that all the neutralizing substances in sera are specific, however, specificity of the neutralizing antibody in serum against JBE virus should be always assured by observing on the neutralization test with the same serum against SLE, louping-ill, EEE and WEE viruses.

There have been no report of the isolation of JBE virus among Javanese and Philippine natives. And the positivity rates of the neutralizing antibody against JBE virus are low (1-2%) among them while horses and bullocks in the same areas show 19-57% and 4-15% positivity rates respectively. Then it is presumable that the virus may be spread in these areas and this opinion is ascertained by post mortem findings³¹⁾ as well. French Indo-China and Central-China are undoubtedly presumed to be infected because they have 44% and about 60% positivity rates respectively.

As for the positive results seen against SLE virus among natives in Philippines and Java, it is pointed out that there have been one or two such findings among Philippines and Annamites, as well as among horses in the southern lands as shown in Table 1 including 7 positive out of 15 (or 47%) horses in Quelpart Island in Southern Korea. But they were low and more likely due to unspecific cross-reaction, excepting some of those cases proving decisively negative against JBE virus but slightly positive against SLE virus. Anyway it may not be concluded that SLE virus is spread in some are as in the Far East. They were all negative against the louping-ill and EEE and WEE

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viruses. It is strongly suggested that the Far East Asia is entirely free from the contamination with those three encephalitis viruses so far.

SUMMARY

The neutralization test against JBE virus was carried out with sera collected from the native adults who had never travelled out of their lands in Saghalien, Manchuria, Central China, Hainan Island and French Indo-China. Positivity rate was found in none (0%) in North Manchurian and Saghalien natives, 30% among South Manchurians, 60% among the natives in Central China, non (0%) in Hainan Island and 44% in French Indo-China. Compared with findings by Mitamura and Kii of positivity rates in human and animal sera collected in Malay, Sumatra, Java, southern Burma and Philippine, our results thus obtained reveal serologically that JBE is a disease wide spread over the area from South Manchuria down to Java and Burma as shown on the map of the Far East Asia. And also the personal communication of the repatriated workers were described that there were epidemics of JBE in South Manchuria between 1945-48).

There have been some sera proving negative against JBE virus while though faintly positive against SLE virus. It might be presumed to have been a sort of cross reaction. Furthermore it was made clear that the Far East is still free from the Iouping-ill and EEE and WEE viruses.

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