A 日本脳炎患者発生及び死亡者数

資料7

STATEMENT SHOWING THE YEARWISE CASES & DEATHS DUE TO JAPANESE ENCEPHALITIS FROM 1978 TO 1985

Name of the States/UTs	1978		1979		1980		1981		1982		1983		1984		1985	
	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
1. Assam	422	213	-	-	360	194	87	46	145*	59	29	5	30	15 Aug	-	-
2. Arunachal Pra.	22	5	-	-	-	-	-	-	-	-	-	-	-	- Aug		
3. Andhra Pradesh	-	-	254	54	289	121	1273	439	419	141	325	163	150	97 Oct.	112	30
4. A&N Islands	NA	NA	NA	NA	9	4	-	-	-	-	-	-	-	-	-	-
5. Bihar	1252	452	109	57	737	336	157	60	229	68	116	57	104	54	269	89 Sep
6. Chandigarh	NA	NA	42	19	-	-	-	-	-	-	-	-	-	- Aug		
7. Delhi	NA	NA	NA	NA	-	-	-	-	1	1	-	-	-	-		
8. Dadar Na. Haveli	NA	NA	NA	NA	-	-	-	-	-	-	-	-	-	- Oct.		
9. Gujarat	NA	NA	NA	NA	-	-	-	-	-	-	-	-	NA	NA		
10. Goa, Daman & Diu	NA	NA	NA	NA	-	-	-	-	35	13	15	1	-	- Nov.		
11. Haryana	5	5	-	-	-	-	-	-	-	-	-	-	-	- Sept		
12. Himachal Pr.	-	-	-	-	-	-	-	-	-	-	-	-	-	- Aug		
13. J&K	NA	NA	NA	NA	-	-	-	-	-	-	-	-	-	- Aug		
14. Karnataka	72	18	920	223	9	5	837	236	150	52	410	112	37	14	55	18
15. Kerala	5	1	-	-	-	-	2	2	NA	NA	-	-	NA	NA		
16. Lakshdweep	NA	NA	NA	NA	-	-	-	-	-	-	-	-	-	May		
17. Madhya Pd.	34	15	-	-	108	72	NA	NA	-	-	NA	NA	NA	NA		
18. Meghalaya	12	12	-	-	-	-	-	-	-	-	-	-	-	-		
19. Mizoram	2	-	-	-	-	-	-	-	-	-	-	-	-	-		
20. Manipur	27	4	-	-	NA	NA	-	-	100	53	35	16	13	20 Oct		
21. Maharashtra	117	34	-	-	21	5	-	-	NA	NA	-	-	NA	NA		
22. Nagaland	12	10	-	-	-	-	-	-	-	-	-	-	NA	NA	26	16
23. Orissa	NA	NA	NA	NA	NA	NA	-	-	56	12	-	-	-	- July		
24. Pondicherry	163	114	65	32	-	-	49	17	-	-	-	-	-	- May		
25. Punjab	NA	NA	NA	NA	-	-	-	-	-	-	-	-	-	- Aug		
26. Rajasthan	20	11	-	-	-	-	-	-	-	-	-	-	-	- Feb.		
27. Sikkim	NA	NA	NA	NA	-	-	-	-	-	-	-	-	-	- Aug		
28. Tripura	33	30	-	-	69	62	19	16	33	25	14	14	NA	NA		
29. Tamil Nadu	412	422	83	4	188	67	1324	290	242	83	623	155	454	138	41	16 Mar
30. U.P.	3550	1117	150	72	1604	530	78	26	637	199	149	58	-	-		
31. West Bengal	1300	592	1222	465	84	40	71	85	1469	555	-	-	1821	815 Dec		
Total:-	7463	2755	2845	926	3478	1436	3894	1167	3516	1251	1716	581	3609	1135	503	169

Note:-NA=Note available =Nil *J E Diagnosis not confirmed, reported as viral encephalitis

B.

Proceedings of the
National Conference on Japanese Encephalitis, 1984, pp 1-9

Epidemiology of Japanese encephalitis in India: A brief overview

F.M. Rodrigues

National Institute of Virology, Pune 411001

Encephalitis due to Japanese encephalitis (JE) virus occurs in most countries of East and South East Asia.

In India, the earliest evidence of JE virus activity, as determined by the detection of specific neutralizing antibodies to JE virus, was obtained through the early serological survey carried out in 1952[1]. The disease was first recognized in 1955, when cases of encephalitis from North Arcot district and neighbouring districts of Tamil Nadu and Andhra Pradesh, admitted to the Christian Medical College Hospital (CMCH), Vellore, were serologically diagnosed as having been caused by JE virus[2,3]. In the same year, JE virus was isolated for the first time in India. The isolation came from wild-caught mosquitoes of this area[4]. The virus was, however, not recovered from man until 1958, when three isolations were made from brain tissue of cases of encephalitis admitted to the CMCH, Vellore[5].

Prior to 1970, cases of JE were recorded only from South India[6]. In 1973, a large outbreak occurred in West Bengal mainly in Burdwan and Bankura districts[7] (Fig. 1 and Table I). In 1976, a second epidemic of JE occurred, which was restricted to Burdwan district only[8].

The end of 1977 and the year 1978 were characterized by the occurrence of extensive outbreaks in new areas in India. In south India, there was a large outbreak in Tamil Nadu, mainly in Tirunelveli district[9]. An outbreak also occurred in Kolar district of Karnataka[10].

In north-eastern India, there were extensive epidemics in Burdwan, Bankura, Birbhum and some additional districts of West Bengal[8]; in the neighbouring Dhanbad district of Bihar and several other districts[11]; and in Dibrugarh district of Assam (NIV, unpublished data).

In north India, Uttar Pradesh experienced its first epidemic of JE. Several districts were affected, chiefly Gorakhpur and Deoria[12].

In 1979, JE epidemics occurred in south India in Kolar and adjoining

This article was presented at the National Conference on Japanese Encephalitis, held at the Indian Council of Medical Research, New Delhi, during November 3-4, 1982.

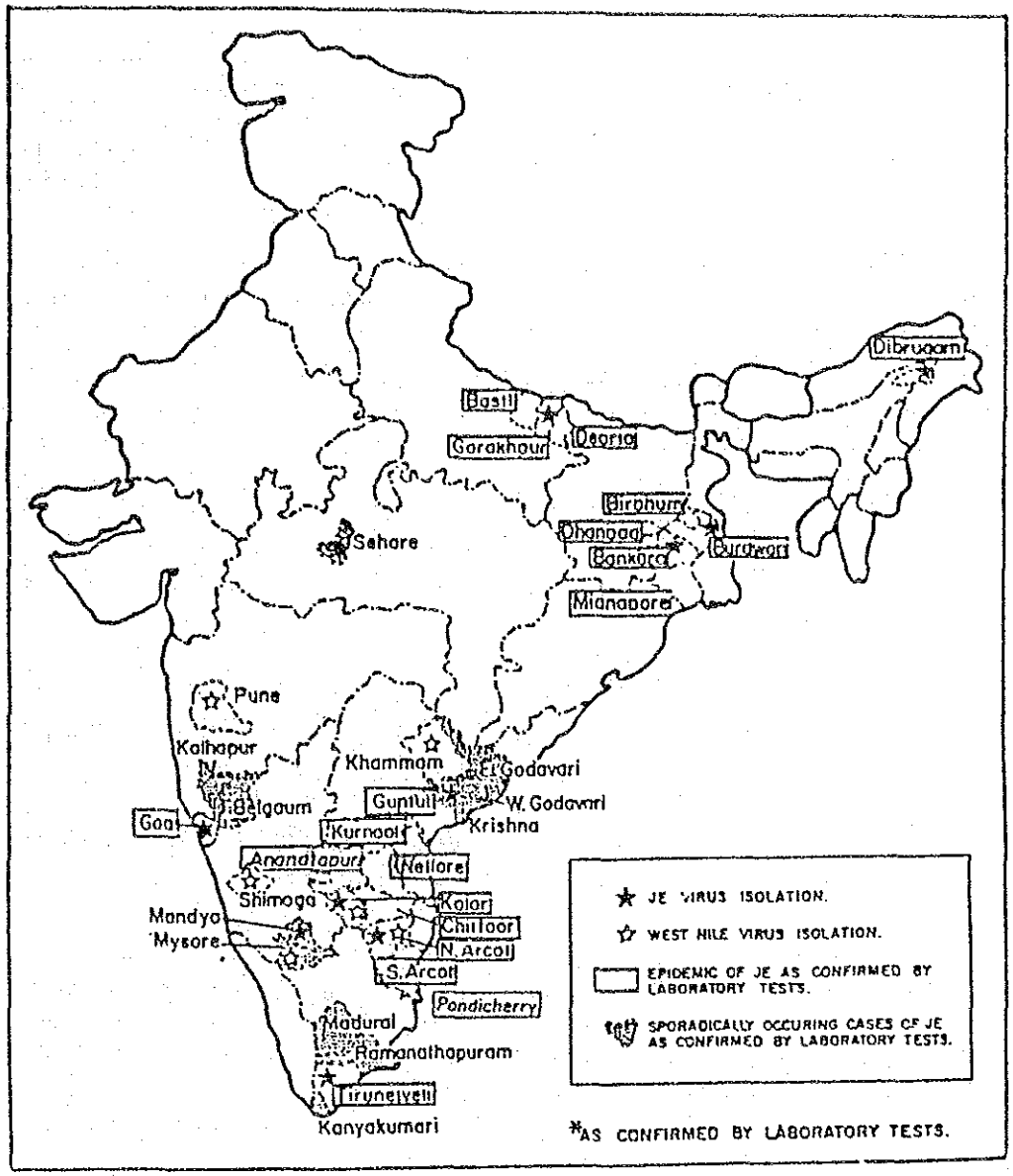


Fig. 1. District-wise occurrence of epidemics of Japanese encephalitis* and sporadically occurring cases of JE in India.

districts of Karnataka and in Andhra Pradesh mainly in Chittoor, Anantapur, Cuddapah and Nellore districts. In West Bengal an extensive outbreak occurred again mainly in Burdwan district.

In November and December, 1979, a serological diagnosis of JE was made in three of eight sporadically occurring cases of encephalitis in Kolhapur district, Maharashtra[13]. This was the first time that laboratory-proven cases had been detected in Maharashtra.

In 1980, although some outbreaks did occur, the incidence of JE in south India was relatively low. In north India, another large outbreak occurred in Uttar Pradesh, again mainly in Gorakhpur and Deoria districts. In north-east India, there was an

Table I. Epidemics of encephalitis in different parts of India—1973-1981

Area and year	Cases	Deaths	Case-fatality rate (%)
Burdwan and Bankura districts, West Bengal, 1973	763	325	42.6
Burdwan district, West Bengal, 1976	307	126	41.0
Tirunelveli district, Tamil Nadu, 1977-78	298	99	33.2
Kolar district, Karnataka, 1977-78	71	18	25.4
Burdwan, Bankura, Birbhum and other districts, West Bengal, 1978	1256	544	43.3
Gorakhpur and Deoria districts, Uttar Pradesh, 1978	1734	515	29.7
Kolar district, Karnataka, 1979	670	158	23.6
Anantapur and Chittoor districts, Andhra, 1979	340	71	20.9
Gorakhpur and Deoria districts, Uttar Pradesh, 1980	1386	455	32.8
Dibrugarh district, Assam, 1980	100	44	44.0
South Arcot district, Tamil Nadu and Union Territory of Pondicherry, 1981	633	151	23.85
Anantapur, Chittoor, Kurnool, Guntur and Prakasam districts, Andhra Pradesh, 1981	789	274	34.7
Kolar district, Karnataka, 1981	487	116	23.8

outbreak in Assam, mainly in Dibrugarh district.

Extensive outbreaks of JE occurred again in south India in 1981. South Arcot district of Tamil Nadu and the Union Territory of Pondicherry were mainly affected. In Andhra Pradesh, the districts mainly affected were Anantapur, Chittoor, Kurnool and Guntur. In Karnataka, Kolar district was again the area mainly affected.

In 1982, laboratory confirmed cases of JE were detected for the first time in the western coastal region of India, *i.e.*, in the Union Territory of Goa[14]. Epidemics of JE have occurred in West Bengal, Uttar Pradesh and Assam.

In Table I are presented relevant data pertaining to the above-mentioned epidemics. The incidence has varied from 71 cases in Kolar district, Karnataka, in 1977-78 to as many as 1734 cases in Gorakhpur and Deoria districts, Uttar Pradesh, in 1978. Case-fatality rates have ranged between 21 per cent in Anantapur and Chittoor districts, Andhra Pradesh in 1979 to 44 per cent in Dibrugarh district, Assam, in 1980.

Distribution patterns

The disease is predominantly rural and mainly affects the lower socio-economic groups. It has a scattered pattern of incidence. On an average, only 1 to 1·5 cases have occurred per village. The ratio of clinically apparent disease to inapparent, *i.e.* subclinical human infections has been estimated in different countries to be between 1:300 to 1:1000. However, limited studies carried out in certain parts of India

indicate that it is much lower in India, *i.e.*, between 1:20 and 1:30 (NIV, unpublished data).

The disease in southern India has predominantly affected children below 15 yr. In West Bengal, Bihar, Assam, Uttar Pradesh and Goa, all age groups were affected. This would appear to indicate that the virus in these areas has been newly introduced into a relatively non-immune population.

In most of the epidemics in India, the incidence has been higher among males.

Seasonal patterns

In southern India, the illness has occurred mainly during the latter half of the year, coinciding with the rainy season and period of high mosquito prevalence. Tamil Nadu receives, in addition to the south-west monsoon, rainfall due to the north-east winter monsoon. The exception was the outbreak in Tirunelveli and other districts of Tamil Nadu in 1977-78, which commenced in November and lasted till April with the peak in February and March.

In West Bengal, the disease has occurred between May and October. This has been shown to be related to the summer monsoon. In Assam, Bihar and Uttar Pradesh, outbreaks have occurred between September and December.

Vectors

Of about 350 species of mosquitoes known to be present in India, JE virus has been isolated from 11 species[15]. A total of 38 strains of the virus have been isolated from different parts of the country (Table II). Of these, 30 strains are from Peninsular India, 7 from West Bengal and one from Assam.

Table II. Japanese encephalities virus isolates from mosquitoes in India

Sl. no.	Mosquito species	No. of isolates	Locality and year
1.	*Anopheles barbirostris*	1	Asansol, 1973
2.	*A. 'hyrcanus'* group	1	Asansol, 1973
		2	Bankura, 1975
3.	*A. subpictus*	3	Kolar, 1979-81
4.	*Mansonia annulifera*	1	Dibrugarh, 1980
5.	*Culex 'bitaeniorhynchus'* group	1	Bankura, 1975
6.	*C. epidesmus*	1	Bankura, 1975
7.	*C. tritaeniorhynchus*	9	N. Arcot, 1962-68
8.	*C. vishnui*	1	Asansol, 1973
		1	N. Arcot, 1963
		3	Kolar, 1978-81
9.	*C. 'vishnui'* group	7	N. Arcot, 1955-56
10.	*C. whitmorei*	1	N. Arcot, 1958
		1	Krishna, 1972
11.	*C. pseudovishnui*	5	Kolar, 1979-81.

JE virus has been isolated several times from *Culex* mosquitoes (Table II). All but 4 of these isolates have been from mosquitoes belonging to the *Culex vishnui* complex, which breeds extensively in rice fields. The *C. vishnui* complex comprises 3 species, *viz.*, *C. tritaeniorhynchus*, *C. psuedovishnui* and *C. vishnui*.

Within the *C. vishnui* complex, most of the isolations have come from *C. tritaeniorhynchus*. All the isolations of JE virus from *C. tritaeniorhynchus* have come from South India. Ecological studies, particularly on habitats, the relative population density, biting habits, host predilection and on the vector potentials have also incriminated *C. tritaeniorhynchus* as a major vector in Peninsular India. Experimental transmission of JE virus has been achieved several times with *C. tritaeniorhynchus* (Table III). Other probable vectors in south India are *C. psuedovishnui* and *C. vishnui* and possibly *C. whitmorei*.

The mosquito vectors in eastern and north-eastern India appear to be different. In West Bengal where extensive studies have been carried out, 3 isolates of JE virus from mosquitoes were obtained during the 1973 outbreak—one each from the *Anopheles 'hyrcanus'* group, *C. vishnui* and *Anopheles barbirostris*[7]. Subsequently, during a follow up study, conducted in 1974-75 in Bankura district, West Bengal, four more isolates of JE virus were obtained from mosquitoes. Two of these came from the *A. 'hyrcanus'* group and one each from the *C. 'bitaeniorhynchus'* group and *C. epidesmus*[16]. Although numerous mosquitoes belonging to the *C. vishnui* complex

Table III. Experimental transmission of JE virus with mosquitoes in India

Species	Survival of virus (days)	Transmission by bite*	Recipient host used
Anopheles 'hyrcanus' group	11	No transmission	Chicks
A. tessellatus	11	4/13	Chicks
Culex fatigans	15	12/19	Chicks
C. tritaeniorhynchus	14	2/3	Pigs
		2/14	Mice
		7/7	Ducks
		2/2	Pond heron
		25/39	Proboscides tested
C. 'bitaeniorhynchus' group	40	18/24	Chicks
		1/1	Ducks

*Number positive/number of transmission attempts

were processed during the 1973 outbreak, no isolation of JE virus was obtained from these mosquitoes with the exception of the one isolation already mentioned. From the studies carried out so far, it is not clear which is the mosquito vector in West Bengal or in any of the other areas of JE virus activity in eastern and north-eastern India. In experimental studies with JE virus and mosquitoes of the *A. 'hyrcanus'* group collected around Pune, these mosquitoes failed to transmit the virus[15].

Experimental transmission of JE virus has been attempted with five species of mosquitoes and has been successful with four of the species (Table III) *viz., C. tritaeniorhynchus,* the *C. 'bitaeniorhynchus'* group, *C. fatigans,* and *A. tessellatus*[15]. The single experiment with the *A. 'hyrcanus'* group, however, needs to be repeated.

The natural cycle of JE virus in India

In India, as in other parts of east and south-east Asia, pigs appear to be involved in the maintenance and spread of JE virus through a pig-mosquito-pig cycle. A significant proportion of pigs in areas of JE virus activity—ranging from 33 per cent in North Arcot district, Tamil Nadu, to 83 per cent in Dibrugarh district, Assam (Table IV)—were found to possess specific neutralizing antibodies to JE virus[17,18] (NIV, unpublished data). The pig sera in Bankura district, West Bengal, Tirunelveli district, Tamil Nadu, and Kolar district, Karnataka, were obtained during or immediately after an epidemic and the high proportion of pigs possessing antibodies to JE virus is of significance. Experimental pig-*C. tritaeniorhynchus*—pig transmission in the laboratory has been successfully carried out[19]. The evidence thus indicates that pigs act as efficient "amplifiers" of the virus in the natural cycle of JE virus. Uninfected mosquitoes in nature become infected on feeding on pigs circulating JE virus in their blood and, after an extrinsic incubation period, transmit the virus to normal uninfected pigs. Thus the cycle keeps on repeating. The

Table IV. HI antibodies to flaviviruses and neutralizing antibodies to JE virus in sera obtained from pigs in areas of JE virus activity

Area and year	HI antibodies		N antibodies	
	No. positive / No. tested	Percentage positive	No. positive / No. tested	Percentage positive
North Arcot district, Tamil Nadu, 1962-1966	25/67	37.3	23*/70	32.9
Bankura district, West Bengal, 1973	102/181	56.3	93/97*	95.9
Tirunelveli district, Tamil Nadu, 1978	216/303	71.3	133/184	72.3
Kolar district, Karnataka, 1978	39/114	34.2	77/146	53.0
Dibrugarh district, Assam, 1979	Not	tested	73/88	82.95

*All these sera possessed HI antibodies to flaviviruses

pigs suffer from a clinically inapparent infection. Under conditions favourable to excessive mosquito breeding, the density of these mosquitoes increases, with consequent increase in the population of infected mosquitoes, which then results in a "spill-over" of the infection to man. Man is a "dead-end". Because of transient and low levels of circulating virus in his blood, mosquitoes do not become infected when feeding on man.

Another finding of interest is that 35 per cent of birds of the species cattle egret and pond heron, respectively, belonging to the family Ardeidae, collected in Andhra Pradesh, possessed neutralizing antibodies to JE virus[20] (Table V). These findings, together with successful experimental pond heron-*C. tritaeniorhynchus*—pond heron and cattle egret-*C. tritaeniorhynchus*-White leghorn chick transmission studies, suggest the possibility that, as in Japan, Ardeid birds may be additional hosts of JE virus[21].

Table V. Neutralizing antibodies to JE virus in Ardeid bird sera, Krishna and West Godavari districts, Andhra Pradesh, 1974 to 1976

Species	No. positive / No. tested	Percentage positive
Bubulcus ibis (Cattle egret)	80/229	34.9
Ardeola grayii (Pond heron)	100/285	35.1
Egretta garzetta (Little egret)	0/14	..
Total	180/528	34.1

The probably natural cycle of JE virus in India is depicted in Fig. 2. Although buffaloes and cattle have been commonly found to possess antibodies to JE virus, experimental transmission studies with buffaloes did not result in successful transmission in India. This indicates that bovines are probably not involved in the epidemiology of Japanese encephalitis.

On epidemiological grounds also, bovines are unlikely to play a role in the maintenance and spread of JE virus in India. India has an extremely large cattle

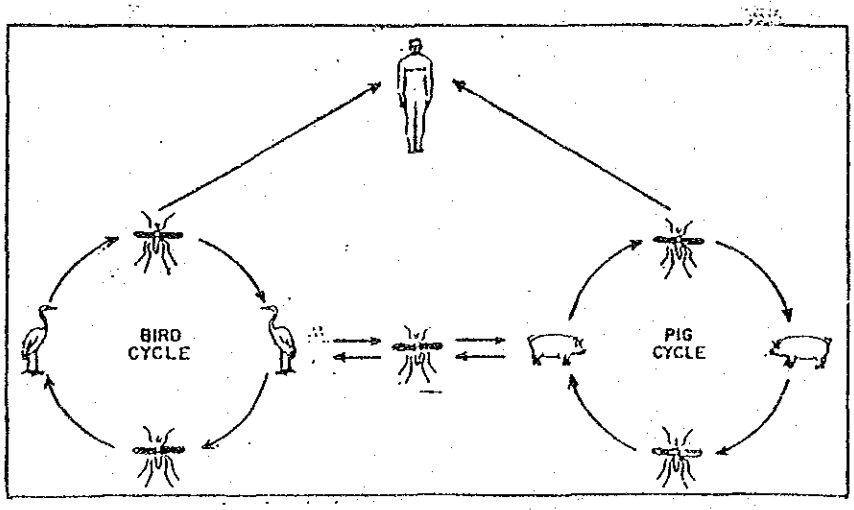


Fig. 2. Probable natural cycle of JE virus in India.

population. Studies in south India have shown that *C. tritaeniorhynchus* preferentially feeds on cattle. If cattle were involved as amplifying hosts of JE virus, there would have been far more extensive annual outbreaks of JE in India than have so far been experienced. In fact, the evidence so far available indicates that cattle may serve as "blocking agents" to JE virus. A large proportion of JE-infected mosquitoes feed on cattle, which serve as "dead-ends" for JE virus. Only a relatively small proportion of these JE-infected mosquitoes remain to feed on pigs and man. However, experimental transmission studies with species of cattle other than buffaloes need to be carried out to support this hypothesis.

Only a small proportion of sera from sheep and goats, obtained from different areas of JE virus activity, have possessed neutralizing antibodies to JE virus. An interesting finding in Tirunelveli district was the detection of neutralizing antibodies to JE virus in 85 per cent of donkey sera collected soon after the 1978 outbreak of JE.

Suggestions for future research

Although, in the past 27 years a great deal of information has been obtained on the epidemiology of JE in India, there still remain several lacunae in our knowledge towards which future research could be directed. Some of the more important aspects for such epidemiological research are :

1. What is the actual clinical to subclinical ratio for JE virus infection in India ?
2. To what extent does previous infection with the closely related West Nile virus affect the incidence and severity of disease due to JE virus infection?
3. What are the factors involved in the occurrence of extensive outbreaks in India only relatively recently, *i.e.*, since the 'seventies', 20 years after JE virus was first recognized as a cause of encephalitis in India?
4. What are the vector mosquitoes in eastern and north-eastern India?
5. What are the natural hosts of JE virus in different areas in India? Are there other hosts besides pigs and Ardeid birds?

References

1. Smithburn, K.C., Kerr, J.A. and Gatne, P.B. Neutralizing antibodies against certain viruses in the sera of residents of India. *J Immunol* 72 (1954) 248.
2. Webb, J.K.G. and Pereira, S.M. Clinical diagnosis of an arthropod-borne type of virus encephalitis in children in North Arcot district, Madras State, India. *Indian J Med Sci* 10 (1956) 572.
3. Work, T.H. and Shah, K.V. Serological diagnosis of Japanese B type of encephalitis in North Arcot district of Madras State, India, with epidemiological notes. *Indian J Med Sci* 10 (1956) 582.
4. Dandwate, C.N., Rajagopalan, P.K., Pavri, K.M. and Work, T.H. Virus isolations from mosquitoes collected in North Arcot district, Madras State, and Chittoor district, Andhra Pradesh, between November 1955 and October 1957. *Indian J Med Res* 57 (1969) 1420.
5. Webb, J.K.G., Pavri, K., George, S., Chandy, J and Jadhav, M. Japanese B encephalitis in South India: isolation of virus from human brain. In: *Asian Paediatrics—The Scientific Proceedings of the First Asian Congress of Paediatrics*, New Delhi, 1961, S.K. Bose, and A.K. Dey, Eds (Asia Publishing House, Bombay) 1964 p 192.
6. Carey, D.E., Myers, R.M., Reuben, R. and Webb, J.K.G. Japanese encephalitis in South India: A summary of recent knowledge. *J Indian Med Assoc* 52 (1969) 10.

7. Chakravarty, S.K., Sarkar, J.K., Chakravarty, M.S., Mukherjee, M.K., Das, B.C. and Hati, A.K. The first epidemic of Japanese encephalitis in India——virological studies. *Indian J Med Res* 63 (1975) 77.

8. Chakraborty, M.S., Chakraborty, S.K., Mukherjee, K.K., Mitra, A.C., Mitra, K.K., Gupta, B. and Sarkar, J.K. Recurrent Japanese encephalitis epidemics in West Bengal. *Indian J Med Res* 72 (1980) 1.

9. Prasada Rao, G.L.N., Rodrigues, F.M., Nambiapan, M., Nagarajan, M., Ghalsasi, G.R., Rodrigues, J.J., Pinto, B.D., Mohan Rao, C.V.R. and Gupta, N.P. Aetiology of the 1978 outbreak of encephalitis in Tirunelveli and other districts of Tamil Nadu. *Indian J Med Res* 76 (1982) 36.

10. Prasad, S.R., George, S. and Gupta, N.P. Studies on an outbreak of Japanese encephalitis in Kolar district, Karnataka State, in 1977-78. *Indian J Med Res* 75 (1982) 1.

11. Mohan Rao, C.V.R., Banerjee, K., Mandke, V.B., Dandawate, C.N., Ilkal, M.A., Anand, B.R., Biswas, P.N. and Ray, M. Investigations of the 1978 epidemic of encephalitis in Asansol, West Bengal and Dhanbad, Bihar. *J Assoc Physicians India* 28 (1980) 441.

12. Mathur, A., Chaturvedi, U.C., Tandon, H.O., Agarwal, A.K., Mathur, G.P., Nag, D., Prasad, A. and Mittal, V.P. Japanese encephalitis epidemic in Uttar Pradesh, India, during 1978. *Indian J Med Res* 75 (1982) 161.

13. Rodrigues, J.J., Shaikh, B.H., Shendarkar, S.P. and Ramdasi, S.G. Laboratory confirmed cases of Japanese encephalitis in Maharashtra State. *Indian J Med Res* 72 (1980) 471.

14. Mohan Rao, C.V.R., Prasad, S.R., Rodrigues, J.J., Sharma, N.G.K., Shaikh, B.H. and Pavri, K.M. The first laboratory proven outbreak of Japanese encephalitis in Goa. *Indian J Med Res* 78 (1983) 745.

15. Dhanda, V. and Kaul, H.N. Mosquito vectors of Japanese encephalitis virus and their bionomics in India. *Proc Indian Natl Sci Acad* B 46 (1980) 759.

16. Banerjee, K., Mahadev, P.V.M., Ilkal, M.A., Mishra, A.C., Dhanda, V., Modi, G.B., Geevarghese, G., Kaul, H.N., Shetty, P.S. and George, P.J. Isolation of Japanese encephalitis virus from mosquitoes collected in Bankura district (West Bengal) during October 1974 to December 1975. *Indian J Med Res* 69 (1979) 201.

17. Carey, D.E., Reuben, R., Myers, R.M. and George, S. Japanese encephalitis studies in Vellore, South India. Part IV. Search for virological and serological evidence of infection in animals other than man. *Indian J Med Res* 56 (1968) 1340.

18. Rodrigues, F.M., Vidyasagar, J., Singh P.B., Ghosh, S.N., Guttikar, S.N., Joshi, M.V. and Gupta, N.P. The 1973 epidemic of Japanese encephalitis in West Bengal: a serological survey of domestic animals. *Indian J Med Res* 64 (1976) 973.

19. Carey, D.E., Reuben, R. and Myers, R.M. Japanese encephalitis studies in Vellore, South India. Part V. Experimental infection and transmission. *Indian J Med Res* 57 (1969) 282.

20. Rodrigues, F.M., Guttikar, S.N. and Pinto, B.D. Prevalence of antibodies to Japanese encephalitis and West Nile viruses among wild birds in the Krishna-Godavari Delta, Andhra Pradesh, India. *Trans R Soc Trop Med Hyg* 75 (1981) 258.

21. Soman, R.S., Rodrigues, F.M., Guttikar, S.N. and Guru, P.Y. Experimental viraemia and transmission of Japanese encephalitis virus by mosquitoes in Ardeid birds. *Indian J Med Res* 66 (1977) 709.

Proceedings of the
National Conference on Japanese Encephalitis, 1984, pp 77-80

Japanese encephalitis virus vaccine manufacture in India: Perspectives

S.N. Saxena

Central Research Institute, Kasauli

A human is infected with Japanese encephalitis virus (JEV) by the bite of mosquitoes which carry the virus. Most of the infected persons show no symptoms but develop immunity. Only a small fraction of the infected individuals develop encephalitis. Information currently available shows that Japanese encephalitis virus activity is widespread in India and control measures are urgently required.

There are three possible ways to control this disease :—

1. Control of the insect vector, which is very difficult.

2. Protection by vaccination of the "amplifying host." This is not at present feasible in India due to lack of accurate information on "amplifying host(s)."

3. Immunization of man. This may be the only effective method of control of the disease at the present moment. An infected human being is not responsible for spread of the disease in the community and hence vaccination against the disease aims only at minimising the number of susceptible people in a given area.

JE vaccines

For immunization of humans, four different kinds of vaccines are known :—

(a) Inactivated chick embryo vaccine;
(b) Live attenuated tissue culture vaccine ;
(c) Inactivated cell culture vaccine; and
(d) Inactivated mouse brain vaccine.

Chick embryo vaccine was developed sometime in 1946 but the vaccine has not been in use. The tissue culture vaccines have shown promising results but there are still many problems to be solved in their preparation for general use. Hence at present the formalin inactivated mouse brain vaccine is being generally used and such a vaccine is manufactured principally in Japan.

Proposed Indo-Japanese collaboration

Initially in 1979 it was decided to explore the possibility of producing JE vaccine at Central Research Institute, Kasauli from suckling mouse brain with Japanese assistance. However, the Japanese expert groups that visited New Delhi and

This article was presented at the National Conference on Japanese Encephalitis, held at the Indian Council of Medical Research, New Delhi, during November 3-4, 1982.

Kasauli during August/Septe... 1981 and again in March, 1982 re... nded the production of this vaccine in weaned mice. According to them the vaccine is currently being manufactured in Japan in weaned rather than suckling mice and such a vaccine when purified by alcohol protamine precipitation method followed by sucrose density centrifugation in K-II zonal centrifuge has been found to be very good and potent.

It has now been decided by the Government of India that the vaccine shall be produced at CRI Kasauli under Indo-Japanese collaboration. The Japanese side shall provide the imported equipments required for the production of the vaccine and shall make available the services of Japanese experts and also arrange for training of Indian scientists in Japan at their cost. The Indian component shall include the provision of adequate scientific and other personnel, indigenously available equipment and other raw materials, undertake certain minor additions/alterations to the existing buildings and above all make arrangements for breeding and maintenance of mice and other animals.

The proposed collaboration covering a 4 yr period from 1982-86 is aimed at creating facilities for annual production of 20 lakh doses of the vaccine by 1986.

Method of production

The Nakayama NIH Strain of virus and healthy Swiss albino mice of 3-5 wk of age shall be used. The brains of the animals inoculated intracerebrally with the virus shall be harvested when moribund. The harvested brains will be emulsified in phosphate buffered saline and centrifuged. The supernate will be treated with protamine sulphate. After another round of centrifugation the supernate will be filtered through membrane filter and later treated with formalin for inactivation of the virus. The virus antigen shall then be purified by the method of sucrose gradient centrifugation using K-II zonal ultracentrifuge. The resulting suspension shall be subjected to in process quality control tests. The bulk material shall be made by collecting above suspensions and diluting them in suitable medium. After pooling and dilution the vaccine will be filled in final containers, freeze dried and sealed to serve as the final product. Appropriate quality control tests as applicable at different stages of production and in final vaccine will be undertaken.

Stability of the vaccine

The fluid vaccine should be stored between 4° to 10°C in the dark and the shelf life of the vaccine under these conditions is 12 months. Considerable loss of potency occurs if the vaccine is frozen or exposed to higher temperatures.

The freeze-dried vaccine when stored at —20°C retains its potency for a period of about 5 yr. It is also found to be more stable at higher temperatures than the fluid vaccine. In a tropical country like ours where storage of vaccines at suitable temperatures is difficult, the preparation and use of a freeze-dried vaccine is more appropriate and hence the decision to manufacture freeze-dried mouse brain JE Vaccine at CRI, Kasauli in India.

Requirements of the vaccine

It has not been possible to make an accurate estimate of the quantity of the

vaccine which will be required annually in our country. It is anticipated that the demand of the vaccine will vary from year to year. Since the demands will also be expected to be met at a very short notice by the manufacturer, it is desirable that stocks of vaccine with a longer shelf life are maintained by the manufacturer.

Cost and targets of production

Approximate cost of the vaccine is likely to be Rs. 8/- per dose. It is assumed that a maximum annual production of 20 lakh doses of the vaccine can be undertaken by CRI, Kasauli with the inputs as proposed. As the vaccine will be produced on a weekly basis using 3-5 wk old healthy mice, about 50,000 mice will be required every week from 1985 onwards to achieve the maximum targetted production of 20 lakh doses from 1986 onwards. But initially in 1984-85 it is anticipated that the production will be only 10 lakh doses using 25,000 mice per week.

Immunization schedule & immunity

At least one month is needed for a human subject to develop immunity after the administration of the initial dose of the vaccine. Hence any preventive vaccination should be completed at least one month ahead of the start of the epidemic, if the same could be roughly predicted. Otherwise, 2 doses of the vaccine at an interval of 7-14 days at any time followed by a booster dose 1-12 months later and subsequent boosters every three to four years can afford sufficient protection and are considered satisfactory. In epidemic situations annual booster dose may be necessary.

Subject of vaccination

There is no well defined age group since in certain states children have been affected more while in other states the disease has occurred mainly in adults. Selection of the subjects has to be done carefully after study of the morbidity statistics in the area concerned.

Efficacy of vaccine

In extensive controlled field trials conducted in Taiwan and Japan, the efficacy rate of the vaccine given in two doses has been estimated to be 80 per cent. Such data for India is not available as no field trials have been undertaken in this country with the imported vaccine but that the vaccine does produce seroconversion has been reported. This does not necessarily indicate protection. However, in view of the low attack rate in this country an extensive controlled field trial with the vaccine is difficult to be undertaken.

Side reactions

Excepting for the common local reactions no serious side effects of the vaccination have been reported. Extensive studies in Japan have failed to report any complications resembling those of post-encephalitis which have been seen after rabies vaccination.

Future projections

Twenty lakh doses of the vaccine in the first year of full production would suffice to provide two initial doses and one booster dose to 6·5 lakh persons. In the subsequent year if epidemics occur, the number of persons who can be given pri-

mary vaccination and first booster dose will proportionately decrease since the second booster may be required at the time of the epidemic. If no epidemics occur, the second booster will be required only after 3-4 yr and under those circumstances the persons that can receive primary vaccination and first booster will be more than in an epidemic situation. In case of increased demands beyond the target of production, other units of production for this vaccine shall to be set up elsewhere for reasons of the constraints of the requirements of mice.

D.

Proceedings of the
National Conference on Japanese Encephalitis, 1984, pp 81-84

Problems of JE immunization in India

Khorshed M. Pavri

National Institute of Virology, Pune 411001

Japanese encephalitis (JE) manifests as a seasonal, cyclic occurrence and is assumed to have established endemicity in several areas. This would imply that a substantially large proportion of the population gets infected and acquires active immunity to JE. However, our studies in Karnataka indicate that even after one or two so-called epidemics, nearly 70–80 per cent of the population remains unaffected and has no detectable neutralizing antibodies to JE virus. Immunization of the population at risk, is, therefore, a desirable goal, at least, in theory if not in practice. Here, an attempt has been made to evaluate some of the problems and consider the feasibility of using vaccine to control JE in different parts of India.

Efficacy of the available vaccine

The most important factor in determining the feasibility of immunization is the availability of an efficiently immunogenic and safe vaccine which is also cost effective. Considering the distant rural areas where the vaccine would be most needed, lyophilization of the vaccine, though more expensive, is essential so as to retain its potency. Dr S. Saxena has presented the perspectives of the vaccine manufactured in India.

After some trials, the Japanese scientists found that the Nakayama NIH strain was most effective and yielded higher neutralizing antibody titres than some other strain(s). They have recommended two doses 1 ml each (0·5 ml for children below 3 yr) to be administered subcutaneously at an interval of 7-14 days. A third injection of the same amount was to be given within one year in order to develop full protection by a booster effect. Yet another booster was recommended after three years. The manufacturers of the BIKEN JE vaccine recommend three injections of 1·0 ml each subcutaneously at 1-2 wk interval for primary immunisation. For booster immunisation a single 1·0 ml injection subcutaneously is recommended 12-18 months after primary immunisation with three injections.

Effectiveness of a vaccine becomes apparent if a commonly occurring or an easily distinguishable disease disappears or is reduced markedly. It is, however, difficult to assess efficacy of a vaccine against

This article was presented at the National Conference on Japanese Encephalitis, held at the Indian Council of Medical Research, New Delhi, during November 3-4, 1982.

JE in a short span of time. In India, JE may occur sporadically throughout the year, or it may manifest itself in an epidemic situation only cyclically. In absence of an adequate surveillance system, backed up by proper laboratory diagnosis, the former situation may often go unrecognized or under-reported and the latter may suffer from inaccurate or over-reporting. Determination of serological response is, therefore, a much better and quicker method of evaluating the effect of JE vaccine.

Experience in India

In Japan, JE virus is the only prevalent mosquito-borne flavivirus. In contrast, in India, we have the closely related West Nile (WN) virus in many areas, in addition to the different serotypes of dengue virus which are most common in urban and sub-urban areas. Potentiation of antibody response to killed flavivirus vaccine is known to occur because of pre-existing antibodies to related viruses.

A trial among the NIV staff was carried out using the killed liquid vaccine with all the required precautions of storage and administration. Neutralization tests were performed both in infant mice by intraperitoneal (ip) route and in adult mice using intracerebral (ic) administration. The latter, more specific test, was less sensitive as compared to the former. Demonstration of N antibody by the ic test was therefore assumed to show higher concentration of N antibodies.

Out of the 111 staff members satisfactorily immunized with two doses, 21 had specific N antibodies in high concentration in their pre-sera while 39 had these in low levels. Of the 39, 28 showed rise in antibody levels and 11 did not. From the remaining 51 individuals, 12 (23·53%) seroconverted with high levels of N antibodies and 10 (19·6%) showed probably specific low levels of antibodies. On the whole, 29/51 (56·86%) did not develop N antibodies. About 3 years later, on securing lyophilized mouse brain vaccine, 18 of these 29 persons were given a further booster. Only 3 of these developed N antibodies.

Another batch of 25 staff members, all of whom without any demonstrable N antibodies to JE virus in their sera, were administered the lyophilized vaccine using the new schedule of three doses. This time, 4 of the 25 (16%) seroconverted with high levels of N antibodies and 7 (28%) with low levels. Thus, the proportion of the individuals who responded with N antibody was 23·53 per cent (high) and 19·06 (low), *i.e.*, 43 per cent in the first trial, and 16 per cent (high) and 28 per cent (low), *i.e.*, 44 per cent in the second trial. The data from adult mouse ic neutralization test employing the homologous Nakayama strain of JE virus revealed approximately 15–20 per cent additional vaccinees with N antibodies for the virus as compared to the P20778 virus adult ic test Rodrigues, F.M. (personal communication).

A closer scrutiny of these results indicated that in persons with low levels of pre-existing antibodies either to JE or WN virus, more than 80 per cent developed specific N antibodies reacting with JE virus. However, the response was poor in those lacking any such previous experience; more than 3 doses of the vaccine would be required for them to develop protective antibodies.

Problems and strategies for administration: A vaccine is used to protect the recipient against a particular disease. In this process, those immunized may be indirectly protected because of reduction in chances of their contact with the infectious agent. If the agent is dependent only on human host for its survival, it may even be totally eliminated. This has been dramatically demonstrated with smallpox and might be possible in future for a disease like measles. In contrast, vaccination of humans would not break the chain of transmission of a mosquito-borne virus like JE which is also zoonotic in nature. Intervention would be possible only if 80-90 per cent of the population at risk is continuously and effectively immunized.

Whom to vaccinate: Almost everyone who comes in contact with measles in unimmunized population gets measles. In contrast, JE exhibits an extremely scattered pattern of incidence; on an average, 1-2 cases are recorded per village although a substantial proportion of population might be nonimmune. Age distribution of JE cases indicates mostly children under 15 yr being affected in southern India. Compare this with the much narrower age group indicated for immunization against measles and poliomyelitis. Even considering the higher limit of less than 5 yr for measles in rural India, it would mean a coverage of 15-20 per cent of the population as compared to about 40 per cent for less than 15 yr. In West Bengal, it is wider still, comprising the age group [illegible]. Determination of age specific seroprevalence rates for JE virus in different areas would be desirable but expensive because of the need to carry out tests for differentiating specific antibodies from the cross-reacting ones. Identification of the risk group will depend on the distinctive epidemiologic features in different areas.

For the present, it would be desirable that the highest priority be given to those on the lowest rung of the socio-economic ladder. This class is recorded as the most vulnerable, high risk group in almost all the epidemics. These are the people who suffer from malnutrition and are especially devoid of agricultural produce during the period of maximum activity of JE virus. Antibody titres in the patients of confirmed JE are found to be much lower than those observed in Japan and some other countries. It is probable that these people might also respond poorly to the inactivated JE vaccine.

When to vaccinate: Duration of effective immunity is yet to be determined in India. Even in Japan, booster doses are needed regularly. Thus 3-4 doses of primary immunization need to be followed by further booster doses. It might perhaps be desirable to carry out primary vaccination during the intervening period when agricultural produce is available in adequate quantities to sustain nutritional requirements of this vulnerable group to enable them to develop adequate level of antibodies. The seasonal pattern of outbreak of JE in different parts of India has been recognized. A general impression is that JE manifests almost in an epidemic form soon after heavy rainfalls preceded by drought conditions.

A strategy to consider would be to give a booster dose to the population at risk immediately on rainfall data without waiting for information regarding the

occurrence of cases of JE. Alternatively, a booster dose may be given in face of an outbreak so as to raise the level of antibodies within a short period.

It is possible that if by chance, the vaccine is given during the incubation period of JE virus infection, the disease may manifest through natural infection. Precaution should be taken to educate the public regarding the killed nature of the vaccine so that its acceptability is not reduced.

Where to vaccinate: The target population live under extreme poverty in far-flung rural areas. The problem will be to decide the correct strategy in a particular area; *viz.*, whether to take the vaccine to the people or to motivate the people to travel long distances to where the vaccine is available. Even the most potent vaccine will be ineffective unless administered under potent condition to those who need it the most.

Logistic considerations: The JE vaccine is to be administered in 3 doses and to an age group much older and wider than that generally accepted for the Expanded Programme of Immunization. A separate—from EPI—schedule for JE immunization in the JE affected States/Union Territories might severely limit the programme due to constraints of resources.

Use of live but attenuated vaccine for the amplifying host like the pig has shown encouraging results in Japan. In India, small numbers of pigs are reared mainly by the scheduled castes and tribes. Immunization of these pigs with one dose schedule may be considered as an appropriate alternative strategy in some particular areas.

To conclude, whatever strategy is applied, its usefulness will be difficult to assess unless we have a strict monitoring system. Its effectiveness will be judged by the reduction in the number of reported cases and deaths and also by the lengthening of the inter-epidemic period.

Mannitol (20 per cent solution) 500 ml *iv*, administered over half to one hour.

Both children and adults:

Artificial hypothermia. Early surgical decompression, where facilities exist.

EPIDEMIOLOGICAL FEATURES

As determined by serological surveys carried out by the NIV between 1955 and 1972, JE virus infection is widespread in India and is particularly high in the southern states of Andhra Pradesh, Tamil Nadu and parts of Karnataka. It was also found to be prevalent in Orissa, Assam and the lower elevations of Arunachal Pradesh as well as in Rajasthan. Until 1973, the disease, however, was not commonly recognized and the occurrence was restricted to southern India. The ratio of inapparent human infections to clinically apparent disease is high for JE. In Japan, it was found to be between 500 and 1000 inapparent infections for every case of JE. In India, it is probably similar to that in Japan as judged by the high proportion of the population possessing antibodies to JE virus in the endemic areas and the relatively low incidence of the disease. Control studies have, however, not been carried out.

The disease in southern India almost exclusively affected children below 15 years, while in West Bengal all age groups have been affected. This would appear to indicate that the virus in this area has been newly introduced into a relatively non-immune population. In most of the epidemics the incidence has been higher in males. In Bankura district, lower socio-economic groups had a higher incidence.

Another feature of JE is the scattered pattern of incidence. On an average, 1 to 1.5 cases occurred per village and there was not more than one case in a household.

The seasonal incidence of JE in humans has not been the same in different parts of India. In southern India, the illness occurred mainly during the later half of the year, coinciding with the rainy season and period of high mosquito prevalence. Tamil Nadu receives, in addition to the south-west summer monsoon, rainfall due to the north-east winter monsoon. The peak incidence of JE was in October to December. The exception was the outbreak in Tirunelveli and surrounding districts in 1977-78 which commenced in November and lasted till April with the peak in February and March.

In eastern India the disease occurred between May and October. In Bankura this was shown to be related to the summer monsoon. In Assam and Uttar Pradesh the recent outbreaks have occurred between September and December. In Uttar Pradesh in 1978, the outbreak followed extensive floods. This could possibly be due to an extensive increase in the vector mosquito density by an increase in breeding sites. However, it was not possible to prove this association by controlled studies.

In 1971, prospective studies were commenced by the NIV in Andhra Pradesh. The aim of these studies was (a) to study the epidemiology of JE in the richly irrigated and fertile Krishna-Godavari delta, and (b) to compare the activity of JE virus in the above mentioned area with the activity in an area located in Khammam district - which was relatively not well irrigated but which was soon likely to come under irrigation from the left bank canal of the Nagarjuna Sagar project - both before and after irrigation.

Several methods were utilized to obtain data on the activity of JE virus, *viz.*, serological surveys

among humans, pigs and birds; mosquito collections and attempts to isolate virus from mosquitoes; sentinel animal studies employing young pigs and chicks; and a survey of records of hospitals and primary health centres to estimate the incidence of encephalitis.

Valuable base-line data were obtained between 1971 and 1975 when the study was temporarily suspended. The JE/WN complex of viruses were found to be prevalent in all three districts. However, the prevalence of JE virus appears to be somewhat lower in Khammam district. A significant proportion of birds of the family Ardeidae were found to possess antibodies to JE virus.

The study will be resumed after canal irrigation is well established in Khammam district.

DIAGNOSIS OF JE

A rapid specific diagnosis of sporadic disease with JE virus is not yet possible. In an epidemic situation, the symptomatology, detailed clinical examination, examination of the CSF and the epidemiological picture can help to arrive at a diagnosis. Under these circumstances, the diagnosis of the aetiology of the epidemic assumes importance. This can be achieved by:

1. Virus isolation from brain biopsy/necropsy specimens to be transported in transport medium (vide Appendix II) or CSF or very rarely from the blood of the patient.

2. Demonstration of virus antigen in the brain of patient.

3. Demonstration of production of specific antibodies in the patient's sera - either by seroconversion or rise in antibody titre against JE virus.

Laboratory tests for diagnosis of JE

The laboratory diagnosis of JE virus can be made by the demonstration or isolation of the virus in the test specimen and by identification of the isolate by serological methods.

In the absence of isolation of the virus, serodiagnosis may be of help. The methods for collection, storage and transport of specimens are described in Appendix I.

Virus detection

Demonstration, isolation and identification of the virus:

1. Demonstration of the virus/viral antigen in the autopsied brain tissue by fluorescent antibody technique (direct and indirect method).

2. Isolation of the virus from autopsied brain tissue, CSF and occasionally from peripheral blood collected during the very early phase of illness. Isolation is usually carried out by intracerebral inoculation of the specimen in infant mice or by infecting cell cultures (Vero, primary MKTC, hamster kidney

3. Identification of the agent is done by serological tests. Usually by the complement fixation (CF) test using antisera to JE, WN, and dengue-2 viruses. Since JE and WN viruses show cross-reactions, specific identification can be made using kinetic CF test at 0 hour (immediate), 3 hours or 18 hours of incubation before adding the haemolytic system. Alternatively, JE/WN monospecific antisera obtained by absorption of cross-reacting antibodies are used. Agar gel diffusion (AGD) tests can also be used for identification of the agent. However, in such cases, antigen has to be prepared from brain tissue.

NATURAL HISTORY OF JE

Most of the arboviral infections are zoonotic diseases having their natural cycle in wild or domestic vertebrates and haematophagous arthropods. They are generally maintained in enzootic forms and appear as focal outbreaks under specific ecological conditions. JE virus infection is no exception.

Arthropod vectors

During the studies in different parts of India, 29 strains of JE virus have been isolated from mosquitoes. Of these, 22 were isolated from peninsular India and 7 from West Bengal. In the earlier part of these studies, the taxonomic status of the species belonging to the *Culex vishnui* complex was not clear and all the species under the group were clubbed as the *"Culex vishnui"* group and the isolations made in 1956 were attributed to the *"C. vishnui"* group. Subsequently, the three species belonging to the *C. vishnui* complex, *viz.*, *Culex tritaeniorhynchus*, *C. pseudovishnui* and *C. vishnui* were distinguished on taxonomical grounds and the isolations made thereafter were attributed to each species.

Of the 13 isolations made after 1956, eight were from *C. tritaeniorhynchus*, three from *C. vishnui* and two from *C. whitmorei*. The ecological studies, particularly on the habitats, the relative population density, biting habits, host predilection and on the vector potentials incriminate *C. tritaeniorhynchus* as the major vector in peninsular as well as in the other parts of India. *C. vishnui (C. annulus)* which is closely related to *C. tritaeniorhynchus* is considered to be the main vector in Taiwan. In India *C. vishnui* may be another important vector as recently JE has been isolated from this species from West Bengal and Kolar district of Karnataka. These mosquitoes as compared to *C. tritaeniorhynchus* have been shown to be attracted more to children, birds and pigs. In Thailand, *Culex fuscocephalus* and in Malaysia, *Culex*

gelidus are also known to be vectors, but apparently of lesser importance. However, there have been no isolations of JE from these species in India. The two isolations from *C. whitmorei* from North Arcot district and Krishna district incriminates it as a possible vector.

During the 1973 epidemic of JE in the Asansol area in West Bengal, three strains of JE virus were isolated - one each from *C. vishnui*, *Anopheles barbirostris* and *A. "hyrcanus"** at the School of Tropical Medicine, Calcutta. During the follow up studies in 1974-75 in Bankura district, 4 more strains were isolated. Two of them were from *A. "hyrcanus"* and one each from *Culex bitaeniorhynchus* and *Culex epidesmus*. Subsequently, 2 strains, one each from *C. vishnui* and *A. subpictus* were isolated from Kolar district. The five isolations from Anophelines have added new dimension and complexity to the natural history of the disease. There has been a report of virus isolation as well as laboratory transmission of virus, in *Anopheles sinensis*, a species closely related to *A. nigerrimus*, in Japan. Transmission of virus by *C. tritaeniorhynchus* and isolation and transmission in *C. pipiens pallens* have also been reported. Recent laboratory experiments undertaken at the NIV demonstrated that *Anopheles tessellatus* is capable of transmitting the virus. However, *A. "hyrcanus"* collected around Pune did not transmit it, although it retained the virus for nine days. The vector potential of *A. "hyrcanus"* needs confirmation, particularly the strain found in West Bengal.

The isolation of virus from *C. bitaeniorhynchus* is noteworthy. Laboratory studies have determined its high vector potential. In Bankura district, the species was found in all types of collections but more frequently found in chicken baited traps, as well as

* *A. hyrcanus* group in India is represented by six species. Of these, *A. nigerrimus* and *A. peditaeniatus* are widely distributed.

human biting collections. The aviphilic and anthropophilic behaviour of the mosquito incriminates it in transmission from bird to bird as well as to man. *C. epidesmus*, which yielded one isolation, is found in northern India. During the year-round studies at Bankura district, this species was found in significant numbers only from July to November and was present in all types of collections. Until more information is available on its habitat, habit and vector potential, it is difficult to evaluate its role in the natural history of the virus.

The available information obviously incriminates mosquito species belonging to *C. vishnui* group. Other species might be acting as complementary vectors. The transmission and maintenance system of JE appears to be complex and may vary from area to area depending upon many ecological factors than mosquitoes alone. With the available information it is not possible to assess the relative role of these factors in the epidemiology of the disease.

The following observations on the bionomics of some vector species might be added. The two main approaches employed at Vellore to study the biting habits of vectors were: (i) the host predilection for various animal baits and (ii) the analysis of mosquito blood meals by precipitin tests to determine the hosts on which they had fed. These studies showed that members of the *C. vishnui* complex are more attracted to cattle than man. Of the three species under the group, *C. tritaeniorhynchus* is least attracted to man. They are less attracted to birds than cattle and again *C. tritaeniorhynchus* is the least attracted. The number of pig-feeds as detected by precipitin tests was low with all the three species. However, they fed well when exposed to pigs in pig-baited traps. The relatively low level of pig-feeds detected in wild-caught mosquitoes probably reflects the relatively low pig population as compared to human and cattle.

Vertebrate hosts

Birds: The birds belonging to the family Ardeidae have been incriminated both in the maintenance and dissemination of JE virus in Japan. In India too, birds have been suspected but until recently no full scale investigations were conducted to assess their role.

The first of a series of investigations was conducted between 1955 and 1965 at Vellore and 1396 birds, representing 71 species, were processed for virus isolation. No strain of JE virus was isolated. Sera from 410 birds, 71 of which belonged to the family Ardeidae, were screened for N antibodies against the virus and no incriminating serological evidence was obtained.

During the subsequent studies carried out in Andhra Pradesh, sera from 286 *Ardeola grayii* (pond heron) and 229 *Bubulcus ibis* (cattle egret), both belonging to the family Ardeidae, were screened for N antibodies against JE and 110 (38.5 per cent) of the former and 85 (37.0 per cent) of the latter showed the presence of antibodies. Experimental viraemia and bird-to-bird transmission of the virus through *C. tritaeniorhynchus* mosquitoes showed that pond herons and cattle egrets develop viraemia in sufficiently high titres to infect mosquitoes and that mosquitoes fed on them effectively transmit the virus.

In West Bengal, in many areas, ducks were found in large numbers living in close association with man under conditions thought to be favourable for virus transmission. A study on experimental viraemia and duck-to-duck transmission through the mosquitoes showed that ducks developed adequate levels of viraemia and the mosquitoes fed on them get infected and effectively transmit the virus. However, when 104 bird sera were tested, only 8 showed the presence of antibodies. No information is available on the immunity status of ducks in the Bankura area. In the Andhra

area only 5.7 per cent of ducks (3 out of 53) possessed N antibodies to JE virus.

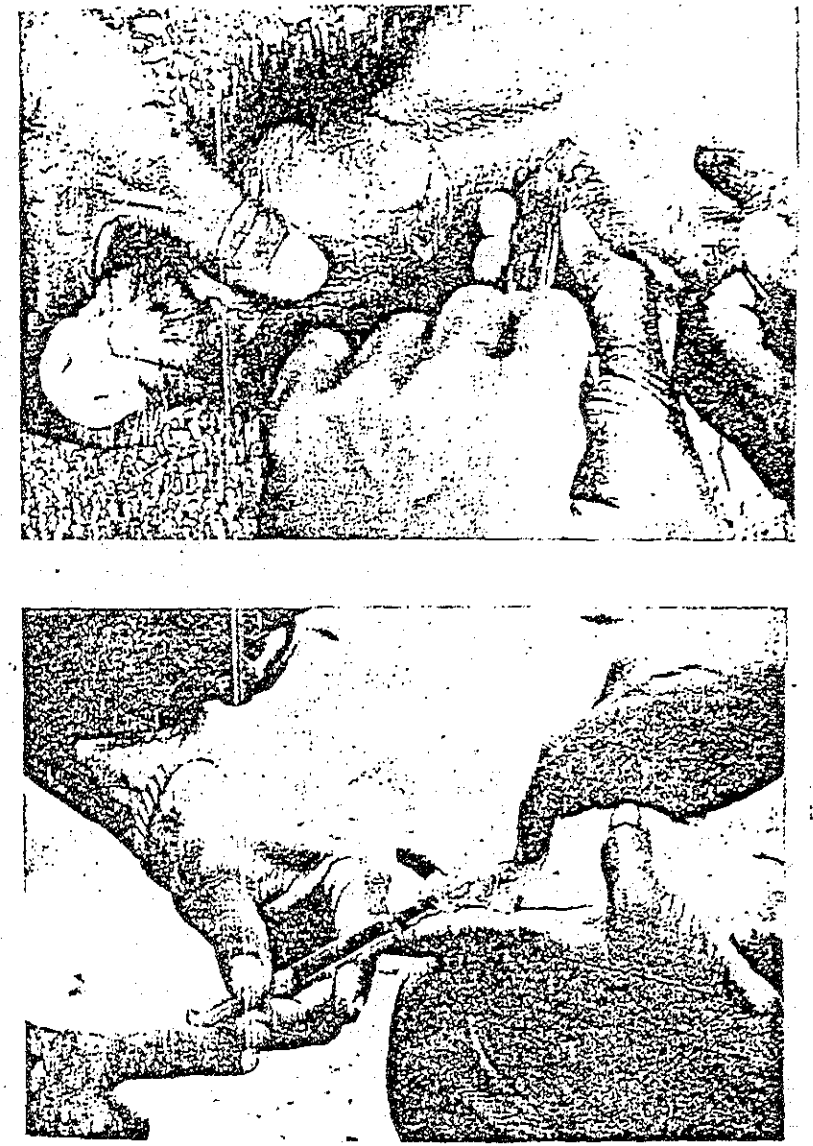
Though the earlier studies did not indicate the involvement of birds, the studies conducted later suggest the involvement of Ardeid birds at least in some areas. The possible role of ducks is yet to be determined with more evidence either by virus isolation or by serological studies.

In a total of 146 bird sera, collected from Asansol (WB) and Dhanbad (Bihar), N antibodies against JE virus were found in 9 out of 34 little egrets, one of 15 cattle egrets, and one of 13 paddy birds. One of the four crow sera and three of the 65 duck sera also showed antibodies against JE virus.

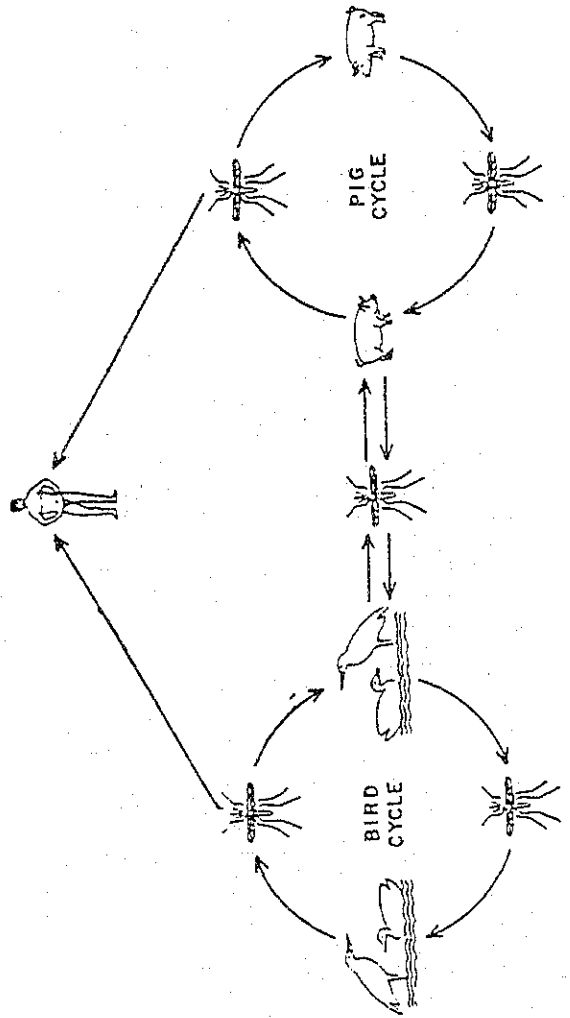
Pigs: Since the beginning of the investigations on the JE natural cycle, pigs have been incriminated as the major vertebrate host for JE virus in Malaysia, Singapore, Taiwan, Korea and Japan. In India, pigs have been implicated in areas around Vellore in Tamil Nadu on the basis of a study conducted to search for extra-human vertebrate reservoirs. About 30 per cent of the pigs were found immune. On experimental infection, pigs developed adequate titres of virus to infect mosquitoes. Subsequently, serological surveys of pigs have been carried out in different parts of India. The percentage of antibody positives in pigs against JE varied from 1.2 to 44 per cent in different parts of the country.

It may be noted that in the Pune region of Maharashtra, JE activity is low. This is also supported by the extremely low incidence of antibodies to JE virus in pigs. In Tamil Nadu which is a JE-endemic area, the proportion of pigs possessing antibodies for JE virus was high (44 per cent).

The percentage of pigs immune to JE in East and South-East Asian countries is much higher than in India, sometimes as high as 100 per cent. Also, in



Blood being collected from a pig (above) and a bird (below), the animals that play an important role in the natural cycle of Japanese encephalitis virus in India



PROBABLE NATURAL CYCLE OF JAPANESE ENCEPHALITIS VIRUS IN INDIA.

India there is a much lower ratio of pigs to the human population than found in other Asian countries with pork-eating populations. This may be the reason for the relatively lower incidence of JE in India. The relative role of pigs vis-a-vis Ardeid birds and other birds remains to be determined. In studies conducted in Bankura, a positive and significant correlation was found between the number of pigs in a given area and the total number of infected cases. A significantly large number of cases also came from scheduled castes and scheduled tribes who lived at the bottom of the socio-economic ladder and were the only communities who reared pigs and lived in close association with them.

Bovines: In a serological survey carried out at Vellore, 57 per cent of buffaloes and 27 per cent of cattle were found to possess N antibodies. Fifty per cent of buffalo sera and 26 per cent of cattle sera collected during and immediately after the 1973 outbreak in Bankura had HI antibodies. N antibodies were detected in 60 per cent of these HI-positive sera. It is evident that cattle and buffaloes become infected with JE virus by the bite of infected mosquitoes. However, two young buffaloes inoculated with large doses of JE virus failed to develop viraemia indicating that these animals might not be involved in the maintenance of the virus.

Bovines are unlikely to play a role in the maintenance and spread of JE. India has an extremely large cattle population. Studies in south India have shown that the vector mosquito for JE, *C. tritaeniorhynchus*, has predilection for cattle. Under the circumstances, there should have been explosive annual outbreaks of JE in India. However, the incidence of human cases of JE in India is much lower than that in Japan and in most countries of East and South-East Asia.

The lower incidence of JE in India may be explained by the predilection of the vector mosquitoes

for cattle. A large proportion of infected mosquitoes feed on cattle which are "dead-ends" for the transmission. Thus the high cattle-pig ratio dampens JE virus activity in nature.

Man and other vertebrates: Isolation of JE virus from human blood is rare and it seems that man is not a suitable host to infect mosquitoes. On the other hand, *C. tritaeniorhynchus*, the major vector though mainly a zoophilic mosquito, may bite man in the absence of the usual host or when its population is high.

Among the animals' sera collected at Vellore and tested for JE neutralising activity, none of the frogs snakes and lizards showed neutralizing antibodies. Of the 20 monkeys tested, none had antibody nor did any of the 156 rodents or the 25 bats. However, viraemia at low levels has been demonstrated in bats *(R.leschenaulti)* for 8-9 days after experimental infection.

The available evidence implies Ardeid bird-mosquito-Ardeid bird and pig-mosquito-pig cycles in nature. Man appears to be only an incidental "dead-end" host (Diagram). The Ardeid bird-mosquito-Ardeid bird cycle may be more stable, persistent and enzootic. The pig-mosquito-pig cycle appears to be temporary and epizootic. The epidemic in man appears to follow pig epizootics. There might be several other vertebrates and mosquitoes involved on the periphery of these cycles as complementary factors. These need careful assessment.

CONTROL MEASURES AGAINST JE VIRUS

In the natural cycle of JE virus, man appears to be a "dead-end". Man to man transmission has not so far been recorded. Among vertebrate hosts, pigs circulate virus in titres high enough to infect large numbers of mosquitoes. The pigs, however, do not show any signs of illness, except abortion as reported in Japan. In India, however, there is no evidence of abortions

in pigs due to JE virus. The role of cattle and buffaloes needs to be considered more as attractants to mosquitoes. Where people share their lodgings with their cattle, mosquitoes attracted to the animals might occasionally bite humans. The role of cattle in the epidemiology of JE would be to support large numbers of mosquitoes by providing them with adequate blood meal. Horses, on the other hand, develop encephalitis and succumb to the infection. In areas where the population of horses is in sufficient number, death of horses may herald JE outbreaks in humans. The role of other equines, such as mules and donkeys, is not known in India.

The control measures, therefore, need to be directed towards: (i) control of the mosquito vectors; (ii) prevention of mosquitoes from biting humans; (iii) vaccination of the humans; and (iv) measures against reservoirs.

Vector control

In India several species of mosquitoes appear to be involved in the ecology of JE virus. It also seems likely that in different parts of the country different mosquitoes may play a major part in the transmission of the virus to humans. It is, therefore, difficult to recommend control measures during the inter-epidemic period.

Considering the vast areas serving as breeding places for the mosquitoes and the inadequate information on the breeding habitats in different areas, larvicidal measures do not appear to be practicable at present. Aspects of vector control which are of the nature of exploratory research are discussed later.

The following practical measures are recommended for implementation particularly by the State and District health authorities in the known "high risk" areas of JE:

F.

BRIEF NOTS ON JAPANESE ENCEPHALITIS

Since 6.11.78, NMEP Directorate has set up a Japanese Encephalits cell to co-ordinate the work of Japanese Encephalits, as to contain it and also to know the extent of recent outbreak of Japanese Encephalitis in the country.

Incidence of Japanese Encephalitis and its trend :

This Directorate has been collecting informations about Japanese Encephalitis incidence from States/UTs In the year 1978 badly affected States were Uttar Pradesh, West Bengal, Bihar, Assam, Tamil Nadu and Maharashtra. In 1979, West Bengal reported higher number of cases but these were less than that of 1978, on the other hand Karnataka reported higher number of cases in comparison to the previous year. During 1978, Andhra Pradesh did not report any case but in 1979 it reported some cases. During 1980, Uttar Pradesh and Bihar reported cases which constituted about 69.2% of total number of cases reported in the whole country but in both the States the total number of cases reported were less than that of 1978. During the year 1981 total 3894 cases and 1167 deaths have been reported. Out of these 89% of cases have been reported from three Southern States of Andhra Pradesh, Tamil Nadu and Karnataka. During the year 1982, Maximum number of cases had been reported from West Bengal and Uttar Pradesh. Total 3516 cases with 1261 deaths have been reported during 1982. Assam, Andhra Pradesh, Bihar, Delhi, Karnataka, Goa, Manipur, Orissa, Tripura, Tamil Nadu U.P. and West Bengal were the affected States. Goa and Orissa reported the JE incidence for the first time. During 1983, Andhra Pradesh, Assam, Bihar, Goa, Karnataka, Manipur, Tamil Nadu and Uttar Pradesh have reported total 1693 cases and 566 deaths.

Statewise incidence/deaths for the year 1978 to 1983 (prov) is appended at Annexure I and II.

Measures taken:

i) One J.E. cell is opened in NMEP Directorate.

ii) All the States/UTs are asked to spray BHC/ DDT on an area 2/3 km. around a case where ever reported.

iii) A detail working note on Japanese Encephalitis regarding causation, transmission, Epidemiology, Entomology, signs symptoms, treatment, vaccine and other preventive measures has been prepared and sent to all the States and Union Territories.

iv) A booklet on Japanese Encephalitis regarding general information and guidelines for investigation, management and preventive measures has been prepared and distributed to all States and Union Territories.

v) Note on Japanese Encephalitis for distribution to General Public & Medical Personals were printed and distributed.

vi) BHC/DDT are supplied from NMEP for the purpose and there is no shortage of insecticide for the control of Japanese Encephalitis outbreaks.

vii) N.I.V. Pune, School of Tropical Medicine, Calcutta, All India Institute of Hygeine & Public Health, Calcutta and NICD, Delhi are involved in the programme for advice and diagnosis of cases.

viii) Vaccines are supplied to the States/UTs on their demand from NICD, Delhi.

ix) Tiffa, Fontan, Leco. & Tiga Machines are supplied for spray operations.

x) Malathion for fogging/ULV is also supplied by the NMEP.

xi) States and Regional Office ROH & FW have been requested to carry out entomological work for J.E. including susceptibility test, vector density etc.

xii) Technical Committee on Japanese Encephalitis had been consituted to review the programme.

xiii) A plan for the financial assistance form Govt of India has been prepared and is under consideration.

STATEMENT SHOWING THE YEARWISE CASES & DEATHS DUE TO J.P.NESE ENCEPHALITIS FROM 1978 TO 1982 ANNEXURE-I

Sl No.	Name of the States/UTs	1978		1979		1980		1981		1982 (Prov)	
		Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
1.	Assam	422	213	-	-	360	194	87	46	145*	59
2.	Arunachal Pd.	22	5	-	-	-	-	-	-	-	-
3.	Andhra Pd.	-	-	254	54	289	121	1273	439	413	141
4.	A&N Islands	NA	NA	NA	NA	9	4	-	-	-	-
5.	Bihar	1252	452	109	57	737	336	157	60	229	68
6.	Chandigarh	NA	NA	42	19	-	-	-	-	-	-
7.	Delhi	NA	NA	NA	NA	-	-	-	-	1	1
8.	D&N Haveli	NA	NA	NA	NA	-	-	-	-	-	-
9.	Gujarat	NA	NA	NA	NA	-	-	-	-	35	13
10.	Goa Daman & Diu	NA	NA	NA	NA	-	-	-	-	-	-
11.	Haryana	5	5	-	-	-	-	-	-	-	-
12.	Himachal Pd.	-	-	-	-	-	-	-	-	-	-
13.	Jammu & Kashmir	NA	NA	NA	NA	-	-	-	-	-	-
14.	Karnataka	72	18	920	223	9	5	837	236	150	52
15.	Kerala	5	1	-	-	-	-	2	2	NA	NA
16.	Lakshadweep	NA	NA	NA	NA	-	-	-	-	-	-
17.	Madhya Pd.	34	15	-	-	108	72	NA	NA	-	-
18.	Meghalaya	12	12	-	-	-	-	-	-	-	-
19.	Mizoram	2	-	-	-	-	-	-	-	-	-
20.	Manipur	27	4	-	-	NA	NA	-	-	100	53
21.	Maharashtra	117	34	-	-	21	5	-	-	NA	NA
22.	Nagaland	12	10	-	-	-	-	-	-	-	-
23.	Orissa	NA	NA	NA	NA	NA	NA	-	-	56	12
24.	Pondicherry	163	114	65	32	-	-	49	17	-	-
25.	Punjab	NA	NA	NA	NA	-	-	-	-	-	-
26.	Rajasthan	20	11	-	-	-	-	-	-	-	-
27.	Sikkim	NA	NA	NA	NA	-	-	-	-	-	-
28.	Tripura	33	30	-	-	69	62	19	16	33	25
29.	Tamilnadu	412	122	83	4	188	67	1324	290	242	83
30.	Uttar Pradesh	3550	1117	150	72	1604	530	75	26	637	199
31.	West Bengal	1303	592	1222	465	84	40	71	35	1469	555
	TOTAL :	7463	2755	2845	926	3478	1436	3894	1167	3516	1261

NOTE : NA= Not Available,-Nil, *Japanese Encephalitis disgnosis not confirmed, reported as viral Encephalitis (clarification sought)

STATEMENT SHOWING THE CASES AND DEATHS DUE TO JE DURING 1984 AND COMPARATIVE DATA DURING CORRESPONDING PERIOD 1983.

Sl. No.	Name of the State/ U.Ts.	1984 provisional cases	Deaths	Report upto	1983 corresponding period Cases	Deaths
.	Assam	30	15	August	4	-
.	Arunachal pradesh	-	-	August	-	-
.	Andhra Pradesh	150	97	October	243	123
.	A & N Islands	-	-	August	-	-
.	Bihar	104	54	July	39	34
.	Chandigarh	-	-	August	-	-
.	Delhi	-	-	September	-	-
.	Dadra, Nagar & Haveli	-	-	September	-	-
.	Gujarat					
0.	Goa, Daman & Diu	-	-	Sept.	15	1
1.	Haryana	-	-	August	4	-
2.	Himachal Pradesh	-	-	August	-	-
3.	Jammu & Kashmir	-	-	August	-	-
4.	Karnataka	37	14	May	130	30
5.	Kerala					
6.	Lakshadweep	+	-	September	-	-
7.	Madhya Pradesh					
8.	Meghalaya	-	-	August	-	-
9.	Mizoram	-	-	June	-	-
0.	Manipur	14	2	October	32	15
21.	Maharashtra	-	-	August	-	-
2.	Nagaland					
3.	Orissa	-	-	July	-	-
4.	Pondicherry	-	-	May	-	-
5.	Punjab	-	-	August	-	-
6.	Rajasthan	-	-	February	-	-
7.	Sikkim			October	11	11
8.	Tripura	454	138	September	108	27
9.	Tamil Nadu	-	-	May	2	2
0.	West Bengal	415	168	October	-	-
	Total	1204	488		611	241

Note: Blank = Not available - = Nil.

資料8－1
バルク製造関連資料

STATEMENT SHOWING JAPANESE ENCEPHALITIS VACCINE PRODUCTION
(13.12.1985)

Ready in hand

Bulk A	(1)	Inactivated JE Vaccine	= 44.5 l	(30/85, 34/85 to 45/85)
	(2)	Under inactivation	= 12.6 l	(46/85 to 50/85)

Bulk B (1) Purified and Concentrated through K II Zonal ultracentrifuge equivalent to

PN Results

1/84 to 5/84 1/85 to33/85 except 30/85	J 85001 = 900 ml J 85002 = 940 ml J 85003 = 700 ml J 85004 = 700 ml J 85005 = 700 ml	= 200.0 l
	Total 3940 ml	

Ready for dilution as final product about 0.2 million doses.

資料８－２

バッチ No.	接種 日付	接種 マウス数 (DL)	接種 スタム番号	採脳 日付	採脳 マウス数 (DL)	採脳 採脳率 (%)	採脳 採脳量 (g)	採脳 一匹当り採脳量(g)	上清量 (ML)	瓶交換日付	無菌試験 (1)	無菌試験 (2)	無菌試験 (3)	ウイルス含量 (log 10)	備考
1/85	1/5	5300	1/84 5.4/0.03ML	1/9	4508	85	1430	0.317	6800	1/23	O.K.	O.K.	O.K.	8.29	
2/85	1/10	5300	〃	1/14	4903	92.5	1546	0.315	7400	1/28	O.K.	O.K.	O.K.	7.57	
3/85	1/15	5500	〃	1/19	4787	87.0	1639	0.342	8200	2/1	O.K.	O.K.	O.K.	7.10	
4/85	1/18	5250	〃	1/22	4866	92.7	1691	0.347	8000	2/5	O.K.	O.K.	O.K.	7.16	
5/85	1/24	5300	〃	1/28	4850	91.5	1678	0.346	8300	2/11	O.K.	O.K.	O.K.	6.65	
6/85	1/31	5300	〃	2/4	4627	87.3	1535	0.332	7500	2/18	O.K.	O.K.	O.K.	7.00	
7/85	2/7	5300	〃	2/11	4860	91.7	1683	0.346	8200	2/25	O.K.	O.K.	O.K.	7.46	
8/85	2/16	2900	〃	2/20	2695	92.9	953	0.353	4700	3/5	O.K.	O.K.	O.K.	8.28 *	V.T. Same day
9/85	2/23	10500	〃	2/27	8740	87.2	2896	0.331	14700	3/13	O.K.	O.K.	O.K.	7.14	
10/85	3/8	5350	〃	3/12	4499	84.1	1490	0.331	7600	3/26	O.K.	O.K.	O.K.	7.00	
11/85	4/4	5150	〃	4/8	3400	66.1	553 A 510 B	0.312	2600 A 2400 B	4/23	O.K.	O.K.	O.K.	8.5 A 7.0 B	A= NBC Protamine Sulphate B= YGK Protamine Sulphate
12/85	4/12	7250	〃	4/16	5816	80.2	1952	0.333	8400 A 500 B 500 C 500 D	4/30	O.K.	O.K.	O.K.	8.42 *	A=NBC Protamine Sulphate-Chs-0.09%, B=NBC $PrSO_4$-CRI=0.08% C=NBC $PrSO_4$-CRI=0.12% D=NBC $PrSO_4$ Takeda=0.09%
13/85	4/15	5700	〃	4/19	5211	91.4	1807	0.347	8950	5/3	O.K.	O.K.	O.K.	7.88 *	NBC Protamine Sulphate-Takeda 0.08%

*V.T was put up on same day

バッチ No.	接種			採脳					上清量 (ML)	瓶交換 日付	無菌試験			ウイルス含量 (log 10)	備考
	日付	マウス数 (匹)	スタム番号	日付	マウス数 (匹)	採脳率 (%)	採脳量 (g)	一匹当り採脳量(g)			(1)	(2)	(3)		
14/85	4/20	4900	1/84 5.4/0.03ML	4/24	4530	92.4	1489	0.329	7100	5/9	O.K.	O.K.	O.K.	8.5 *	NBC $PrSO_4$ - CRI - 0.08%
15/85	4/22	4900	〃	4/26	4421	90.2	1528	0.345	6700	5/	O.K.	O.K.	O.K.	8.5 *	NBC $PrSO_4$ - CRI - 0.08%
16/85	4/27	4850	〃	5/1	4310	88.9	1432	0.332	7000	5/16	O.K.	O.K.	O.K.	8.16 *	NBC $PrSO_4$ - CRI - 0.08%
17/85	4/29	4850	〃	5/3	4105	84.6	1440	0.351	6700	5/18	O.K.	O.K.	O.K.	8.3 *	$PrSO_4$ - YGK 0.08%
18/85	4/3	4800	〃	5/7	3890	81	1285	0.330	6300	5/21	O.K.	O.K.	O.K.	8.67 *	$PrSO_4$ YGK 0.08%
19/85	5/10	4897	〃	5/14	3924	80.2	1372	0.35	6500	6/24	O.K.	O.K.	O.K.	7.23 *	$PrSO_4$ YGK 0.08%
20/85	5/31	9500	〃	6/5	8021	84.7	2660	0.33	13600	6/24	O.K.	O.K.	O.K.	8.23 *	$PrSO_4$ YGK 0.08%
21/85	6/7	4750	〃	6/11	4290	90.4	1492	0.34	7330	6/29	O.K.	O.K.	O.K.	INVALID TEST	$PrSO_4$ YGK 0.08%
22/85	6/10	2780	〃	6/14	2577	92.7	774	0.30	35855	6/29	O.K.	O.K.	O.K.	8.38 *	$PrSO_4$ YGK 0.08%
23/85	6/14	4400	〃	6/18	3895	88.6	1295	0.332	6363	7/6	O.K.	O.K.	O.K.	7.58 *	~~$PrSO_4$ YGK 0.08%~~ 〃 〃 〃
24/85	6/17	2460	〃	6/21	2347	95.4	773	0.33	36865	7/6	O.K.	O.K.	O.K.	7.62 *	~~$PrSO_4$ YGK 0.08%~~ 〃 〃 〃
25/85	6/21	4550	〃	6/25	4202	92.4	1385	0.33	6767	7/16	O.K.	O.K.	O.K.	8.0 *	~~$PrSO_4$ YGK 0.08%~~ 〃 〃 〃
26/85	6/24	2250	〃	6/28	2063	91.7	718	0.347	3333	7/16	O.K.	O.K.	O.K.	8.2 *	~~$PrSO_4$ YGK 0.08%~~ 〃 〃 〃
27/85	6/29	1840	〃	7/3	1652	89.8	569	0.345	2727	7/17	O.K.	O.K.	O.K.	8.45 *	~~$PrSO_4$ YGK 0.08%~~ 〃 〃 〃

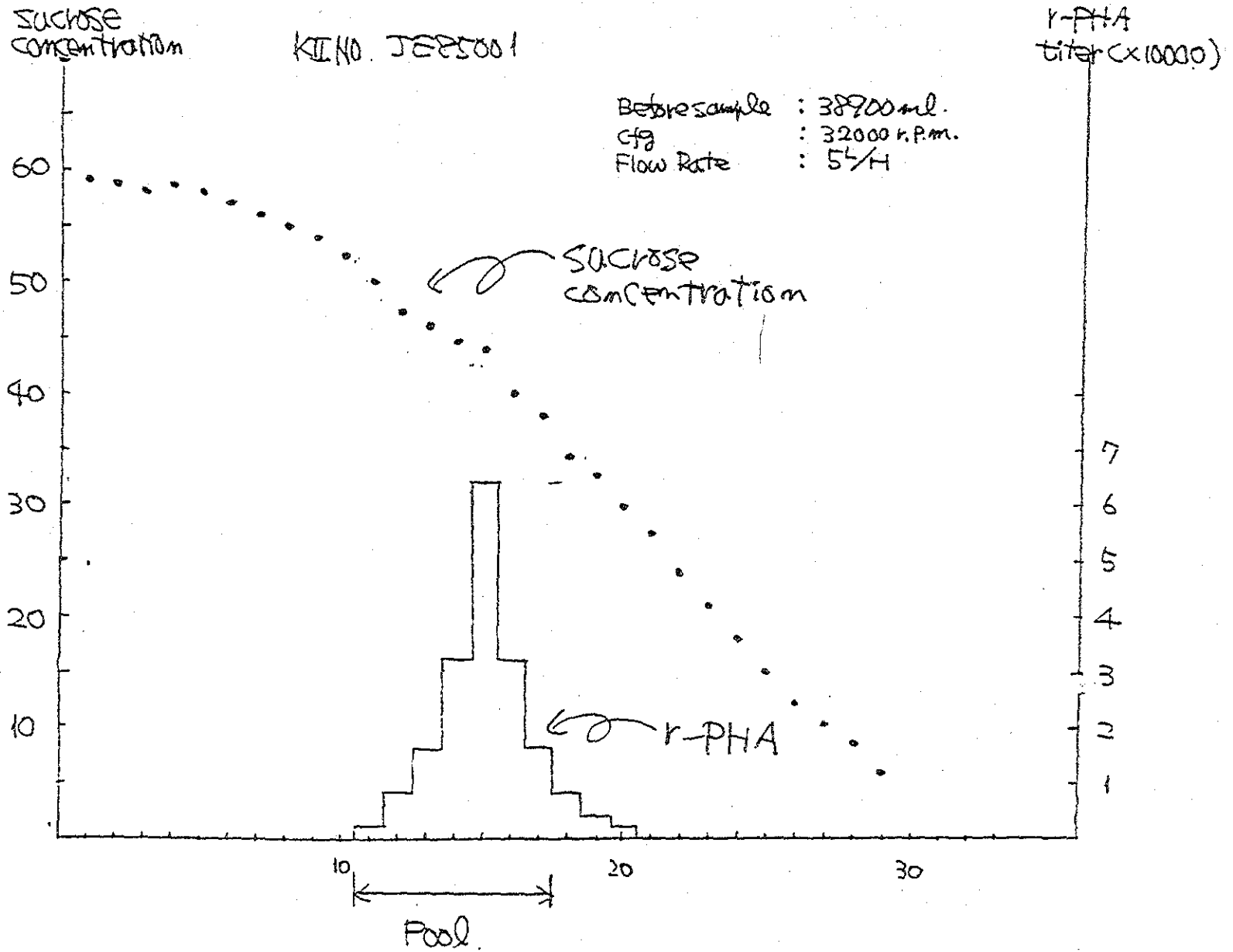
* V.T. was put up on the same day.

バッチ No.	接種 日付	接種 マウス数(匹)	接種 スタム番号	採脳 日付	採脳 マウス数(匹)	採脳率(%)	採脳量(g)	一匹当り採脳量(g)	上清量(ML)	瓶交換日付	無菌試験 (1)	無菌試験 (2)	無菌試験 (3)	ウイルス含量 (log 10)	備考
28/85	7/6	1940	1/84 5.4/0.03ml	7/10	1791	92.4	653	0.36	3131	7/24	O.K.	O.K.	O.K.	* 6.88	$PrSO_4$ YGK 0.08%
29/85	7/12	1950	〃	7/16	1825	93.6	620	0.339	3030	7/30	O.K.	O.K.	O.K.	* 7.80	$PrSO_4$ 〃 YGK 〃 0.08%
30/85	7/20	1900	〃	7/24	1776	93.5	614	0.346	2828	8/7	O.K.	O.K.	O.K.	* 8.00	$PrSO_4$ 〃 YGK 〃 0.08%
31/85	7/27	1890	1/85 5.13/0.03ml	7/31	1596	84.5	542	0.34	2626	8/14	O.K.	O.K.	O.K.	** 6.23	$PrSO_4$ 〃 YGK 〃 0.08%
32/85	8/3	1900	1/85 4.83/0.03ml	8/7	1658	87.3	591	0.356	2828	8/21	O.K.	O.K.	O.K.	* 7.87	$PrSO_4$ 〃 YGK 〃 0.08%
33/85	8/9	1950	〃	8/13	1760	90.3	605	0.344	2929	8/28	O.K.	O.K.	O.K.	* 8.12	$PrSO_4$ 〃 YGK 〃 0.08%
34/85	8/17	3440	〃	8/21	2983	86.7	957	0.321	3939	9/4	O.K.	O.K.	O.K.	* 8.25	$PrSO_4$ 〃 YGK 〃 0.08%
35/85	8/24	2800	〃	8/28	2476	88.4	837	0.338	4000	9/16	O.K.	O.K.	O.K.	7.67	$PrSO_4$ = 0.08%
36/85	8/31	3380	〃	9/4	2878	85.2	1038	0.36	5100	9/18	O.K.	O.K.	O.K.	8.40	〃 〃
37/85	9/6	2680	〃	9/10	2361	88.1	812	0.344	3900	9/24	O.K.	O.K.	O.K.	8.33	〃 〃
38/85	9/16	2860	〃	9/20	2486	87.0	762	0.307	3000	10/4	O.K.	O.K.	O.K.	8.76	〃 〃
39/85	9/20	3450	〃	9/24	3218	93.2	1149	0.357	5500	10/8	O.K.	O.K.	O.K.	8.41	〃 〃
40/85	9/30	1930	〃	10/4	1852	96.0	529	0.286	2350	10/18	O.K.	O.K.	O.K.	7.67	〃 〃
41/85	10/5	1950	〃	10/9	1748	89.6	609	0.348	2800	10/24	O.K.	O.K.		8.575	〃 〃

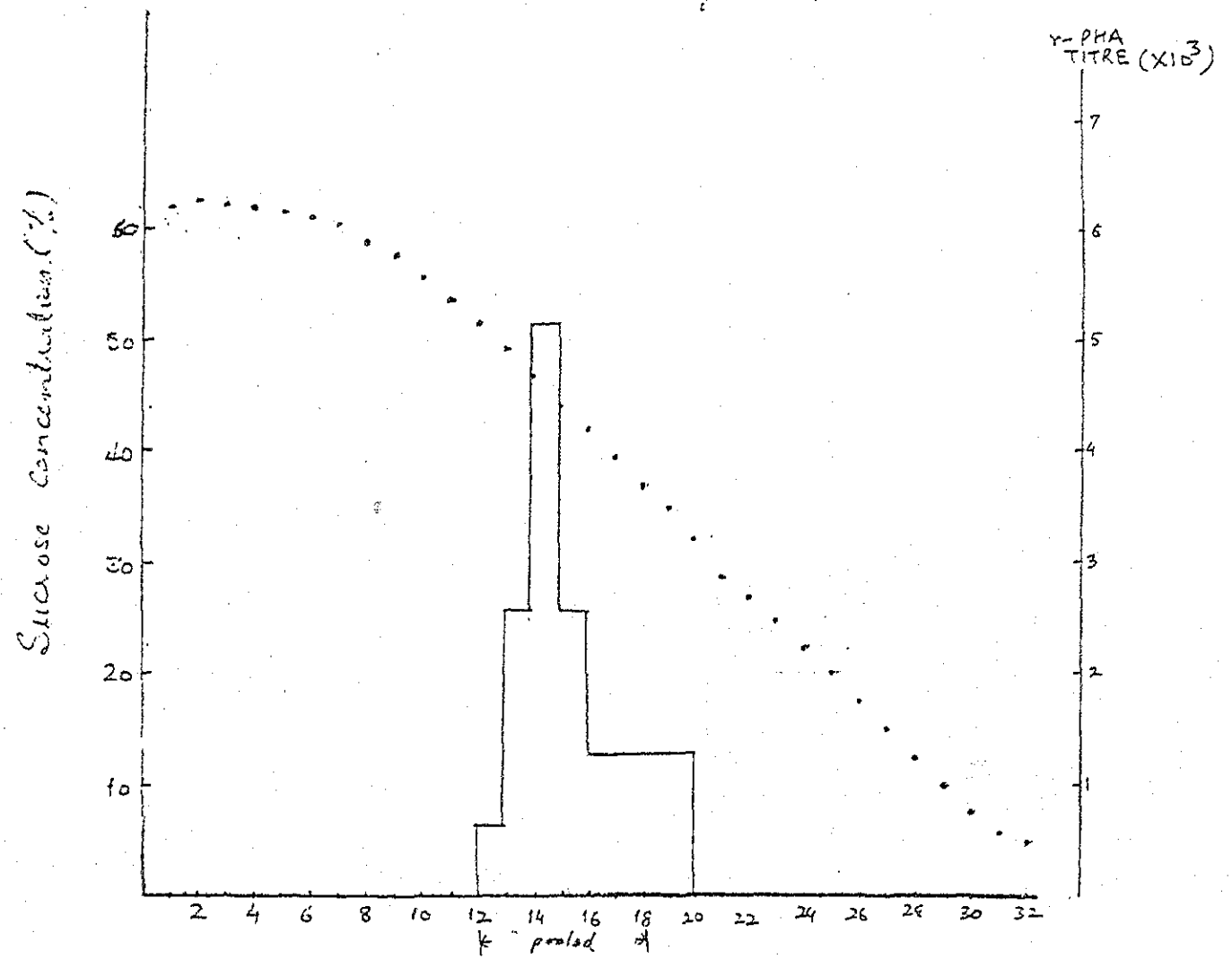
* V.T. was put up on the same day.
** V.T. was put up on a sample held at -20℃ for 24 hours.

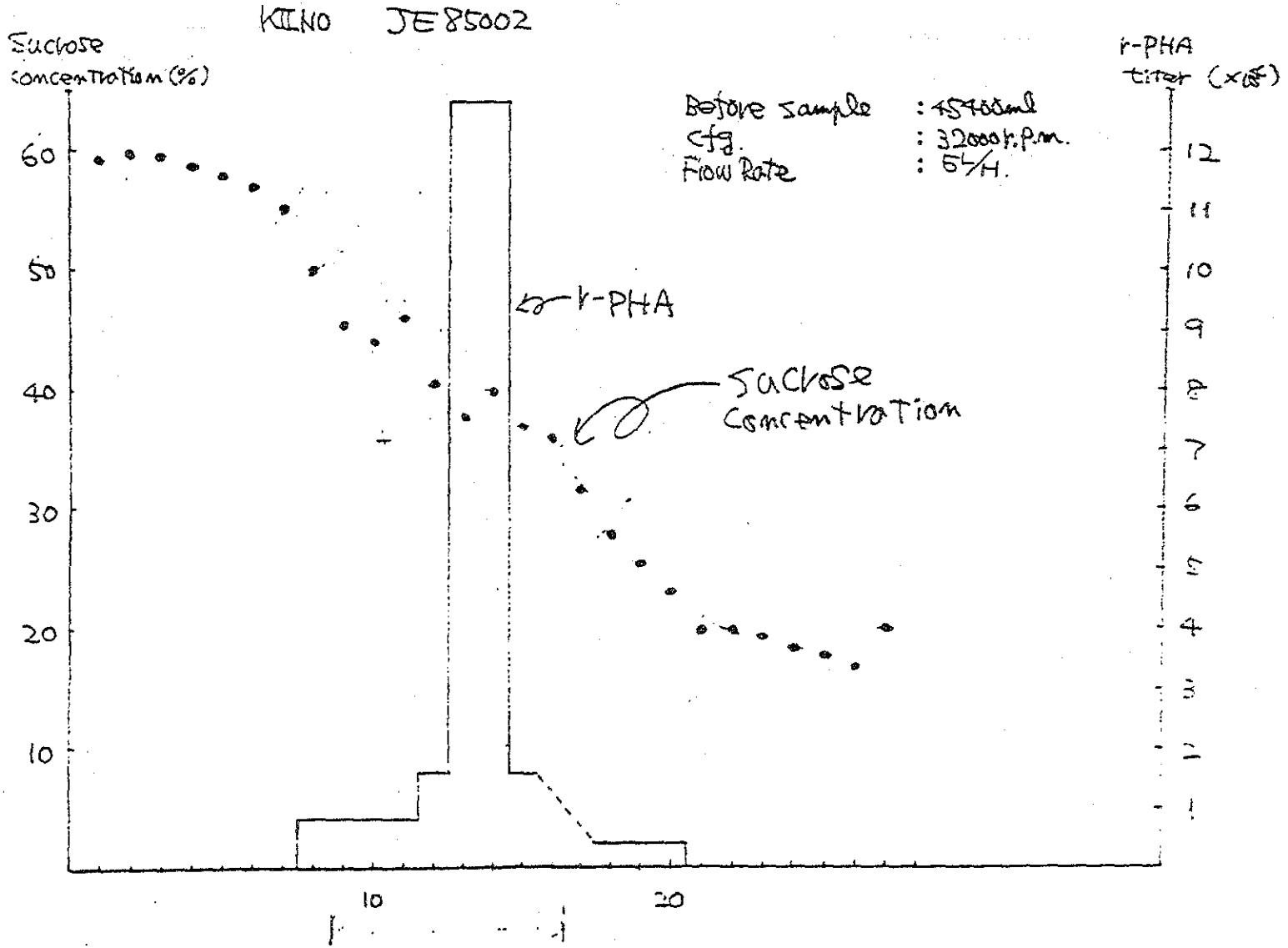
バッチ No.	接種			採脳					上清量 (ML)	瓶交換日付	無菌試験			ウイルス含量 (log 10)	備考
	日付	マウス数 (匹)	スタム番号	日付	マウス数 (匹)	採脳率 (%)	採脳量 (g)	一匹当り採脳量(g)			(1)	(2)	(3)		
12/85	10/11	1900	1/85 4.83/0.03ml	10/15	1772	93.3	630	0.356	2850	10/29	O.K.	O.K.		8.125	$PrSO_4$ = 0.08%
13/85	10/18	1900	〃	10/22	1768	93.0	612	0.346	2850	11/6	O.K.	O.K.		8.33	〃 〃
14/85	10/26	1950	〃	10/30	1881	96.4	672	0.357	3200	11/14	O.K.	O.K.		8.41	〃 〃
15/85	11/2	1800	〃	11/6	1690	93.9	562	0.33	2600	11/20	O.K.	O.K.		7.83	〃 〃
16/85	11/11	1900	〃	11/15	1596	84	510	0.319	2300	11/29	O.K.	O.K.		8.43	〃 〃
17/85	11/16	1800	〃	11/20	1707	94.8	545	0.319	2500	12/5	O.K.	O.K.		7.87	〃 〃
18/85	11/22	2400	〃	11/26	2073	86.4	610	0.294	2950	12/12					〃 〃
19/85	11/30	1600	〃	12/4	1443	90.2	460	0.318	2000						〃 〃
20/85	12/7	2000	〃	12/11	1896	94.8	635	0.335	2900						〃 〃

資料８－３－１

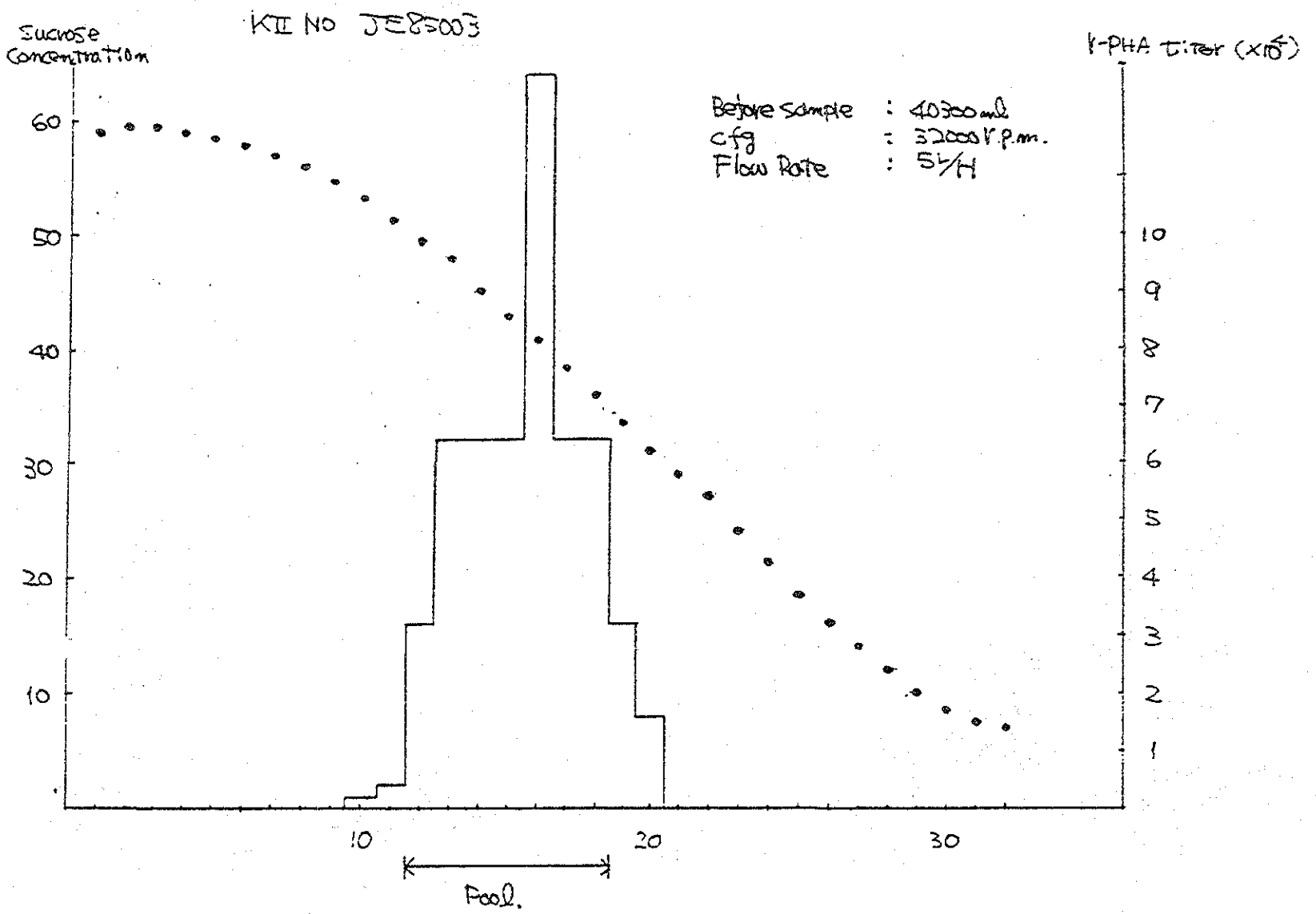

Sucrose concentration
KII NO. JE85001
r-PHA titer (×10000)
Before sample : 38900 ml.
cfg : 32000 r.p.m.
Flow Rate : 5L/H
Sucrose concentration
r-PHA
60
50
40
30
20
10
7
6
5
4
3
2
1
10
20
30
Pool

4.10.85.


Glucose concentration (%)
γ-PHA TITRE (X10³)
60
50
40
30
20
10
7
6
5
4
3
2
1
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
time pooled


KINO JE85002
Sucrose concentration (%)
r-PHA titer (×10⁴)
Before sample : 4540ml
Cfg. : 32000r.p.m.
Flow Rate : 6ℓ/H.
r-PHA
Sucrose Concentration
60
50
40
30
20
10
12
11
10
9
8
7
6
5
4
3
2
1
10
20

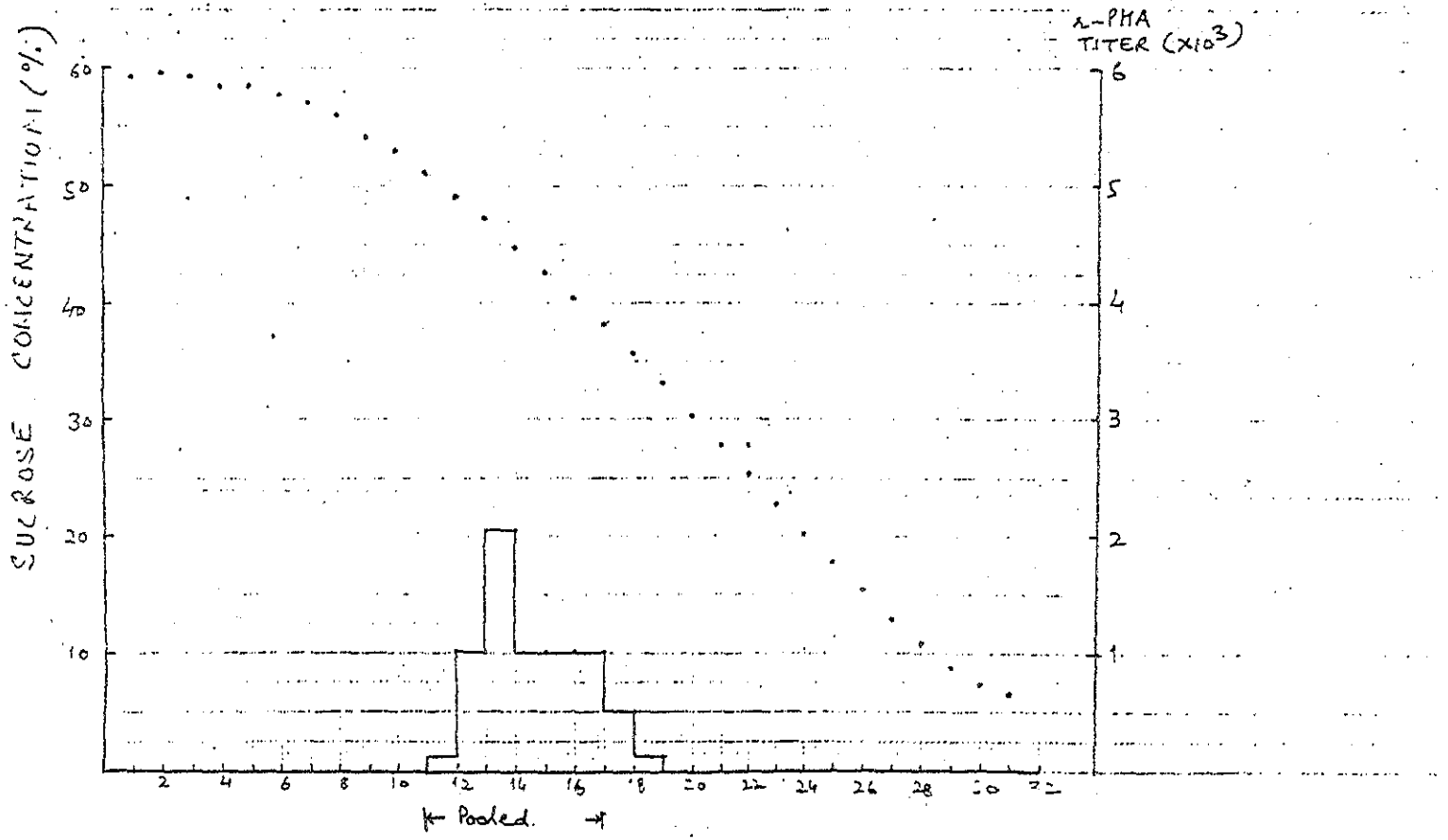
資料８－３－４


KII NO JE85003
Sucrose Concentration
HA titer (x10⁴)
Before sample : 40300 ml
cfg : 32000 r.p.m.
Flow Rate : 5ℓ/H
60
50
40
30
20
10
10
9
8
7
6
5
4
3
2
1
10
20
30
Pool.

KII No. JE 85005

28.4.85

Sample volume = 41,000 ml
Cfg = 32,000 rpm
Flow rate = 5L./hour



Fractions-pool

prepare 10x and 20x diluted suspensions for determination of protein nitrogen content (PN) of fractions-pool

e.g. for micro-Kjeldhl method
15 ml of fractions-pool + 135 ml of PBS ⟶ 150 ml of 10x suspension
50 ml of 10x suspension + 50 ml of PBS ⟶ 100 ml of 20x suspension

* Lowry method is also applicable to determination of PN with a small amount of diluted suspensions

after determining PN of original fractions-pool, make a diluted suspension containing 100 μg PN/ml with M/100 PBS (pH 7.1 - 7.2). add Tween 80 to a final concentration of 0.05 v/v % in the above suspension

Filtration through membrane filters (Pre, RA & HA types)

add thimerosal to a final concentration of 0.01 w/v % in the filtrated suspension

Bulk material

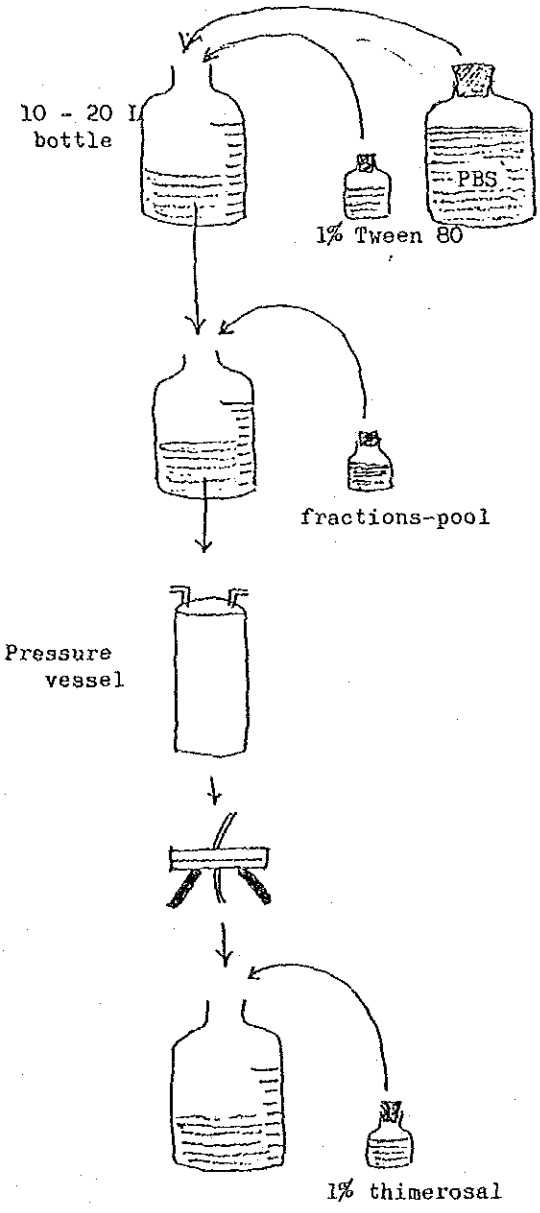
Tests: a. Sterility tests
b. Inactivation test (TC)
c. Test for protein nitrogen content
d. Preliminary potency test
A test sample corresponding to final product should be prepared to contain 8 μg PN/ml using M/100 PBS (pH 7.1 ± 7.2)

Final bulk

Final product

Materials and apparatus for dilution and filtration

1. 20 L of M/100 PBS (pH 7.1 - 7.2). sterilized by autoclaving.
2. One L of 1% Tween 80 solution in PBS. sterilized by autoclaving.
3. One L of 1% thimerosal solution in PBS. sterilized by filtering through Millipore membranes.
4. Three 10 - 20 L bottles. sterilized by autoclaving.
5. Pressure Vessel for filtration. sterilized by autoclaving.
6. Filtration apparatus (293 mm) - Prefilter, RA & HA membranes - sterilized by autoclaving.
7. Transferring devices (siphon). sterilized by autoclaving.



prepare an estimated somewhat large volume of diluent consist of 1 part of 1% Tween 80 and 19 parts of PBS

mix fractions-pool with calculated volume of the above diluent

add 1/100 vilume of 1% thimerosal to filtrated suspension