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When Japanese Encephalitis Virus Invaded Eastern Hemisphere – The History of the Spread of Virus Genotypes

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1. Introduction

1.1 Japanese Encephalitis expansion

Japanese Encephalitis (JE) was first clinically identified in 1871 in Japan and known as "summer encephalitis". In 1924, during an outbreak of encephalitis, important studies were carried out at the Tokyo Research Institute of Infectious Disease (Mackenzie et al., 2007). Subsequently, a virus was isolated of a brain of a patient deceased of Japanese summer encephalitis and named "Japanese encephalitis type B" (JEB) in order to distinguish it, by that time, from another intensively circulating pandemic encephalitis, the "von Economo encephalitis", initially named "Encephalitis Lethargica" (von Economo, 1931), and named afterward "type A encephalitis". Then, in 1933, the virus responsible of JEB was reisolated and ultimately characterized in 1934, when it was experimentally inoculated into monkey brain and successfully reproduced the disease (Rosen, 1986; Showa in Seiichi & Teizo, 2002[1]; Hayashi, *in* Asim A. Jani, 2009[2]).

From Japan, until the late 1990s, Japanese encephalitis virus (JEV) was known to actively circulate in South East Asia, extending its eastern range towards Korea, Chinese mainland, Taiwan and Philippines, and then further West towards India and Pakistan. Major epidemics occurred in the 1960s and JE appeared endemic within the Indochinese Peninsula including Cambodia, Laos, Thailand and Vietnam, and further on to Malaysia, Burma, Singapore (rare cases), Brunei (Erlanger et al., 2009). Then, within the following four decades, JE occupied subsequently most of the Asian continent from Pakistan to Sri Lanka on the east of its range (Igarashi et al., 1994; Solomon et al., 2000; Nga et al., 2004; van den Hurk et al., 2009) and then Bangladesh, Nepal (Terai region). Furthermore, in the mean time, JEV emerged among unaffected area of Asia as in Papua New Guinea, Far East Russia (maritime Siberia), and subsequently crossed the Torres Strait toward Northern Australia where it was isolated and emerged for the first time within the Australian continent (Hanna

[1] Seiichi Iwao, Teizo Iyanaga, *Dictionnaire historique du Japon, Volume I*, Maisonneuve & Larose, 2002 (ISBN 9782706815751) p. 441

[2] http://emedicine.medscape.com/article/233802-diagnosis

et al., 1999). Limited outbreaks were also reported from the two Western Pacific Islands of Guam and Saipan, respectively in 1947-48 and 1990, but the enzootic cycle might be not sustainable and the virus was therefore very probably introduced there (Richards et al., 2010; Fisher et al., 2010).

1.2 Japanese Encephalitis epidemiology

In nature, JEV is essentially transmitted by *Culex* mosquito species to wild and domestic birds and pig herds. In the transmission cycle, humans are accidental and dead-end hosts. The most prevalent vector for human infection is *Culex tritaeniorhynchus* which breeds in pools of stagnant water such as rice fields (Keiser et al., 2005; Richards et al., 2010). However, about fifteen other mosquito species belonging to genus *Anopheles*, *Aedes*, *Armigeres*, *Mansonia* and *Culex* and other species of true flies are recognized to carry the virus, but all of them are not equally competent to transmit the virus to new hosts (Mackenzie et al., 2007). Moreover, pigs are attractive hosts that are generally asymptomatic and are important virus amplifiers. As a consequence, human living close industrial or familial piggeries are at higher risk of transmission (Nitatpattana et al., 2011).

1.3 Japanese Encephalitis syndrome

In terms of human morbidity and mortality, JE is one of the most important and widespread causes of arboviral encephalitis worldwide, with an estimated 35,000 to 50,000 cases and 10,000 deaths annually in Asia (Tsai, 2000). It is estimated that three billion people are at risk of infection. Human infections are generally asymptomatic (1 in 1000 cases), while 25% of symptomatic infections will present brain inflammatory signs and, among them, a quarter may result in permanent neurological and psychiatric sequel with a 25% mortality rate (Vaughn & Hoke, 1992; Solomon & Vaughn, 2002).

1.4 Japanese Encephalitis Virus

JEV is a *Flavivirus* of the *Flaviviridae* family and a member of the Japanese encephalitis eponym serogroup, including ten antigenically related virus species as: Alfuy, Koutango, Kokobera, Kunjin, Murray Valley encephalitis, Japanese encephalitis, Stratford, Usutu, West Nile and St. Louis encephalitis viruses. JEV consists of a lipo-glicoprotein envelope surrounding a nucleocapsid of a single-stranded positive-sense RNA of 11-Kb nucleotide. A single open reading frame is flanked by two 5′ and 3′ untranslated regions (UTRs), and carry genes coding for structural proteins including a capsid (C), a membrane (cleavage product of a pre-membrane protein PrM), an envelope (E), and seven nonstructural (NS) proteins (Sumiyoshi et al., 1987). Based on nucleotide sequencing of C/PrM and E genes, four virus genotypes have been distinguished (Chen et al., 1990; Chen et al., 1992): genotype I (GI), genotype II (GII) and genotype III (GIII) are distributed all over the geographical area of Asia, and genotype IV (GIV) includes isolates from Eastern Indonesia (Solomon et al., 2003). In addition, a JEV strain named Muar, isolated once in Singapore from a patient who originated from Malaysia, may represents a fifth genotype (GV) (Hasegawa et al., 1994; Uchil & Satchidanandam, 2001). Although this fifth genotype remained uncertain, it has been recently argued that it would be the most genetically different from the other JEV four genotypes (GI to GIV) (Mohammed et al., 2011). Furthermore, this fifth genotype is believed to be the oldest of the JEV lineage which would have originated from an ancestral virus in the mid 1500s in the Indonesia-Malaysia region. As suggested by Solomon et al. (2003), the

JEV is supposed to have evolved in other genotypes from the most divergent and probably oldest genotypes (GIV and GV), that have only be found in Indonesia-Malaysia region, and that JEV has spread from this region across Asia.
Although it was classically accepted that genotypes I and III occurred mostly in epidemic regions (temperate region of Asia), while GII and GIV were associated with endemic disease (tropical region), and ultimately postulated that differences in strain virulence could explain the clinical epidemiology (Chen et al., 1990; Chen et al., 1992; Williams et al., 2000), limited experimental assay on mice were not conclusive (Solomon et al., 2003). Moreover, several more recent observations tend to discard this hypothesis and suggest that genotypes could be found indifferently within former epidemic or endemic area. Thus, two JEV strains belonging to GI were isolated in the Australasian region (Pyke et al., 2001) and strains of the genotype II have been shown to use to circulate in Korea before 1970's (Schuh et al., 2010, 2011).

1.5 Japanese Encephalitis vaccine

Currently, three JEV vaccines are in use (Guirakhoo et al., 1999 ; Chang et al., 2000): (i) a formalin-inactivated, mouse brain-derived vaccine, based on the wild-type Nakayama or Beijing-1 strains, has been used internationally since the 1960s; (ii) a cell culture-derived inactivated vaccine; (iii) a cell culture-derived live attenuated vaccine, in China only. All vaccine types were developed from GIII JEV strains. Vaccine campaigns dramatically reduced the burden of the disease particularly in Japan, Korea, Taiwan, Sri Lanka, Thailand and Vietnam.

2. Materials and methods

2.1 Data collection

Among 563 wild strains investigated, 512 Japanese encephalitis virus strains could be fully characteristics from the available literature, GenBank Nucleotide database (www.ncbi.nlm.nih.gov/nuccore/), and possibly from Virus Sequence Database (http://kcdc.labkm.net/vsd/) and the database from Journal of General Virology (vir.sgmjournals.org/content/vol90/issue4/images/data/827/.../TableS1.xls). The literature review was achieved by using both generalist web search engines such as Google and Google Scholar, and scientific web search engines such as PubMed, Springerlink, ScienceDirect and Web of Science. It aimed at identifying an exhaustive panel of different JEV strains, and also at gathering a maximum of information on them as for date of sample collection and/ or virus isolation, site of collection, country, host, genotypes, sequence used for genotyping, GenBank accession number, and original reference when available. Keywords used for searching were: Japanese encephalitis virus; genotypes; genome; strains; phylogeny; molecular; epidemiology. Several different combinations and different suffixes were used; for instance, we used genom(e) as well as genom(ic). Then, articles were selected depending on their degree of relevance for the study, which were initially evaluated through the examination of the abstract. Then, to be selected, each article needed to meet the following criteria:

- Study of wild strain of Japanese encephalitis virus and
- Availability of the main features of the strain(s) including circumstances of viral isolation (i.e.: country, host, year of isolation and/or collection), and virological and molecular characteristics (i.e.: genes of interest, sequences).

The year of sample collection was a key data for the temporal analysis of virus genotypes dispersion and a particular attention was required to identify the year (and the site) of sampling collection, the year of virus isolation, and the year of publication.
However, for the selection, no particular attention was paid to the year of publication of articles. Nevertheless, since molecular techniques used for genetic, phylogenetic and epidemiologic studies are quite recent, the most recent articles from mid 90's to 2011 were deeply reviewed. These articles were especially interesting because they integrate isolates that were previously characterized, to increase the relevance of their analysis. Language accepted was English. When available, abstract in English of studies published in other languages were integrated to our review when they provided isolate name, of which characteristics were presented in GenBank database.
All available sequences of interest for genotyping were crossrefered with the corresponding one of GenBank, allowing checking for accuracy of the data presented in those articles, and consequently imprecision or even mistakes were identified and corrected.
The number of times that each strain was cited was recorded, also JEV strain descriptions were carefully compared as many studies often used the same strains and, in some instances, with different name.
On the one hand, this analysis aimed at enriching the total amount of specific data for each strain. On the other hand, it was used to identify erroneous data introduced by authors during the process of data collection from the original sources.

2.2 Identification of homogeneous regions

Countries were grouped together into distinct regions depending on biological, physical, anthropogenic and geographic factors. First, geopolitics criteria were used to associate several neighbouring states such as India and Sri Lanka. Secondly, since the epidemiology of JEV is strongly dependant on agroclimatic features (Solomon et al., 2000; Bi et al., 2003; Keiser et al., 2005), land use and climatic conditions were taken into account. In the context of climate change and rapid expansion of human activities, many studies have been conducted for the last two decades and have provided a large amount of updated information on these topics. On the one hand, regions that are characterized by the same vegetation and the same climatic conditions were defined as homogenous ecoregions (Olson et al., 2001). On the other hand, land use, and in particular irrigated and paddy fields areas, was considered, given the strong relationship existing between rice field density and mosquito populations, especially *Cx. tritaeniorhynchus* (Richards et al., 2010), and given the role of flooded rice system on JE outbreaks (Akiba et al., 2001; Keiser et al., 2005). Regarding both ecoregions and land use, we determined regions, which were also consistent with those proposed by Solomon et al. (2003). Moreover, since China is a very large country consisting in multiple different biomes ranging over the territory, for which the classification has been subject to several studies (Ni, 2001), it was divided in four parts, two of them being then integrated into already defined regions, one of them constituting one single region. The last area, including the Tibet, Xinjiang and Qinghai Provinces, was considered irrelevant for our study because JE is absent and no strain was collected there[3]. Grouping of Chinese provinces was achieved following precipitation variation, land use and ecoregions.

[3] Fischer et al., 2010 In: Travelers' Health - Yellow Book, Chapter 2: Japanese Encephalitis; available at wwwnc.cdc.gov/travel/yellowbook/2010/chapter-2/japanese-encephalitis.aspx

3. Results

3.1 Statistics on data collected

45 relevant articles from 1984 to 2011 were used for the data retrieval, of which most of the first author affiliation was Japanese, Chinese, Indian, Australian, South Korean and US American (Fig. 1).

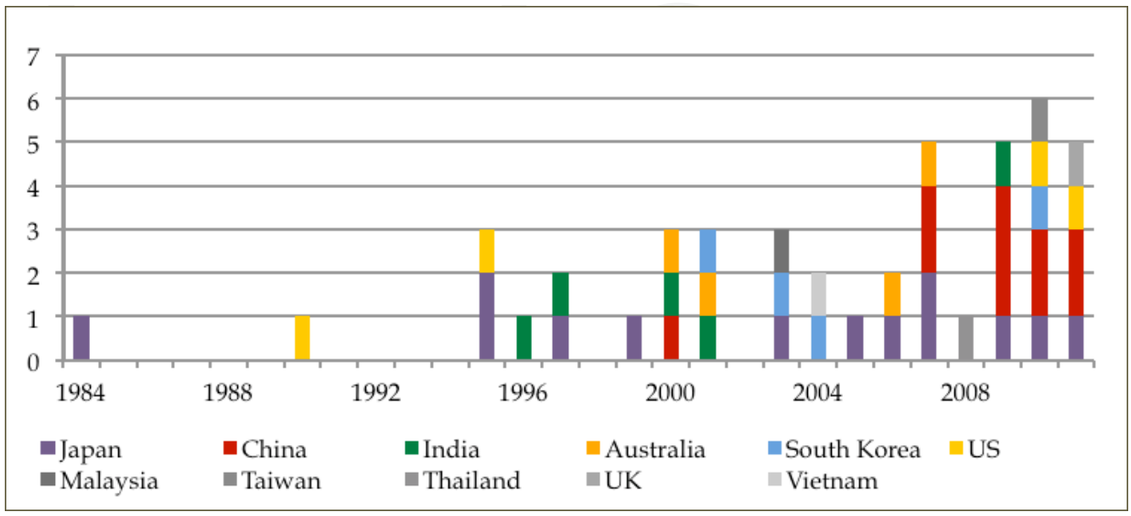

Fig. 1. Temporal distribution of the 45 selected articles and the country of their first author affiliations

A total amount of 2113 strains were integrated to these articles, among which 591 were strictly different wild strains. Vaccine strains were all excluded. Number of appearance of strains among studies ranged from 1 to 44, Beijing-1 being the strains the most cited. On the 591 strains available, 530 (89.7%) were referenced in the GenBank Nucleotide database.

In parallel, 886 sequences of 686 different strains were extracted from the GenBank Nucleotide database. Therefore, thanks to the cross-reference between all resources available, 564 distinct strains were investigated. Among them, the year of collection of 537 (95.2%), the country of collection of 562 (99.6%) and genotype of 526 (93.3%) strains were available. Complete information on 1/ the year of collection, 2/ the location of collection and 3/ genotypes was achieved on 500 (88.7%) strains. As we considered 20 years-time period, the amount of strains reached 511 (90.9%). Furthermore, while parts of countries or whole countries were integrated to larger region (see below 3.2 Region identification), three Chinese JEV strains that failed at being localized in an administrative division were unuseful. Eventually, 508 (90.1%) wild strains were integrated to our study on the spatiotemporal evolution of JEV genotypes in Asia.

Strains were collected from 1935 to 2009 in 16 countries of South Asia (India, Nepal and Sri Lanka), East Asia (China, Japan, South Korea, Russia, Taiwan, the Philippines, and Vietnam), of Southeast Asia (Cambodia, Thailand, Indonesia and Malaysia) and Northern Australasia (Papua New Guinea, the Torres Strait islands of northern Australia and northern Australia mainland) (Fig. 2). Number of strains collected in each country ranges from 1 to 158 (Japan). With Japan, China and Taiwan were the countries where larger amount of strains was collected, respectively accounting for 28.1%, 24.4% and 16.4% of the 562 strains for which location was available.

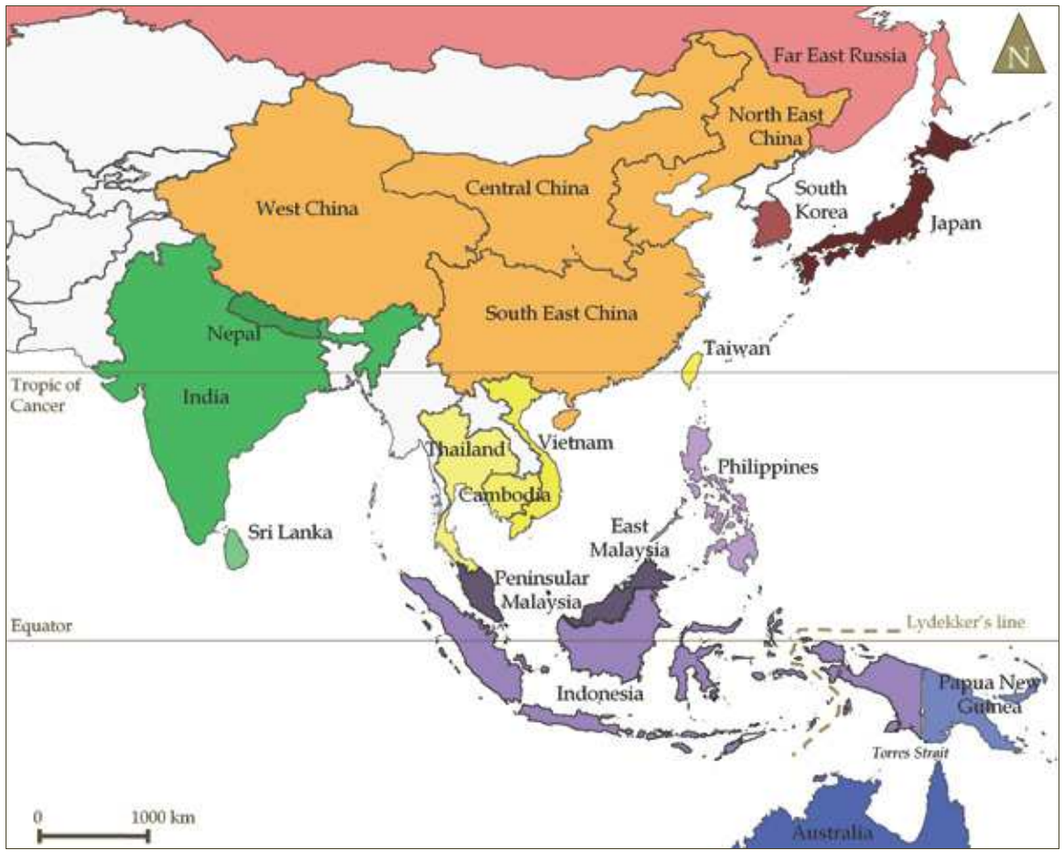

Fig. 2. Map of Asia: the 16 countries from which 562 JEV strains were collected between 1935 and 2009. Each shade of colour corresponds to one of the seventeen countries

3.2 Region identification

Regarding biotic, abiotic and anthropogenic features, we classified the 16 countries into six different regions (Fig. 3). First, the biogeoraphic realms separated the countries in three areas: Paleoarctic, Indo-Malay and Australasia realms (Olson et al., 2001). The Paleoarctic realm comprised Russia, Japan, Korea and northern part of China and was considered as the first region.

Within the Indo-Malay realm, Indian peninsula was separated from the Indochinese peninsula following a geographic east-west separation. On the east, the Indian peninsula included India, Nepal and Sri Lanka, plus Bangladesh, Buthan and Pakistan, and represented the second region. Further west, the Indochinese peninsula comprising Thailand, Cambodia and Vietnam plus Laos and Burma, and South China and Taiwan, were considered as the third region characterized by tropical climate and large cover of rice fields (Xiao et al., 2006). Then, peninsular Malaysia, east Malaysia and Indonesia constituted, with the Philippines, the fourth region. However, since Indonesia is a very fragmented country composed by very numerous islands stretching over 4,500 km from east to west and over 1,500 km from north to south, we considered the Lydekker's line to separate Australasia and the Oriental realm. Indeed, the Lydekker's line snaking through the Indonesian islands represents one of the ecological boundaries between the Sunda Shelf and the Sahul Shelf, which illustrates a great difference of biodiversity between ecosystems under similar

climatic conditions (Cox, 2001; van den Bergh et al., 2001). Consequently, on the opposite side of the Lydekker's line, northern Australian mainland, Torres Strait Islands, Papua New Guinea were associated with the West Indonesian provinces of the Western New Guinea region, and therefore constituted the fifth region.

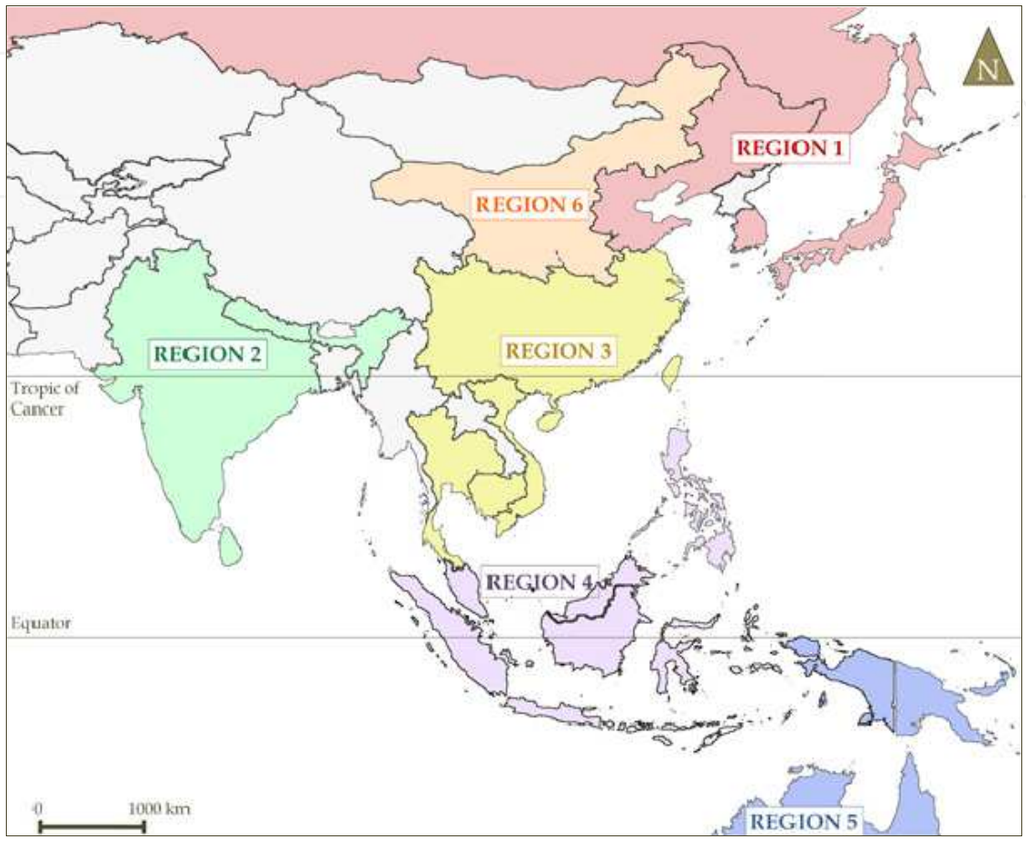

Fig. 3. Map of Asia: the six regions.
Region 1 in red; Region 2 in green; Region 3 in yellow; Region 4 in purple; Region 5 in blue, Region 6 in orange

China: as presented above, we defined an area on the south of the country as belonging to the same region than Vietnam, Thailand and Cambodia. This region was part of the Indo-Malay realm (Olson et al., 2001) and was mostly characterized by high precipitation and a tropical climate with high rice crop production (Frolking et al. 2002; Xiao et al., 2005). South China included the 14 (51.2%) following provinces (or other administrative divisions): Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hainan, Hunan, Hubei, Jiangsu, Jiangxi, Shanghai, Sichuan, Yunnan and Zhejiang. Further north, 6 provinces (22.2%) (Gansu, Henan, Inner Mongolia, Ningxia, Shanxi and Shaanxi) represented the sixth and last region and were characterized by lower rice crop production in temperate climate and higher altitude of Central China. Finally, the last 7 (26.0%) northern provinces of the country (Beijing, Hebei, Heilongjiang, Jilin, Liaoning, Shandong and Tianjin) were associated with Far East Russia, Japan and Korea peninsula, all under temperate environment (Olson et al., 2001). Altogether, these six regions were used to describe the temporal evolution of the JE genotypes (Fig. 3).

3.3 Historic of the JEV genotypes in Asia

Over the 80 years of survey, from 1930's to 2000's, JEV have been actively circulating in Asia (Fig. 4). Historically, JEV was first identified in 1935 in Japan (Table 1).

Regions	Countries	Genotypes					Number of strains
		I	II	III	IV	V	
Reg. 1	Japan	1991	1959	1935			158
Reg. 1	Far East Russia			1943			1
Reg. 1	South Korea	1991		1946			37
Reg. 1	North East China	2002		1949			15
Reg. 2	India			1956			23
Reg. 2	Sri Lanka			1969			3
Reg. 2	Nepal			1985			3
Reg. 3	South East China	1979		1954			100
Reg. 3	Taiwan	2008		1958			92
Reg. 3	Vietnam	2001		1962			18
Reg. 3	Thailand	1979	1983	1964			40
Reg. 3	Cambodia	1967					2
Reg. 3	Hong Kong		2000				1
Reg. 4	Malaysia	1994	1968	1965		1952	18
Reg. 4	Indonesia		1978	1979	1980		15
Reg. 4	Philippines			1984			1
Reg. 5	Australia	2000	1995				10
Reg. 5	Papua New Guinea		1997				3
Reg. 6	Central China	2004		1954			18

Table 1. Year of collection of the first strain of each genotype in the 6 regions and 19 areas of Asia

Then the virus was detected in Far East Russia, South Korea and China (1940's), then in Malaysia, India and Taiwan (1950's), then Vietnam, Thailand, Cambodia and Sri Lanka (1960's), Indonesia (1970's), Philippines, Nepal (1980's), Australia, Papua New Guinea (1990's) and finally in Hong Kong in early 2000's. Thus, after the first isolation of the virus in 1930's, a large amount of strains was isolated during the following decades. Their genotype

was usually defined through the comparison of sequences between representatives of each genotype and the newly collected strains, from which the degree of homology was extracted. Often in studies, a phylogenetic tree is also constructed. Thus, from 1930's to present (2009), many strains have been collected and have clustered within genetic groups.

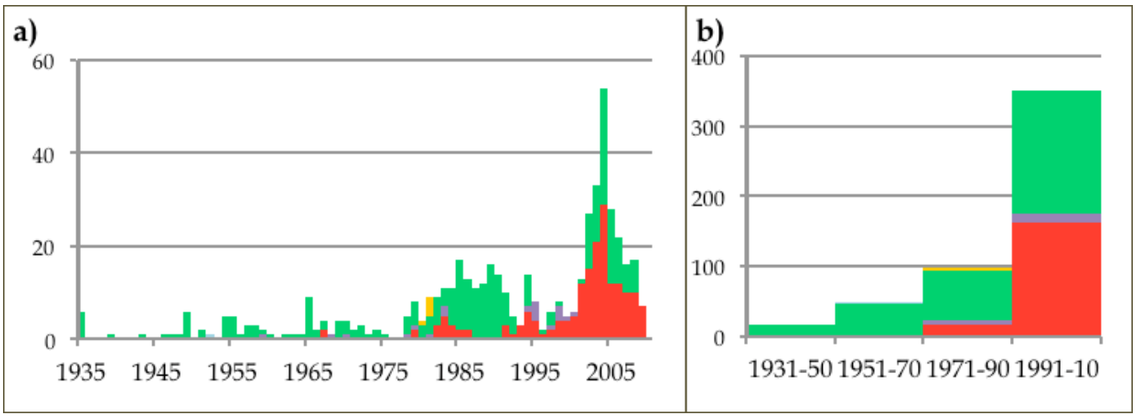

Fig. 4. Number of strains collected and their genotypes: a) from 1935 to 2009; b) during the four 20-years time periods (1931-1950, 1951-1970, 1971-1990 and 1991-2010).
Genotype I in red; Genotype II in purple; Genotype III in green; Genotype IV in gold; Genotype V in light blue (one single strain, 1952)

In figure 4, it appears that until the 80's, no more than 10 strains were collected each year. Then, from 80's to early 90's, a first peak of 17 strains was recorded, in 1985, mainly due to strains from regions 1 and 3. After a 7-years period with relatively constant and low strains collection, another pick was recorded, in 2004. Much higher than the previous one, this peak reached 54 strains, which were collected in region 1 and 2, but above all in region 3 (40 (75.5%) strains, of which 23 (57.5%) originated from South East China). By considering 20-years time periods, the amount of collected strains clearly increased, seemingly in an exponential way.

Spatially, genotypes that have been identified differed between regions and every genotype have not been found in each Asian region yet. As an example, although India-Sri Lanka-Nepal Region (Region 2) has displayed one single genotype (III), strains of the five genotypes have been collected from the Indonesia-Philippines-Malaysia Region (Region 4) (Table 1). In fact, Region 4 is the only one that has been showed to house all the genotype and the only region where genotypes IV and V have been identified.

Moreover, genotypes evolved in time, with genotype III being the predominant genotype until early 1990's, and being progressively replaced by genotype I by then, especially in China, Taiwan, Korea and Japan (i.e. regions 1, 3 and 6) (Fig. 4 and 5).

Contrary to regions 1 and 3 where many strains have been collected (respectively 186 and 240, counting for 85.7% of the 497 strains reported), relatively few strains were found in the remaining four regions (Fig. 5). Thus, region 1 and 3 predominantly contribute to the yearly variation of the number of strains collected. As these two regions comprise the most developed countries with the most efficient epidemiological survey, the variation is expected to reflect the real incidence of JE. Consequently, the figure 5 shows that JEV has intensively circulated in regions 1 and 3 during the last decades.

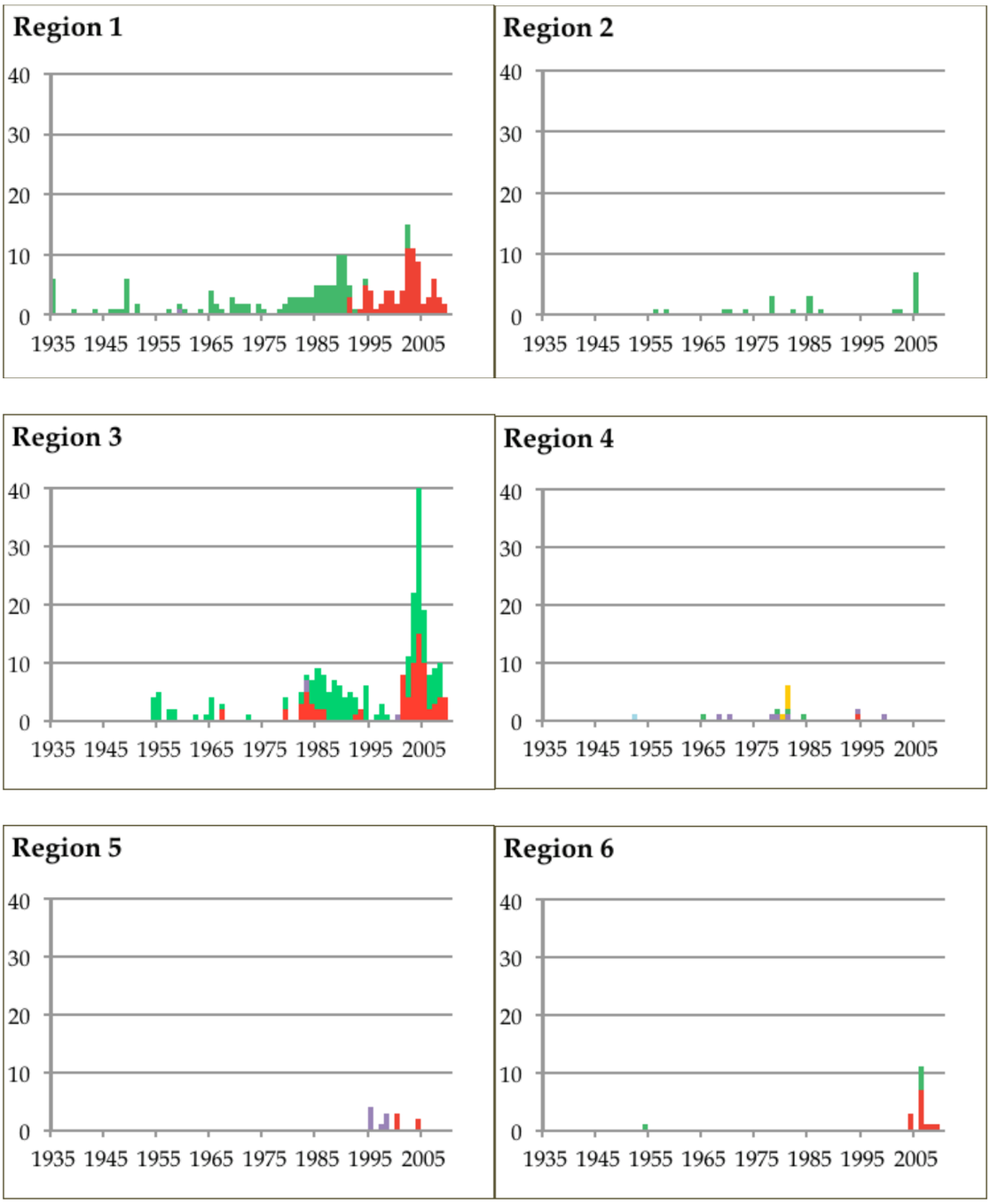

Fig. 5. Temporal evolution of the number of strains collected and their genotype among the fully characterized within the 6 regions. The colour code of the 5 genotypes is the same than in Fig. 4

In region 2 and 4, whereas few strains was collected and isolated, JEV seems to have circulated from early 1950's. Interestingly in region 2, in five instances, each one approximately separated by 5-10 years, a few and lowly variable strains was collected, suggesting a periodic increase of the amount of JEV strains, but quite constant. In region 4, a

peak period of circulation was recorded between 1975 and 1985, suggesting that apart from this 10-years period, JEV circulated less actively even if additional strains were still isolated before and after. In region 6, one single strain was isolated before 1955, and after about 50 years without any other strain, several have been collected since 2004. In region 5, the first strain was isolated in 1995. From then, strains have been regularly isolated until 2004.

3.3 Dispersion of the genotypes

The following four figures show how JEV genotypes changed during the four 20-years time periods (Fig. 6a, b, c, d).

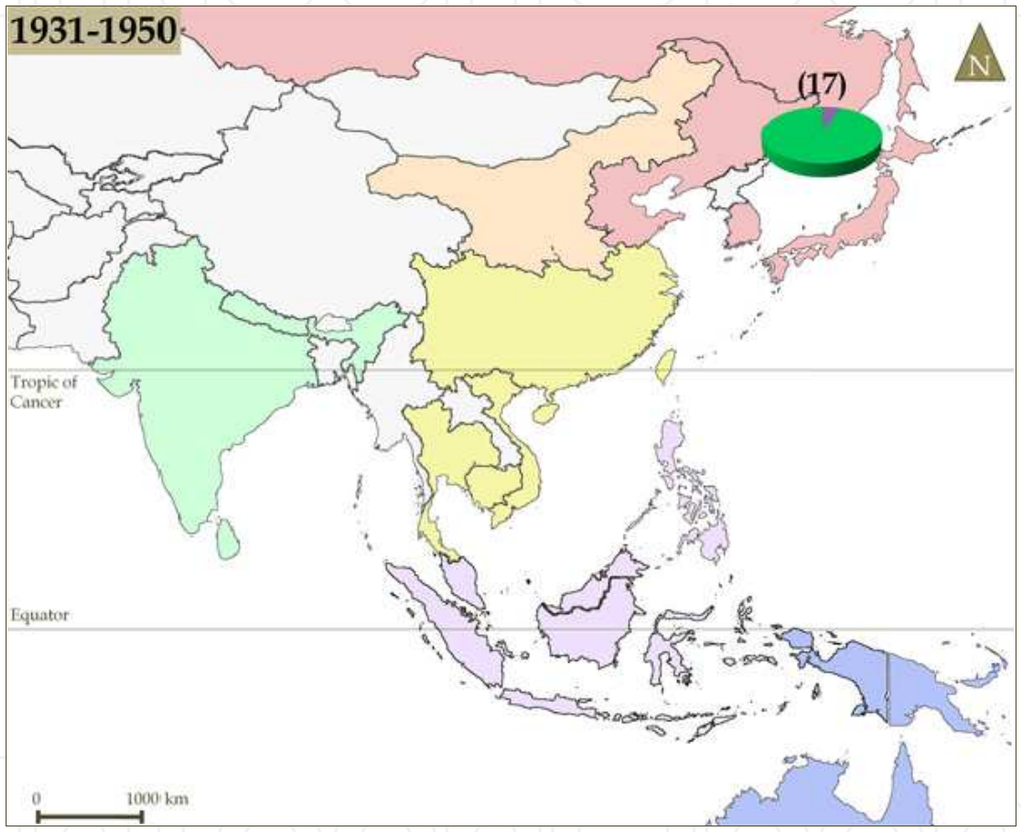

Fig. 6a. Evolution of the proportion of each genotype identified in every region from 1931 to 1950. The total amount of strains collected in every region is in brackets. Regions are coloured as in Fig. 3. Pie charts represent the proportion of each genotype in every region. The colour code used for the genotypes is the same than in Fig. 4 and 5. When no pie chart is presented, no strain was collected in the corresponding region

Between 1931 and 1950 (Fig. 6a), JEV strains were isolated from region 1 only. Among the seventeen strains characterized, about 95% belonged to genotypes III, the remaining ones belonging to genotype II. Then, from early 1950's, strains have been isolated from every areas of Asia, apart from Australasia. On the 47 strains isolated between 1951 and 1970, more than 95% still belonged to genotype III (Fig. 6b). Interestingly, the first, and single, isolation of strain from genotype V was performed in region 4. Rather than illustrating a dispersion of JEV from further north region of Asia towards south-eastern and southern

Asia, our results suggest that from 1931 to 1970, JEV did circulate in the whole continental Asia. Indeed, the proportion of the genotype III did not change within the 40-years period of time (stabilized around 95%), and if considered as control region, the region 1 displayed the same profile: numerous strains from genotype III and a few from genotype II.

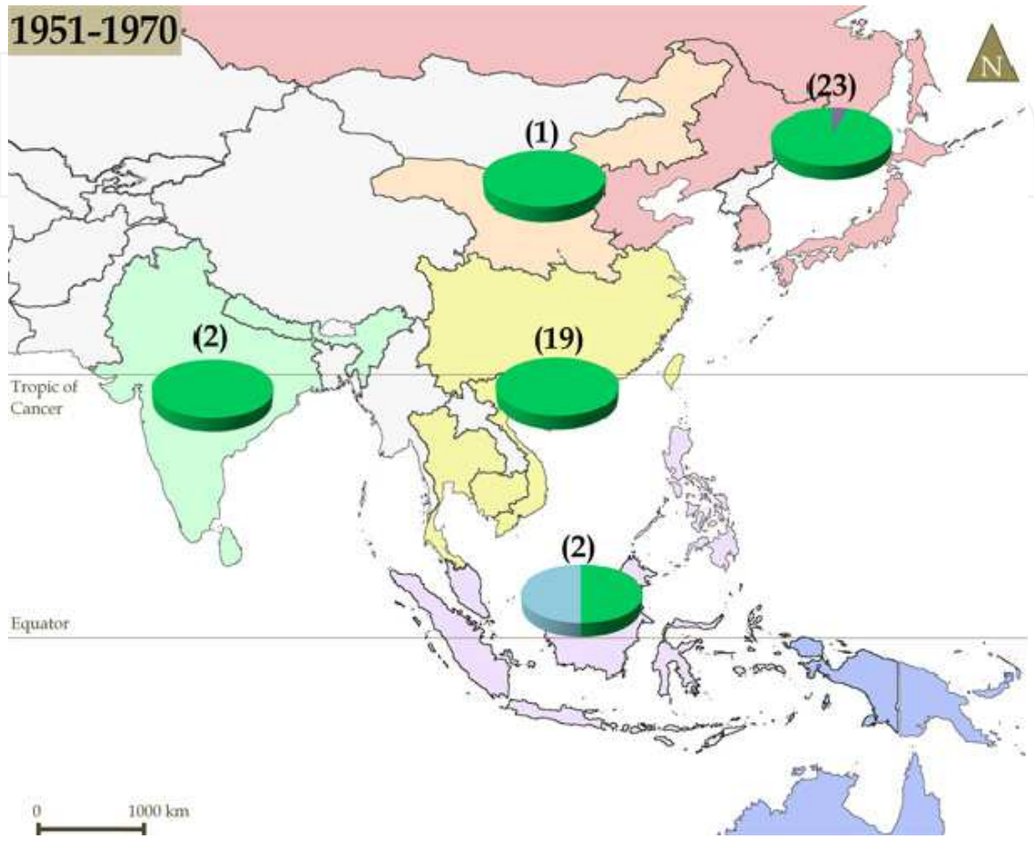

Fig. 6b. Evolution of the proportion of each genotype identified in every region from 1951 to 1970. The legend is the same than if Fig. 6a

Between 1970 and 1990 (Fig 6c), 98 strains were collected, doubling the amount of strains collected between 1951 and 1970. While strains still originated from regions 1, 2, 3 and 4, none was isolated from region 6. Also, for the first time, several strains of genotype IV were collected in region 4. Although genotype II had only been found in the northern region 1 since 1930's, several strains were collected both in region 3 and 4.
More interestingly, proportion of genotype III started to decrease from 95% to 70% between 1971 and 1990, while the new genotype I appeared in region 3 and reached 17%, becoming the second most important JE genotype in Asia. This tendency was then confirmed during the last 20-years time period, between 1990 and 2010 (Fig. 6d), when genotype I reached 46% against 50% for genotype III. However, this change did not occur widely in Asia. Indeed, in region 2, genotype III has ever been the only one that could be identified, and genotype I has seemed to be geographically limited to Eastern areas of Asia and in Australasia. By considering these five eastern regions only, genotype I and III became equally important (50%-50%). Furthermore, temperate areas (regions 1 and 6) were markedly different than tropical areas (regions 3, 4 and 5).

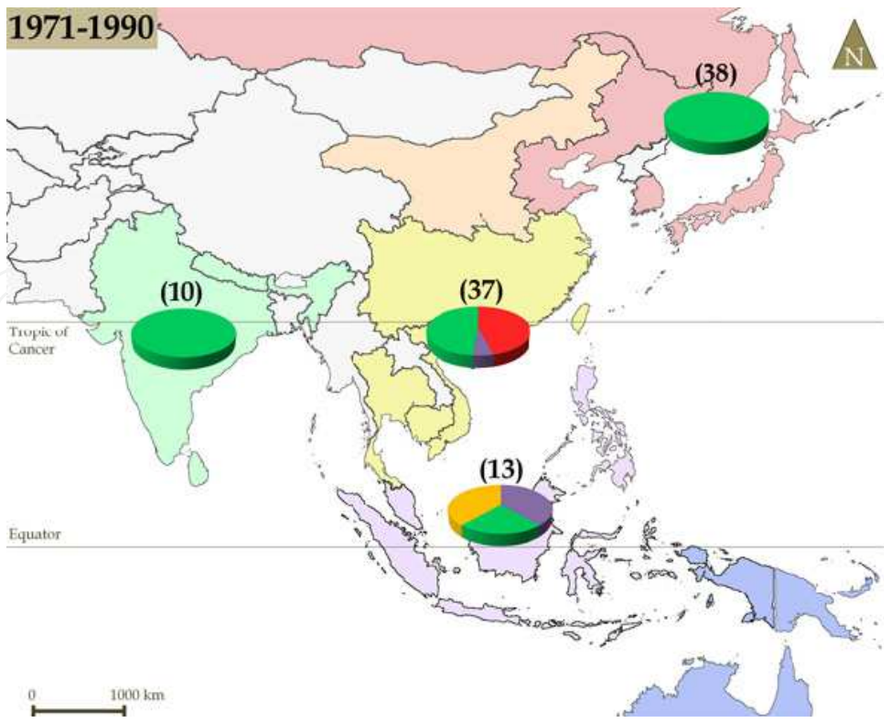

Fig. 6c. Evolution of the proportion of each genotype identified in every region from 1971 to 1990. The legend is the same than if Fig. 6a

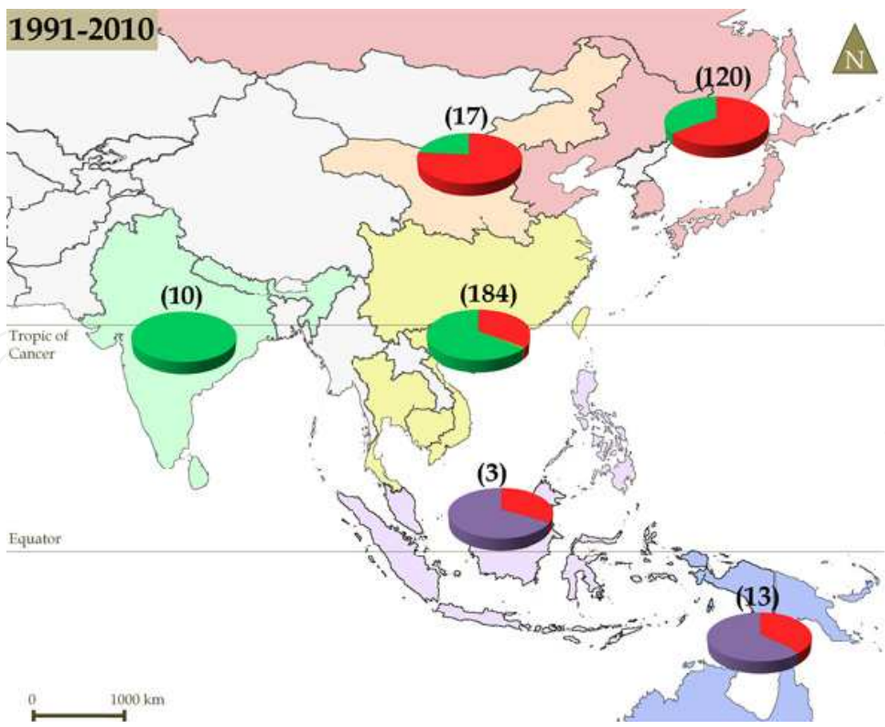

Fig. 6d. Evolution of the proportion of each genotype identified in every region from 1991 to 2010. The legend is the same than if Fig. 6a

Whilst in these three tropical regions, genotypes I, II and III respectively counted for 35.5%, 5.5% and 59%, in temperate regions (1 and 6) genotypes I and III respectively counted for 66% and 34%, making genotype I the most important genotype.
On top of that, for the really first time, JEV strains were obtained from the Australasia region (region 5), firstly in Papua New Guinea and Torres Strait Islands, and secondly in Australia mainland. Genotype II was the first isolated and then genotype I appeared, in lower proportion however (38% against 62% for genotype II during this period).

4. Discussion

More than 500 JEV strains were used to understand the spatio-temporal evolution of JEV genotypes since the disease was detected. Characteristic of strains such as the year of collection and isolation, the exact place it originated from and the genotype it belongs to are not always properly presented in articles. Also, retrieval of the original source of strain isolation very often failed, partially because of the use of more recent references. As a consequent, the scientist who detected a strain for the really first time becomes progressively forgotten. However, the original source of strain sequencing was better conserved. Moreover, a doubt remains on the year. The year of isolation is more often cited in studies (for instance Pyke et al., 2001) while in GenBank database, the year of collection is sometimes given (for instance strains LY5P-09 and YN114, respectively presented in Zhang et al., 2011 and Wang et al., 2010). Even, in other articles, the term year alone is used and serves as an element of description of the history of the strains (for instance Yun et al., 2003). Thus, in our study, we considered the year presented in studies as being the year during which the strains was proven to circulate.
Also, in time, sequencing techniques have improved in the same time that database of sequenced strains enlarged. At that time, more strains were available and molecular and statistical tools allowed reinforcing phylogenetic analysis. However, in some instances, genetic characterization of strains led to different results. As an example, strain B2239, collected in 1984 in Thailand (GenBank accession number U70391) clustered in genotype II (Pyke et al., 2001) and I (Nabeshima et al., 2009). Furthermore, strains JKT6468, collected in 1981 in Indonesia (GenBank accession numbers AY184212, U70407 or L42162) was used in 21 different articles and was suggested to belong to genotype II once (Yang et al., 2004), III once (Yun et al., 2003), IV fifteen times (Huang et al., 2010; Fan et al., 2010). Consequently, we choose to keep the genotype that was the most often and most recently obtained. Finally, we had to eliminate several incomplete strains to our database and used the other to evaluate the spatio-temporal evolution of the genotype over the Asian and Australasian continents.
Since its detection in 1930's, Japanese Encephalitis Virus has continuously circulated in Asia for about seven decades. First isolated in Japan, many JEV strains have been collected since. Despite large vaccination campaigns in developed countries such as Japan, Taiwan or even Thailand, the disease has never been eliminated from any country and human cases still regularly occurs. Furthermore, JE progressively spread in new Asian areas and progressively enlarge its distribution area. Restricted to north-eastern areas of Asia at first, the disease then invaded a majority of the countries of South East Asia, South Asia, East Asia and finally Australasia. Of course, the disease might have not been detected from developing countries in early period because of a lack of health services and epidemiological survey. Especially, the differences in survey effort, virus isolation techniques and public

health services very probably explain why no strains had been characterized before 1950's outside region 1. As evidence, before 1970, most of strains (52.8%) were isolated from human hosts, suggesting that public health services were the first tool used to identify the virus. Thus, in countries where health care was not as well-developed as, for example, in Japan, several human cases may have failed at being diagnosed and virus at being detected. On the contrary, since 1970's, the proportion of strains that have been isolated from mosquitoes and pig has strongly increased, reaching 81.1% while the proportion of strains extracted from humans has fallen to 17.6%. Regarding the serious effects of JE on human health, better attention has been progressively paid to the disease. Thus, this important change of strains sources illustrates the epidemiological survey effort that has been developed in Asia, also confirming that the number of strains collected increased with time partly thanks to improving survey. Therefore, it is more likely that less human cases stayed undetected from 1970's and most of areas where JE outbreaks occurred were recorded.

Even if epidemiological survey improved in time the number of strains that were yearly collected may have not totally reflected the number of strains really circulating until 1970's. However, in a few countries where survey effort has been constantly maintained since the first identification of the JEV, the variation of strains amount is expected to be close to what really happened. Thus, Japan, Korea, India or even Australia can be considered as witness of the temporal evolution of the total amount of JEV strains collected, whereas that does not cover every region. Since it appeared that the increase of collected strains was exponential, the effect of epidemiologic survey effort cannot be easily distinguished from the real increase in JEV circulation. Yet, the examination of the temporal evolution of the amount of strains detected in Japan shows that a few strains were annually present from 1930's to mid-1980's and during 1990's. On the contrary, two peaks of abundance of strains, signs of epidemic manifestations, occurred between mid-1980's and 1992, and then between 2000 and 2009. Moreover, the second peak was higher than the previous one, enforcing the assumption of a general increase of the number of strains circulating. Consequently, JEV most intensively circulated during the last two decades, especially in region 1, 3 and 5, where it appeared for the first time. In region 4 and 6, weaker epidemiologic survey effort were made, and strains, which have been scarce and have temporally scattered there, may have failed at illustrating the real circulation of JEV (see also Solomon et al., 2003). In Central China, drier climate with less developed irrigated agriculture such as rice (Frolking et al., 2001), less important livestock and human population compared to other parts of China do not provide the best conditions for the JEV vectors to breed, and for the JEV to amplify and be transmitted to human. Also, provinces of this area are poorer than coastal provinces. Therefore, until 1990's and the rise of molecular techniques of sequencing, less attention could have been paid to this region for decades, despite an existing risk. In region 2 however, the effort of survey seems to have been maintained since 1970's, and the periodic and constant re-emergence of JEV strains is likely to point out a less intense activity of the virus in South Asia compared the East Asian countries. Also, genotype III is the only one to have ever been found in this region, suggesting that the strains pool little evolutes. Future constant efforts in every country of the Asian region would provide a better insight. As an effective tool, the use of sentinel animals in survey would improve the assessment of the risk of JEV transmission to human. In particular, serological survey on domestic pig herds, which are the main reservoir amplifying hosts of the virus and are

widely raised in Asia, would be a means to monitor the JEV activity (Nitatpattana et al., 2011).

However, evidence of recent introduction of the virus was demonstrated. In Australia, intensive surveillance has existed for long. Thus, JEV strains are unlikely to have not been detected before mid-1990's, and the disease has obviously extended its geographical range. The way the virus is introduced is subject to debate. On the first hand, some researchers believe that infected vectors, mostly mosquitoes, may be transported by cyclonic winds. This theory was presented to explain the crossing of the Torres Strait towards Australia mainland (Ritchie & Rochester, 2001) or the several introductions of strains from China towards Japan (Nga et al., 2004). On the other hand, the virus may be transported in long distance by migratory birds, as it has also been suspected to explain the numerous introductions of JEV strains in Japan from China or in northern areas of Asia from the Indochinese peninsula (Nga et al., 2004). Whatever the reason is, these findings show that JE is an emerging-remerging disease which may colonize new areas, whilst trades of goods, as well as transport of people are in constant development and increase the probability of the virus to be introduced in vectors or animals.

In parallel of the increasing of JEV strains and dispersion towards new Asian areas, genotypes changed. After that genotype III remained the major genotype until 1990's, it is very obvious that genotypes I, which emerged in every region of Asia except in South Asia (Nepal, India and Sri Lanka), became the very major genotype in northern temperate regions (i.e. regions 1 and 6 essentially constituted by Japan, South Korea, and North East China, none strain having been isolated from Far East Russia since 1943). Genotype I originated from the Indochinese peninsula in late 1960's and circulated at first in Cambodia, Thailand and South East China by the end of the 1970's, before it enlarged its geographical distribution to further north and southeast areas. Genotype I also indicates a difference of activity between temperate and tropical regions. Indeed, while genotype I invaded tropical Asia (Indochinese peninsula, Philippines, Indonesian-Malaysian region and Australasia) without becoming the main genotype, the major genotype has clearly shifted from genotype III to genotype I in Japan (Ma et al., 2003), South Korea (Yun et al., 2010), North East China (Wang et al., 2007a) and Central China (Wang et al., 2007a), since early 1990's. Furthermore, whereas JEV strains of genotype I were detected, none isolate was obtained from human patients before the second half of the 2000's. Unfortunately, by then, strain has been detected from human, throwing the efficiency of the vaccine used until then into question (Wang et al., 2010). Indeed, existing live or attenuated vaccines used to be derived from genotype III strains (Zhang et al., 2011). Since the infectivity and pathogenecity of JEV genotype I strains may be different to genotype III, the vaccines may become less protective for human, therefore pointing out the need to evaluate them both.

By considering the current map of JEV genotype distribution, it appears that the Indonesia-Malaysia-Philippines region was the source of introduction into Australasia. Also, this region has always been the one where several genotypes cohabited. Eventually, the five genotypes were recognized there. Thus, our data are consistent with the hypothesis of Solomon (2003) suggesting that genotypes IV and V are the most ancestral ones. This region may be the original source of the virus, which spread first towards northern areas, then was detected in Japan, and then spread to other Asian regions. A gradient of dispersion from region 4 to region 3, then to region 6 and 1 further north, and to region 2 further west, and another to region 5 further southeast is indeed probable (Solomon et al. 2006).

5. Conclusion

Although Japanese Encephalitis has been known to recently spread over South Asia since its clinical identification in the late 1870s, followed by the discovery of the etiological agent in the early 1930s, considering the molecular clock, Japanese Encephalitis virus appears to have spread and diversified - as genotypes – across South East Asia more than 200 years ago (Solomon et al., 2003). Actual dispersion of the different JEV genotypes do not present a clear pattern of distribution within the South Asian endemic region; also a temporal and spatial analysis appears to be the most appropriate way to understand the actual distribution of JEV and its significance in term of risk of expansion (emergence) and vaccine strategies.

We provide here some insight for such understanding as for the dynamics of genotype diffusion (i.e.: one genotype rising in a given territory and taking over a previously prevailing genotype). The immunological pressure through vaccination campaign – with a vaccine generated from one single serotype - could drive such actual pattern, while also agricultural practices (ex.: pig farming), changes in the main vector (ex.: *Culex quinquefasciatus*) could theoretically participate to such a distribution. However more study need to be done to identify the fundamentals of genotype emergence within a given territory. Ultimately, taking into account these observations, each JEV genotype dynamic much be considered and understand at a local level with respect to environmental factors of natural and anthropic origins.

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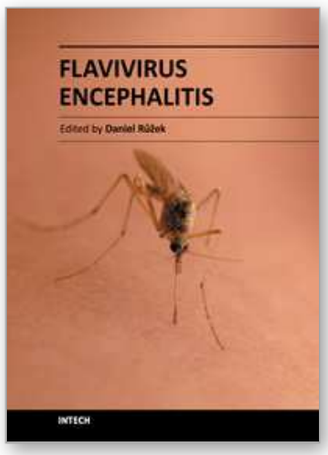

Flavivirus Encephalitis

Edited by Dr. Daniel Ruzek

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Encephalitis is an inflammation of the brain tissue associated with clinical evidence of brain dysfunction. The disease is of high public health importance worldwide due to its high morbidity and mortality. Flaviviruses, such as tick-borne encephalitis virus, Japanese encephalitis virus, Murray Valley encephalitis virus, or St. Louis encephalitis virus, represent important causative agents of encephalitis in humans in various parts of the world. The book Flavivirus Encephalitis provides the most recent information about selected aspects associated with encephalitic flaviviruses. The book contains chapters that cover a wide spectrum of subjects including flavivirus biology, virus-host interactions, role of vectors in disease epidemiology, neurological dengue, and West Nile encephalitis. Special attention is paid to tick-borne encephalitis and Japanese encephalitis viruses. The book uniquely combines up-to-date reviews with cutting-edge original research data, and provides a condensed source of information for clinicians, virologists, pathologists, immunologists, as well as for students of medicine or life sciences.

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