Seroepidemiological Studies on Japanese Encephalitis: A Systematic Review

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Abstract

Background Japanese encephalitis (JE) is one of the major mosquito-borne infectious diseases in the Western Pacific region, accounting for 20-30% of mortality cases. The JE virus (JEV) seroprevalence fluctuations indicate that continuous research is important for prevention and control activities. By mapping JEV seroprevalence by age stratification, the population profile for immunity and susceptibility can be identified to aid in vaccination programme planning. Thus, the aim of this study is to determine the trend of age-specific JEV seroprevalence.

Method Systematic search was conducted on all studies conducted on JEV seroprevalence between the years 2010 until 2019. The two search engines used were PubMed and Web of Science. Eligible criteria were set and articles were screened according to the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines. Three investigators cross-checked all articles assigned. Data were extracted into Excel sheet and results were tabulated in tables and graphs accordingly.

Result Four studies from four countries (Taiwan, Sri Lanka, South Korea, India) met the eligibility criteria. The papers show an increasing trend of JEV seropositivity in all countries as

their populations reach older age cohorts. Nonetheless, there were slight downtrend notches seen among young adults in Taiwan and India before increasing again after reaching more mature ages. South Korea has the highest seroprevalence rate (97.8% to 98.3%) among the compared countries; this is most likely because it was the earliest to introduce the JEV vaccine in 1967 which was later made mandatory in early 1980s, while India has the lowest seroprevalence rate (12.9% to 18.1%). Among the old-vaccination-naïve population, seropositivity is commonly derived from natural infection.

Conclusion Decreases in reported JE cases are mainly due to immunisation. As JEV is expected to remain in nature and the zoonotic chains, the risk of infection will persist. Hence, it is important to apply JEV vaccination protocols in national immunisation programmes with priority given to those at the young childhood stages.

Keywords: Japanese encephalitis, immunogenicity, seroprevalence, seropositivity, vaccination.

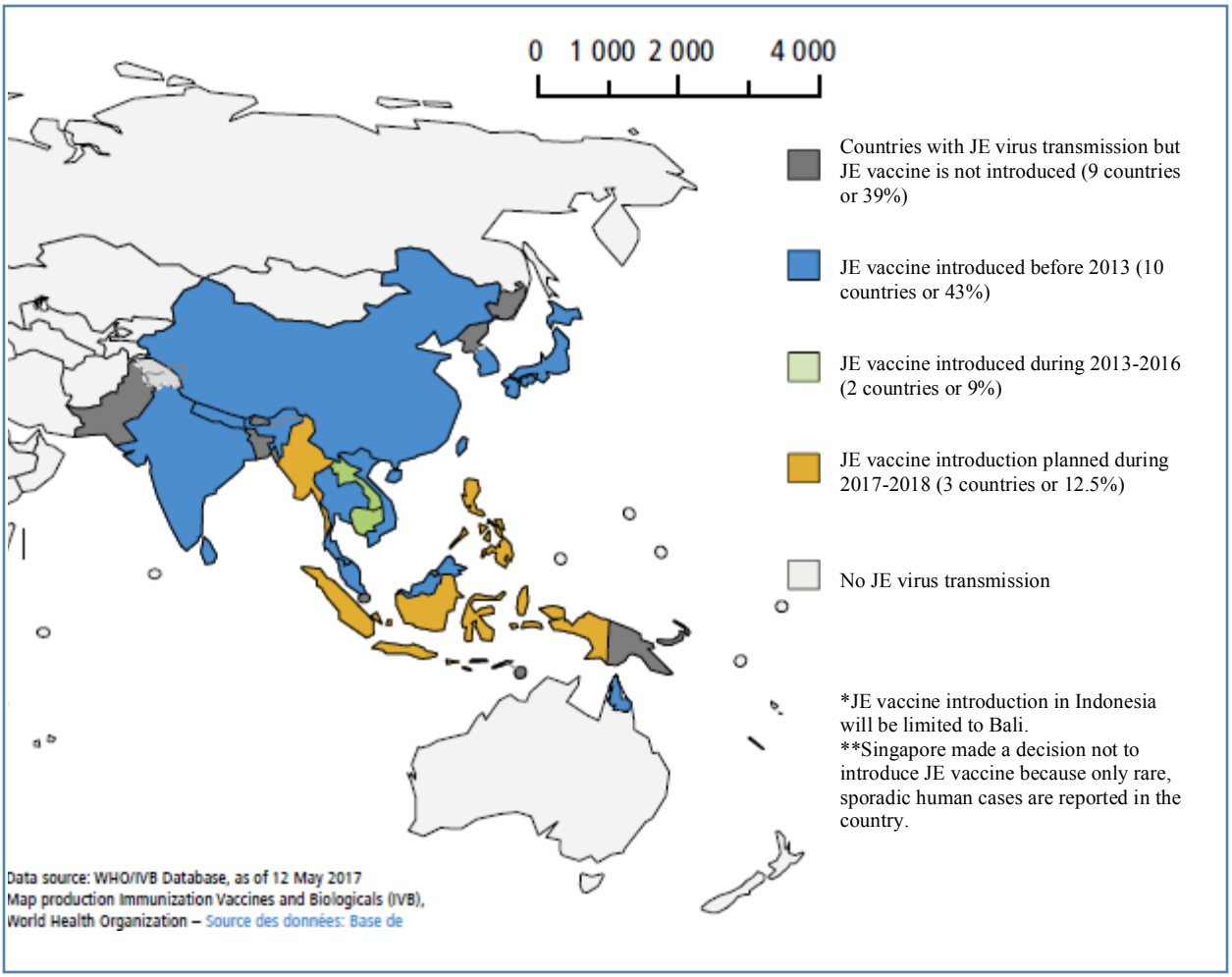
Introduction

The Japanese encephalitis virus (JEV) is an important cause of encephalitis in Southeast Asia and the Western Pacific region. Its transmission is primarily by the vector mosquito *Culex tritaeniorhynchus* through zoonotic cycles, mostly between pigs and wild birds, with humans as incidental dead-end hosts. As the amplifying hosts tend to be most abundant in agricultural areas such as farms and rice paddies where flooding irrigation attracts wading birds, most infections occur in rural areas.[1] Although most infections are asymptomatic, the manifestation of this virus can range from non-specific febrile illness to multiple neurological complications.[2]

A systematic review of Japanese encephalitis (JE) disease burden estimated approximately 68 000 JE cases to typically occur annually (overall incidence: 1.8 per 100 000), of which only about 10% are reported to the World Health Organization.[3,4] Approximately 33 900 (50%) of these cases occurred in China and about 51 000 (75%) cases were among children aged 0-14 years (incidence: 5.4 per 100 000). It was estimated that 55 000 (81%) cases occurred in areas with well-established or developing JE vaccination programmes, while 12 900 (19%) cases were

from areas with minimal or no JE vaccination programmes.[5] In a 2015 surveillance, WHO received reports of 4,087 JE cases from 20 (83%) of 24 endemic countries (Figure 1) of which 87% of these cases were reported from four countries; China, India, Nepal, and Vietnam.[6] In addition, the Advisory Committee on Immunization Practices (ACIP) reported that among those annual cases, an estimation of 20%–30% of patients died, and 30%–50% of survivors had neurologic or psychiatric sequelae.[7]

The introduction of JE vaccination is therefore important to reduce the disease's burden, particularly its incidence as it may cause severe complications such as encephalitis that could lead to permanent neurologic or psychiatric sequelae.[4] JE vaccination's nationwide implementation ~~s~~ince the 1980s has increased the seroprevalence of JEV in some endemic countries, with higher seropositivity seen in adults and the elderly as compared to those of younger ages, suggesting natural infection or natural boosting of immunity through exposure to wild virus.[8,9] The dynamics of JEV seroprevalence fluctuations indicate that continuous research is important for future prevention and control activities of JEV infection. By stratifying JEV seroprevalence by age categories, a human population profile of JEV's immunity and susceptibility can be identified and therefore, influence decision-making on national or subnational vaccination programmes. To the authors' knowledge, there is a lack of systematic review studies done on JE seroepidemiology in recent years. Thus, the aim of this study is to determine the trend of age-specific JEV seroprevalence in JE endemic countries using results available from studies conducted in the past decade.



Data Source: World Health Organization (1)/Immunization Vaccines and Biologicals database; May 12, 2017.

Methodology

Search Strategy

A systematic search was conducted throughout November and December 2019 for all reported studies on JEV seroprevalence published from the year 2010 until 2019. The literature search was performed in two databases; PubMed and Web of Science according to the Preferred Reporting Items for a Systematic Review and Meta-analysis (PRISMA) guidelines. The keywords used are:

"Japanese encephalitis"

AND

"Seroprevalence" OR "seropositivity" OR "seroepidemiology" OR "serosurvey" OR "immunity" OR "antibody"

Selection Criteria

The target population of this search was subjects whom had been investigated for JEV status by serology. The inclusion criteria from the database searches were (a) quantitative studies that fulfil the keywords and terms searched, (b) availability of full text article, (c) original articles, and (d) English-language articles. The exclusion criteria in this search were on the basis of (a) types of article: case study articles, review paper articles, non-peer reviewed articles; (b) clinical JE studies (serostatus of patient with confirmed JE); (c) vaccine trial studies; and (d) non-human seroprevalence studies (pigs, horses, birds, chickens, or mosquitoes). Articles were then identified through titles and subsequently, abstracts were screened using the eligibility criteria. The flow of the article search is described in Figure 2.

Operational Definition

Seroprevalence in this context refers to seropositivity with (a) neutralizing antibodies to JEV (50% plaque reduction neutralization titres ($PRNT_{50}$) against JEV with a titre of ≥1:10, (b) anti-JEV serum IgM and IgG (ELISA), or (c) micro virus neutralization >1:20.[4,10,11,12]

Data extraction tool

All researchers independently extracted information from each article into an Excel sheet. The data was customised into (a) code, (b) author and year of study publication, (c) country of study, (d) province or district of study, (e) population source, (f) sample size, (g) type of assay used, (h) assay threshold or dilution, (i) year of survey done, (j) age cohort, (k) median age, (l) JEV vaccination dose received, (m) seroprevalence, and (n) year of vaccine introduction. A second reviewer crossed-checked the articles assigned and provided comments in the table. Data was presented in tables and a graph to provide a visual summary of the changing seroprevalence by age over time. For standardisation, the median age of each age cohort was determined for further analysis. In cases where the median cannot be determined but the mean value was available, the mean value was considered for graph tabulation.

Quality Assessment Tool

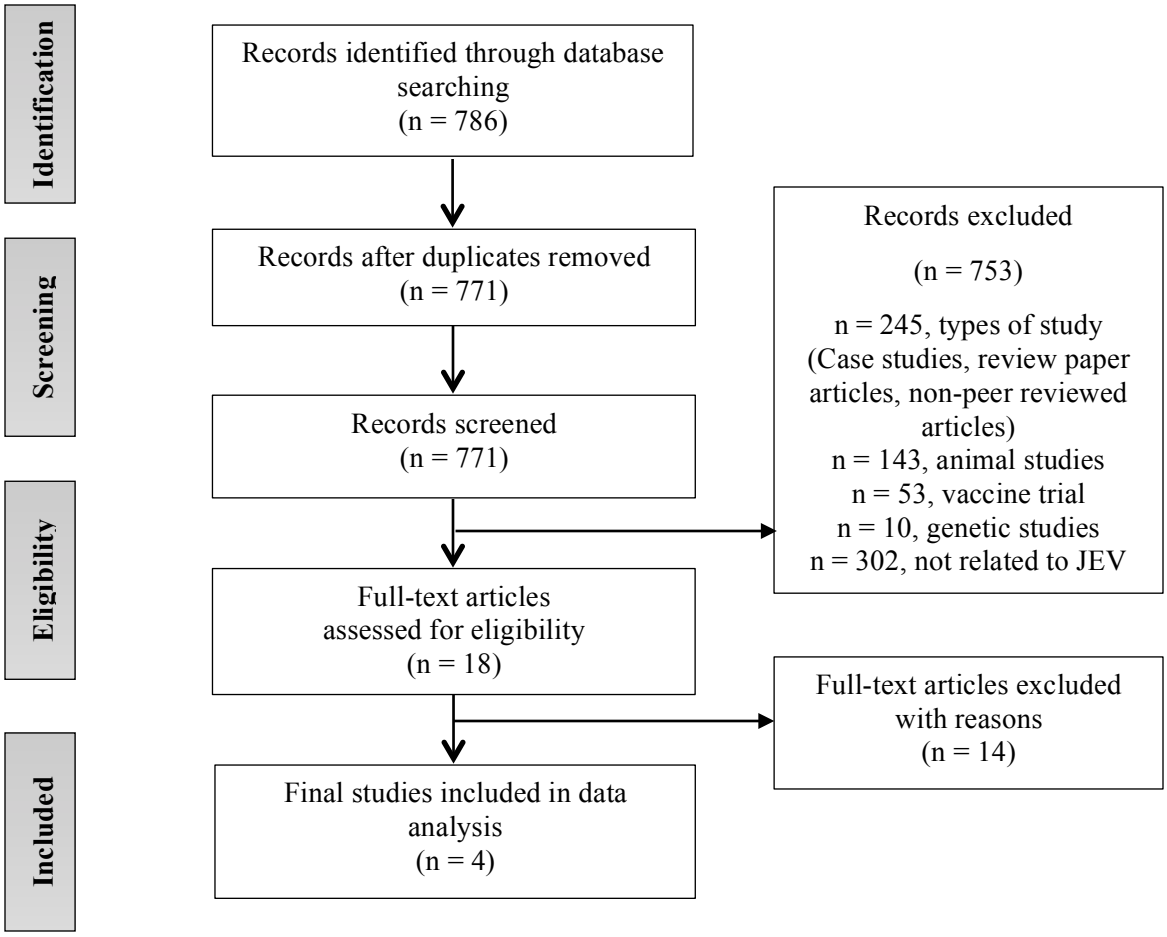


Figure 2: Results of literature search and evaluation of identified studies according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines

Results

Four studies met the eligible criteria (Figure 2). These studies were conducted in Taiwan, Sri Lanka, South Korea and India and were coded according to the year of study publication (Table 1). Study code 1 represented the whole country of Taiwan since data was collected from a nationwide population based survey. Meanwhile, the other three studies (study codes 2, 3 and 4) presented data stratified by sub-geographic regions with study code 3 consisting of ten provinces in South Korea and the other two studies included only one district in the country.

Study references, countries and population sources, study assays and their thresholds extracted from the selected studies are summarised in Table 1. The first study was conducted by Taiwan's Centre for Disease Control in 2014.[13] In the following year, one study was conducted by the Centre of Dengue Research at a Sri Lankan tertiary education centre.[11] In 2016, the third study took place in South Korea by its Centre for Disease Control and Prevention, while the fourth study was conducted by the Indian National Institute of Virology in 2017.[12,14]

The timing and seasons of blood sample collection varied across all studies and ranged from year 2002 until 2014. The plaque reduction neutralisation test (PRNT) was used to determine seroprevalence in two studies (study codes 1 and 3), while the other two studies used enzyme-linked immunosorbent assay (ELISA) IgG and micro-virus neutralisation assay respectively to profile neutralising antibody responses (study codes 2 and 4). In the two studies that used the PRNT test, a titre of $\geq$1:10 was considered seropositive. On the other hand, the study that used the micro-virus neutralisation assay considered cases to be positive if it had a dilution of $\geq$1:20, while the study that used ELISA IgG considered cases of positive seroprevalence as those with an immune status ratio (ISR) of more than five.

Table 2 summarises the age-stratified seropositivity rates obtained by the four studies by study year. Since the introduction of the JEV vaccination program in Taiwan in 1968, there were multiple changes in the JEV doses given to its population ranging from two doses to three doses and finally four doses according to birth cohorts. This study that took place in 2002 showed that the eldest age group (age 50 to 90) had the highest seroprevalence rate of 86% followed by the adolescence age group (16 to 21) with a seropositivity of 74%, while the adult age group (33-39) had the lowest seroprevalence amongst all age groups at 54%.

Comparatively, study code 2 conducted in Sri Lanka had lower JEV seroprevalence rates. In 2003, the children age group (age 6 to 15) had lower neutralised antibodies compared to the elder group (more than 16 years), and this trend persisted until 2014 although the seroprevalence rates of both groups were seen to be higher in the latter year (28% vs 44% in 2003 and 40% vs 78% in 2014, respectively). Study code 3 which was conducted in South Korea showed a very high average seroprevalence rate (98%) among its adult and elderly populations. Among all age cohorts, those belonging to the 30-39 year group may have received one JEV vaccination dose, those aged 40-49 years might not been vaccinated, while the rest of South Korea's elderly cohort never had any JEV vaccination in their life. The last study (code 4) represented an Indian population with relatively low JEV neutralised antibody rates compared to other Asian populations being reviewed. In 2012, the age cohort of 45-64 years scored the highest seropositivity (18.1%), followed by 0 to 14 years (15.6%), 15 to 44 years (15.4%) and finally those more than 65 years (12.9%).

The trend for age-stratified seroprevalence was tabulated in a graph (Figure 3). In general, there is an increasing trend of JEV seropositivity in all countries as their populations reach older ages. Nonetheless, there was a slight downtrend notch of seroprevalence among young adults in Taiwan and India before it increased again after the subjects reached a more mature age. It is also obvious that South Korea has the highest seroprevalence rate (97.8% to 98.3%) for all age cohorts compared to other countries, while India has the lowest seroprevalence rate (12.9% to 18.1%.

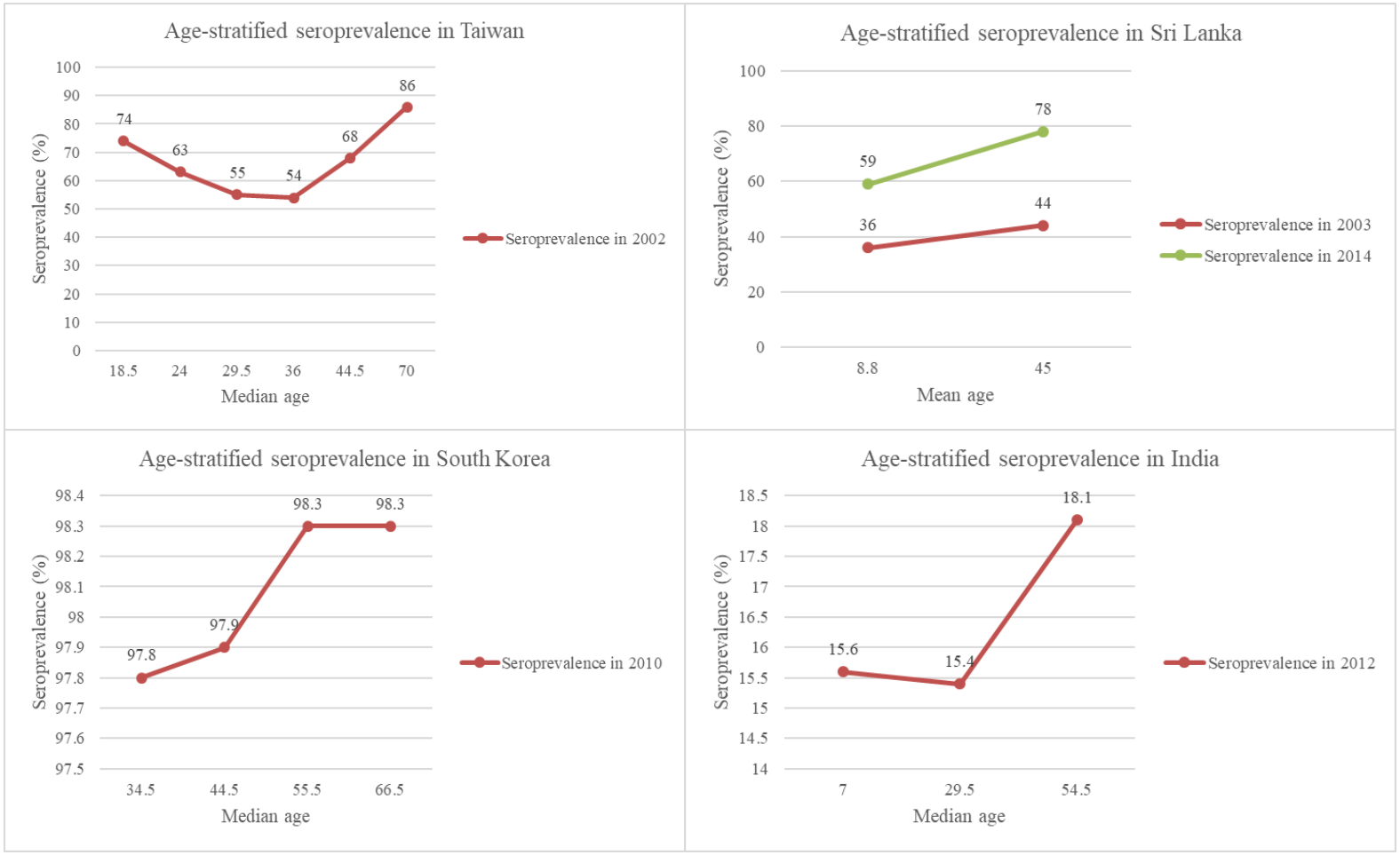
Table 1: Details of the included studies

Code	Author and year	Country	Province/district	Population source	Sample size	Assay	Threshold/ dilution
1	Hsu LC et al, 2014	Taiwan	-	Nationwide population	6594	$PRNT_{50}$	Not mentioned
2	C Jeewandara et al, 2015	Sri Lanka	Colombo	Population attended a primary health care facility in the suburban areas of Colombo	1689	ELISA	ISR>5
3	Lee EJ et al, 2016	South Korea	Seoul Gyeonggi Gangwon Chungbuk Chungnam Jeonbuk Jeonnam Gyeongbuk Gyeongnam Jeju	Population in 10 provinces	945	$PRNT_{50}$	$\geq$1:10
4	Balakrishnan et al, 2017	India	Alappuzha	Population in Alappuzha	1125	Microvirus neutralization assay	$\geq$1:20

Table 2: Age stratified seroprevalence of Japanese encephalitis virus according to period of survey and vaccine introduction

Code	Country/ district	Year of survey	Age cohort	Median age	JEV vaccination dose received	Seroprevalence	Year vaccine introduction
1	Taiwan	2002	16-21	18.5	4 doses	74%	1968
			22-26	24	4 doses	63%	
			27-32	29.5	3 doses	55%	
			33-39	36	2 doses	54%	
			40-49	44.5	-	68%	
			50-90	70	-	86%	
2	Sri Lanka	2003	6-16	8.8[a]	Mostly vaccinated; 1 or 4 doses	28-44%	1988
			>16	45[a]	-	>44%	(Became national
		2014	6-16	8.8[a]	Mostly vaccinated; 1 or 4 doses	40-78%	immunization
			>16	45[a]	-	>78%	program in 1988)
3	South Korea	2010	30-39	34.5	1 dose	97.8%	1967
			40-49	44.5	May have been vaccinated	97.9%	(Became mandatory
			50-59	55.5	-	98.3%	in early 1980s)
			60-69	66.5	-	98.3%	
4	India Alapphuza	2012	0-14	7	-	15.6%	2008
			15-44	29.5	-	15.4%	
			45-64	54.5	-	18.1%	
			>65	*	-	12.9%	

[a]mean age for age cohort *unable to determine



: Age-stratified seroprevalence of Japanese encephalitis virus according to country and year

Discussion

Since the first JE case was recorded in 1891 in Japan, there are currently 24 endemic countries with the risk of JEV transmission identified. Twelve (50%) of the 24 countries had a JE immunisation programme in 2016; 10 (42%) programmes were implemented nationally or subnationally in all risk areas, and two (8%) were subnational and did not include all risk areas.[6,15]

Of the four countries being studied, Taiwan recorded the greatest number of age cohorts, enabling the authors to review its JEV age-stratified seroprevalence a little closer. The period of study survey in study code 1 began thirty-four years after the country initiated its mass vaccination programme. The continuous rescheduling of the immunisation policy has resulted in various birth cohorts receiving distinct doses of the JE vaccine. This study revealed that in 2002, the age-group-specific seroprevalence was the lowest among those 33 to 39 years of age (54%) than among adolescents (74%) or old people (86%). These results might have been caused by the loss of antibodies because they either received the last dose long ago, received less than four doses of the vaccine, or received no vaccine at all. Conversely, a high seropositive rate of JEV neutralising antibodies were found among people who were not vaccinated which is the cohorts born before 1952. This is likely caused by a high frequency of early natural infection and accumulated exposure which induces a stronger immunological memory response than that induced by vaccination.[13,16]

In study code 2, the differences between JEV seroprevalence according to time period can be seen since the study included two years of data in its survey (2003 and 2014) despite a lacking in distinction of age-stratification cohorts. It clearly demonstrated that JEV seroprevalence of both median age groups had increased most likely due to the gap between the year the surveys were done with the year that the mass vaccine campaign was introduced in Sri Lanka where they initially targeted higher-risk areas and later expanded the program in 1988.[17]

In contrast, the JEV seroprevalence was found to be the highest in South Korea (study code 3) with an average of 98%. This is most likely because it was the earliest country to introduce the JEV vaccine in 1967, and the vaccine was later made mandatory in the early 1980s. Interestingly, old South Koreans who had no history of vaccination were also able to maintain a very high rate of JEV seropositivity (98.3%). Undoubtedly, this must be because of the natural infection that

had led to this phenomenon.[12] On the other hand, either vaccination or natural infection or both must have contributed to the very high seroprevalence rate among the rest of South Korea's age cohorts.

Data from WHO for 2016 confirmed that all countries included in this review are covered for JE national vaccination with an exception of India where the JEV vaccination programme only covers high risk and sentinel areas (7).[6] Vaccination campaigns were introduced in 2008 in the studied district, a date much later compared to the other studied districts of the three other included countries as shown in Table 2. Similar to other endemic districts in India, the live attenuated SA-14-14-2 vaccine against JEV was introduced as routine immunisation under the Universal Immunisation Program in 2011. Realising that JE poses a great burden to their public health, the government of India announced the introduction of one dose of JE vaccine for adults in endemic districts in its national vaccination programme in 2014.[18] Hence, the age cohorts included in this study had never received any JEV vaccination. This probably explains why study code 4's population have the lowest JEV seroprevalence amongst all.

A number of serological methods were used in studying antibody responses to JE, including neutralisation, haemagglutination inhibition (HI), complement fixation (CF), enzyme-linked immunosorbent assay (ELISA), and the indirect fluorescent antibody test (IFA). However, antibodies measured by HI, CF, ELISA and IFA do not correlate with protection. Only neutralising antibodies correlate with protection. The neutralisation test is the most specific measure of antibody, i.e. little cross-reaction with other flaviviruses. To measure neutralising antibody titres, the plaque reduction neutralisation test (PRNT) is most often used. The essence of the test is that when JEV is grown on a cell monolayer, it causes plaques to form. The number of plaques formed is reduced if the virus has been mixed with serum containing neutralising antibodies, and this reduction in plaques gives a measure of the antibody titer (WHO1). Most studies recommend an end-point of 50% reduction in PRNT, but no international standard for the exact procedure or choice of end-points has been established.[19]

There are few limitations noted in this study. All blood samples taken for seroprevalence analysis were collected in a twelve-year range (2002 to 2014) with inconsistencies in the definition of age cohorts in each study. The dosage and type of JEV vaccination given were also not the same either inter- or intra-nationally. All these resulted in difficulty to produce a standard

comparison. Finally, the assays used for JEV neutralising antibody detection were also not standardised in this review. $PRNT_{50}$ is the most reliable method for detecting vaccine-induced JEV antibodies, while ELISA is more suitable for diagnosis because the chance of confusion with vaccine-induced antibodies is relatively low.[20]

Despite these limitations, findings in this study indicate that JEV seroprevalence increases with age and its high seropositivity rate can be maintained with thorough implementation of national or universal vaccination programs. More specifically, this study also indicated that protective immunity remains high in adults who received vaccinations during childhood up until the age of at least 30 years before natural immunity becomes superior in the older population. The data compiled highlighted consistent changes in age-stratified JEV seroprevalence, hence proving that increasing age does increase a person's susceptibility to the disease. In other words, it may aid in the identification of vulnerable populations. Although there is contradicting evidence on higher seroprevalence among vaccine-naïve populations, vaccination remains highly recommended until today. Such situation is supported by many studies; a study in Japan reported that naïve populations with detectable neutralising antibodies had higher annual infection rates compared to populations with vaccination histories. This signifies that in Japan, JEV remains present and active in nature; thus, continuing the JEV vaccination program is indispensable to prevent JE infection in humans.[21]

Conclusion

Although JE remains a prominent public health concern in the Western Pacific region, reported cases have decreased mainly due to JE immunisation. As JEV will remain in nature and the zoonotic chains, the risk of infection will persist. Hence, it is important to apply JEV vaccination protocols in national immunization programs with priority given to those at the young childhood stages.

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