British Microbiology Research Journal
6(3): 126-134, 2015, Article no.BMRJ.2015.065
ISSN: 2231-0886

SCIENCEDOMAIN *international*
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Sero-prevalence of Japanese Encephalitis (JE) among Nepalese Children

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SK, SRB, DJ, NA, UTS, DA and BPU designed the study. Authors UTS and PA performed the statistical analysis. Authors SK, SA, PMN and RK wrote the protocol and prepared the first draft of the manuscript. Authors SK, SA and RK managed literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2015/15442
Editor(s):
(1) Vijay Kumar Eedunuri, Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, USA.
Reviewers:
(1) Anonymous, USA.
(2) Or Cohen-Inbar, Department of Neurosurgery, university of Virginia, USA.
(3) Anonymous, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=829&id=8&aid=7450

Original Research Article

Received 26th November 2014
Accepted 11th December 2014
Published 24th December 2014

ABSTRACT

Background: Japanese encephalitis (JE) is one of the major public health problems particularly in the Terai regions of Nepal. Children upto the age of 15 years are more susceptible for JE. The present study was conducted to aim the sero-prevalence of JE among children of Nepal.
Methods: The samples were collected and processed at National Public Health Laboratory (NPHL). In this study, children of age upto 15 years defined with Acute Encephalitis Syndrome (AES) were studied. A total 1009 samples, were collected and tested by MAC ELISA technique for the detection of Anti JEV-IgM.
Results: From the processed samples, 107 (10.61%) were found positive for Anti JEV-IgM. Among

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total JE seropositive cases, 58 (54.21%) were male and 49 (45.79%) were female and found no significant difference in distribution of disease among gender (χ^2=1.03, P>0.05). The majority of seropositive cases (40.18%) were found in the children of 6 to 10 years of age. The highest number of seropositive cases (34, 29.31%) were detected from Mid Western Development Region (MWDR) followed by Far Western Development Region (25%). Analyzing month wise seasonal variation, the sero-positivity rate was highest in the month of August (28.89%) followed by September (18.37%) and no positive cases were found in the month of March.

Conclusions: In conclusion, the higher prevalence of JE among Nepalese children indicates alarming seasonal endemicity of the disease.

Keywords: Acute encephalitis syndrome; Japanese encephalitis; children; Nepal.

1. BACKGROUND

Japanese Encephalitis (JE) is a disease caused by the Japanese Encephalitis Virus (JEV), a single stranded RNA virus and a member of the JE serological group of flaviviruses and is transmitted by culicine mosquitoes (primarily C*ulex tritaeniorhynchus*), with pigs and birds as amplifying hosts [1]. Patients with JE typically present a few days of non-specific febrile illness followed by headache, vomiting and a reduced level of consciousness, often heralded by convulsion and may progress to a serious infection of the brain (encephalitis) [2]. Encephalitis due to JE virus (JEV) was reported in Japan as early as 1871, but JEV was isolated from a clinical case in the first reported epidemic in Japan in 1924 [3]. Then it spread from East Asia to South East Asia (SEA) and then to South Asia. Nakayama strain of JEV was first isolated from human cases in 1935 [4].

JE is one of the most important endemic encephalitis in the world especially in Eastern and Southeastern Asia and there are approximately 50,000 cases and 15,000 deaths annually [5]. The epidemiological pattern and the geographical distribution of JE have been found changing throughout Asia. There is the reduction of JE cases in the developed countries such as China, Japan and Korea with widespread use of JE vaccine and proper vector control systems.

In Nepal, JE is endemic in 24 districts (20 terai and 4 inner terai districts) starting from Jhapa in the east to Kanchanpur in the far west; however, the sporadic cases from other districts including Kathmandu valley have been reported in recent years. Between 1978 and 2003, nearly 26667 cases and 5381 deaths due to acute encephalitis have been reported in Nepal. Some hyper endemic district of Nepal represent the paddy field ecosystem with abundant *Culex* species and amplifying host pigs and migratory birds indicating the potential epidemics in these districts. High humidity, summer temperature of 24-38ºC and paddy field ecosystem of the terai region are the favorable conditions for breeding of *Culex* mosquitoes. Therefore, a high prevalence of JE has been identified in the terai and inner terai where cross-border transmission is also possible around the border areas [6].

JE still remains the major public health problem though it can potentially be controlled and prevented on large scale by effective vaccination. Despite the effort towards its prevention, it still is in existence causing the death and also has emerged as an important cause of disability in children in Asia. The present study has been designed to describe the sero-prevalence of JE among Nepalese children with seasonal variation from different geographical regions of Nepal. The understanding of burden of the diseases among the children is very essential to make strategy for JE vaccine introduction in to the routine childhood immunization programs and also useful in monitoring and evaluating such programs.

Nepal is a landlocked country with an area of 147,181 square kilometers. Nepal is one of the richest countries in the world in terms of bio-diversity due to its unique geographical position and altitude variation. The elevation of the country ranges from 60 meters above sea level to the highest point on earth, Mt. Everest at 8,848 meters, all within a distance of 150 kilometers resulting in climatic conditions from sub-tropical to arctic. Nepal is commonly divided into three physiographic areas: Mountain, Hill and Terai. Nepal is divided into 5 Development Regions, Far West Development Region (FWDR), Mid West Development Region (WDR), West Development Region (WDR), Central Development Region (CDR)

and East Development Region (EDR), 14 Administrative Zones and 75 Districts.

2. METHODS

2.1 Study Area

The surveillance study was descriptive laboratory based conducted from January to December 2010 in 48 districts of Nepal, covering all five development regions and different geographical regions (mountains, hills and terai).

2.2 Sample Collection, Transportation and Processing

Samples (serum and CSF) were collected from 48 districts of Nepal, stored and transported to National Public Health Laboratory (NPHL), Teku, Kathmandu with the technical support of World Health Organization Program for Immunization Preventable Diseases (WHO-IPD) Nepal under its JE surveillance activities. Children below 15 years of age defined with acute encephalitis syndrome (AES) were considered as the study population. According to clinical case definition by World Health Organization (WHO), AES is defined as acute onset of fever and a change in mental status including symptoms such as confusion, disorientation, or inability to talk and/or new onset of seizures excluding febrile convulsions in a person of age at any time of year. A total 1009 (Serum 694 and CSF 315) samples were collected during the year 2010 from suspects of AES for testing at the NPHL.

Primary data collection was made through standard questionnaire at the concerned health institutes where patients were visited. The collected data were obtained through WHO-IPD lab line list at NPHL. Following aseptic techniques, 3 ml of venous blood was collected by venipuncture from children of age below 15 years with AES cases during acute phase illness from different medical centers of 48 districts and was transferred in a labeled, clean and dry test tube. The blood was allowed to clot for 15 minutes at room temperature and then at 4ºC to retract the clot. The test tube containing the blood sample were centrifuged and serum was transferred into a screw capped (labeled) vial and transported to IPD/Surveillance medical offices. The samples were transported to NPHL in an ice box following standard reverse cold chain protocol. The received samples were stored at -20ºC until tested and their corresponding forms were checked thoroughly, data entered into the computer. Same procedure was followed for the samples which were directly received at the NPHL. CSF samples were collected by attending medical officers and was transferred in a provided screw capped (labeled) vial and transported to the NPHL as for serum sample mentioned above. All the collected samples were tested for the presence of anti-JEV IgM following the MAC-ELISA technique developed by Armed Force Research Institute of Medical Sciences (AFRIMS), Department of Virology, Bangkok , Thailand [7].

2.3 Ethical Approval

The samples used in this study were from routine clinical specimens. Because acquiring the samples did not involve direct patient contact and did not interrupt routine clinical care, written consent was not required. However, verbal consent for this study was taken from the guardians of participating children.

2.4 Statistical Analysis

Data collected by laboratory analysis and questionnaire were managed and analyzed by using SPSS-11.5 version and Microsoft Excel. Chi- square test was applied wherever applicable. P values < 0.05 were considered as statistically significant.

3. RESULTS

During the study period, a total of 1033 AES cases were reported among children and 1009 samples were tested and evaluated.

Among total processed cases, 107 (10.61%) were found to be sero-positive while 902 (89.39%) were reported to be sero-negative. Sex-wise distribution of JE positive cases among total suspected cases showed a slightly higher positivity in female than male showing no significant difference while sex-wise distribution among total positive cases showed higher positivity in male (54.2%) than female (45.8%) as depicted in Table 1. The majority of sero-positive cases were found in the age of 6-10 years; however, no statistically significant difference was found in the age-wise distribution of sero-positive cases as shown in Table 2.

The highest positive cases of 29.31% were found in Mid-Western Development Region (MWDR)

followed by Far-Western Development Region (FWDR) with 25% positive cases (Table 3). The highest rate of positive cases (28.89%) was reported in the month of August followed by September to December as shown in Table 4.

The highest cases (24.3%) were reported from Kathmandu followed by Dang and Surkhet as depicted in Table 5.

Geographical distribution of positive cases depicted that Hill region was most vulnerable region with 57.01% positive cases and only 0.94% sero-positivity was found in Mountain region (Table 6).

4. DISCUSSION

The present study reveals the sero-prevalence of JE using MAC ELISA technique for the detection of JE specific IgM to be 10.61%. IgM capture MAC ELISA technique has been proved to be a reliable serological method for the diagnosis of JE [8,9]. However, different investigators elsewhere performed different techniques to reveal the sero-prevalence of JE.

Table 1. Sex-wise distribution of JE cases among children in Nepal

Gender	Total cases	Seropositive (%)	Chi square test
Male	593	58(9.78)	
Female	416	49(11.78)	χ^2= 1.03, P>0.05
Total	1009	107(10.61)	

Table 2 Age-wise distribution of JE cases among children in Nepal

Age group	Total cases	Seropositive (%)	Chi square test
Neonate (<28 days)	46	3 (6.52)	
Infant (1month-year)	118	4 (3.39)	
2-5 years	256	30 (11.72)	χ^2= 8.72, P>0.05
6-10 years	349	43 (12.32)	
11-15 years	240	27 (11.25)	
Total	1009	107 (10.61)	

Table 3. Regional distribution of JE cases among children in Nepal

Region	Total cases	Sero-positive (%)	% of total positive cases
CDR	663	56(8.45)	52.34
EDR	21	1(4.76)	0.94
FWDR	24	6(25.00)	5.61
MWDR	116	34(29.31)	31.77
WDR	185	10(5.41)	9.34
Total	1009	107(10.61)	100.00

Table 4. Monthly distributions of JE cases among children in Nepal

Month	Total cases	Sero-positive (%)	% of total positive cases
Janurary	33	1(3.03)	0.93
Feburary	49	3(6.12)	2.81
March	43	0(0.00)	0.00
April	53	3(5.66)	2.81
May	124	2(1.61)	1.87
June	58	2(3.45)	1.87
July	141	8(5.67)	7.48
August	90	26(28.89)	24.30
September	196	36(18.37)	33.65
October	74	13(17.57)	12.15
November	129	12(9.30)	11.22
December	19	1(5.26)	0.93
Total	1009	107 (10.61)	100.00

Table 5. District-wise distribution of JE cases among children in Nepal

Districts	Total cases	Sero-positive	% of total positive cases
Achham	1	0	0.00
Arghakhanchi	7	2	1.87
Baglung	5	0	0.00
Bhaktapur	24	1	0.94
Bara	81	4	3.74
Bardiya	4	1	0.94
Banke	19	6	5.61
Bhojpur	1	0	0.00
Chitwan	45	0	0.00
Dhading	3	0	0.00
Dhanusha	16	1	0.94
Dadeldhura	1	0	0.00
Dang	29	13	12.15
Gorkha	7	0	0.00
Gulmi	15	2	1.87
Jhapa	18	1	0.94
Kailali	8	2	1.87
Kapilvastu	3	1	0.94
Kaski	36	0	0.00
Kavre	10	2	1.87
Khotang	1	0	0.00
Kanchanpur	14	4	3.74
Kalikot	1	0	0.00
Kathmandu	266	26	24.30
Lalitpur	47	6	5.61
Lamjung	4	0	0.00
Mahottari	16	2	1.87
Makwanpur	30	2	1.87
Myagdi	2	0	0.00
Nawalparasi	40	0	0.00
Palpa	25	2	1.88
Parsa	54	2	1.88
Parbat	5	0	0.00
Pyuthan	1	0	0.00
Ramechhap	2	0	0.00
Rasuwa	1	0	0.00
Rautahat	27	2	1.88
Rolpa	2	1	0.94
Rukum	4	0	0.00
Rupandehi	5	2	1.88
Saptari	1	0	0.00
Sarlahi	27	4	3.73
Sindhupalchowk	6	1	0.94
Sindhuli	4	3	2.80
Surkhet	57	13	12.15
Sunsari	1	0	00
Syangja	12	0	0.00
Tanahu	21	1	0.94
Total	1009	107	100.00

Table 6. Geographical distribution of JE cases among children in Nepal

Geographical region	Total cases	Sero-positive (%)	% of total positive cases
Hill	593	65 (10.29)	57.01
Terai	408	45 (11.03)	42.05
Mountain	8	1 (12.50)	0.94
Total	1009	107 (10.61)	100.00

JE infection in its prodromal phase is of very nonspecific type, so it is possible that by the time patient reports and JE is suspected and sample is collected, IgM falls and becomes undetectable by ELISA. Thakare et al. [10] had suggested that MAC-ELISA negative samples should also be tested for JEV specific IgG subclass before ruling out the possibility of JE.

Of the total 1009 reported AES cases, 593 were male (58.78%) and rest 416 were female (41.23%). Among total cases of male and female the positivity for anti JE-IgM was seen higher in female (11.78%) than male (9.78%). The finding is in contrast with the findings of other investigators in Nepal who reported higher sero-prevalence especially in male children. Positivity for anti JE-IgM in female may be due to sexual disparity and also due to social discrimination and nutritional status in socioeconomically poor community [11]. The predominance of male cases was usually expected because males are mostly involved in the outdoor activities than female, leading to greater exposure to JE infected mosquitoes. However, male children approaches health care facilities better than females and also may be due to exposed body parts of the males leading to mosquito bites. Females are restricted to the household work (indoor activities) and also there is no explanation for the higher positivity in female [11]. Among total positive cases the positivity was found to be higher in male (54.2%) than female (45.8%). The result coincides with the result of Rayamajhi [12] who reported 69% male and 31% female. The results also agree with the result of Shrestha et al. [13], who demonstrated 56.25% male and 43.75% female. In the study carried out by Khinchi et al. [14] people affected with JE were 54% male and 46% female.

Analysis of the total number of cases by age groups revealed maximum number of JE positive cases from the age group of 6-10 years, which constitutes 12.32% of total positive prevalence. In the study carried out by Rayamajhi et al. [15] children of age 2-10 years of age showed higher prevalence. Children of age 2-10 years are more active and they always roam outside, play around the water logged area and rice fields, increasing the risk for bitten by the mosquitoes. The increase in physical activity, poor immune status or waning of immunity makes them vulnerable to other viral encephalitis [12]. Rao et al. [16] reported that the age group 1-14 years including infants had been affected but nearly 86.8% of them was from 1-9 years age group in Andhra Pradesh epidemic during 1999.

Analysis by patient age in disease endemic areas revealed that 60% of patients infected with JE were of age below 15 years [17]. Seventy six percent in this study were under nine years of age. Potula and Badrinath, [18] in their study found 71.2% patients less than 10 years. Rayamajhi, [12] in their study found 58.4% patients below 9 years of age. No or low degree of knowledge about the disease and hygienic conditions, and low immunity may be the reasons for high prevalence among the children. Children of the age group below 5 years show very few positive cases because they are under strict supervision of their parents for their protection until they are grown up.

Out of 1009 tested AES cases, majority of cases were found from Central Development Region (CDR) (663) followed by Western Development Region (WDR) (185), Mid-Western Development Region (MWDR) (116), Far-Western Development Region (FWDR) (24) and Eastern Development Region (EDR) (21). The highest number of JE positive cases was reported from the CDR, which accounts for 52.34% of the total JE positive cases followed by MWDR and WDR with 31.78% and 9.35% respectively. Higher number of cases from CDR may be due to good reporting system, a number of health centers situated in this region and also may be due to high level of public awareness [19].

Specimens for suspected AES cases were obtained throughout the year. In 2010, the month wise distribution revealed that the highest number of specimens were collected and tested in September (196), of which 36 were found to

be positive for anti-JE IgM which accounts for 33.65% of the total JE positive cases. During August, 26 (out of 90 AES cases) were confirmed as JE positive which constitutes 24.29% of the total positives. After the month September, the number of JE positive cases followed a decreasing pattern. The possible reason for the high incidence of disease in the month August, September and October may be because these months are the favorable month for the mosquito breeding and also paddy cultivation. Moreover in August and September, heavy rain changes most of the dry areas to water-lodged areas which are appropriate for mosquito breeding [19]. Khinchi et al. in their study found most of the cases occurred after rainy season in September and November in 2007 and July and August in 2008 [14]. According to Shrestha et al. in a study from Nepal, he also found that the cases start to appear in the month April-May and reach its peak during late August and early September and start to decline from October [13]. Study done by Bista *et al.* showed, upsurge of cases take place after the rainy season (monsoon) [20]. Kabilan et al. [21] from India gave similar report of having maximum number of patients with encephalitis during the month of July, August and September. Specimens from suspected cases of JE were obtained throughout the year but no positive case was obtained during the month of March.

Specimens suspected for AES were reported from 48 districts of Nepal. However JE positive cases were detected only from 27 districts. Eight districts that recorded single positive case were Bhaktapur, Bardiya, Dhanusha, Jhapa, Kapilvastu, Rolpa, Sindhupalchowk and Tanahu. No positive cases were reported from 21 districts. District wise distribution of AES cases showed maximum number of AES cases from Kathmandu (266) followed by Bara (81), Surkhet (57), Parsa (54), Chitwan (45) and so on. The higher detection rate of JE sero-positivity was found in Kathmandu. The finding is in contrast with the findings of other investigators in Nepal who reported higher sero-prevalence especially in the terai region of Nepal. The higher number of cases in Kathmandu indicates the availability of vectors even in Kathmandu. Although Kathmandu is the capital city, immigration rate is high because people from different districts migrate to the Kathmandu for the purpose of higher education, jobs, health care facilities etc.

The change in climate, dense population, unmanaged solid waste, nearby dumping sites, heavily polluted river, facilitates the breeding of vector mosquitoes might be the factors of higher sero-prevalence. Presence of *Culex tritaeniorhynchus* in Kathmandu (According to MoH source) and isolation of JEV by Ogawa et al. (1992) from a pig raised in Kathmandu may be indicators of the real presence of disease. The regular contact of the inhabitants of Kathmandu with people from different part of the country also signals towards the high positive cases [19].

During the year 2005, 85% children in Banke and 81% in Kailali district were vaccinated against JE. The children of second highest risk districts Kanchanpur, Bardiya, Dang and Rupandehi have got also JE vaccination during the year 2006. This vaccine was used in children under 15 years of age of 12 districts of JE risk and high-risk areas of Nepal. In Kailali and Banke districts it was found to be about 13% coverage in children population targeted, in Dang district it was 100% coverage, and in Bardiya district it was 73% coverege but in Rupandehi and Kanchanpur districts it was only about 40% and 41% respectively. JE vaccine coverage was very low in Rupandehi and Kanchanpur districts during 2005 and 2006 and very high coverage during the year 2008 [22]. According to Jeffrey et al., [23] the shift in the proportional distribution across ecologic regions in 2006 is likely caused by the mass immunization campaign using the live attenuated SA 14-14-2 vaccine that was conducted in the Terai in July/ August 2006 that targeted all of the population > 1 year of age in four highly endemic districts and the > 1 to < 15-year population in two endemic districts. Another possible reason for the shift may be because the Terai experienced a large JE epidemic in 2005 (591 laboratory-confirmed cases) that may have conferred widespread immunity resulting in fewer susceptibility.

Based on this study, the number of AES cases and higher positive cases were obtained in the hill region of Nepal. It may be because of the mass vaccination program conducted in the Terai region. Based on the studies conducted in Nepal, the Government of Nepal has approved the use of the SA 14-14-2, a live attenuated JE vaccine. The study conducted by Bista et al., Tandam et al. and Ohrr et al. suggest that the vaccine may be efficacious and safe [20,24,25]. The other reason may be, the persons from hill and

mountain may have migrated to the endemic area for work and also transmission taking place by people travelling during the incubation period from endemic places to the non-endemic places.

5. CONCLUSION

This study demonstrates that JE is still prevalent among Nepalese children especially up-to 15 years of age but other age group cannot be excluded. Although most of the cases were seen in the Central Development Region (CDR) during the month of August to November, an extensive study to cover whole area of country is of importance to know the overall scenario of Nepal. There is a need for longitudinal surveillance of JE virus on molecular level to recommend the type of vaccine effective to control the JE in Nepal.

ACKNOWLEDGEMENT

The authors express their thanks to all participating children and we are also grateful to Department of microbiology, National Public Health Laboratory, Teku, Kathmandu for providing a platform for this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=829&id=8&aid=7450