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Epidemio-entomological Study of Japanesse Encephalitis in Bhaktapur District, Nepal

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ABSTRACT

A descriptive cross-sectional questionnaire survey on Japanese encephalitis was conducted in Bhaktapur district. Mosquitoes were collected for six months by means of dark activated rechargeable CDC light trap. Out of 884 mosquitoe individuals collected, 73.41 percent were *Culex* species. The highest numbers of mosquitoes were collected in July and August. There was no significant variation in *Culex* vectors abundance during six months in three study sites Tathali, Bode and Bhelukhel. Significant correlation was found between level of knowledge and attitude towards JE prevention. Fair level of practice towards JE prevention but having no good attitude shows that though people are supportive towards JE control.

Keywords: KAP, Zoonotic disease, breeding sites, *Culex,* vector

INTRODUCTION

Japanese Encephalitis (JE) is a mosquito-borne fatal disease caused by the Japanese Encephalitis Virus (JEV).JE is transmitted to humans when bitten by the flavivirus infected female vector mosquitoes. The female mosquitoes get infected by JEV after feeding on a viremic host (pigs, cattle egret, and wild birds). The zoonotic cycle is from large water birds like cattle egrets to vector mosquitoes while swine (pig) act as amplifying hosts. Thus man acquires infection by mosquito bites only when they come up to this enzootic cycle (Konishi *et al.* 1998, Rao *et al*. 2000). Approximately 50,000 epidemic cases of JE were reported annually in Asia, including Nepal (Kanojia *et al*. 2003).

JE is endemic to Tarai region of Nepal (Joshi 1983) but evidence of JE cases were also reported from hilly districts including Kathmandu valley (Pant *et al*. 2006). A few publications describe the presence of JE outside the Tarai regions, and outbreak of JE in Kathmandu valley was conformed in 40 persons including 30 cases that had no history of travel outside the valley during the incubation period (Partridge *et al*. 2007). Principal vector *Cx. tritaeniorhynchus* was found abundant in paddy field (Darsie & Pradhan1989) and isolation of JEV by Ogawa *et al*. (1992) from a pig raised areas in Kathmandu valley incoluding twenty four cases of JE were reported in Bhaktapur district from 2007 to 2012(NZFHRC 2012).

Bhaktapur district covers 96.57 square kilometer (80.1%) of agricultural land and paddy is a major crop that provides favorable place for the JE vector *(*Shrestha *et al*. 2014*)*. Amplifying hosts like pig and cattle egret were also common in the district but people's perception about JE is scanty. Different studies on community participation programme like Knowledge, Attitude and Practice (KAP) have revealed that direct interaction with community members plays a vital role in controlling disease spread (Borante *et al*. 2010). In view of this, JE vector survey and KAP study was carried out to determine the mosquito distribution and public's perception regarding vector control programmes.

MATERIALS AND METHOD

Study area

Three locations of previously identified JE cases namely Tathali VDC, Bhelukhel tole of Bhaktapur Municipality and Bode tole of Madhyapur Municipality were selected for sampling. Rice field, pond, ditches, bushes, standing water along the road side were surveyed for mosquito collection from July to December, 2012.

Study design

Mosquitoes were sampled from regular visit, twice a month to each area around the paddy fields and pig enclosures by means of dark activated rechargeable CDC light trap from dawn to dusk. Mosquitoes were identified to genus and species following taxonomic key of Darsie and Pradhan (1989). Hundred persons from each site were randomly selected for questionnaire survey on socio-demographic characters and KAP analysis.

Data analysis

Two way ANOVA table was used to analyze the variation of number of *Culex* species between study sites in six months. The relationship among demographic characters,

knowledge, attitude and practices were analyzed with Chi-mean score by using Statistical Software Package for Social Science (SPSS) programme, version 16 for windows. Scoring system was used for analysis. A correct answer was given 1 score and 0 score for a wrong answer. The score varied from 0–12 points and was classified into 3 levels on the basis of Bloom's cut off Point, 60-80%.

RESULTS

Eleven species of *Culex* were reported in this study. The collected *Culex* vectors comprised 16 percent in Tathali, 65.5 percent in Bode and 18.6 percent in Bhelukhel (Fig. 1). Most of the species collected were female (n = 836). *Cx. quinquefasciatus* was the dominant species in Tathali (58.1%) and Bode (57.1%). *Cx. tritaeniorhynchus* was not reported from Tatahli. Occurrence of recorded species of three study sites are given in the table 1.

Table 1. Occurrence of *Culex* species in sampling sites

Culex spp.	Tathali	Bode	Bhelukhel
Cx. tritaeniorhynchus	0	1.0	9.8
Cx. bitaeniorhynchus	8.6	8.6	17.9
Cx. vishnui	2.9	2.9	9.0
Cx. gelidus	1.0	1.0	7.3
Cx. quinquefasciatus	58.1	57.1	5.7
Cx. fuscocephala	17.1	17.1	24.4
Cx. edwardsi	7.6	7.6	15.4
Cx. hutchinsoni	1.0	1.0	8.2
Cx. whitei	1.9	1.9	2.43

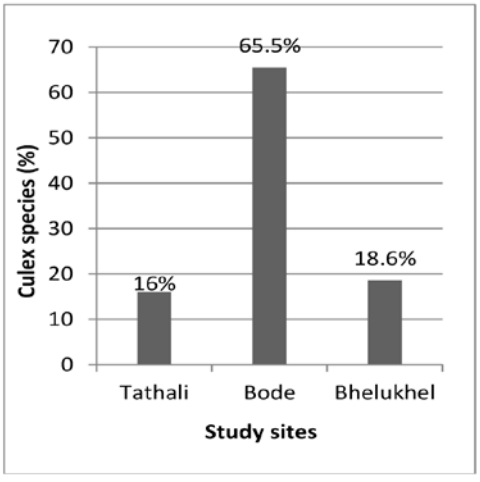

Fig. 1. *Culex* mosquito species in Bhaktapur district

The seasonal prevalence of mosquitoes collected is summarized in table 2. Highest number of mosquitoes were collected from Tathali in July (75/105) followed by August (15/105) while in Bode 274/421 of the total were collected in August with 4.91% (21/421) principal JE vector, *Cx. tritaeniorhynchus*. In Bhelukhel, highest population density was recorded in July (44/121) and *Cx. tritaeniorhynchus* was the second most abundant species 6.5% (8/121). *Cx. fuscocephala* was common throughout the survey period with its highest peak in September (7/121).

Table 2. Month wise distribution of vector mosquitoes (July to December, 2012)

Culex spp.	Area	Jul.	Aug.	Sep.	Oct.	Nov.
Cx. tritaeniorhynchus	Tathali	-	-	-	-	-
	Bode	6	21	5	0	0
	Bhelukhel	8	2	2	0	0
Cx. bitaeniorhynchus	Tathali	3	2	0	4	0
	Bode	20	47	1	0	0
	Bhelukhel	6	6	5	0	2
Cx. vishnui	Tathali	3	0	0	0	0
	Bode	8	59	3	0	0
	Bhelukhel	4	5	2	0	0
Cx. pseudovishnui	Tathali	0	0	0	0	1
	Bode	9	24	5	0	4
	Bhelukhel	1	4	4	0	0
Cx. gelidus	Tathali	45	8	5	1	1
	Bode	9	9	0	0	4
	Bhelukhel	0	4	2	0	1
Cx. quinquefasciatus	Tathali	13	4	1	0	0
	Bode	11	74	32	0	0
	Bhelukhel	18	4	11	0	2
Cx. fuscocephala	Tathali	7	1	1	0	0
	Bode	8	39	13	0	1
	Bhelukhel	4	5	7	1	1
Cx. edwardsi	Tathali	1	0	0	0	0
	Bode	8	0	1	0	0
	Bhelukhel	3	2	5	0	0
Cx. whitmorei	Tathali	3	0	0	0	0
	Bode	1	1	0	0	0
	Bhelukhel	0	0	2	0	1
Total	Tathali	75	15	7	5	2
	Bode	80	274	60	0	9
	Bhelukhel	44	32	40	1	7

The overall view of Tables shows that highest density of mosquitoes was collected during July and August. There was remarkable decline of mosquitoes from October-November and absent in December. Almost same seasonal pattern was observed in three sites. Thus no significant variation was observed in *Culex* abundance in three study sites (F value is less than tabulated value, Table 3). *Cx. quinquifasciatus* played major role in elevating combined population density. *Cx. tritaeniorynchus* was collected only during July, August and September from all three sites.

Table 3. Seasonal fluctuation of vector mosquitoes (Two Way ANOVA)

Source of Variation	Sum of square	d.f	Mean sum	F-ratio
Between sites (SSC)	967.17	2	4837.585	F1=0.282
Between months (SSR)	27265.82	5	5453.164	F2=0.317
Residuals	171744.51	10	17174.451	

Knowledge, Attitude and Practice on Epidemiology of JE

There were 300 respondents (100 from each site). The respondents were divided into Different age groups. Majority of participants were female and were found ≤20 years of age (table 4).

Table 4. Distribution of the respondents by socio-demographic characteristics

Characteristics	Tathali (%)	Bode (%)	Bhelukhel (%)
Gender			
Male	48	37	39
Female	52	63	61
Age group (years)			
≤20	32	18	22
21-30	24	38	28
31-40	20	27	24
41-50	8	8	14
≥51	16	9	12
Mean	31.8	32.2	33.1
S.D.	1.4	1.2	1.3

Knowledge of the respondents

Knowledge regarding mosquito breeding site and mosquito-borne disease is shown in table 5. The knowledge on JE symptom was very poor among the participant of the study sites. The mean knowledge was 0.45, 2.74 and 0.57 with a S.D. of 0.312, 0.456 and 0.297 in Tathali, Bode and Bhelukhel respectively.

Table 5. Knowledge of mosquito breeding site and vector borne diseases

Level	Tathali (%)	Bode (%)	Bhelukhel (%)
Breeding site			
Yes	76	79	69
No	24	21	31
Vector borne diseases			
Know	36	43	33
Don't know	64	57	67

Distribution of knowledge on JE transmission showed that 89% respondents in Tathali, 58% in Bode and 82% in Bhelukhel had low level of knowledge (Table 6).

Table 6. Distribution of knowledge level towards JE

Level	Tathali (%)	Bode (%)	Bhelukhel (%)
JE Symptoms			
High (10-12)	2	3	2
Moderate (7-9)	2	13	1
Low (0-6)	96	84	97
Total	100	100	100
Mean	0.45	2.74	0.57
S.D.	0.312	0.465	0.297
JE Transmission			
High (10-12)	7	14	13
Moderate (7-9)	4	28	5
Low (0-6)	89	58	82
Total	100	100	100
Mean	1.4	8.1	4.23
S.D.	0.539	0.729	0.297

Attitude towards JE

Majority of respondents considered JE as a non severe disease. It was noted that greater number of respondents agreed that preventive measures were needed to prevent from mosquito borne diseases. Almost all respondents agreed that communities should actively participate in controlling of JE vector. More positive attitude was observed in Bode site with 46% while it was found less in Bhelukhel with 26% only. The mean attitude score for Tathali, Bode and Bhelukhel was 8.1, 18.53 and 12.25 out of a possible 30 points with a S.D. of 0.835, 0.911 and 0.861 respectively (Table 7).

Table 7. Distribution of attitude levels towards JE of the respondents

Level	Tathali (%)	Bode (%)	Bhelukhel (%)
Positive (27-35)	22	46	26
Neutral (22-26)	4	17	17
Negative (7-21)	74	37	57
Total	100	100	100
Mean	8.1	18.53	12.25
S.D.	0.835	0.911	0.861

Practice about JE

Distribution of respondents on the basis of presence of Household pig, raising ducks and paddy cultivation is given in table 8. In all three study sites most of the respondents were found with fair level of practice regarding JE prevention. The mean practice score for the respondents of Tathali, Bode and Bhelukhel was 4.35, 4.67 and4.23 out of possible 10 scores with a S.D. of 0.672, 0.570 and 0.561 respectively (Table 9).

Table 8. Practice adopted by the respondents

	No.	No.	No.
Household pig			
Have	5	4	5
Don't have	95	96	95
Total	100	100	100
Raising ducks			
Have	5	16	10
Don't have	95	84	90
Total	100	100	100
Paddy cultivation			
Have	90	63	45
Don't have	10	37	55
Total	100	100	100

Table 9. Distribution of practice level towards JE prevention

Level	Tathali (%)	Bode (%)	Bhelukhel (%)
Good (7-10)	15	12	7
Fair (4-6	52	67	64
High (0-4)	33	21	29
Total	100	100	100
Mean	4.35	4.67	4.23
S.D.	0.672	0.57	0.561

Table 10. Association between gender and practices on JE prevention (n=300)

Level	Tathali		Bode		Bhelukhel	
	Male	Female	Male	Female	Male	Female
Poor	15	18	7	14	10	19
Fair	23	29	24	43	26	38
Good	10	5	6	6	3	4
Total	48	52	37	63	39	61
P-value	0.29		0.597		0.834	

(P value are based on chi-square analysis showing significance).

Practice behavior in relation to sex of the respondents was presented in Table 10. Beside Tathali where male had better practice than female, proportionally both sexes had nearly equal practice behavior against JE in Bode and Bhelukhel sites. The table shows that there is no significant impact of sex of the respondents with the level of practice behavior in all three sites.

Table 11. Association between age and level of practice behavior against JE (n=300)

Level	Tathali					Bode					Bhelukhel				
	<-20	21-30	31-40	41-50	>50	<-20	21-30	31-40	41-50	>50	<-20	21-30	31-40	41-50	>50
Poor	7	6	7	3	10	4	4	4	3	0	6	6	6	7	5
Fair	20	15	12	2	3	11	20	20	4	8	13	17	17	7	6
Good	5	3	1	3	3	3	3	3	1	1	3	1	1	0	1
Total	32	24	20	8	16	18	38	27	8	9	22	28	24	14	12
P value	0.04					0.677					0.395				

The result showed that the mean score of practice were found to be statistically different between resident's age in Bode and Bhelukhel but not in Tathali. Majority of them had fair level of practice in all age group (Table11).

P-value less than 0.05 in table 12 shows that practice towards JE prevention did not lead to correlate with knowledge, based on scoring analysis.

Table 12. Association between knowledge and behaviour against JE prevention (n = 300).

Level	Tathali		Bode		Bhelukhel	
	Yes	No	Yes	No	Yes	No
Poor	5	28	10	11	9	20
Fair	19	33	46	21	30	34
Good	5	10	8	4	5	2
Total	29	71	64	36	44	56
P value	0.098		0.211		0.115	

Table 13 shows that the attitude of the respondents from Bode and Bhelukhel were not very good in comparison to their practice in terms of JE prevention.

Table 13. Association between attitude and behavior against JE prevention (n=300)

Level	Tathali			Bode			Bhelukhel		
	Positive	Neutral	Negative	Positive	Neutral	Negative	Positive	Neutral	Negative
Poor	1	2	30	8	2	11	5	4	20
Fair	17	1	34	30	15	22	17	12	35
Good	4	1	10	8	0	4	4	1	2
Total	22	4	74	46	17	37	26	17	57
P value	0.023			0.136			0.233		

DISCUSSION

In Nepal, the isolates of JE virus have been obtained only from *Cx. tritaeniorhynchus*. However, in our closest neighboring country India, the virus has been isolated from 16 mosquito species viz. *Cx. tritaeniorhynchus, Cx. quinquefasciatus, Cx. bitaeniorhynchus, Cx. pseudovishnui, Cx. whitmorei, Cx. indiana, Cx. infula, Cx. fuscocephala, Cx. epidesmus, Anopheles peditaeniatus, A subpictus, Mansonia annnulifera, M indiaca and M uniformis*. Hench their role in JE epidemiology in Nepal seems to be feeble. Among the 11 species of vector collected *Cx. quinquefasciatus,* the principal vector of LF was reported in highest number. Though the species is known as poor vector of JE, few isolates of JE virus have been made from this species in India and Vietnam (Sirivanakarn 1976). The species was found maximum in July and August. The result can be compared with a study conducted by Byanju *et al.* (2012) which reported the highest population density of *Cx. quinquefasciatus* in July and lowest in September in Jaukhel VDC of Bhaktapur district. Neupane *et al*. (2009) reported the similar result in Chitwan district. Dominance of the species correlates with unhygienic environmental condition, inadequate drinking water and poor management of cattle sheds. Das *et al*. 2004 revealed the similar result for the proliferation of *Cx. quinquefqsciatus* and *Aedes* species.

In Nepal, so far, JE virus has been isolated only from *Cx. tritaeniorhynchus* and during this study highest density of the species was found in Bhelukhel which was 9.7% of the total and showed increase in abundance during July and August corresponding to rice field in the district. The similar result was obtained in Kathmandu valley (Shrestha 2011).Study conducted in Gorakhpur, India also found rice fields contributing towards the building up of population density of the species (Kanojia *et al*. 2003). House spraying with residual insecticides, elimination of breeding sites and intermittent paddy irrigation may help in controlling larval population of *Cx. tritaeniorhynchus* (Kanojia *et al*. 2003).

Cx. gelidus which found to be suspected JE vector in Nepal was recorded with 0.95%, 0.95% and 7.3% from Tathali, Bode and Bhelukhel respectively. All three sites represent large proportion of rice cultivation and few numbers of piggeries (Shrestha *et al*. 2014). This species breed in habitat like cow dung pit, ground pools containing much weeds, marshy tracts etc (Gubler *et al*. 1989). Five other *Culex* species viz. *Cx. edwardsi, Cx. thileria, Cx. hutchinsoni, Cx. whitei* and *Cx. hitmorei* formed 10.41% of the total collected mosquitoes, which are known to be important JE vector in South Asian countries (Kanojia 2007). These species are unlikely to play important role in JE transmission in district in view of its poor population density.

Community knowledge regarding vector breeding sites of mosquito is comparatively good (>50% from each site). In the similar study conducted by Kumar and Gururaj (2002) in Karnataka state of India the percentage of the respondents who were aware about breeding site was found very poor. The study suggested that this kind of ignorance of breeding site could lead to epidemics in that area. Borante *et al*. (2010) stated that there was a high negligence (70%) of stagnant water collection in Puducherry promoting the breeding of mosquitoes that inclined the population density of mosquitoes. People from the present study area also did not have the habit of removing the stagnant water nearby their houses. They mentioned that it is not needed to do it regularly as it happens in every rainy season. Dhaduk *et al*. 2013 revealed that this type of people's perceptions promotes prevalence of mosquito breeding and more larval niches in that area.

People's knowledge on JE is found to be similar to KAP study conducted by Dhimal *et al*. (2014) in highland and lowland of Nepal. Only a small proportion (<20%) of the respondents knew about the JE symptoms and its transmission. Most of them were supposing JE as another name of malaria and mentioned headache and fever as obvious symptoms. People had no clear idea how disease

is transmitted. They did not perceive pigs and wading birds as amplifying host of JE and thought that there is no problem in rearing indoor pig and ducks. To improve this kind of knowledge Kumar and Gururaj (2002) suggested the need of regular active surveillance and awareness programme through TV and radio. The knowledge of JE symptoms and its transmission is less among females than males which is similar to findings of Sharma *et al*. (1992) and Snehalata *et al*. (2003) from India. In contrast Teetipasatit (2005) stated that male and female of household leader had no relationship with preventive behavior on dengue hemorrhagic fever. Though female had less knowledge, they had good practice toward JE prevention. This might be because it is believed in the population that females should take care of the households while the males have other responsibilities (Ahmed 2007).

KAP study conducted by Dhimal *et al.* (2014) in high and low land of Nepal regarding dengue, the knowledge regarding mosquito borne diseases was found quite lower than their practice behavior. Thus significant association was not found between knowledge and level of practice behavior against JE prevention in all the three study sites.

Practice towards JE prevention among the people was not satisfactory. Whatever the prevention measure community had adopted most of them were general household sanitation. Based on scoring analysis, the result suggested that attitude did not lead to correlate with practice which was similar to Limros (2006). Fair level of practice towards JE prevention but having no good attitude shows that though people are supportive towards JE control they perceived disease as low risky due to lack of awareness as indicated by Dhimal *et al.* (2014).

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