1. INTRODUCTION

The outbreak of infectious diseases is increasing day by day due to the geographical situation and socio-ecological diversifications of Nepal. The majority of people in rural areas of Nepal are illiterate and socio-economically weak. Vector-borne diseases are the one that are more destructive to human health. Nepal has also been found endemic for different types of vector-borne diseases. Of the total population about 64.6% people is at the risk of vector borne diseases (Joshi 2004). Mosquitoes are the most important arthropod vectors that affect the well being of humans and domestic animals and are capable of transmitting different types of viral, bacterial and parasitic agents which are responsible for diseases such as malaria, yellow fever, dengue, filariasis and encephalitis. Encephalitis is of different types e.g. St. Louis encephalitis (SLE), Western Equine encephalitis (WEE), LaCrosse encephalitis (LAC), Japanese encephalitis (JE), Eastern Equine encephalitis (EEE) and West Nile virus (WNV)] etc. The relation between mosquito to man is inevitable that affects economic and human health costs (Utz et al. 2003, CDC 2010). For the understanding of the epidemiology of the vector-borne disease and their control the study of diversity, prevalence of vector in an area is important.

Arboviruses are the viruses that multiply in certain blood sucking arthropods as well as in certain vertebrates. It is a non contagious mosquito transmissible virus of human and animals. One of the arboviral diseases, Japanese encephalitis (JE) is a leading cause of mosquito borne viral encephalitis through large parts of Asia with temperate and subtropical or tropical climate. It is one of the most important zoonotic diseases worldwide caused by arboviruses. The JE virus was first isolated from the brain of the patient with fatal encephalitis in Tokyo in 1935 (Joshi 2004). People’s Republic of China (PRC), Korea, Japan, Southeast Asia, the Indian subcontinent, and parts of Oceania are the area from where most of the sporadic and epidemic cases of JE are reported annually (Joshi 2004). About 3 billion (60%) people of the world’s population live in JE endemic regions (Joshi et al. 2004). Primarily the disease affects children below 15 years (Umenai et al. 1985). Approximately 75% cases occur in children aged 0-14 years (Joshi 2004). However, adults too get infected in populations where the virus is newly introduced. In those countries where children are immunizes against JE, the disease incidence is shifted toward the elderly (Vaugh and Hoke 1992). JE usually is severe, resulting in a fatal outcome in 25% of cases and about 30% of survivors develop serious permanent sequelae

(Theodore 1996, Burke et al. 1985). The disease is more common in male than in female may be due to greater exposure (Akiba et al. 2001 and Partridge et al. 2007).

1.1 Vector and its behavior

Thirty species of mosquitoes belonging to 5 genera of *Culex*, *Anopheles*, *Aedes*, *Mansonia* and *Armergeries* harbor the viruses of JE. Culicine mosquitoes, notably *Cx. tritaeniorhynchus, Cx. vishnui, Cx. gelidus* along with some Anopheles have also been incriminated as the vector of JE (Darsie and Pradhan 1990). *Culex tritaeniorhynchus* species is suspected to be the principal vector of JE in Nepal because JEV isolates have been obtained only from a pool of *Cx. tritaeniorhynchus* females and also because the female *Culex tritaeniorhynchus* is found abundant in rice field ecosystem of the endemic areas during the season (Darsie and Pradhan1990). These mosquitoes are known to live in close association with vertebrate hosts specially pigs and wading birds and are prolific in paddy field ecosystem which is the favorable place for the completion of their life cycle. The zoonotic cycle is from large water birds 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). Environmental factors, behavioral patterns of human populations and vector distribution provide the favorable conditions for disease outbreaks. Temperature between 24ºC to 38ºC with high humidity and rice field land mass of the tropical region promote the breeding of JE vector mosquito species for completion of their life cycle. Precisely larvae of these mosquitoes prefer to breed in irrigated rice field, shallow ditches and pools, ponds in partial shed or full sun (Pradhan 1981). These mosquitoes are zoophilic feeding primarily on vertebrate hosts, exophagus principally on vertebrate hosts other than human (Gubler et al. 1989). They begin to bite at dusk and during evening hours until dawn. The rice field is the most important larval breeding places. For the first time the mosquito-borne mode of JE transmission was proved with the isolation of JE virus in 1983. Later in other field studies that also established the role of aquatic birds and pigs in the viral enzootic cycle (Bista and Shrestha 2001).

The female mosquitoes get infected by JEV after feeding on a viremic host (pigs, wild birds etc.). And after 9-12 days of incubation period, they can transmit the virus to other hosts (Darsie and Pradhan 1989). Human get the JEV infection when bitten by the flavivirus infected vector mosquitoes. The virus passes to human through their saliva during feeding. It is carried to brain and begins to affect CNS and causes inflammation of brain tissue, known as encephalitis. Patient with JE typically present a few days of non specific febrile illness followed by headache, vomiting and a reduced level of progress to a serious infection of the brain (encephalitis) (Solomon1998). Furthermore, person starts to develop symptoms like high fever, headache and stiff neck etc. In severe cases it may progress to paralysis, coma and death. Man is considered as an incidental 'dead end' host. After infection it does not develop a level of viremia so that it can infect the mosquitoes. The virus transmission continues unnoticed through mosquitoes. The virus does not cause any disease among its natural host pig but abortion, still birth and miscarriage are more common. Man to man transmission has not so far been recorded.

1.2 Japanese encephalitis in Nepal

As mentioned earlier, Japanese encephalitis (JE) is one of the most important arboviral diseases in Nepal. It is seasonally endemic to terai region, which borders the northern states of India, Uttar Pradesh and Bihar. The disease was first recorded in Nepal in 1978 as an epidemic in Rupandehi district of the Western Development Region (WDR) and Morang of the Eastern Development Region (EDR) (Bista 1992, Bista and Shrestha 2001). Since then, it has been reported in animal reservoirs and in humans throughout the Terai region (Bista 1992, Bista and Shrestha 2001, Pant et al. 2006, Akiba et al.1997, Joshi 1983, Wierzba et al. 2008). At present the disease is endemic in 24 districts (Singh and Gurung 2002) namely Jhapa, Morang, Sunsari, Saptari, Siraha, Udayapur, Dhanusa, Mahottari, Sarlahi, Sindhuli, Rautahat, Bara, Parsa , Makawanpur, Chitwan, Nawalparasi, Rupandehi, Kapilvastu, Palpa, Dang, Banke, Bardia Kailali, Kanchanpur. Among them, 10 districts namely Jhapa, Morang, Sunsari, Parsa, Rupandehi, Dang, Banke, Bardia, Kailali and Kanchanpur are affected most (Bista and Shrestha 2001).The endemicity of JE in these districts of Nepal represent the paddy field ecosystem, with abundant *Culex* species and amplifying hosts like pigs and migratory birds indicating the potential epidemics in these districts. The transmission and outbreak of disease positively related with lack of continuous surveillance, unmanaged agricultural practice, inadequate environmental sanitation, monsoon rain, irregular vector monitoring etc.

Joshi et al. (2004) recorded a total number of 8874 cases and 1264 deaths in the study conducted from 1998 to 2003 in Nepal. The average case fatality rate was 14.2 percent in aggregate since 1998. The study revealed that the highest number of cases were reported in 1999 which was 2924 cases and the second highest were in 2001 which was 1888 cases. A total of 49,385 cases and 7,492 deaths with the average CFR rate of 15.2% has been observed in Nepal from 1978 through 2011 (EDCD 2005, Joshi 2011). Almost 50% cases occur in 15 years or younger age group with high incidence in 5-15 years (Dhakal 2011). More than 50% of morbidity and 60% mortality of JE cases occur in this age group. The CFR were 36.0%, 38.0%, 35.2%, 31.2% in 1990, 1991, 1992 and 1993 respectively (Joshi et al. 1995). Since 1978 total of 26,667 cases and 5,381 deaths with average case fatality rate of 20.2% have been reported (Bista and Shrestha 2001). Cases of JE begin to appear in the April-May period and reach its peak during August to early September and declines from October (Bista and Shrestha 2001). Majority of cases take place after the rainy season (monsoon).

1.3 Situation of JE in Kathmandu valley

JE cases are found endemic mainly in tropical climate areas of the terai region. Temperate and cold climates of hills and valleys also proved its existence. A few publications describe the presence of JE outside the Terai regions, and outbreak of JE in Kathmandu valley in the hill region was confirmed in 40 residents of the Kathmandu valley, including 30 cases that had no history of travel outside the valley during the incubation period (Partridge et al. 2007). Presence of *Cx. tritaeniorhynchus* in Kathmandu (Annual report, 2001) and isolation of JEV by Ogawa et al. (1992) from a pig raised in Kathmandu valley are indicators of the real presence of disease. To inhibit the forthcoming JE epidemics study on vector fauna, vector control and community education towards the disease in these areas are the need of the day.

In view of evidence of JE endemicity in Kathmandu valley, a somewhat broader comprehensive view of the current status of JE in relation to reported cases and species composition of vector mosquitoes and its seasonal prevalence in Bhaktapur district is urgently needed to help in ourselves and other concerned health workers to deal with forthcoming JE epidemics. Total 9, 8, 3, 1, 1 and 2 numbers of cases have been recorded during 2007, 2008, 2009, 2010, 2011 and 2012 respectively from the district (NZFHRC 2012). Thus the area should not be considered as risk free area. No detail information is available on the epidemio-entomological aspect of JE in the district which is known for its endemicity in Kathmandu valley. Surveys regarding disease and its vector should be conducted to control disease burden. One of the important components of vector-borne disease control program is to impart awareness about mosquito bite prevention in the general community along with vector density. Community participation program like KAP (Knowledge, Attitude and Practice) of community serve as a baseline survey to make ourselves and other concerned health workers to deal with forthcoming JE epidemics.

1.4 Objectives

General objective

To identify KAP of community members in relation to JE and its vector abundance in three study sites of Bhaktapur district.

Specific objectives

1. To study the relationship of JE prevention practices with age, gender, education, marital status, level of knowledge and attitude of the people of the three study sites.
2. To compare fluctuation in population size of vectors of JE.

1.5 Rational and justification of the study

Every person in Nepal is at the risk of vector-borne diseases like JE. Although twenty-four districts are considered to be at the risk sporadic cases have also been reported from other non endemic districts including Kathmandu Valley (Partridge et al. 2007). In 2007, a total of 360 AES cases were reported from 40 hill or mountain districts. Of the 344 reported AES cases for which diagnostic samples were obtained, 90(26%) were laboratory conformed as JE from 21 hill and 3 mountain districts. Among laboratory confirmed JE cases, CSF samples were collected from 13(14%) patients and serum samples from 77(86%) patient (Akiba et al. 1997). The largest number of AES and laboratory conformed JE cases were reported during the monsoon month of August (23 cases) and September (33 cases). Since JE is transmitted to humans via the bite of infected mosquitoes, this may have epidemiological significance. This is the reason, JE is mostly a disease of rural agricultural areas like Bhaktapur where vector mosquitoes live and grow in close association with the main vertebrate hosts. In the district, since 2007 total of 24 cases have been reported. In 2012, of the 6 reported AES cases 2 were proved as JE (NZFHRC 2012). This type of outbreak of JE in non-endemic district makes it a serious public health problem.

Earlier studies conducted in Bhaktapur also found rice fields contributing towards the building up of population density of vectors (Shrestha 2011). The presence of JE vectors *Culex tritaeniorhynchus, Cx. vishnui*, *Cx. pseudovishnui* and *Cx. gelidus* in the valley including Bhaktapur district (Darsie and Pradhan 1990) and amplifying hosts has been confirmed in the hill region (Zimmerman et al. 1997). Additional studies are also needed to confirm the presence of environmental and ecological conditions that promote JE virus transmission. Gathering of information on the level of knowledge and practice along with species composition and seasonal fluctuation regarding JE and its vector as well as regular surveillance of vector is the prerequisite way to control the vector-borne diseases like JE. To get success in the community based program, the most important step is to know the community’s perceptions towards disease, vector mosquito, breeding habits of mosquitoes and the attitudes held by them in the control efforts. A sound knowledge base about vector-borne diseases and method of vector control is required among the community (Rao et al. 2000). Therefore, KAP study is one way to find out the actual situation of JE of that area. It provides the valuable insights necessary for guiding the implementation of JE health promotion programs and reduces risk behavior. It is necessary to gather information, thus appropriate communication program are to be used to make the education strategy effective (Kumar and Gururaj 2002).

The epidemio-entomological study is needed in order to determine the species composition, relative abundance and fluctuation of mosquitoes in relation to occurrence of JE in the district. Therefore, keeping this in mind, through a KAP, an attempt has been made to analyze the community’s perception towards disease, the breeding habit of mosquito and their participation in control activities, monitoring of area to determine the transmission cycle and collection of mosquitoes by means of dark activated rechargeable CDC light trap to study species composition and fluctuation of mosquitoes in Bhaktapur district, with particular emphasis in Bhelukhel tole, Bode tole and Tathali VDC.

2. LITERATURE REVIEW

2.1 Knowledge Attitude and Practice (KAP)

Ahmed (2007) demonstrated that the demographic data were not correlated with practice scores except for gender. Knowledge was significantly associated with practice. Hence people who have high knowledge regarding dengue fever will have a good practice in prevention of dengue fever. Measures against mosquitoes are probably only used when people experience a mosquito nuisance. Therefore proper prevention programs need to be developed to make the community more aware which then will motivate people to modify their behavior.

Borante et al. (2010) conducted a community based cross sectional study on knowledge of mosquito-borne diseases in peri urban areas of Puducherry. The study revealed that awareness and knowledge towards mosquito borne disease was low among females, illiterate and economically backward respondents. Majority of respondents knew about the chemical method as a measure for prevention and control of the mosquito-borne diseases.

Dhakal (2011) revealed in their study that heavy rice farming under flooded irrigation, inflated pig husbandry without good sanitation, seasonal fluctuation and lack of awareness are the responsible factor for the geographical expansion of JE. They confirmed the expansion of JE by the presence of vector mosquito and pig infection with JEV from the previously silent hill and mountain districts. Dowling (2011) conducted 242 paired entomological surveys and KAP questionnaires in six socio-economically diverse neighborhoods in residential Washington, D.C. metropolitan area. Study found that certain demographic groups have lower rates of source reduction practice and mosquito-related knowledge, potentially putting them at greater risk of exposure to mosquito-borne disease. They noted that household income affected residents' knowledge and attitudes regarding mosquitoes, but that greater general knowledge of mosquitoes and concerned attitudes did not lead to higher levels of source reduction practice.

Dumre (2004) demonstrated that 4.3% JE positive cases from Kathmandu, 4.1% from Kapilvastu, 3.7% from Rupandehi, 3% from Kanchanpur, 2.9% from Sunsari, 2.6% from Bara and 2.5% from Nawalparasi were confirmed. He concluded that higher number of cases from Kathmandu district may be due to the poor reporting system, a number of health institutions concentrated in this area and low level of public awareness. The regular contact of the inhabitants of Kathmandu with people from different part of the country also signals towards the high positive cases.

Environmental Health Project (EPH 2003) surveyed on three important mosquito-borne diseases in Nepal namely malaria, Japanese encephalitis and Kalazaar through a series of study. Of the total respondents 76%, 68% and 23%, were unaware of Kalazaar, Japanese encephalitis and Malaria respectively. Increased awareness was associated with reduced risk behavior like use of bed net. Socio-economic status, literacy and radio ownership etc. were the related factors that increase the knowledge and awareness of vector-borne diseases (VBDs).

Gurung and Singh (2003) studied on the risk factor associated with JE in western district of Nepal during 2002. Total of 142 numbers of confirmed JE cases and 142 controls from three western district of Nepal namely Banke, Bardia and Dang were interviewed. Joshi (2004) mentioned in his research work carried out in Kanchanpur district in 2003 that most of respondents were unaware about the JE and its preventive measures. The study revealed that use of mosquito net, knowledge towards disease transmission, symptoms and its vector was very low. And non immunization status, presence of household pigs, non use of mosquito net and sleeping outdoor were the risk factors to occur JE.

Hairi et al. (2003) conducted a study on knowledge, attitude and practices (KAP) on dengue among selected rural communities in the Kuala Kangsar district. They found that the knowledge on dengue of community was good. Cross tabulations were done between knowledge and practice, knowledge and attitude, and practice. There was no significant association seen between knowledge and practice. However, there was a significant association seen between knowledge and attitude towards *Aedes* control (p=0.047).

Joshi et al. (2004) carried out a KAP study in Nepal during 2003. They studied that knowledge regarding mosquito borne disease is less among female than male. More than 50% of respondents were aware towards stagnant water as a mosquito breeding site. Most of them used to spray insecticide as a control measure. Joshi (2011) investigated that source reduction (for instance, removing stagnant water), importing natural predators like dragonflies, biological control etc are the important methods to control and prevent from mosquito-borne diseases.

Joshi (2011) conducted a study in an association between demographic variables and mosquito exposure risk in Nepal and found that higher adult mosquito infestation rate correlates with lower socio-economic status.

Kay et al. (2000) reported that elimination of standing water by residents in their own yards can be a cost-effective means of controlling vector mosquitoes in urban areas. Kubo et al. (1996) and Pandey et al. (2004) revealed that in terai; most of the people have farming as their major occupation. Presence of paddy fields also provides a suitable environment for mosquito-breeding. The open border system with India can also contribute to high number of JE cases in these areas. The economic activities in these areas were related to farming, fishery, cattle raising and jungle roaming for woods. Extensive outdoor activities, longer stay outside the home and exposed body parts due to poor clothing put them at a high risk of mosquito bites (Kubo et al. 1996, Pandey et al. 2004).

Kumar and Gururaj (2002) conducted a survey regarding mosquito-borne diseases in an urban and rural area in Karnataka state of India. The study showed most of the study population had knowledge that mosquito bite is the cause for malaria but unaware about JE, kalazar, dengue etc. Television was found to be the best source of acquiring knowledge as a source medium amongst the study population. Pandit et al. (2010) studied on awareness and practice against mosquito bite in Gujarat in the month of June 2009. They revealed that almost all had knowledge about mosquito breeding places in opposite to biting time. Since the insecticide treated bed net is a good weapon to fight against mosquito-borne diseases, only 39% and 10% of households were using net and insecticide respectively against mosquito bite.

The attitude score was positively correlated with knowledge score ($p<0.001$) but attitude, unlike knowledge, was not found to be associated with elimination of breeding places in the study conducted by Limros (2006) in Konkrailat District, Sukhothai Province. For community level cooperation against dengue infection, attitude was associated with campaign ($p=0.001$) and frequency of information with spray use ($p<0.001$) and coil use ($p=0.022$).

Snehalata et al. (2003) studied the mosquito problem and personal protection measures used in rural and urban communities in Pondicherry region of India and revealed that human knowledge, attitude and practice of various methods of personal and household protection against mosquito bites vary in different endemic region of tropical countries. The most popular method used against mosquito bite was mosquito coil in urban and rural areas. Sabesan et al. (2010) reviewed the data at macro-level. They demonstrated that the occurrence of endemic JE is largely determined by the factors Such as irrigation, agriculture activities, and animal husbandry practices. They noted that the epidemic JE is mainly due to fluctuation of climate conditions in the region. Both rainfall and altitude found to be promoted the outbreak of JE in the epidemic districts. JE immunization of the epidemic areas may be an effective preventive measure. Whereas besides the selective immunization of children in the endemic areas, regular monitoring of vector population and viral activity, and implementing appropriate integrated methods of vector control reduce the transmission in the endemic.

2.2 VECTOR

Darsie and Pradhan (1990) reported 168 species and sub species in 16 genera of Mosquitoes in Nepal. Their publications continued the record of *Culex tritaeniorhynchus*, the principal vector and other suspected vectors of JE from different parts of the country. Darsie and Pradhan *(*1990) revealed that *Culex gelidus* was also believed to be the suspected vector of Japanese encephalitis and was the most abundant mosquito in Sunsari district. It is associated closely with man and his domestic animals. Their immature live in puddle, pools, rice fields and marshy depressions having abundant vegetation. Their preferred hosts are bovines and swine but they also readily attack man.

Das et al. (2004) conducted an epidemiological investigation of JE in Orissa, India. Anophelian mosquitoes were dominant species and *Culex vishnui* group (the known vector of JE) constituted 19% of the total collection. They reported that of the total *Culex tritaeniorhynchus* collected, 23.5% of these mosquitoes had human blood and 5.8% had blood meals from pigs.

Culex tritaeniorhynchus and Culex pseudovishnui were abundant in shallow pools and in fallow rice fields. Heavy rainfall might have been detrimental to the larvae at the time when their surveys were conducted by Hill (1970).

Jeong et al. (2003) collected a total of 3,663.3 females of malaria vector *Anopheles sinensis* and 31,425 females of Japanese encephalitis vector C*ulex tritaeniorhynchus* per trap night from June to September for the years respectively during the field survey on the prevalence and seasonal abundance of the dominant mosquito species in a large marsh near coast of Uslan. *Anopheles sinensis* was found to be the most abundant (53.4%) in species ratio followed by *Culex tritaeniorhynchus* (43.0%), *Culex inatomii* (1.6%) *Ochleratatus dorsalis* (1.3%) and C*ulex pipiens pallens* (0.5%).

Jomon and Thomas (2010) conducted an entomological study to determine species composition, relative abundance, seasonal population fluctuation in Kottayam district of India and revealed that out of 16 species, 10 were *Culex viz*., *Cx. bitaeniorhynchus, Cx. epidesmus, Cx. fuscocephala, Cx. gelidus, Cx. infula, Cx. psedovishnui, Cx.quinquefasciatus, Cx. tritaeniorhynchus, Cx. vishnui*, and *Cx whitmorei* 9 are known vectors in India. *Culex tritaeniorhynchus* was the predominant and wide spread species. *Culex quinquefasciatus* was the second most predominant species. *Cx. gelidus* and *Cx. bitaeniorhynchus* occupied the third position in abundance. Most of the species exhibited pronounced seasonal population fluctuation.

Joshi et al. (1983) made a major contribution by reporting 59 species of Culicine including 28 new country records were reported during the entomological survey in Nepal. The new records included the *Culex tritaeniorhynchus;* the principal vector of JE and *Culex whitmorei* the other suspected vector of JE.

Macdonald et al. (1967) and Hill (1970) mentioned that rice field, ground pools, fish ponds and ditches were found to be preferable breeding places of *Culex tritaeniorhynchus* in Sarawak. In the absence of paddy fields it was found in other types of ground water. In south India this species was found in large numbers in well, ditches and ponds but rice fields were undoubtedly the major breeding source. It may be due to providing a much larger water source than any other type of ground water surface. LaCasse and Yamaguchi 1995 found *Culex tritaeniorhynchus* breeding chiefly in ground pools, ponds, ditches and paddy fields but not the most importantly in paddy fields in Japan. Its larvae was reported from different habitats such as paddy field, field with rice (paddy) plants and follow field flooded with water and ploughed but not yet planted with rice plants.

Peters and Dewar (1956) were the first to record certain Culicine species including *Cx. vishnui*, *Cx. fuscocephala*, *Cx gelidus*, *Cx bitaeniorhynchus* and *Cx barraudi* the suspected vectors of JE in Nepal. Neupane et al. (2009) had reported 12 species of suspected JE vector including *Culex quinquefasciatus*, *Cx. fucocephala*, *Cx. tritaeniorhynchus*, *Cx. gelidus*, *Cx. bitaeniorhynchus*, *Cx. whitmorie* and *Cx. sinensis* from 10 villages of Chitwan district of Nepal during August-September 2007 to March-April, 2008. The study revealed that in Champanagar VDC of Chitwan *Cx. quinquefasciatus* was abundant both in pre-monsoon and post monsoon with highest resting density (human) in post-monsoon.

Ree et al. (1976) conducted an excellent review on literature of Japanese encephalitis referring to biology and epidemiology of *Culex tritaeniorhynchus* in Korea. Similar study was conducted by Gloud et al. (1974) in Thialand on its role and bionomics. Samuel et al. (1980) conducted an sero- entomological study of JE in Utter Pradesh, India. The study showed that *Cx. tritaeniorhynchus* was the predominant species followed by *Cx. vishnui*.

Shrestha (2011) carried out an entomological study in Tokha and Gothatar of Kathmandu, Hattiban of Lalitpur and Balkot of Bhaktapur district. The study reported 404 *Cx. tritaeniorhynchus* and 55 *Cx. gelidus* from the study areas. The highest vector abundance was recorded in September 2009.

2.3 JE in Global context

JE is a seasonal disease, with most cases occurring in temperate areas from June to September. Further south in subtropical areas, transmission begins as early as March and extends until October. Transmission may occur all year in some tropical areas (eg Indonesia). Although considered by many in the west to be a rare and exotic infection, JE is numerically one of the most important causes of viral encephalitis worldwide, with an estimated 50,000 cases and 15,000 deaths annually (Tsai 1998, Solomon et al. 1998).

Solomon et al. (2003) described epidemics of encephalitis in Japan from the 1870s onwards. Major epidemics were reported about every 10 years with more than 6000 cases reported in the 1924 epidemic (Miyake 1964). During 1970s and 1980s, JE was endemic to only a few countries of east Asia like Japan, Korea and China. Then, it spread from

East Asia to south east Asia (SEA) and then to south Asia. The virus was isolated in Japan in 1935, and has been recognized across Asia since then.

In the past 50 years, the geographical area affected by JEV has expanded. The timing of the first reported cases or new epidemics in each area gives an impression of the relentless spread of JE (Solomon et al. 2003). The disease has occurred on the western pacific islands with outbreaks in Guam in 1947 (Hammon et al.1958) and Saipan in 1990 (Paul et al.1990). In that year in Saipan, 10 cases occurred among a population of 40,000 and the prevalence of antibody to JEV among 234 lifelong Saipan residents surveyed after outbreak was 4.2% while the sero-prevalence in pigs was 96% (n=52).

In Thailand, 1,500-2,500 cases were reported annually. An antibody prevalence survey conducted in Thailand during 1989 studied 3,089 blood samples of children (aged 6 months to 14 years). Out of which 27.45% of children possessed neutralizing antibody to JEV (Rajanasuphot et al. 1992).

Chokephaibulkit et al. (2001) conducted a perspective study of childhood encephalitis in Bangkok from 1996 to 1998. Among 26 (65%) children with identifiable viral agents, JE was reported in 6 children.

Strickman et al. (2000) studied distribution of dengue and JE among the school children of rural and suburban Thai villages where, out of the 1,477 children, 33/1000 had recent dengue and 7/1000 had recent JE infection. In a study an acute undifferentiated fever caused by infection with JEV, JE was reported in 22 (14%) individuals out of 156 adults, indicating JE as an important cause of acute fever in Asia (Watt and Jongsakul 2003). This study was carried out in Chiangrai Regional Hospital, Thailand.

Umenai et al. (1985) reported that in recent years the epidemiological pattern and geographical distribution of JE had changed in Asia. In Taiwan, Japan, South Korea and China clinical cases of JE have decreased dramatically. This has been possible through the integrated control effort comprising of human vaccination program, water management, and immunization of pigs, systematic piggery and community awareness program. Epidemics of JE were reported in many provinces of China in the early 1950s and with almost all provinces being affected by now (Yu 1995). There is currently 10,000-20,000 cases/year, although in the early 1970s it was over 80,000 cases per year (Vaughn and Hoke 1992).

Hanna et al. (1995) reported that gradual spread of disease to other non-Asian regions for example, Torres Strait of Australian mainland had been reported recently. The first outbreak of JE (two clinical cases) in Australia was reported in 1995. These cases were identified on an island in the Torres Strait. No new case of JE was notified in Australia in 2002. An entomological investigation of an outbreak of JEV in the Torres Strait, Australia in 1998 recovered 43 isolates of JEV from adult mosquitoes (42 from *Culex sitiens* and one from *Ochlerotatus vigilax*) and also identified 2 confirmed human JE cases in that area and Cape York Pexinsula in Northern Queensland (Johansen et al. 2001). Because of these outbreaks, mosquito borne arboviruses causing human diseases have been considered as important public health issues in Australia also.

Vaughn and Hoke (1992) reported since, most of the JE cases (35-50,000/year) are reported from the Asian countries especially south east Asian countries more research activities are also focused in these countries. In northern temperate region of Asia, JEV causes larger summer epidemics, whereas in southern tropical regions, it causes endemic disease year round. Cross sectional serological surveys have shown that in rural Asia, most of the populations are infected with JEV during childhood or early adulthood. About 10% of the susceptible population is infected each year; however, most infections are asymptomatic.

In Taiwan, the first clinical case of JE was recorded in 1931. The case incidence rate of JE during 1966 to 1977 showed a sharp decrease from 2.05/10,000 in 1967 to about 0.03/10,000 in 1997 reflecting the efficacy of JE vaccination started in 1968 onwards in Taiwan **(**Wu et al. 1999). Korea demonstrated more than 1000 JE cases/year before 1969 but after the vaccination started in 1960 onwards the number of cases decreased dramatically. Vaccine coverage reached almost 100% in the 3-15 years age group in 1985. The widespread use of Vaccine in children has been associated with a higher incidence of JE in those over 15 years (Vaughn and Hoke 1992).

Kanojia et al. (2003) revealed that general mosquito population showed bimodal pattern of peak occurrence during February and October in both the peaks, *Cx. tritaeniorhynchus* contributed maximum. Substantial densities of *An. subpictus* and *An. peditaeniatus* during JE season suggested the supportive role of these species. Other vector species *viz., Cx. quinquefasciatus, Cx. pseudovishnui, Cx. vishnui, Cx. gelidus, Cx. fuscocephala, Cx.*

bitaeniorhynchus, *Ma. annulifera, Ma. Uniformis* and *An. barbirostris* perhaps had a limited role in the transmission of JE virus due to their extremely poor density.

2.4 National issue

In Nepal JE was first reported in Rupandehi district of Western Development Region (WDR) during 1978 (Joshi 1986, Bista and Shrestha 2001). Subsequently, JE epidemics occurred in Morang district of Eastern Nepal from adjoining Bihar state of India and thus, the disease is gradually spread into other districts in the successive years. At present JE is endemic in twenty four districts of Nepal namely Jhapa, Morang, Sunsari, Saptari, Siraha, Udayapur, Dhanusa, Mahottari, Sarlahi, Sindhuli, Rautahat, Bara, Parsa , Makawanpur, Chitwan, Nawalparasi, Rupandehi, Kapilvastu, Palpa, Dang, Banke, Bardia Kailali, Kanchanpur. Among them, 10 districts namely Jhapa, Morang, Sunsari, Parsa, Rupandehi, Dang, Banke, Bardia, Kailali and Kanchanpur are affected most (Bista and Shrestha 2001).

Dhakal **(**2011) reviewed that after the first report of JEW from Kathmandu valley in 1997, the disease was also reported from different hilly as well as mountain region which was previously silent. The paper reported that till now 54 districts have shown the JE cases and is its geographical diversification. Bista and Shrestha (2001) reported that a total of 26,667 cases and 5,381 deaths occurred from JEV infection with 20.2% average case fatality rate (between the periods of 1978-2003). Pant et al. (2006) reported a total of 371 cases of JE in Nepal in 2004.

Zimmerman et al. (1997) reported the first proven outbreak of JE in Kathmandu valley and re-analyzed 44 pig sera (providing collected for hepatitis E serology from free roaming swine of Kathmandu valley and stored frozen) for IgM test against JE virus infection and found 24 of 44 pigs positive. They reported the presence of both JE vectors and pigs that were already confirmed in hill region. The endemicity of JE in Kathmandu valley was first studied by Patridge et al. (2007). Of the 344 reported AES cases for which diagnostic samples were obtained, 90(26%) were lab confirmed as JE from 21 hill and 3 mountain districts. Among 40 cases with confirmed residency in the Kathmandu valley, 30 had no history of travel outside the valley.

A study conducted during 2004-2006 revealed that a total of 108 lab confirmed JE cases were reported from hilly as well as mountain district except Kathmandu valley (Bhattachan et al. 2009). Two and four cases of JE from mountain district and 76 and 153 cases from hill district was reported during 2006 and 2007 respectively total 54 districts was detected with JE cases (Pant et al. 2006).The number of JE cases and deaths that occurred due to JE in Nepal during the thirteen-year period (1978 to 1990) correlated well with the findings of India (Kubo et al. 1996). This was also proved by Kubo's antigenic study and is attributable to the free and frequent travel of people of both countries through open border. The plain areas (<1000m) were seen to be endemic, while the hills (1000-3000m) and mountains (>3000m) seen to be affected sporadically in Nepal (Kubo et al. 1996).

Joshi et al. (2004) and EDCD (2005) mentioned that conditions in the terai region are most favorable for the breeding of *Culex* mosquitoes. Although the disease has been reported throughout the year from endemic areas, epidemics occur during the monsoon season- starting in the April/ May period, peaking during August and September, declining in October, and leveling off in November. Joshi et al. (2004) also mentioned that between 1993 and 1997, the districts affected with JE ranged from Jhapa district in the eastern Terai region to Kanchanpur in the Far West. Almost 85% of cases came from eight districts namely Kailali, Banke, Bardia, Rupandehi, Parsa, Morang, Sunsari and Jhapa. Banke reported 30 percent of the total, Kailali 20%, Morang 10%, Bardiya 7%, and almost 6.5% from Rupandehi. Sunsari, Jhapa and Chitawan each reported less than 5%.

Solomon et al. (2003) reported the JE case clustered in the terai where it seemed to shift to the Kathmandu valley in subsequent years. They mentioned spatial; pattern of JE cases during the 2005 epidemic in Nepal was significantly associated with low precipitation of irrigated land. Dumre (2005) noted the highest no. of AES cases were reported from Kailali district (435, 14.7%) followed by Dang (10.3%), Bardia (9.3%), Kathmandu (8.8%), Banke (7.4%), Kachanpur (5%), Kapilvastu (3.8%), Nawalparasi (3.8%) and Sunsari (3.7 %). More than 66 % (1535 cases) AES cases were reported from these 9 districts. The highest number of AES cases (839, 28.4 %) was reported from MWDR and geographically, 20 terai districts reported 75.6 % of the total AES cases.

JE situation in Bhaktapur: 2007 to 2012

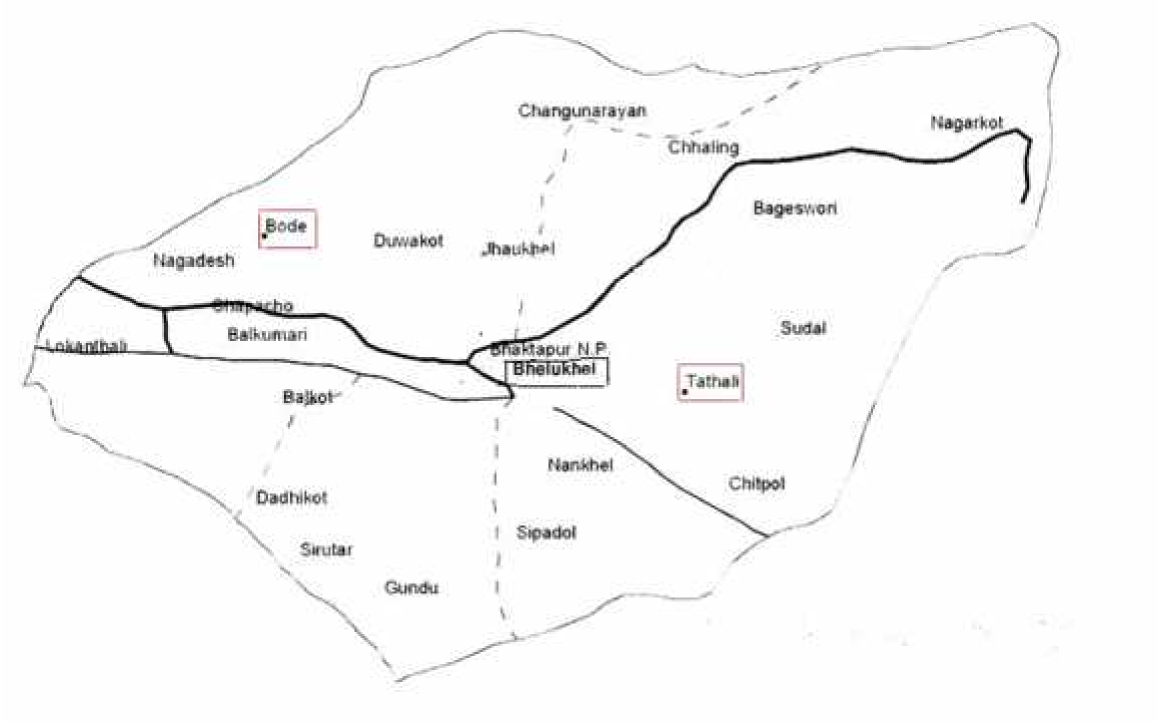
Year	Place	Ward no.	Sex	Age	Vaccine	Outcome
2007	Nankhel VDC	2	M	27	No	Cured
	Bhaktapur municipality	1	M	27	No	Cured
	Bhaktapur municipality	7	F	8	No	Cured
	Bhaktapur municipality		M	22	No	Death
	Bhaktapur municipality	5	F	0/5	No	Cured
	Bhaktapur municipality	8	M	32	No	Death
	Nankel VDC	2	M	0/2	No	Improving
	Nankhel VDC	8	F	2	No	Cured
	Nankhel VDC		M	6	No	Cured
2008	Bhaktapur municipality	12	F	13	No	Improving
	Sudal VDC	2	M	0/10	No	Cured
	Thimi municipality	14	F	4	No	Cured
	Thimi municipality	14	F	0/3	No	Cured
	Thimi municipality	16	M	13	No	Improving
	Bhaktapur municipality	4	M	0/2	No	Improving
	Nagarkot VDC	4	M	12	No	Death
	Duwakot VDC	4	F	6	No	Cured
2009	Sirutar VDC	3	M	18	No	Improving
	Bhaktapur municipality	17	M	0/4	No	Cured
	Thimi municipality	14	M	24	No	Cured
2010	Thimi municipality	15	M	14	No	Improving
2011	Thimi municipality	14	M	8	No	Cured
2012	Thimi municipality	14	M	1-4	No	Cured
	Thimi municipality	15	F	15+	No	Cured

Source: NZFHRC; 2012

3. MATERIALS AND METHODS

3.1 Study Area

Bhaktapur, the city of devotees is situated at 27º36' to 27º44' latitude and 85º21' to 85º 32' longitudes (CBS, Nepal 2001). It is situated at west of Bagmati zone and lies about 13 km eastward of Kathmandu covering an area of 119sq km with an average population of 303,027 in the national census 2011 (City population 2012). Bhaktapur is listed as a world heritage by UNESCO for its rich culture, temples and wood, metal and stone artwork and it comprises two municipalities viz. Bhaktapur municipality and Madhyapur Thimi municipality and sixteen VDCs namely, Bageswori, Balkot, Changunarayan, Chhaling, Chitpol, Dadhikot, Duwakot, Gundu, Jhaukhel, Kautunje, Nagarkot, Nankhel, Sipadol, Sirutar, Sudal, and Tathali. Agriculture is the primary occupation of the people. The main crops grown are rice, wheat, maize and different types of vegetable crops. Some of the farmlands are occupied by some local as well as people outside the valley for cattle farming. The climate of the district is sub-tropical cool weather type with 75% annual average humidity. The temperature in general is maximum 32°C and minimum 20°C and average rainfall is 1400 millimeter most of which falls during June to August. Three study sites namely Tathali VDC, Bhelukhel tole of Bhaktapur municipality and Bode tole of Madhyapur Thimi municipality were selected for the study.



Map showing study areas of Bhaktapur district

Tathali

Tathali village develop committee lies towards the eastward of Bhaktapur municipality with an estimated population of 4,520 with total 751 numbers of houses. It lies at a distance of 5km from Bhaktapur Durbar Square. Most of the farmlands are used for the main agricultural product. Some of the farmlands are used by non local people for pig farming. A typical family in the village maintains livestock in their compound. Compound is made up of a human residence and a shed for the animals. Thus people live in close proximity to their livestock, i.e. within a distance of 5-6 m.

Bode tole

Bode tole of Madhyapur Thimi municipality is located at 10 km eastward of Kathmandu and 4 km northwestern corner of Bhaktapur and can be reached by 30 minutes on foot from Sankhadhar choke. The area is situated on the elevated land and thus one has to climb up to reach this area. Most of the inhabitants are farmers. Beside vegetable cultivation and rice field, few number of traditional indoor cattle shed was also present. One piggery at the center of the area was present with poor sanitation. It was situated just behind the two slouther houses. Another two piggeries could also be seen from this area which was located at the downhill side of southeast direction. Manohara river passes just by these two piggeries. People in this area maintains livestock in their compound including cattle, chicken, ducks etc. thus, the people live in close contact to their livestock generally doors and windows were kept open until the people retired for the day.

Bhelukhlel tole

Bhelukhel tole, ward no 14 of Bhaktapur municipality is situated at the centre of the district. It is just 5 min walk from Bhaktapur Durbar Square. Bhelukhel tole has its own cultural value. Sluggish Hanumante River which lies towards the eastern side of the area was the destination of untreated sewage and most of thebresidents in this area empty personal garbage and waste into the river. The indoor and outdoor pig rearing was very common in the past in this area. During the time of survey only few piggeries were present in this area. Many pigs were roaming around the area especially near by the Hanumante river side. Rice field, pond, ditches, bushes, stagnant water along the road side etc. are frequently visible around the houses which are known to be important breeding site of *Culex* mosquitoes.

3.2 Study period

The study was carried out for six months from July 2012 to December 2012.

3.3 Study design and data collection

The study was designed in two different phases:

1. Epidemiological investigation
2. Entomological investigation

3.3.1 Epidemiological investigation

The study design was a descriptive cross-sectional study concerning Knowledge, Attitudes, and Practices (KAP) of Japanese encephalitis prevention among the people of Tathali, Bode and Bhelukhel. The standard questionnaire with observational checklist was the tool for data collection.

Interviews through KAP questionnaire

A structured questionnaire was designed and administered to 300 randomly selected households, 100 respondents from each site**.** Only one person per household was interviewed**.** The questionnaire included children, adult and old of both sexes, aged between 15-65 years. Respondents were interviewed through structured questionnaire collecting information on their knowledge, attitudes, and practices (KAP) regarding JE, its vector and control measures. KAP questionnaire was divided into 5 different parts.

Socio-demographic There were 7 questions in this part including age, sex, income, education, marital status, JE history and source of information.

Knowledge regarding JE

12 questions were asked in this part including symptoms, transmission, treatment, prevention, vector mosquitoes etc. 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 as follows: Bloom's cut off point, 60-80%.

High level (80-100%)	10-12 scores
Moderate level (60%-79%)	07-09 scores
Low level (less than 59%)	00-06 scores

Attitude regarding JE

This part includes the attitude of the people towards JE in the aspect of prevention and it was assessed by using Likert's scale. There were 7 statements which included both positive and negative. The rating scale was measured as follows:

Positive Statement		Negative Statement	
Choice	Scores	Choice	Scores
Strongly agree	5	strongly agree	1
Agree	4	Agree	2
Neither agree nor disagree	3	Neither agree nor disagree	3
Disagree	2	Strongly disagree	4
Strongly disagree	1	strongly disagree	5

The scores varied from 7 to 35 and all individual answers were summed up for total scores and calculated for means. The scores were classified into 3 levels i.e. Positive Attitude 27-35 scores, Neutral Attitude 22-26 scores, Negative Attitude 7-21 scores.

Practice regarding JE

12 items were analyzed and the score in practices varied from 0 to 12, and were classified into 3 levels. These 12 items were all assessed as zero-one indicator variables. These variables were given value o for "no" and value 1 for "yes". They were good practice, fair practice and poor practice (Bloom's cut off point, 60-80%).

Good Level	10 – 12 scores
Fair Level	07 – 09 scores
Poor Level	00 - 06 scores

Observation:

In this part as a passive observer, the environmental condition and activities done by the people of the study sites were examined and conclusions were drawn from what have been observed.

Data Analysis and Statistic Application

Data was entered and analyzed. The relationships among demographic characters, knowledge, attitude and practices were analyzed with Chi-square test of association, by using Statistical Software Package for Social Science (SPSS) program, version 16 for windows.

Ethical Considerations

Verbal consent was taken from all the respondents before data collection process. The respondents were explained in detail the full description of the research and asked in local language.

Secondary data collection

Record of total JE cases in Nepal and previous year cases in Bhaktapur district were obtained from secondary data record of NZFHRC, Kathmandu.

3.3.2 Entomological investigation (Light trap operation)

Materials

S.N.	Materials	S.N.	Materials
1.	CDC light trap (Bio Quip Products, Inc. 2321 E Glaowick St. Rancho Dominguez, CA 90220, USA)	6.	Hand lence
2.	Batteries	7.	Ethyl acetate
3.	Thermo-Hygrometer	8.	Data sheet
4.	Vials	9.	Marking pen
5.	Entomological pin	10.	Standard taxonomy key (Darsie and Pradhan, 1990)

Three aforementioned areas were selected for regular mosquito collection. The area with piggeries, traditional cattle shed, poultry, past records of JE cases and cultivation of paddy around the area was chosen for study. Piggeries were located close to human habitations, thus allowing mosquitoes to pass from one to the other.

Collection technique

Adult mosquitoes were collected using dark activated rechargeable CDC light trap fitted with double ring fine mesh collection bags (Bio Quip Products, Inc. 2321 E Glaowick St. Rancho Dominguez, CA 90220, USA), to assess prevalence, mosquitoes were collected twice a month in each site throughout the month in July to December 2012 using dark activated, rechargeable CDC light trap specifically, one light trap was placed outside houses within the same compound per village at dusk and was collected at dawn the next day. The light trap was turned on overnight. Samples were collected in the morning after each night.

Meteorological data

The relative humidity and average temperature for the period of the study was recorded by means of hygrometer.

Killing method

The screened bag from the light trap was removed and the mosquitoes were anesthetized with the help of ethyl acetate. These mosquitoes were picked up and were kept in vials. The vials were transported to the Tribhuvan University, Natural History Museum, Swayambhu for identification.

Identification

All the sampled mosquitoes were morphologically studied, and identified to the species level by using standard key by Darsie and Pradhan (1990) with the help of standard light microscopy, hand lence etc. Mosquitoes collected at each station were counted by species. The relevant collecting data such as collection number, locality, date of collection etc. was noted on a collection form along with the individual species identified. All mosquito specimens are deposited in Natural History Museum, Tribhuvan University, Swayambhu, Kathmandu.

3.4 Data Analysis and Statistic Application

Collected meteorological data and entomological data during the six month of study period were entered in excel. The data was presented in table, bar graph and line graph. The variation in the number of vectors between different study sites and month wise survey was analyzed by using two- way ANOVA table.

3.5 Limitation of the study

As descriptive study design was used, descriptive designs do not attempt to generalize the findings to populations outside the study participants. Therefore, findings of this study could not be generalized beyond the participants of the study.

3. RESULTS

The result is presented in two parts:

1. Epidemiological investigation (KAP study)

2. Entomological investigation

4.1 KAP Study

KAP study was conducted at three sites of Bhaktapur district namely Tathali, Bode and Bhelukhel. Altogether 300 respondents participated in this study.

Table 1: Distribution of the respondents by socio-demographic characteristics

Characteristics	Tathali		Bode		Bhelukhel	
	No.	%	No.	%	No.	%
Gender						
Male	48	48%	37	37%	39	39%
Female	52	52%	63	63%	61	61%
Age group (years)						
≤20	32	32%	18	18%	22	22%
21-30	24	24%	38	38%	28	28%
31-40	20	20%	27	27%	24	24%
41-50	8	8%	8	8%	14	14%
≥51	16	16%	9	9%	12	12%
Mean	31.8		32.18		33.1	
S.D.	1.425		1.15		1.297	
Marital Status						
Single	33	33%	40	40%	16	16%
Married	63	63%	60	60%	84	84%
Widowed/Divorced	0	0%	0	0%	0	0%
Education Level						
Primary	17	17%	7	7%	13	13%
Lower secondary	14	14%	12	12%	13	13%
Secondary	25	25%	9	9%	22	22%
Graduate/Postgraduate	16	16%	31	31%	21	21%
Uneducated	28	28%	31	31%	31	31%
Income (Rs. /month)						
None	0	0%	0	0%	0	0%
≤1000	0	0%	0	0%	1	1%
1001-5000	7	7%	10	10%	33	33%
5000-10000	57	57%	39	39%	40	40%
>10000	36	36%	51	51%	37	37%
History of JE						
Yes	0	0%	0	0%	0	0%
No	100	100%	100	100%	100	100%

Demographic characteristics

The results of socio-demographic characteristics are shown in Table 1. Majority of participants were females. The respondents were grouped age wise. In Tathali VDC majority of respondents (32%) were found ≤20 years of age. In both Bode and Bhelukhel site greater number of the respondents belonged to 21-30 years of age group with 38% and 28% respectively. The married respondents in Tathali, Bode and Bhelukhel were 63%, 60% and 84% respectively. Uneducated respondents in Tathali (28%) were less than in Bode (31%) and Bhelukhel (31%). Among the educated respondents most of them had secondary and graduate level of education. Almost all were employed and economically active. All the respondents from three sites mentioned that they have no history of JE.

The information media through which respondents got information about JE is tabulated in Table 2. Radio and television was the main source of information. The table shows that 7% of respondents from Tathali, 12% of respondents from Bode and 25% of respondents from Bhelukhel mentioned radio as the major source of information regarding JE.

Table 2: Source of information regarding JE.

Source of information	Tathali		Bode		Bhelukhel	
	No.	%	No.	%	No.	%
None	66	66%	59	59%	28	28%
T.V.	5	5%	10	10%	20	20%
Radio	7	7%	12	12%	25	25%
Health personal	1	1%	5	5%	8	8%
Miking	1	1%	1	1%	2	2%
Neighbor	2	2%	2	2%	1	1%
Teachers	4	4%	2	2%	1	1%
Children	1	1%	1	1%	0	0%
All of above	2	2%	0	0%	2	2%
Radio +TV	8	8%	4	4%	7	7%
Radio, Teachers+TV	2	2%	1	1%	4	4%
Health Personal+Teachers	1	1%	1	1%	2	2%
Total	100	100%	100	100%	100	100%

Knowledge on JE

Table 3: Distribution of the respondents by their knowledge level

Level	Tathali		Bode		Bhelukhel	
	No.	%	No.	%	No.	%
JE Symptoms						
High (10-12)	2	2%	3	3%	2	2%
Moderate (7-9)	2	2%	13	13%	1	1%
Low (0-6)	96	96%	84	84%	97	97%
Total	100	100%	100	100%	100	100%
Mean	0.45		2.74		0.57	
S.D.	0.312		0.465		0.297	
JE Transmission						
High (10-12)	7	7%	14	14%	13	13%
Moderate (7-9)	4	4%	28	28%	5	5%
Low (0-6)	89	89%	58	58%	82	82%
Total	100	100%	100	100%	100	100%
Mean	1.4		8.1		4.23	
S.D.	0.539		0.729		0.297	

As shown in table 3, participants answered a total of 12 close ended, multiple choice questions regarding JE. Each correct response was given 1 mark with a total of 12 marks. The knowledge on JE symptom was very poor among the participants 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. Distribution of knowledge on JE transmission showed that 89% respondents in Tathali, 58% in Bode and 82% in Bhelukhel had low level of knowledge.

Knowledge of respondents towards vector mosquito is tabulated in Table 4. The table shows that the knowledge of standing water as a mosquito breeding site was high among the respondents of three sites. Almost 76%, 79% and 69% of participants of Tathali VDC, Bode and Bhelukhel mentioned water as breeding site of mosquitoes. But the knowledge towards JE vector was very poor. Comparatively more people of Bode with 79% were aware about mosquito-borne disease than Tathali (76%) and Bhelukhel (69%). The distribution of knowledge on mosquito biting time shows that more than 85% of respondents from all study sites answered that mosquito bites during night time.

Table 4: Distribution of the respondents by their knowledge regarding vector mosquito

Level	Tathali		Bode		Bhelukhel	
	No.	%	No.	%	No.	%
Breeding site						
Yes	76	76%	79	79%	69	69%
No	24	24%	21	21%	31	31%
Total	100	100%	100	100%	100	100%
Vector borne diseases						
Know	36	36%	43	43%	33	33%
Don't know	64	64%	57	57%	67	67%
Total	100	100%	100	100%	100	100%
Mosquito biting time						
Night	86	86%	92	92%	97	97%
Day	0	0%	0	0%	1	1%
Day and night	14	14%	8	8%	2	2%
Don't know	0	0%	0	0%	0	0%
Total	100	100%	100	100%	100	100%

Attitude towards JE

Participants answered a total of 7 questions which had a total score of 35. 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 JE vector. More positive attitude was observed in Bode site with 46% while it was found negative in Bhelukhel (57%). 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 5).

Table 5: Distribution of the respondents by the attitude level towards JE

Level	Tathali		Bode		Bhelukhel	
	No.	%	No.	%	No.	%
Positive (27-35)	22	22%	46	46%	26	26%
Neutral (22-26)	4	4%	17%	17%	17	17%
Negative (7-21)	74	74%	37	37%	57	57%
Total	100	100%	100	100%	100	100%
Mean	8.1		18.53		12.25	
S.D.	0.835		0.911		0.861	

Practice about JE

Table 6: Distribution of the respondents by the practice behavior against JE prevention

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

As shown in table 6, the presence of household pig as a practice, in Tathali, Bode and Bhelukhel site was found to be 5%, 4% and 5% respectively. Raising of ducks was found 16% in Bode while 5% and 10% in Tathali VDC and Bhelukhel respectively. Majority of respondents of all study sites were agriculture based. Ninety percent of respondents of Tathali VDC mentioned that they had paddy cultivation proportionally which was greater than Bode (63%) and Bhelukhel (45%).

Practice towards JE

Table 7: Distribution of respondents by the practice level towards JE prevention

Level	Tathali		Bode		Bhelukhel	
	No.	%	No.	%	No.	%
Good (10-12)	15	15%	12	12%	7	7%
Fair (7-9)	52	52%	67	67%	64	64%
High (0-6)	33	33%	21	21%	29	29%
Total	100	100%	100	100%	100	100%
Mean	4.35		4.67		4.23	
S.D.	0.672		0.57		0.561	

Participants answered a total of 12 questions regarding JE. Each correct response was given one mark with a total of 12 marks and the score was summed up and set as three levels, poor practice, fair practice and good practice. 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 and 4.23 out of possible 12 scores with a S.D. of 0.672, 0.570 and 0.561 respectively (Table 7). Preventive methods against mosquito bites included use of insecticide sprays, professional pest control, screen windows, prevent of water stagnant, use of mosquito repellant cream etc.

Comparison of practice score between grouping variables

Table 8: 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 values are based on chi-square analysis showing significance)

Practice behavior in relation to sex of the respondents was presented in Table 8. 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 the sex of the respondents had not association with the level of practice behavior in all three sites (p>0.05). As shown in table 9, there was no clear association of marital status with practice behavior (p>0.05) in all three study sites.

Table 9: Association between marital status and practices on JE prevention (n=300)

Level	Tathali		Bode		Bhelukhel	
	Single	Married	Single	Married	Single	Married
Poor	6	27	10	11	3	26
Fair	21	31	24	43	10	54
Good	6	9	6	6	3	4
Total	33	67	40	60	16	84
P value	0.087		0.07		0.108	

Table 10: Association between education status and practice behavior against JE (n=300)

Level	Tathali					Bode					Bhelukhel				
	Primary	Lower Secondary	Secondary	Graduate	Uneducated	Primary	Lower Secondary	Secondary	Graduate	Uneducated	Primary	Lower Secondary	Secondary	Graduate	Uneducated
Poor	7	7	3	2	14	2	1	6	6	6	6	2	6	3	12
Fair	8	5	15	12	12	4	11	10	19	23	6	10	15	15	18
Good	2	2	7	2	2	1	0	3	6	2	1	1	1	3	1
Total	17	14	25	16	28	7	12	19	31	31	13	13	22	21	31
P value	0.033					0.409					0.379				

Table 11: Association between age and level of practice behaviour 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				

Variation in practice level was found to be statistically associated with the education status and age of the respondents in Tathali ($p<0.05$) but no statistical difference was observed in Bode and Bhelukhel ($p>0.05$). All age group of respondents were observed equally active in practice against JE (Table 10 and 11).

Table 12: Association between knowledge status and level of practice 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
χ^2	0.098		0.211		0.115	

Table 13: Association between attitude and level of practice behavior against JE prevention (n=300)

Level	Tathali			Bode			Bhelukhel		
	Positive	Neutral	Negative	Positie	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		

Table 12 reveals that in all the three study sites there is no significant difference between knowledge and level of practice behavior against JE prevention ($p>0.05$). As presented in table 13, attitude had statistically significant association in level of practice in Tathali ($p<0.05$) and no significance difference was observed in Bode and Bhelukhel ($p>0.05$).

4.2 Entomological study

A total of the 884 mosquitoes belonging to 6 genera (*Culex, Anophelese, Aedes, Armegeries, Mansonia and Malaya*) and 21 species were collected during the six months of study period (July 2012-December 2012). Of the 884 mosquitoes, 73.41% were *Culex* mosquitoes, of which 15.99%, 65.46% and 18.55% were recorded from Tathal, Bode and Bhelukhel respectively. (Figure 1). 94.5% of the species collected were female. Sex-wise distribution of vector mosquitoes is shown in appendix 2.

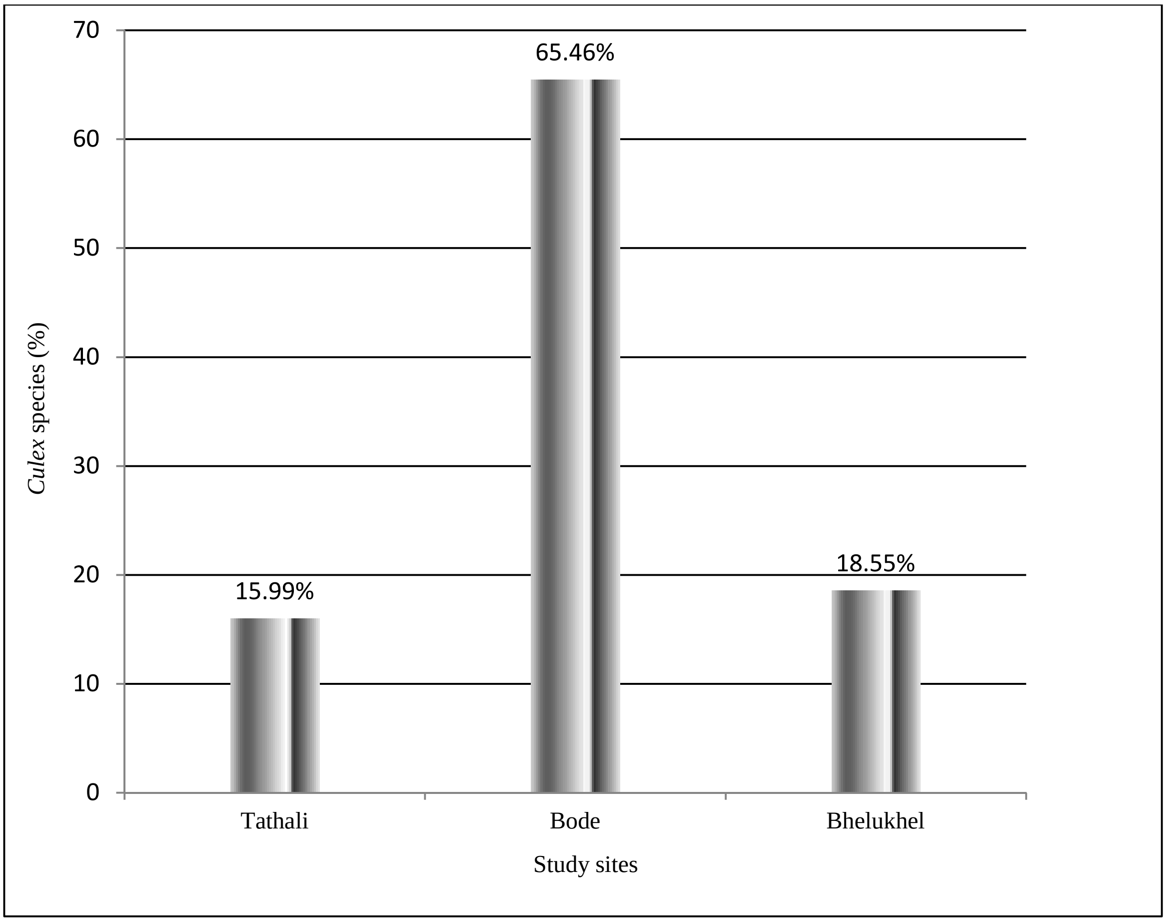


Figure1: *Culex* species collected during study period

Monthwise prevalence of *Culex* species in Tathali

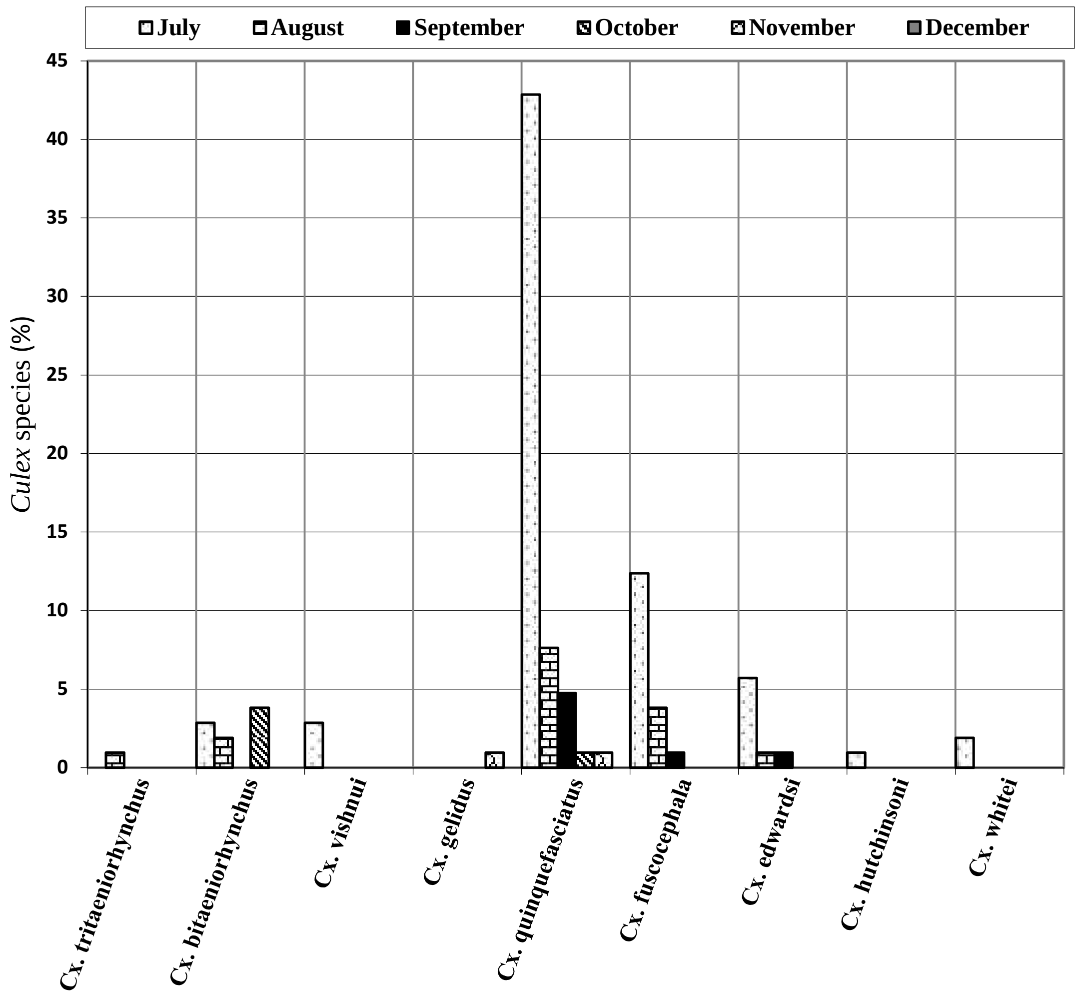


Figure 2: Monthwise prevalence of *Culex* species in Tathali

A total of 105 *Culex* species were collected from Tathali during the entire study period. The highest number of mosquitoes were collected in July followed by August. *Cx. quinquefasciatus* was the dominant species and was collected in every month of study period except December with its highest peak in July (42.95%). *Cx. fuscocephala* was the second dominant species and was attracted in greatest number during July (12.38%) and August (3.81%). During July each of *Cx. vishnui* and *Cx. bitaeniorhynchus* constituted 2.86% of the total. *Cx. tritaeniorhynchus,* the principal vector of JE in Nepal was found only in July and constituted 0.95% (1/105). *Cx. gelidus* was found only in November comprising 0.95% (1/105). No specimen was collected in December.

Monthwise prevalence of *Culex* species in Bode

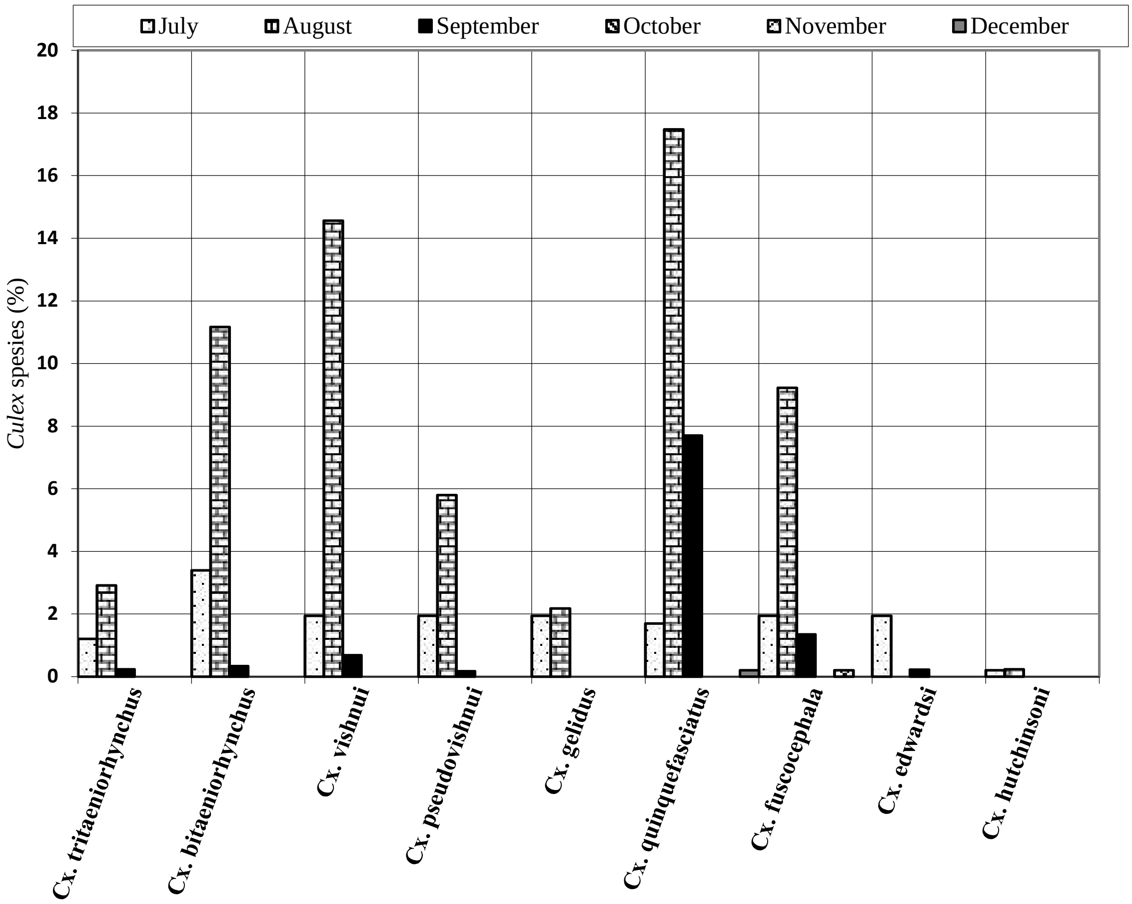


Figure 3: Monthwise prevalence of *Culex* species in Bode

Of the total 421 *Culex* mosquitoes collected from Bode, the highest number of mosquitoes was collected in August (Figure 3). Mosquitoes collected during that period comprised 17.48% *Cx. quinquefasciatus,* 14.56*% Cx. vishnui,* 11.1*7% Cx. bitaeniorhynchus, 9.*22% *Cx. fuscocephala* and *2.91% Cx. tritaeniorhynchus.* The second highest numbers of mosquitoes were collected in July. *Cx. bitaeniorhynchus* was the predominant species during July comprising 3.93% (14/421). During this period *Cx. tritaeniorhynchus,* the principal JE vector constituted 0.57% (5/421). Each of *Cx. vishnui, Cx. pseudovishnui, Cx. gelidus, Cx. quinquefasciatus* and *Cx. fuscocephala* constituted nearly 0.9% of the total 421. The numbers of mosquitoes were gradually decreased from September.

Monthwise prevalence of *Culex* species in Bhelukhel

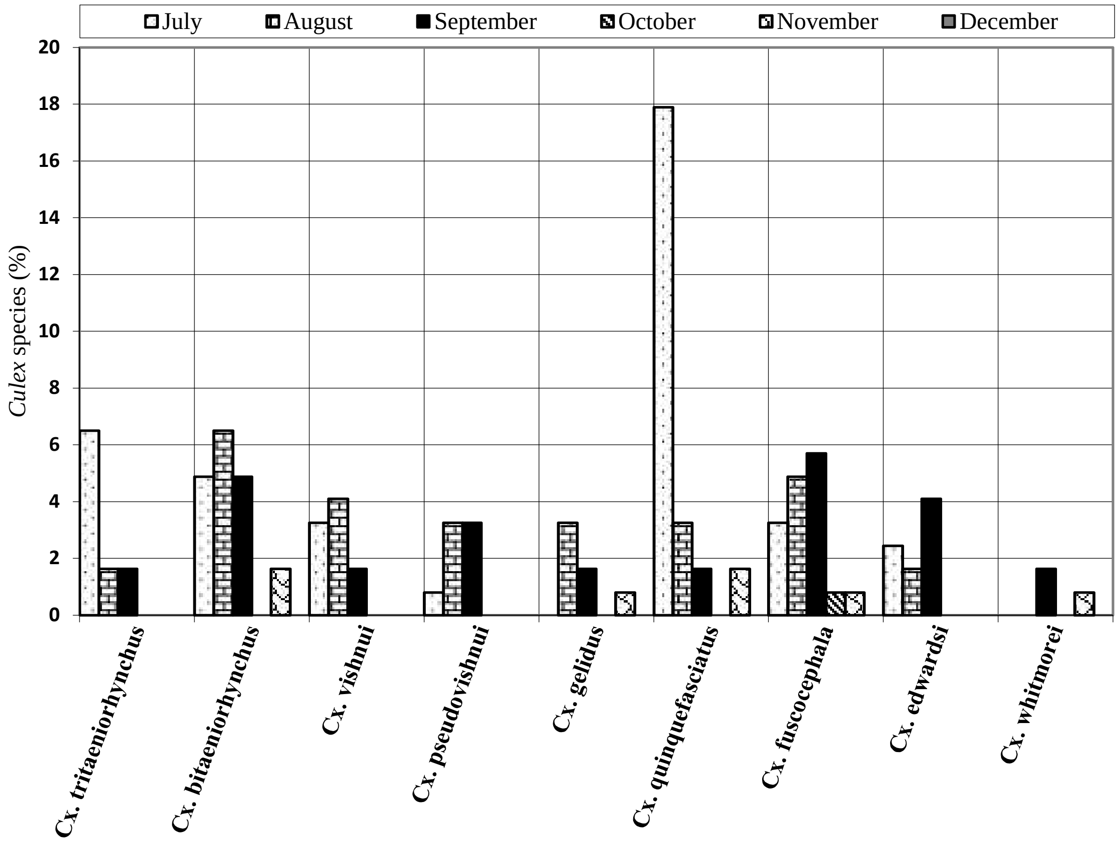


Figure 4: Monthwise prevalence of *Culex* species in Bhelukhel

Figure 4 shows the population density of mosquito reordered from Bhelukhel. A total of 123 mosquitoes were collected from this site. Highest population density was recorded in July (39.02% of 123). In this month *Cx. quinquefasciatus* contributed a dominant species comprising 17.88% (22/123). The principal vector *Cx. tritaeniorhynchus* recorded to be the second most abundant species constituting 6.5% (8/123). Other species *Cx. bitaeniorhynchus, Cx. vishnui and Cx. fuscocephala* constituted 4.9%, 3.25% and 3.25% of 123 respectively. Second highest density was recorded in August. During this period Cx. *bitaeniorhynchus* showed a tall peak with 6.8% (8/123). Comparatively good numbers of mosquitoes were collected in September as well. Among the all species collected, *Cx. fuscocephala* was found in every month except December, with its peak abundance in September (5.7%). *Cx. tritaeniorhynchus* was found the most abundant in July with 6.5% (8/123). This species was collected 1.63% (2/123) in each month of August and September. No mosquito was collected in December.

Total month wise prevalence of *Culex* species in three study sites

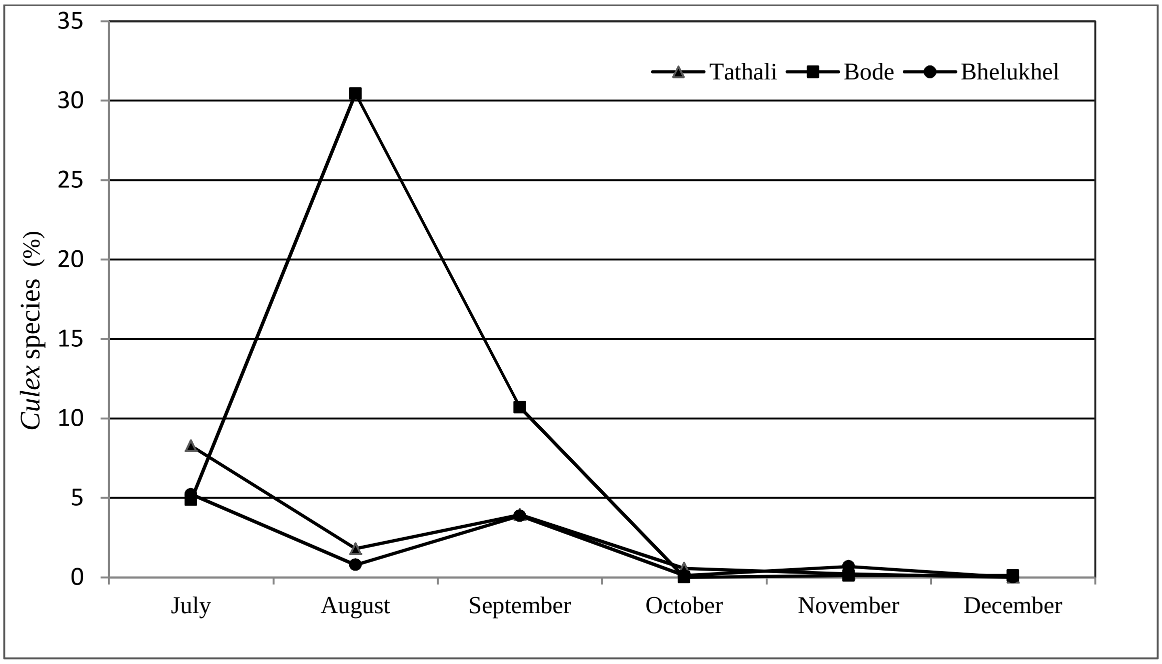


Figure 5: Total *Culex* species collected in three study sites

The combined number of mosquitoes collected by the light trap of three study sites is presented in Figure 5. Out of total vector mosquitoes collected, the highest population density was recorded from Bode site in August (40%, 258/649). During the study period the minimum *Culex* mosquitoes was collected from Tathali. No significant variation was observed in vector abundance in three study sites in six different months of study period.

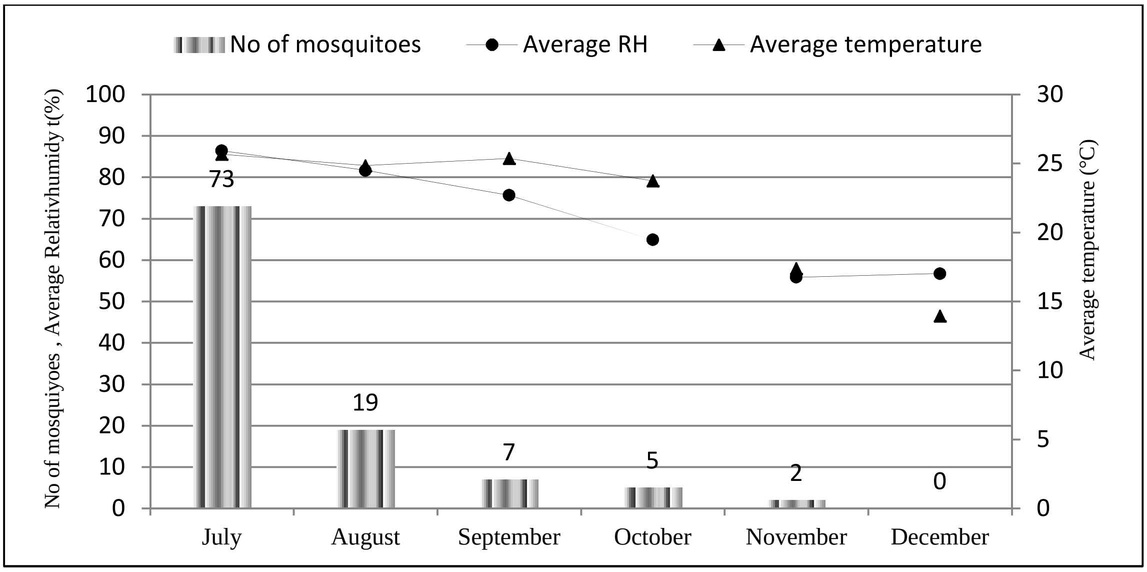


Figure 6: *Culex* species in relation to average temperature and Relative Humidity in Tathali

As shown in Figure 6, where the vector distribution in association with the meteorological data of Tathali site is presented, the maximum number of 73 vector mosquitoes was collected in July. The average temperature at that time was recorded as 25.66°C and average Relative Humidity 86.38%. No *Culex* species was found in December when the temperature was 14°C and relative humidity was 58%.

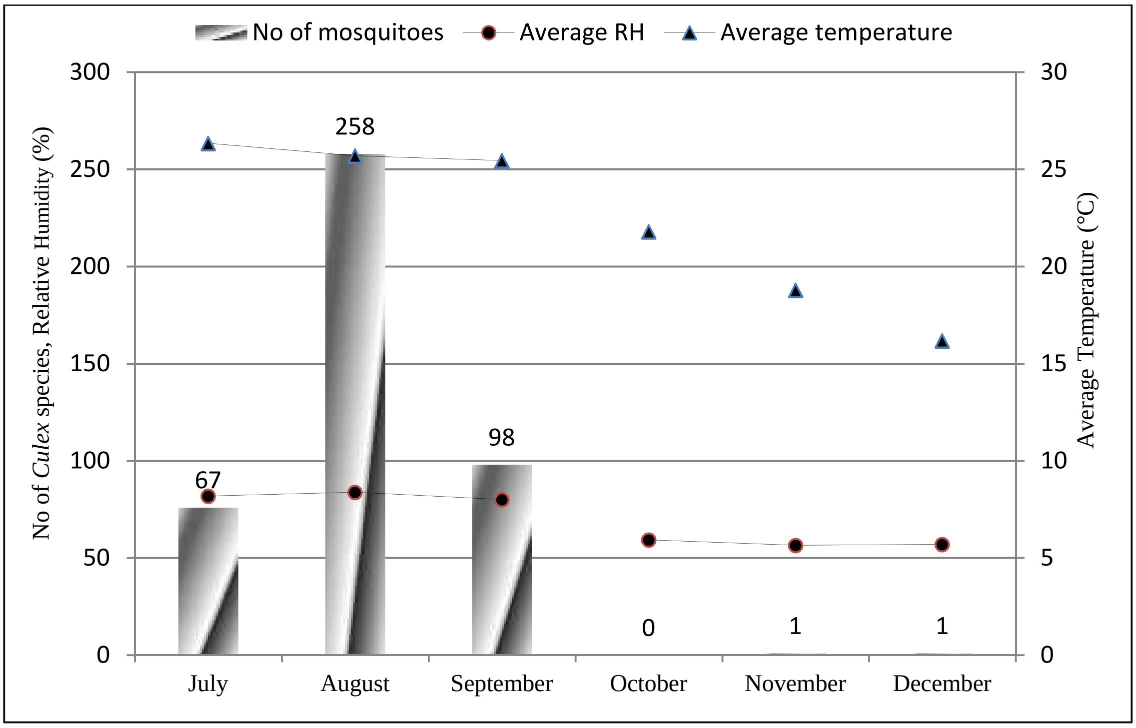


Figure 7: *Culex* species in relation to average temperature and Relative Humidity in Bode

The prevalence of vector mosquitoes and meteorological data of Bode site is presented in Figure 7. The total mosquito collected in July was 67 and the maximum number reached to 258 in August when the average temperature was recorded 25.69ºC with average Relative Humidity 83.75%. The average temperature and average RH during October was recorded as 22.73ºC and 68.83% respectively and no mosquito was collected during this month.

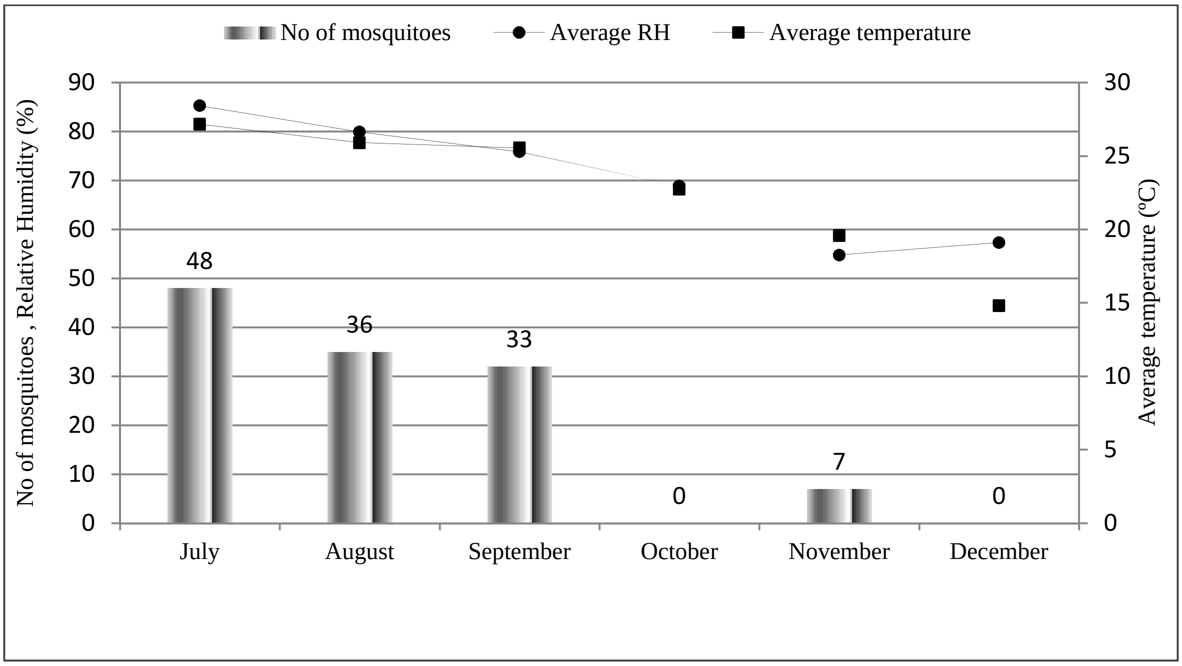


Figure 8: *Culex* species in relation to average temperature and Relative Humidity in Bhelukhel

A total of 48 vector mosquitoes were collected in July. The average temperature of July was recorded as 27.15ºC and RH 85.25%. In the month of October the mosquito number abruptly decreased to 0. At that time the average temperature and RH was recorded to be 22.73ºC and 68.83% respectively. The mosquitoes were recollected in November when the average temperature and average RH was recorded to be 19ºC and 54.75% respectively (Figure 8).

5. DISCUSSION

KAP Survey

Japanese encephalitis is one of the major arboviral zoonotic diseases worldwide. The high fatality rate and frequent residential neuropsychiatric sequalae in survivors make JE a significant public health problem. In the context of Nepal, before 2006, there was a single large cluster of JE cases located in the far–west and mid–west terai of Nepal. After then it shifted with cluster to the hilly regions including Kathmandu valley where it confirmed its endemicity. Lack of awareness programme, inadequate surveillance of environmental factor and vector abundance are the related factor for continuous outbreak of disease. The present epidemio-entomological study was conducted in Bhaktapur district which is known for its JE endemicity in Kathmandu valley. The study was carried out for six months of period from July 2012 to December 2012.

A KAP questionnaire conducted in Tathali, Bhelukhel and Bode sites showed that female participants were more than male. The study showed that the respondents' knowledge about JE symptoms was very poor but comparatively higher knowledge was found towards JE transmission. In the present study, no relation was observed between respondent's income and their knowledge regarding disease. Opposite result was found in KAP questionnaire survey carried out in US by Dowling 2011 who mentioned respondent's income as a good indicator of knowledge towards mosquito-borne diseases. In Dowling's study knowledge towards JE was found unrelated to the source reduction practice and same result was found in this study as well. No literature was found regarding KAP on JE in Nepal.

In the present study, radio and television was found to be the most important source of information and this is similar with the study by Hairi et al. (2003). Study conducted in Nepal regarding Malaria showed that respondents labeled radio (58.1%) and television (25.4%) as the major source of information regarding mosquito-borne diseases (Joshi et al. 2004). Study from Puduchhery showed that about 75.93% of the study population was aware about mosquito-borne diseases through television followed by healthcare providers and newspaper (Borante et.al 2010). This shows that mass media such as radio and television was a very important source to disseminate information and this could be used to generate better awareness among the public with regards to disease prevention and control (Anita et al. 2005).

Regarding knowledge about vector breeding sites, more than 70% stated stagnant water as a commonest breeding place. Similar results have been reported from Nepal conducted by Joshi et al. 2004.This result was similar to the study carried out by Borante et al. (2010) but contrary to the study conducted by Kumar and Gururaj (2002) in which a large number of people did not know where the mosquitoes breed during their study on community perception regarding mosquito-borne diseases in Karnataka, India.

The present study reveals that knowledge regarding JE is less among females than males which is similar to findings of Sharma et al. (2001) from Delhi, India. Similar results have been reported by Sharma et al. (1992) from Delhi, India and opposite findings have been revealed by Snehalata et al. (2003) from Zambia. In the present study household income did not appear to be a good indicator of knowledge and attitudes towards JE. Contrast finding has been given by Dowling (2011) from US. Females had good practice than males in practice of JE prevention. This might be because it is believed in the population that females should take care of the households while the kids and male have other responsibilities (Ahmed 2007). This study was not consistent with the study made by Teetipsatit (2005) that male and female of household leader had no relationship with preventive behavior on dengue hemorrhagic fever.

The present study reflected that nearly 80% respondents from all three study sites used to eliminate breeding sites and used mosquito nets. This practice of source reduction was not associated with the knowledge score of the respondents. Similar result was given by Dowling (2011) from US. Total knowledge was unrelated to source reduction practice in the study. Koenraadt et al. (2006) found similar results in Thailand, where general knowledge of mosquitoes was not important to source reduction practice. Rather, Koenraadt et al. (2006) suggested that education campaigns should emphasize specific knowledge of mosquito prevention practices.

The results of this study showed that the demographic data were not associated with level of practice scores. Therefore, the demographic characteristics investigated in this study were not significantly associated with level of practice but in Tathali VDC it was found that age and education status had significant association with level of practice behaviors of JE prevention among the respondents. The result was found similar with the study carried out by Ahmed (2007) in Male, Maldives. In the present study, no relationships existed between the level of education status and the practice of JE prevention. Study from Male, Maldives revealed the similar result. The study mentioned that for this type of result one reason might be educated people will have more of other responsibilities and have less time to practice. JE history was not found among the respondents of all three study sites thus no significant association between JE history among the family members and level of practice behaviors among the respondents. Ahmed (2007) also reported that dengue history was not an important confounder in his study analysis of KAP on Dengue. The mean attitude score was found to be 8.1, 18.53 and 12.25 from a possible 30 points with a standard deviation of 0.835, 0.911 and 0.861 in Tathali, Bode and Bhelukhel site respectively. The respondents from Bode site were observed with more positive attitude. This may be due to comparatively more literate number in that site.

This study had no significant association with knowledge and level of practice behavior against JE prevention in all the three study sites. This was similar that had been achieved by Hairi et al. (2003) where they had conducted a study on Knowledge, Attitude and Practices (KAP) on dengue among selected rural communities in the Kuala Kangsar district and found out that there was no significant association seen between attitude and preventive practice on dengue. The significance difference between the attitude and practice behavior ($p<0.05$) in Tathali VDC was found which was similar to the study done by Limros (2006) who conducted a study on preventive behaviors against dengue infection among family health leaders in Kongkrailat District, Sukhothai Province found out that attitude showed no correlation with breeding place prevention. In contrast large gap was observed in both Bode and Bhelukhel sites. The results obtained from these sites showed that there was no significant association between attitude and practice. This was found to be consistent with the findings by Sharma et al. (1992). The result suggested that attitude did not lead to correlate with practice, based on scoring analysis.

During the study period rice field, pond, ditches, bushes, standing water along the road side etc. were found around the houses which are known to be important breeding sites of *Culex* mosquitoes. It was also found that some of them had habit of removal of stagnant water present nearby their household. This practice was found comparatively more in Bode site. It may be because of higher literacy rate. In Bhelukhel site it was also observed that number of pigs were roaming around the area especially near by the river side. People living in these areas might be at risk of getting JE.

Entomological study

An entomological study was conducted during the six months of period from July 2012 to December 2012. It gives a clear picture of relative abundance, species composition and seasonal fluctuation of Japanese encephalitis vector mosquitoes in the study areas. A total of 884 numbers of mosquitoes belonging to 6 genera and 21 species were collected. *Cx. tritaeniorhynchus* is the principal vector for Nepal. The higher number of *Culex* mosquitoes were collected from Bode, Thimi (n=412) and lesser number was recorded from Tathali VDC (n=106). The vector abundance was higher in July- September months (wet and rainy season) and found to be the lowest in November and December. The study of mosquito population in Kathmandu valley revealed the similar result (Shrestha 2011).

In the present study from three study sites the highest number of mosquito was collected in July and August when the average temperature was recorded between 25.65ºC-26ºC with average RH 79.10%-86.69%. (Joo and Kang 1992), reported that the month of the highest average mosquito catch in night was July, when the temperature was between 18.6ºC- 34.8ºC and the average RH 59%.-76%. The main factors contributing to the appearance and change in the density of mosquito was considered to be breeding sites such as paddy cultivation, swamps, rainfall, temperature and relative humidity etc.

During this study period, *Cx. tritaeniorhynchus* consisted 3.74% of the total collection. Higher population density was observed in August in Bode site. *Cx. tritaeniorhynchus* has been incriminated as a major JE vector in Nepal including other south Asian countries (Kanojia et al. 2003). This species is known to breed in paddy fields and its abundance is related to rice cultivation. The species showed its tall peak during the paddy cultivation period in Kathmandu valley (Shrestha 2011). Similar result was observed in Bellary district, Karnataka, India (Kanojia 2007). Study conducted in Gorakhpur, India also found rice fields contributing towards the building up of population density of JE vectors (Kanojia et al. 2003). *Cx. tritaeniorhynchus* was found predominant in outdoors in Warangal and Karim Nagar district of Andra Pradesh, India (Das et al. 2004).

Cx. quinquefasciatus was found to be the predominant species in all three sites collected during study period. It constitutes 6.89% in Tathali, 16.75% in Bode and 3.40% in Bhelukhel. The species was found in maximum number in Gorakhpur district, India (Kanojia et al. 2003) and it stood fourth most abundant species in Bellari district 2003. A few isolates of JE virus have been made from this species in India and Vietnam but the species is known as poor vector of JE (Sirivanakarn 1976).

Cx. gelidus has been recognized as the most important vector of JE in Sri Lanka, Thailand, Malaysia, Vietnam and Sarawak (Macdonald et al. 1967). This species breed in habitat like cow dung pit, ground pools containing much weeds, marshy tracts etc (Gubler et al. 1989). The adult densities of *Cx. tritaeniorhynchus* and *Cx. gelidus* were found the highest in Tokha and Gothatar which consists of higher number of cattelsheds, little paddy cultivation and no piggery. The density was found relatively low in Balkot and Hattiban with large proportion of paddy cultivation (Shrestha 2011). In the present study *Cx. gelidus,* important vector of JE was recorded with good number from Bode (n=19 and Bhelukhel (n=16) but relatively fewer recorded from Tathali (n=1). All three sites represent large proportion of rice cultivation and few numbers of piggeries.

Cx. fuscocephala, an efficient JE vector in Thailand (Gould et al. 1974) was also collected in good number from all three sites. This species may differ in its susceptibility in different geographical areas as it failed to prove its transmission capacity though it got infected with JE virus (NIT unpublished data). *Cx. bitaeniorhychus* and *Cx. vishnui* was also found in appreciable density from Bode and Bhelukhel site between August to September. This species is known to breed in large ground pools always filled with dense mass of filamentous green algae (Sirivanakarn 1976). Two JE viruses have been isolated from *Cx. bitaeniorhychus* in Bankura west Bengal, India (Banerjee et al. 1975). Similar report was recorded for *Cx. pseudovishnui.* In the epidemic investigation this species was found to be the most abundant species including *Cx. tritaeniorhynchus* (Gupta et al. 2004).

Other *Culex* species viz. *Cx. edwardsi, Cx. thileria, Cx. hutchinsoni, Cx. whitei and Cx. whitmorei* formed 10.41% of the total collected mosquitoes. These species are also regarded as JE vector in different countries including India (Dhanda and Kaul 1980, Dhanda et al. 1977 and Samuel et al. 2000). Their role in transmission seems very feeble (Kanojia 2003). The vector abundance was maximum (30.44%) in August as recorded from Bode site. Similarly the maximum number of vector was recorded as 8.26% and 5.24% in July month from Tathali and Bhelukhel site respectively. Almost all individual species showed a rapid decline in the population from July to December as average temperature falls from 26.38ºC to 14.97ºC and average RH from 84.46% to 56.98%. From the statistical analysis, variable vector mosquito distribution was recorded in three study sites. Vector species was found highly distributed in Bode site. No significant variation was observed in vector abundance in three study sites in six different months (F value is less than tabulated value).

6. CONCLUSION AND RECOMMENDATIONS

Conclusion

Based on the findings from this study it can be concluded that knowledge about mosquito breeding places and mosquito biting time was satisfactory but the majority of respondents were unaware about vector-borne diseases. Similarly most of them were not familiar with the symptoms and mode of transmission regarding JE. Most of the people did not see JE as a threat to their community, thus proper prevention programs need to be developed to make the community more aware. The study revealed that practice regarding JE is satisfactory. However, significance association was not found among the knowledge, attitude and practice. There is a need to increase health promotion activities in Bhaktapur district to increase knowledge towards JE and its control. The study showed that mass media such as television was a very important source of information and this could be used to generate better awareness among the public with regards to disease prevention and control. Further, the distribution of *Culex* species was abundant in July and August. *Cx. quinquefasciatus* the most dominant species recorded in all three sites. The principal JEV *Cx. tritaeniorhynchus* was collected in higher number during August from Tathali and Bode site and during July from Bhelukhel site. Thus can be assumed the higher possibility of JE transmission in July and August. The identification of high risk areas and season using this tool within every centre is of paramount importance.

Financing disease control depends on political will. Partially, this will depends on better educated and awared populations that are attaining a higher culture that allows them to demand a better standard of living such as clean environment. The better integrated clean communities do not have the large mosquito populations that mosquito-rich habitat communities do. A concentrated effort at the time of the year when vector densities are increasing may have more impact. Messages which inform people about the biology of vector mosquitoes of JE and the role that this mosquitoes play in transmitting disease may motivate people to take actions to reduce or control main types of mosquito breeding sites. Input from community members is needed here. It is undisputed fact that it is necessary to know which larval habitats and host predominates in the area to select control strategies. Since the importance of any particular larval habitat host is area specific, a source reduction campaign for example will have a different impact from one country or area to another.

Recommendations

1. For travelers to areas with JE, as well as people living in areas with this disease, the risk of being bitten by mosquitoes indoors could be reduced by utilization of windows and doors that are screened.
2. JE control measures should be integrated into a general mosquito control program with cooperation at regional and national levels. This approach best suits the expectations and needs of the population for an improvement in their quality of life. It also corresponds to the expectations of political decision-makers concerned about ensuring sustainable development for the populations they represent.
3. The training of staff involved in mosquito control should be stressed.
4. Environmental management that promotes the elimination of vector breeding sites should be a priority in control programs. Programs that involve the creation of strategic partnerships should include intersectoral participation of public and private corporations with a strong component of community participation, as well as participation of different ministries, universities, nongovernmental organizations, among others.
5. Last, but not the least, we all need to be united to wrestle against this deadly disease

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APPENDIX 1

EPIDEMIO-ENTOMOLOGICAL STUDY OF JAPANESE ENCEPHALITIS IN BHELUKHEL, BODE AND TATHALI OF BHAKTAPUR DISTRICT

(KAP Questionnaire)

Full name of Respondent: ……………………………………..

Part I: Socio-demographic characteristics

Age ………….

Sex: Male …………Female ……………

Marital Status Married ………. Single …………Divorced ………..Widow………….

Education Level Primary …………. Secondary ………. Graduate …………Postgraduate……………..

Annual Income None………..1000-5000 …………. 5000-10000 …………………

Above 10000…………….

Have you ever heard about Japanese encephalitis (JE)? Yes …….. No………….

Select the sources from which you got the information

T.V ……. Radio ………….. Newspaper …………. Magazines ………. Health personal ………

Friends ……………………..Teacher ………………others …… …………

Part I I: Knowledge about JE

Knowledge of symptoms	Yes	No	Do not know
1. Is fever a symptom of JE?			
2. Is headache a symptom of JE?			
3. Is sore neck a symptom of JE?			
4. Is paralysis a symptom of JE?			
5. Is nausea a symptom of JE?			
6. Is vomiting a symptom of JE?			
7. Is Diarrhea a symptom of JE?			
8. Is skin rash a symptom of JE?			
9. Is muscle pain a symptom of JE?			
10. Is bleeding a symptom of JE?			
11. Does JE lead to coma?			
12. Does JE lead to death?			

Knowledge towards JE vector	Yes	No	Do not know
1. Do you know any disease transmitted by mosquito?			
2. Je vector mosquitoes breed in water?			
3. Windows screen and bed net reduces mosquito bites?			
4. When are the mosquitoes likely to feed or bite?	**Night**	**Day**	**Do not know**
5. When are the mosquitoes likely to breed (breeding site)?			

Knowledge of transmission	Yes	No	Don't know
1. Do you know how does it transmit?			
2. Can JE be transmitted through cough?			
3. Can JE be transmitted by blood transfusion?			
4. Is JE transmitted through food and water?			
5. Does ordinary person to person contact transmit JE?			
6. Can mosquito transmit JE?			
7. What is the vector of JE?			
8. Do flies transmit JE?			
9. Do cockroaches transmit JE?			
10. Do all mosquitoes transmit JE?			
11. Do pigs transmit JE?			
12. Is JE infectious disease?			

Part III: Attitude towards JE

Attitude towards JE	Strongly Agree	Agree	Neither agree nor disagree	Disagree	Strongly Disagree
1. Is JE a serious illness?					
2. Are you at the risk of JE?					
3. Can JE be prevented?					
4. Is controlling the breeding habitats of Mosquito a good strategy to prevent JE?					
5. Standing waters are breeding habitats of Mosquitoes?					
6. Rice fields, ponds, pools, puddles, grassy ditches are breeding habitats of mosquito?					
7. Communities should actively participate in controlling JE?					

Part IV: Practice regarding JE

Variables	Yes	No	Do not know
1. Use of insecticide sprays to reduce mosquitoes			
2. Use of mosquito coil to reduce mosquitoes			
3. Use of mosquito repellant cream to reduce mosquitoes			

4. Use of smoke to reduce mosquitoes			
5. Use of screen windows to reduce mosquitoes			
6. Covering body with clothes			
7. use of fans			
8. Elimination of standing water			
9. use of mosquito net			
10. use professional pest control to reduce mosquito			
11. use mosquito eating fish			
12. prevent water stagnation			

1. Do you perform following activities as occupation?	**Yes**	**No**	**Do not know**
Raising pigs			
paddy cultivation			
raising ducks			
2. Have you or any of your family members been diagnosed with JE?			
3. Do you know about JE vaccination?			
4. Have you been vaccinated against JE?			
5. Government sprays insecticides for controlling mosquitoes?			

Part V: Observation Check list

	Yes	**No**
1. The surrounding environment is kept neat and tidy.		
2. Standing water around the houses.		
3. Fish farming near by the house		
4. Pond, pools, grassy ditches, rice field around the houses.		
5.Pig farming		

APPENDIX 2

Number of *Culex* mosquitoes captured using light trap in Bhaktapur district during the study period

Tathali

Mosquito species	July	August	September	October	November	December
Cx. tritaeniorhynchus	0	1	0	0	0	0
Cx. bitaeniorhynchus	3	2	0	4	0	
Cx. vishnui	3	0	0	0	0	0
Cx. pseudovisisnui	0	0	0	0	0	0
Cx. gelidus	0	0	0	0	1	0
Cx. quinquefasciatus	30 (15)	6 (2)	5	1	1	0
Cx. fuscocephala	9 (4)	3 (1)	1	0	0	0
Cx. edwardsi	6	1	1	0	0	0
sCx. Theileri	0	0	0	0	0	0
Cx. hutchinsoni	1	0	0	0	0	0
Cx. whitei	2	0	0	0	0	0
Cx. whitmorei	0	0	0	0	0	0

BODE

Mosquito species	July	August	September	October	November	December
Cx. tritaeniorhynchus	5	12	2	0	0	0
Cx. bitaeniorhynchus	13 (1)	46	3	0	0	0
Cx. vishnui	8	60	6	0	0	0
Cx. pseudovisisnui	8	21	5	0	0	0
Cx. gelidus	8	9	0	0	0	0
Cx. quinquefasciatus	6 (1)	70 (2)	68	0	0	1
Cx. fuscocephala	8	38	6	0	0 (1)	0
Cx. edwardsi	5 (3)	0	2	0	0	0
Cx. theileri	0	0	0	0	0	0
Cx. hutchinsoni	0 (1)	1	0	0	0	0
Cx. whitei	0	0	0	0	0	0
Cx. whitmorei	0	0	0	0	0	0

Bhelukhel

Mosquito species	July	August	September	October	November	December
Cx. tritaeniorhynchus	8	2	2	0	0	0
Cx. bitaeniorhynchus	6	8	5 (1)	0	2	0
Cx. vishnui	4	5	2	0	0	0
Cx. pseudovisisnui	1	4	3 (1)	0	0	0
Cx. gelidus	0	4	2	0	0 (1)	0
Cx. quinquefasciatus	22	4	2	0	2	0
Cx. fuscocephala	4	6	4 (3)	1	0 (1)	0
Cx. edwardsi	3	2	4 (1)	0	0	0
Cx. theileri	0	0	0	0	0	0
Cx. hutchinsoni	0	0	0	0	0	0
Cx. whitei	0	0	0	0	0	0
Cx. whitmorei	0	0	2	0	1	0

(Note: Figure in bracket indicates number of male mosquitoes)

APPENDIX 3

Data analysis

Hypothesis testing

The hypothesis for variation in distribution of mosquitoes is created as,

1. Between three study sites

i. Null Hypothesis: Ho:$\mu 1 = \mu 2 = \mu 3$

i.e. there is no significant difference in the distribution of mosquitoes between three study sites.

ii. Alternative Hypothesis: H1:$\mu 1 \neq \mu 2 \neq \mu 3$

i.e. there is significant difference in the distribution of mosquitoes between three study sites.

2. Between six months

i. Null Hypothesis: Ho: $\mu 1 = \mu 2 = \mu 3 = \mu 4 = \mu 5 = \mu 6$

i.e. there is no significant difference in the distribution of mosquitoes between six months.

ii. Alternative Hypothesis: H1:$\mu 1 \neq \mu 2 \neq \mu 3 \neq \mu 4 \neq \mu 5 \neq \mu 6$

i.e. there is significant difference in the distribution of mosquitoes between six months.

Analysis of *Culex* mosquitoes

Table 1: Distribution of *Culex* mosquitoes

	Tathali	Bode	Bhelukhel	Total
July	73	76	48	197
August	19	258	35	312
September	7	98	32	137
October	5	0	1	6
November	2	1	7	10
December	0	1	0	1
Total	33	434	123	663

Two way ANOVA Table

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	

APPENDIX 4

Data of Temperature and Relative Humidity recorded during Six months of study period

Date	Time	Temperature (◦c)		Relative Humidity (%)	
		Max	Min	Max	Min
18-Jul	7.00pm	27.5	27.5	89	89
19-Jul	Mor	23.3	23	99	95
	Day	26.6	25	75	75
	Evening	25.7	25.5	94	92
20-Jul	Mor	24	24	75	74
	Day	26.4	26.3	93	92
	Evening	26.1	26	90	90
21-Jul	Mor	25	24.8	90	90
	Day	27.1	27.1	85	85
	Evening	26.8	26.5	87	86
22-Jul	Mor	21	20.5	86	86
	day	26.7	26.3	82	82
	evening	25.6	25.2	85	85
23-Jul	mor	24.2	24	87	87
	day	25.9	25.5	85	85
	evening	26.1	26	85	84
24-Jul	mor	24.8	24.5	86	86
	day	36.2	26	84	84
	evening	25.9	25.8	84	83
25-Jul	mor	25.4	25	84	84
	day	25.7	25.6	84	83
	evening	26.1	26	83	83
26-Jul	mor	24.8	24.7	84	83
	day	27.2	27.1	81	79
	evening	29.4	28.6	74	71
27-Jul	mor	25.2	24.7	84	82
	day	27.3	26.4	81	74
	evening	26.2	26.1	81	80
28-Jul	mor	25.2	25	82	81
	day	26.5	26.5	82	82
	evening	26.7	26.3	83	83
29-Jul	mor	25.3	25.1	86	85
	day	26.8	26.7	85	84
	evening	26.9	26.8	88	87
30-Jul	mor	25.2	24.9	88	84
	day	27.2	27	82	82
	evening	36.7	26.4	86	84
31-Jul	mor	26	25.4	86	84
	day	27.2	27	88	88
	evening	26.8	26.7	86	85
1-Aug	mor	25.5	25.4	84	86
	day	25.7	25.6	86	86
	evening	26.1	26	86	85

2-Aug	mor	24.8	24.8	88	86
	day	26.1	25.8	86	86
	evening	26.6	25.9	89	85
3-Aug	mor	26.3	25.2	84	83
	day	25.7	25.5	88	83
	evening	25.5	25.4	84	83
4-Aug	mor	25.7	25.4	83	83
	day	26.2	26	83	82
	evening	26.1	26	82	82
5-Aug	mor	25.5	25.3	83	82
	day	23.2	26	83	81
	evening	25.8	25.7	82	81
6-Aug	mor	25.3	25.2	82	82
	day	26.2	26.1	82	80
	evening	27.1	26.5	83	81
7-Aug	mor	23	22.7	90	85
	day	26	25.8	86	85
	evening	26.7	26	84	84
8-Aug	mor	23.7	23.4	89	86
	day	26.3	25.8	84	83
	evening	25.5	25.5	84	83
9-Aug	mor	24.6	24.5	83	82
	day	24.6	24.4	82	82
	evening	24.8	24.7	81	80
10Aug	mor	24	23.9	81	80
	day	25	25.3	83	81
	evening	25.2	24.9	83	81
11Aug	mor	25.4	25	82	81
	day	26	25.8	81	81
	evening	25.8	25.6	82	81
12Aug	mor	25	24.8	81	80
	day	26.1	25.9	82	82
	evening	25.8	25.7	82	81
13Aug	mor	25.1	25	81	81
	day	26.4	26	81	80
	evening	26.2	26.1	80	79
14Aug	mor	25.6	25.4	80	80
	day	26.6	26	81	80
	evening	26.6	26.5	80	79
15Aug	mor	26	26	80	80
	day	26.8	26.6	81	81
	evening	25.4	25.3	83	83
16Aug	mor	26.1	25.5	82	80
	day	27.1	27	81	80
	evening	27.1	27.1	82	82
17Aug	Mor	26.4	26.3	81	81
	Day	27.7	26.8	81	80
	evening	26.8	26.6	80	79

18Aug	mor	26.1	26.1	80	79
	day	27.6	26.6	81	80
	evening	27	27	80	80
19Aug	mor	26.4	26	80	80
	day	26.9	26	80	80
	evening	26.7	26.4	80	80
20Aug	mor	25.9	25.7	80	79
	day	26	25.8	81	77
	evening	26.2	25.9	81	79
21Aug	mor	25.4	25.1	80	79
	day	26	25.9	80	80
	evening	26	25.9	81	80
22Aug	mor	25	24.3	84	78
	day	25.3	25.3	81	79
	evening	25.3	25.2	80	79
23Aug	mor	24.6	24.4	79	78
	day	25.36	24.9	80	79
	evening	24.9	24.9	80	78
24Aug	mor	24.6	24.2	82	78
	day	25.9	25.6	79	77
	evening	25.5	25.3	73	80
25Aug	mor	24.7	24.6	84	80
	day	26.7	26.1	81	79
	evening	26.4	26.3	79	79
26Aug	mor	25.1	24.8	80	79
	day	26.2	25.9	80	79
	evening	26	25.9	80	77
27Aug	mor	25.3	24.7	80	79
	day	26.3	25.8	87	84
	evening	26.1	25.9	85	80
28Aug	mor	25.1	25	82	81
	day	26.9	26.1	82	78
	evening	26.7	26.4	81	80
29Aug	mor	25.9	25.8	81	80
	day	26.2	26.1	80	80
	evening	26.6	26.3	80	80
30Aug	mor	25.8	25.4	81	80
	day	26.8	26.5	80	78
	evening	26.7	26.4	86	79
31Aug	mor	25.7	25.4	79	79
	day	26.3	26	79	78
	evening	27.2	26.8	78	76
1-Sep	mor	26	25	79	78
	day	27	25.8	79	75
	evening	27.2	24.8	82	78
2-Sep	mor	25.6	21	81	79
	day	26.4	25.6	80	72
	evening	26.4	26	66	65

3-Sep	mor	21.3	25	84	72
	day	26.1	25.6	79	77
	evening	26.3	26	78	77
4-Sep	mor	25.2	25	78	78
	day	26.6	26.1	78	76
	evening	26.4	26	77	76
5-Sep	mor	24.3	24.3	81	79
	day	26.8	26.3	77	71
	evening	26	25.8	78	76
6-Sep	mor	26.7	26.3	73	72
	day	27.2	26.5	77	74
	evening	26.9	26.7	73	70
7-Sep	mor	25.5	25.1	74	74
	day	26.8	26	75	72
	evening	27	26.9	75	74
8-Sep	mor	26.2	25.9	75	74
	day	27.1	26.9	75	74
	evening	26.6	26.5	75	75
9-Sep	mor	26	25.7	75	74
	day	26.9	26.8	75	75
	evening	26.6	26.3	77	75
10Sep	mor	25.7	25.3	77	75
	day	26.2	26	78	77
	evening	27.5	26.6	81	75
11Sep	mor	25.6	25.4	77	75
	day	26.8	26.7	74	73
	evening	26.2	25.9	80	75
12Sep	mor	25.5	25	80	76
	day	27.2	26.4	78	75
	evening	25.7	25.5	78	78
13Sep	mor	24.8	24.7	80	79
	day	26	25.9	80	80
	evening	25.3	25.4	83	81
14Sep	mor	23.9	23.7	81	81
	day	26	25.5	81	79
	evening	25.7	24.9	81	80
15Sep	mor	24.6	24.8	80	79
	day	25.7	25.2	82	79
	evening	24.2	24.1	80	80
16Sep	mor	24.4	24.1	80	78
	day	24.7	24.5	81	80
	evening	25	24.6	81	81
17Sep	mor	24	23.7	81	79
	day	23.7	22.9	85	79
	evening	23.9	23.7	82	80
18Sep	mor	23.3	23.2	85	83
	day	23.9	23.7	83	81
	evening	23.7	23.2	81	81

19Sep	mor	23.1	23	82	81
	day	24.8	23.8	84	82
	evening	24.4	24.1	84	82
20Sep	mor	23.9	23.7	82	82
	day	25.4	24.8	82	81
	evening	25.2	24.6	76	75
21Sep	mor	24.5	24.3	80	80
	day	25.5	25.4	80	79
	evening	25.4	25.2	79	77
22Sep	mor	24.5	24.3	79	78
	day	27.1	26.7	79	76
	evening	27.4	26	84	76
23Sep	mor	24.7	24.5	78	76
	day	26.2	25.6	79	73
	evening	27.4	26.6	81	73
24Sep	mor	25	24.9	76	75
	day	26	26	78	73
	evening	25.8	25.7	76	76
25Sep	mor	24.6	24	76	75
	day	26	26	77	76
	evening	26	25.5	78	75
26Sep	mor	24.6	24.3	73	71
	day	26	25.9	73	72
	evening	25.8	25.7	74	74
27Sep	mor	24.7	24.5	75	74
	day	25.7	25.5	75	73
	evening	26.1	26	75	73
28Sep	mor	24.9	24.8	74	74
	day	25.5	25.4	75	74
	evening	25.6	25.4	74	74
29Sep	mor	25	25	75	74
	day	25.1	25	75	75
	evening	26.3	26.2	74	74
30Sep	mor	25.5	25.1	76	73
	day	26.7	26.4	73	68
	evening	27.5	26.3	81	75
1-Oct	mor	25.1	24.8	75	74
	day	26.1	25.9	75	73
	evening	26.1	25.8	74	71
2-Oct	mor	25.2	25.1	73	73
	day	26.1	25.7	74	71
	evening	27	26.9	73	70
3-Oct	mor	22.9	22.8	73	71
	day	25.4	25	72	68
	evening	27.3	26.7	65	65
4-Oct	mor	25.6	25.1	69	66
	day	26.6	25	69	66
	evening	25.8	25.4	70	70

5-Oct	mor	24	23.7	68	65
	day	24.2	24	68	64
	evening	24.8	24.7	69	67
6-Oct	mor	23.6	23.3	69	68
	day	26.2	25.8	68	68
	evening	24.7	24.6	70	69
7-Oct	mor	23.2	22.5	68	67
	day	24.4	23.8	68	66
	evening	25.6	25.2	66	64
8-Oct	mor	22.1	22.1	70	69
	day	24.8	24.2	69	63
	evening	24.9	24.4	69	67
9-Oct	mor	22.8	21.3	67	65
	day	23.8	23.7	67	67
	evening	23.7	23.6	69	69
10Oct	mor	22.4	21.9	72	71
	day	24.3	24.2	68	65
	evening	24.9	24.6	66	65
11Oct	mor	22.8	22.2	68	67
	day	24.1	23.8	70	69
	evening	24	23.8	71	71
12Oct	mor	21.6	21.3	71	71
	day	24.3	23.8	66	64
	evening	24.6	24.5	63	62
13Oct	mor	21.9	21.5	67	66
	day	23.2	22.6	67	64
	evening	23.5	23.4	66	63
14Oct	mor	20.8	20.6	67	66
	day	23.6	23.2	63	61
	evening	22.6	22.5	65	64
15Oct	mor	19.6	19.4	66	65
	day	22.6	22.1	64	61
	evening	23.2	22.4	61	60
16Oct	mor	20.5	20.4	64	64
	day	22.7	22.5	64	62
	evening	22.7	22.6	62	62
17Oct	mor	20.1	20	65	65
	day	23.6	23.3	64	63
	evening	23.7	23.4	62	61
18Oct	mor	19.9	19.5	65	64
	day	23.2	22.6	62	60
	evening	23.4	23.5	62	62
19Oct	mor	22.6	22.5	62	62
	day	20.1	20	61	61
	evening	23.1	22.9	66	64
20Oct	mor	20.4	20.1	66	66
	day	23	23	64	64
	evening	23	22.9	66	64

21Oct	mor	19.9	19.5	63	61
	day	21.8	21.4	63	60
	evening	21.8	21.6	65	64
22Oct	mor	19.7	19.6	65	64
	day	23.2	22.5	63	63
	evening	23.3	23.2	64	62
23Oct	mor	19.8	19.5	62	62
	day	22.6	22.4	60	59
	evening	22.7	22.6	61	62
24Oct	mor	19	18.6	60	60
	day	22.5	22.4	60	60
	evening	20.9	20.8	62	61
25Oct	mor	17.9	17.8	61	61
	day	22.5	22	60	60
	evening	22.2	21.3	61	58
26Oct	mor	17.8	17.7	61	60
	day	21.9	21.4	59	59
	evening	20.7	20.6	58	57
27Oct	mor	17.8	17.6	60	60
	day	21.8	21.6	59	58
	evening	20.6	20.5	60	60
28Oct	mor	18.1	17.8	59	58
	day	20.4	19.9	58	57
	evening	21.1	20.9	57	56
29Oct	mor	17.4	17.2	59	59
	day	20.1	19.5	57	52
	evening	21.2	19.9	56	55
					56
30Oct	mor	17.8	17.1	57	52
	day	21.9	20	56	54
	evening	22.3	21.6	55	
31Oct	mor	17.9	16.7	57	57
	day	20	19.8	56	55
	evening	21.2	20.8	56	55
1-Nov	mor	17.9	17.3	56	55
	day	21	20.2	55	50
	evening	21.5	20.4	55	50
2-Nov	mor	19.2	18.1	53	53
	day	21.5	20.7	52	48
	evening	21.8	21.5	52	51
3-Nov	mor	17.8	16.2	56	54
	day	19.2	18.2	55	54
	evening	20	18.8	52	50
4-Nov	mor	17.8	16.4	54	53
	day	20.5	19.5	55	52
	evening	22.5	21.7	55	55
5-Nov	mor	18.8	18.7	54	54
	day	20.6	18.8	57	51
	evening	23.3	20.7	54	52

6-Nov	mor	18.7	14.1	58	56
	day	19.4	18.6	56	54
	evening	19	18.7	55	54
7-Nov	mor	17.1	16	57	57
	day	19.7	19.7	56	54
	evening	20.2	20	56	55
8-Nov	mor	17.3	16.9	55	55
	day	18.9	17.4	56	55
	evening	19.3	18.6	56	55
9-Nov	mor	16.5	16.4	58	57
	day	17.9	17.4	57	55
	evening	20.3	20.1	56	53
10Nov	mor	14.7	14.3	61	60
	day	17.5	17.4	58	58
	evening	20.2	20.1	58	57
11Nov	mor	16.3	15.8	57	57
	day	18.4	18	57	57
	evening	18.6	18.5	58	58
12Nov	mor	16.3	15.6	59	58
	day	18.1	17.2	57	54
	evening	18.2	17.5	59	58
13Nov	mor	14.6	14.2	59	59
	day	16.8	16.4	57	57
	evening	17.9	17.4	60	57
14Nov	mor	14.7	13.8	58	56
	day	17.7	17	55	51
	evening	18	17.6	57	56
15Nov	mor	14.2	13.6	58	56
	day	17.5	17	55	57
	evening	17.9	17.8	57	57
16Nov	mor	15.2	14.7	57	57
	day	18.1	17.5	57	55
	evening	18.9	18.4	57	54
17Nov	mor	16	15.7	57	56
	day	18.4	18	56	57
	evening	20.3	19.3	55	51
18Nov	mor	16	15.1	56	53
	day	18.8	17.5	57	53
	evening	18.8	18.5	53	54
19Nov	mor	16.2	14.1	54	53
	day	19.1	17.5	55	52
	evening	19.4	19	55	53
20Nov	mor	15.7	14.7	54	54
	day	18.8	18.5	55	57
	evening	19.2	19	53	52
21Nov	mor	15	13.9	55	54
	day	17.8	17.2	57	54
	evening	19	18.8	53	53

22Nov	mor	14.5	13	54	54
	day	16.1	14.2	54	53
	evening	16	16	54	54
23Nov	mor	15.7	15.5	55	52
	day	15.6	15.5	56	54
	evening	17	16.8	55	54
24Nov	mor	15	14.8	58	57
	day	17.7	17	57	57
	evening	19	16.6	56	56
25Nov	mor	13.9	12.2	60	59
	day	13.9	13	62	58
	evening	13.5	132	62	60
26Nov	mor	16.5	16.2	59	58
	day	16.7	15.5	58	55
	evening	16.8	16.6	59	57
27Nov	mor	13.8	13.3	57	57
	day	16.7	15.8	56	56
	evening	16.3	16	57	56
28Nov	mor	13.9	13.3	57	57
	day	16.6	15.9	57	57
	evening	16.7	16.2	57	57
29Nov	mor	13.8	13.6	57	57
	day	16.4	16.2	58	56
	evening	16.9	16.3	56	55
30Nov	mor	13.4	12.7	57	57
	day	16.3	14.7	57	57
	evening	16.2	16	57	56
1-Dec	mor	12.9	12.6	57	56
	day	15.6	15.3	56	55
	evening	15.8	15.6	57	56
2-Dec	mor	12.7	11.9	57	56
	day	15.7	15.7	58	58
	evening	15.8	15.8	59	58
3-Dec	mor	12.9	12.3	58	57
	day	14.9	14.4	58	58
	evening	13.3	14.9	57	56
4-Dec	mor	13.4	12.8	57	57
	day	15.8	15.4	56	55
	evening	19.3	19.1	47	44
5-Dec	mor	17.8	17.3	52	51
	day	16.5	15	55	53
	evening	20.1	20	48	48
6-Dec	mor	13.5	13.1	57	56
	day	15.9	15.4	57	57
	evening	15.5	15.2	56	54
7-Dec	mor	14.6	14.2	62	61
	day	17.2	16.8	60	59
	evening	17	16.6	58	57

8-Dec	mor	13	12.3	61	60
	day	17.8	17.2	61	61
	evening	12.7	17.5	61	61
9-Dec	mor	15.5	14.2	62	61
	day	17	16.8	61	60
	evening	18	17.7	60	60
10Dec	mor	15.4	15.4	61	61
	day	17.1	16.8	62	61
	evening	17.8	17	61	60
11Dec	mor	13.4	13.2	62	61
	day	16.4	16.3	57	57
	evening	16.9	16.8	58	58
12Dec	mor	13	12.4	59	58
	day	14.6	14	58	56
	evening	15.5	14.8	56	55
13Dec	mor	12.7	12.1	59	58
	day	14.7	14.5	57	56
	evening	16	15.4	56	56
14Dec	mor	12.4	12.2	58	58
	day	15	14.8	55	55
	evening	16	15.3	57	56
15Dec	mor	11.8	11.2	57	57
	day	13.9	13.4	55	54
	evening	14.8	14.8	55	55
16Dec	mor	12.1	11.8	55	55
	day	14.8	14.5	56	55
	evening	15.9	15.6	55	52
17Dec	mor	12.3	11.9	56	55
	day	14.7	14.2	57	56
	evening	15.9	15.6	57	57
18Dec	mor	12.8	12.6	57	57
	day	14.8	14.1	59	58
	evening	16.2	16	56	56
19Dec	mor	12.9	12.6	59	58
	day	15.8	15.3	58	58
	evening	15.8	15.4	58	58
20Dec	mor	12.8	12.6	60	59
	day	15.2	14.7	59	57
	evening	16.32	16.1	57	56
21Dec	mor	12.2	11.9	58	57
	day	13.6	13.4	58	57
	evening	15.4	15.2	58	57
22Dec	mor	11.8	11.7	59	59
	day	13.8	13.7	59	59
	evening	15.5	15.4	59	59
23Dec	mor	11.4	11.1	58	52
	day	14.5	13.9	59	59
	evening	15.3	15.2	58	58

24Dec	mor	11.2	11.2	58	59
	day	13.7	12.9	57	55
	evening	15.1	14.8	55	55
25Dec	mor	11.2	11.2	56	56
	day	13.1	12.3	56	53
	evening	15.1	14.6	55	55
26Dec	mor	11.1	10.9	55	55
	day	13.4	12.2	54	53
	evening	13.8	13.6	55	55
27Dec	Mor	10.2	10	56	55
	Day	13.3	11.2	59	56
	evening	13.5	13.4	57	57
28Dec	Mor	10.5	10.2	57	57
	Day	13.5	12.7	58	57
	evening	14.8	14.6	56	56
				56	
29Dec	Mor	11.2	11	57	56
	Day	13.2	12.9	56	56
	evening	14.7	14.5		56
				56	
30Dec	Mor	11.9	10.9	58	56
	Day	11	11.7	58	57
	evening	13.6	13.5		57
31Dec	Mor	11.9	11.8	58	57
	Day	14.4	13.1	58	55
	evening	14.7	14.6	58	58

APPENDIX 5

MOSQUITO IDENTIFICATION KEY

Identification key to *Cx. tritaeniorhynchus*

1. Lower mesepimeral setae absent
2. Proboscis with distinct pale-scaled band
3. Tarso- meres with basal and apical pale bands
4. Wing without pattern of pale-scaled spots or streaks
5. Erect scales on vertex mostly dark; anterior surface of hind femur pale-scaled with narrow black-scaled ring apically
6. Scutum covered with dark coppery gold scale.
7. Anterior 0.7 of scutum covered with beige, yellow, golden or dark scales
8. Anterior surface of fore- and mid-femora entirely dark-scale
9. Midfemur entirely dark-scaled or speckling of pale scales not forming definite stripe
10. Post spiracular area without scales on lower anterior aspec
11. Erect scales in center of vertex pale yellow, dingy white or all dark
12. Abdominal terga II-VI with basal pale-scaled bands only
13. Abdominal terga II-VI with bands or patches of pale scale
14. Abdominal terga dark-scaled, with or without pale-scaled band

(Darsie and Pradhan, 1990)

Identification key to *Cx. gelidus*

1. Lower mesepimeral setae absent
2. Proboscis with distinct pale-scaled band.
3. Tarso- meres with basal and apical pale bands
4. Anterior surface of fore- and mid femora without speckling of pale scales
5. white- scaled patch on scutum dense, extending to wing root, dark-scaled posterior to that wing veins R,, R4+5 and Cu with narrow scales
6. Erect scales in center of vertex of head whitish
7. anterior 0.7 of scutum covered with white scales
8. Abdominal terga II-VI with basal pale-scaled bands only
9. Abdominal terga II-VI with bands or patches of pale scales
10. Abdominal terga dark-scaled, with or without pale-scaled bands
11. Wing without pattern of pale-scaled spots or streak

(Darsie and Pradhan, 1990)

Identification key to *Cx. fucocephala*

1. One or 2 lower mesepimeral setae present.
2. Proboscis without distinct pale-scaled band.
3. Tarsomeres without pale bands at joint.
4. Anterior surface of mid femur without median longitudinal pale-scaled stripe.
5. Abdominal terga without basal transverse, pale-scaled bands.
6. Pleuron with striking pattern of dark and pale integumental stripes.

(Darsie and Pradhan, 1990)

Identification key to *Cx. quinquefasciatus*

1. One or 2 lower mesepimeral setae present
2. Proboscis without distinct pale-scaled band
3. Tarsomeres without pale bands at joints
4. Anterior surface of mid femur without median longitudinal pale-scaled stripe
5. Abdominal terga with basal transverse pale-scaled bands
6. Pleuron without striking pattern of dark and pale integumental stripes
7. Integument of thoracic pleuron without dark stripe; scutal integument yellowish or pale brown

(Darsie and Pradhan, 1990)

Identification key to *Cx. bitaeniorhynchu*

1. One or 2 lower mesepimeral setae present
2. Proboscis without distinct pale-scaled band
3. Tarsomeres without pale bands at joints
4. Wing without pattern of pale-scaled spots or streaks
5. Abdominal terga dark-scaled, with or without pale-scaled band
6. Abdominal terga II-VI with bands or patches of pale scale
7. Abdominal terga II-VI with apical or apical and basal pale-scaled bands
8. Abdominal terga II-IV with broad apical bands of pale scales; abdominal terga heavily speckled with pale scales
9. Wing with mixed pale and dark scales
10. Hind tarsomeres with apical and basal pale bands about same length

(Darsie and Pradhan, 1990)

Identification key to *Cx. vishnui*

1. One or 2 lower mesepimeral setae present
2. Proboscis without distinct pale-scaled band
3. Tarsomeres without pale bands at joints
4. Wing without pattern of pale-scaled spots or streaks
5. Abdominal terga II-VI with basal pale-scaled bands only
6. Abdominal terga II-VI with bands or patches of pale scale
7. Erect scales in center of vertex pale yellow, dingy white or all dark; anterior 0.7 of scutum covered with beige, yellow, golden or dark scales
8. Midfemur entirely dark-scaled or speckling of pale scales not forming definite stripe; postspiracular area without scales on lower anterior aspec
9. Anterior surface of fore- and midfemora entirely dark-scaled
10. Erect scales on vertex pale yellow in center, dark-scaled posterolaterally; hind-femur marked otherwise; scutum with scales paler
11. Speckling of pale scales usually present on femora and proboscis; scutum with scales brown and pale mixed in varying degrees; hindfemur without dark- scaled apical band, usually with dark subapical band extending basally to form stripe

(Darsie and Pradhan, 1990)

Identification key to *Cx. whitmorei*

1. One or 2 lower mesepimeral setae present
2. Proboscis without distinct pale-scaled band
3. Tarsomeres without pale bands at joints
4. Wing without pattern of pale-scaled spots or streaks
5. Abdominal terga dark-scaled, with or without pale-scaled band
6. Abdominal terga II-VI with bands or patches of pale scale
7. Abdominal terga II-VI with basal pale-scaled bands only
8. Wing with mixed pale and dark scales
9. Hind tarsomeres with apical and basal pale bands about same length
10. Erect scales in center of vertex of head whitish; anterior 0.7 of scutum covered with white scales
11. Anterior surface of fore- and mid femora extensively speckled with pale scales
12. Pale- scaled patch on scutum thinner, grayish-white, extending posterior to wing root in 4 lines
13. Wing veins R, R4+s and Cu with broad scales

(Darsie and Pradhan, 1990)

Identification key to *Cx. edwards*

1. One or 2 lower mesepimeral setae present
2. Proboscis without distinct pale-scaled band
3. Tarsomeres without pale bands at joints
4. Wing without pattern of pale-scaled spots or streaks
5. Abdominal terga dark-scaled, with or without pale-scaled band
6. Abdominal terga II-VI with bands or patches of pale scale
7. Abdominal terga II-VI with basal pale-scaled bands only
8. Erect scales in center of vertex pale yellow, dingy white or all dark; anterior 0.7 of scutum covered with beige, yellow, golden or dark scale
9. Mid femur with longitudinal stripe of bale scales on anterior surface
10. Post spiracular area with small patch of semi-erect scales on lower anterior aspect .
11. Longitudinal pale-scaled stripe on anterior surface of mid femur complete
12. Pale scales present on base of costa at least to humeral cross vein

(Darsie and Pradhan, 1990)

Identification key to *Cx. pseudovishnui*

1. Lower mesepimeral setae absent
2. Proboscis with distinct pale-scaled band.
3. Tarso- meres with basal and apical pale bands
4. Wing without pattern of pale-scaled spots or streaks
5. Abdominal terga dark-scaled, with or without pale-scaled band
6. Abdominal terga II-VI with bands or patches of pale scale
7. Abdominal terga II-VI with basal pale-scaled bands only
8. Erect scales in center of vertex pale yellow, dingy white or all dark; anterior 0.7 of scutum covered with beige, yellow, golden or dark scale
9. Mid femur with longitudinal stripe of bale scales on anterior surface
10. Post spiracular area with small patch of semi-erect scales on lower anterior aspect
11. Anterior surface of fore- and mid femora entirely dark-scaled
12. Erect scales on vertex pale yellow in center, dark-scaled posterolaterall
13. Hind- femur marked otherwise
14. Scutum with scales paler
15. Scutum with yellow to silvery scales
16. Femora and proboscis never speckled with pale scales
17. Hind femur with dark band apically, contrasting with pale- scaled areas

(Darsie and Pradhan, 1990)

Identification key to *Cx. theileti*

1. One or 2 lower mesepimeral setae present
2. Proboscis without distinct pale-scaled band
3. Tarsomeres without pale bands at joints
4. Anterior surface of mid femur with median longitudinal pale-scaled stripe
5. Post spiracular area and base of prealar knob with distinct pale-scaled patches

(Darsie and Pradhan, 1990)

Identification key to *Cx. whitei*

1. One or 2 lower mesepimeral setae present
2. Proboscis without distinct pale-scaled band
3. Tarsomeres without pale bands at joints
4. Wing without pattern of pale-scaled spots or streaks
5. Abdominal terga dark-scaled, with or without pale-scaled band
6. Abdominal terga II-VI with bands or patches of pale scale
7. Abdominal terga II-VI with basal pale-scaled bands only
8. Erect scales in center of vertex pale yellow, dingy white or all dark; anterior 0.7 of scutum covered with beige, yellow, golden or dark scale
9. Mid femur with longitudinal stripe of bale scales on anterior surface
10. Post spiracular area with small patch of semi-erect scales on lower anterior aspect
11. Anterior surface of fore- and mid femora with speckling of several pale scales at least on apicodorsal surface

(Darsie and Pradhan, 1990)

Identification key to *Cx. hutchinsoni*

1. One or 2 lower mesepimeral setae present
2. Proboscis without distinct pale-scaled band
3. Tarsomeres without pale bands at joints
4. Anterior surface of mid femur without median longitudinal pale-scaled stripe
5. Abdominal terga with basal transverse pale-scaled bands
6. Pleuron without striking pattern of dark and pale integumental stripes
7. Integument of thoracic pleuron with dark stripe across mesokatepisternum and mesepimeron; scutal integument reddish brown

(Darsie and Pradhan, 1990)