CHAPTER - I
INTRODUCTION

1.1 Background

Nepal is a land locked country bordered by India in east, west and south and China in the North. It lies within the sub-tropical to the mountainous region at 26^{o}22' to 30^{o}27' N latitudes and 80^{o} 4' to 88^{o}12' E longitudes with an altitude that ranges from 90m to 8848m. The country land mass stretches 885 km from East to West and has a non-uniform width of 193 km North to South. Geographically Nepal represents a transitional mountain area between the fertile Gangetic plain of India and arid plateau of China. The topography of Nepal is divided into three ecological regions terai, hill and mountain. The narrow strip of flat alluvial terrain along the southern border known as the terai is the extension of the Gangetic plan and comprises about 23% of the country, including most of the fertile and forest areas. A number of broad dune valleys lie between the Siwaliks and Mahabharat range of hilly region. The terai area receives heavy precipitation, ranging between 180 and 225 cm. the relative humidity varies between 80 percent and 90 percent during the monsoon but declines in other months. The climatic conditions of in different parts of the country especially tropical and sub-tropical region is favorable for the breeding of Culex mosquitoes, the proven vectors of Japanese encephalitis and Filariasis and for the availability of the various amplifying host of the certain diseases.

In recent years Vector borne diseases have emerged as a serious public health problem in countries of the South-East Asia region, including Nepal. Many of these particularly Dengue fever, filariasis, malaria and Japanese Encephalitis now occur in endemic form causing considerable morbidity and mortality. JE is a common mosquito borne viral encephalitis found in Asia. Japanese encephalitis Virus belongs to the genus Flavivirus of Togoviridae family and causes JE disease in the tropical and subtropical countries. The virus is included into the same group of dengue virus. Due to the wide distribution and serious in nature of the disease caused by the Togoviridae which multiply in the cytoplasm, show considerable variations in the length of periods for their multiplication cycle. Although the temporal differences are relatively minor within each group, it is an acute infection of the central nervous system caused by *flavivirus* related to San Luis Encephalitis virus seen in North America, and is widespread throughout Asia; (Anderson and Brust, 1995) Viruses in the genus *Flavivirus* (Family Flaviridae) are single-stranded, positive sense, RNA viruses found on every continent except Antarctica (Gould *et al.,* 2003). They can be divided into four groups based on ecological niches and phylogenetic analyses: mosquito-borne, tick-borne, insect-specific, and no-known-vector groups (Gould *et al.,* 1974) Most flaviviruses are arthropod-borne (arboviruses), maintained in nature by hematophagous arthropod transmission between susceptible vertebrate hosts, and many are human and veterinary pathogens. Arthropod-borne flaviviruses include the mosquito-borne and tick-borne groups, containing viruses capable of replicating in vertebrate and invertebrate cells (Cook and Holmes, 2006). Insect-specific flaviviruses are able to replicate solely in invertebrate cells and have been isolated from insect cell lines and numerous species of field-collected mosquitoes (Lobo *et al.,* 2009). No-known vector flaviviruses are a paraphyletic group containing viruses that have only been found infecting vertebrate hosts, namely bats and rodents (Gaunt *et al.,* 2001). Some examples of mosquito-borne flaviviruses that cause significant impacts on human health are dengue virus, yellow fever virus, and West Nile virus (WNV).

Arbovirus transmission cycles have three essential components: 1) the virus, 2) the hematophagous arthropod vector, and 3) the vertebrate host (Kuno and Chang, 2005). Arboviruses are maintained in nature by propagative biological transmission, where the virus replicates in the vector and vertebrate host, and remains in the same developmental form (Higgs and Beaty, 2005). Thus, arboviruses must be capable of infection and replication in two disparate systems: the poikilothermic invertebrate vector and the homeothermic vertebrate host (Higgs and Beaty, 2005). Arboviruses are maintained by ongoing transmission between arthropod vectors and vertebrate hosts by sustained infections in the vector, host, or both and by perpetuation through adverse seasons by using various survival mechanisms (Kramer and Ebel, 2003, Higgs and Beaty, 2005). Some arboviruses survive trans-seasonal periods by vertical and/or horizontal transmission strategies. Vertical transmission refers to the transfer of a pathogen from a parent to his or her progeny (Fine, 1981). This can occur by a female arthropod infecting her offspring through transovarial transmission, where the virus infects the germ layer of the developing egg, or transovum transmission, where virus is on the egg surface, or by a male arthropod infecting progeny via seminal fluid (Higgs and Beaty, 2005). Horizontal transmission between male and female vectors can occur by venereal transmission during copulation or between female vectors via a viremic vertebrate host (Higgs and Beaty, 2005) or by co-feeding on a nonviremic host (McGee *et al.,* 2007). Arboviruses in the genus *Flavivirus* are primarily maintained in nature through biological transmission between blood feeding arthropods and susceptible vertebrate hosts, but vertical and horizontal transmission strategies appear to play a role as well. Vertical transmission of a flavivirus was first described in Senegal with isolation of Koutango virus from a male *Aedes aegypti* Since then, there have been numerous descriptions of flaviviruses isolated from larvae or male mosquitoes, including Japanese encephalitis virus from *Culex tritaeniorhynchus* (Rosen *et al.,* 1978), yellow fever from *Ae. aegypti* (Fontenille et al., 1997), and WNV from *Culex univittatus* (Miller et al., 2000), and also laboratory studies have demonstrated vertical transmission of flaviviruses (Nayar *et al.,* 1986). Apparently, flaviviruses are vertically transmitted at the time of oviposition, during fertilization via the micropyle, as the fully developed egg passes through the oviduct (Higgs and Beaty, 2005). This is much less efficient compared to "true" transovarial transmission, where the virus infects the developing egg (Kramer and Ebel, 2003).

Horizontal transmission of arthropod-borne flaviviruses between adult mosquitoes has also been documented. Venereal transmission was demonstrated with dengue-infected males transmitting to females but females did not sexually transmit virus to males and Saint Louis encephalitis virus was shown to be generally transmitted from male to female mosquitoes (Nayar *et al.,* 1986)There are few data regarding the transmission dynamics of insect-specific flaviviruses, which have been found to replicate only in invertebrate cells. The inability to infect and replicate in vertebrate cells indicates that this group of flaviviruses has a distinct transmission cycle compared to the arthropod-borne flaviviruses, which are maintained between arthropod vectors and vertebrate hosts. There is evidence that vertical transmission plays an important role in the transmission of insect-specific flaviviruses. Kamiti River virus was first isolated from *Aedes macintoshi* larvae and pupae collected from flooded dambos in Kenya (Sang *et al.,* 2003) and laboratory experiments conducted with *Aedes aegypti* mosquitoes, orally exposed to KRV, indicated that vertical transmission was possible (Lutomiah *et al.,* 2007). *Culex flavivirus* and *Aedes* *flavivirus* were both detected in adult males and females during mosquito field surveys in Japan (Hoshino *et al.,* 2009). Cell fusing agent virus, first isolated from an insect cell line, was recently detected in male and female mosquito pools collected in Puerto Rico. Detection of insect-specific flaviviruses in all life stages, including adult mosquitoes of both sexes, suggests vertical transmission as a probable mechanism of viral maintenance in nature (Cook *et al.,* 2006).

The different transmission modes of flaviviruses are strongly correlated with phylogeny (Cook and Holmes, 2006), providing valuable insight into vector-pathogen relationships. The mosquito-borne flavivirus group can be divided into two distinct categories (Gaunt *et al.,* 2001). The first category contains the neurotropic viruses, associated with encephalitic disease in humans, which are maintained in transmission cycles between *Culex* species and bird reservoirs. The second category contains the nonneurotropic viruses, which are maintained in transmission cycles involving *Aedes* species and primate hosts, and are more associated with hemorrhagic clinical manifestations in humans. These correlations provide evidence of the importance of the vector species and host species in flavivirus evolution (Gaunt *et al.,* 2001). Flaviviruses are RNA viruses and thus demonstrate higher mutation rates and greater genetic plasticity, compared to DNA viruses, because of their error-prone polymerase and lack of proofreading capacity (Holland and Domingo, 1998). This provides RNA viruses with a mechanism to adapt to the selective constraints imposed by particular environments, vector species, and host species. Components of arbovirus transmission cycles that also contribute to genetic variation include: 1) mosquitoes feed several times during their life, 2) virus titers can reach high levels in the mosquito and vertebrate host, and 3) viral infections in mosquitoes are persistent, resulting in a very dynamic system (Gould *et al.,* 2003).

Clinically apparent infection takes place in one out of 200–300 infected patients (Pugachev *et al.,* 2003). The disease is characterized by a wide range of presentations, as both the symptoms and the clinical course can differ broadly among patients. They range from mild flu-like symptoms to considerable neurologic symptoms, such as rigors, convulsions, polio-like flaccid paralysis, seizures or encephalomyelitis. Severe clinical cases are likely to have life-long neurological sequelae. Mostly children and young adults are affected (Halstead and Jacobson, 2003). The annual incidence and mortality estimates for JE are 30,000–50,000 and 10,000, respectively (Solomon, 2004). However, there is considered to be severe under-reporting of JE and one study estimated the annual incidence at 175,000 per year (Tsai, 2000). JE outbreaks occur in cycles that may be linked to climatic patterns and the immune status of the populations. It remains a public health problem due to its high fatality rate in different countries. The environmental interactions are important in the various effects of temperature and rainfall. To tackle with problems on JE, it is needed to develop a model which can predict JE case as a function of these environmental factors.

The great majority of cases and death occur in World Health Organization (WHO) regions of South-East Asia and the Western Pacific. In 2002, the estimated global burden of JE was 709,000 disability adjusted life years (DALYs) lost (WHO, 2004). At present there are no established antiviral treatments against JE. Interferon alpha was the most promising drug in small open-label trials, but it failed to affect the outcome in children with JE (Solomon *et al.,* 2003).

JE is principally a disease of rural agricultural areas and primarily a zoonotic diseases infecting mainly Vertebrate animals, e.g. pigs, horses, birds etc. Pigs cooding birds and ducks have been incriminated as important vertebrate amplifying hosts for JE virus due to viremia in them. Man is involved in transmission cycle as an accidental host and plays no role in perpetuating the virus.There has been a changing pattern in the epidemiology of JE. On the one hand, primarily due to extensive vaccination campaigns, JE has been almost eliminated in many economically advanced countries of East Asia and South-East Asia (i.e. Japan, Republic of Korea and Taiwan) and the burden of JE has been substantially reduced in many other endemic countries (Halstead and Jacobson, 2003). On the other hand, intensified transmission has been observed in other parts of South-East Asia and the Western Pacific, most likely due to an expansion of irrigated agriculture and pig husbandry, as well as changing climatic factors. Water resource development and management, in particular flooded rice production systems, are considered among the chief causes for several JE outbreaks (Amerasinghe and Ariyasena, 1991). Conversely, the occurrence of the disease has changed considerably over the past time Siberia and the Republic of Korea in the North, to most parts of China and the

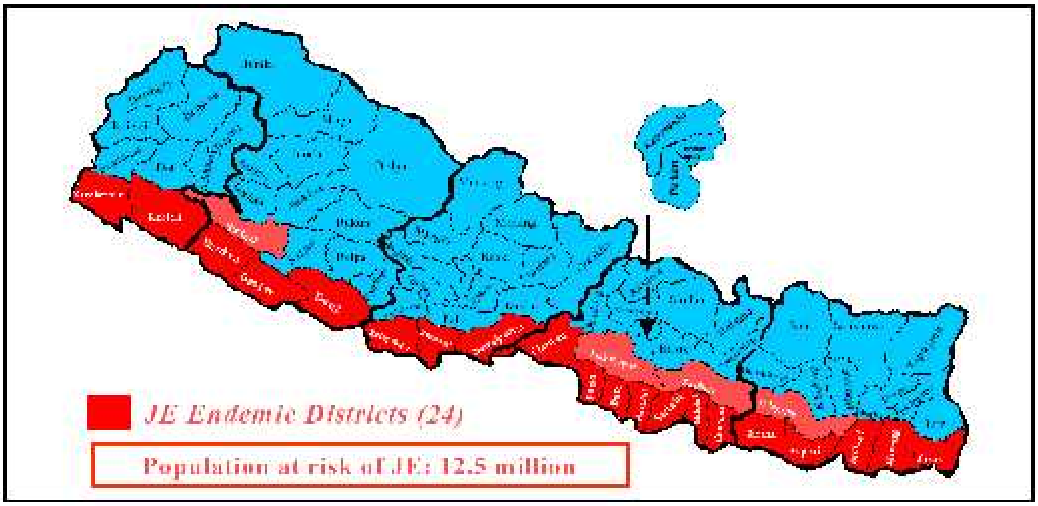
Philippines in the East, Papua New Guinea in the South, and India and Nepal to the West (Broom *et al*., 2003). Recent outbreaks of JE have been reported Southward in Australia, and westward in Pakistan (Solomon *et al.,* 2000). Currently, approximately 90% of the world's rice 56% is produced in Asia. In most of 58 the countries, where JE outbreaks have been reported, rice is not only a staple food, but rice growing also is a major economic activity and key source of employment and income generation. Efforts to further enhance the high annual rice production in these areas are essential to maintain food security. It has been estimated that in the next 25 years the demand for rice will rise by 65% in the Philippines, 51% in Bangladesh, 45% in Viet Nam and 38% in Indonesia. Thailand, for example, is already in the planning stages of designing new rice-irrigation schemes for year round irrigation (Consultative Group on International Agricultural Research *et al.,* 1998). Hence, there is considerable concern in public health circles, as the intensification of rice production systems as well as the extension of the flooded surface area, particularly in semi-arid areas, contributes greatly to increased frequencies and intensities of JE outbreaks.

Human activities are often responsible for the introduction or spread of mosquito species in regions where they were previously unknown. Establishment of vector species in new areas may have important consequences for transmission of vector-borne parasites. Knowledge of the spatial and temporal dynamics of mosquito vectors is important for both efficient mosquito control programs and for emphasizing personal protection measures for the public. Vector-borne disease systems are complex, with numerous factors contributing to the interactions between vector, vertebrate host, and pathogen (Moore, 2008). Environmental variables are important to consider when predicting vector-borne disease risk. Climate is a major factor contributing to the distribution and abundance of arthropods, the duration of arthropod life cycles, the dispersal and evolution of arboviruses, and the vector transmission efficiency of arboviruses to vertebrate hosts (Gould and Higgs, 2009). Specifically, warm temperatures tend to enhance pathogen transmission by 1) increasing vector populations, 2) increasing the frequency of blood feeding and oviposition, thus increasing vector-host contact, and 3) increasing the rate of pathogen development in the vector, resulting in a shorter extrinsic incubation period (Reisen, 2010). Most vector-borne transmission occurs during the warmest periods of the year, but there is a threshold above which elevated temperatures can have negative effects on vector survival (Reisen 1995, 2010). Precipitation is another important environmental variable affecting mosquito-borne disease systems, as it determines the quantity and quality of larval habitats and therefore adult population size (Reisen *et al.,* 2007). Recent studies have indicated that periods of drought can enhance arbovirus transmission (Monath 1980, Jacob *et al*., 2009) by bringing avian hosts into close contact with vector mosquitoes. Landuse patterns can also play a role in the quantity and quality of larval breeding habitats (Winters *et al.,* 2008a, 2008b, Jacob *et al.,* 2009, Liu *et al.,* 2009, Liu and Weng ,2009). For example, irrigated agricultural areas can serve as productive larval habitats (Reisen *et al.,* 1992a, Gates and Boston, 2009), as well as urban areas with bird baths and swimming pools left un-maintained (Reisen *et al.,* 2009).

JE was first recorded in Nepal in 1978 as an epidemic in Rupandehi district of western Nepal and Morang of eastern Nepal. There is little information on the occurrence of this disease in the densely populated Kathmandu valley. JE 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. Incidence was 2.1/100,000 and the case fatality was 20% (8/40) **(**Partridge *et al***.,** 2007.

Mosquitoes of the *Culex vishnui* subgroup (Diptera: Culicidae) are the most important vectors of JE and ricefields are their most productive breeding sites, where predominant species of this subgroup are *Cx tritaeniorhynchus* Giles, *Cx pseudovishnui* Colless and *Cx vishnui* Theobald. The species *Cx tritaeniorhyncus* is suspected to be the principal vector of JE in Nepal as females feed outdoors beginning at dusk and during evening hours until dawn. Larvae are found in flooded rice fields, marshes, and small stable collections of water around cultivated fields. It infects vertebrate hosts, primarily birds and swine, in an enzootic cycle (Endy and Nisalak, 2002). Multiple host contacts in a gonotrophic cycle increase the chance of acquiring and transmitting the pathogen (DeFoliart *et al.,* 1987). Multiple feeding within the same gonotrophic cycle increases the potential for human-vector contact bringing them into the proximity of humans. Patterns of disease transmission are influenced by the vector abundance. In order to understand adequately the dynamics of vector-borne disease, one must understand how and why vector populations change over time and environmental factors. It is often hypothesized that the abundance of mosquitoes in a house is associated with the distribution of livestock and humans. The relationship of 13 abiotic variables with the abundance of *Cx vishnui* subgroup immatures was investigated in transplanted rice fields for 3 years (1991–94) covering three different crop seasons. The results from the multiple regression model suggested paddy height (– ve), water temperature (+ ve), dissolved oxygen (– ve), ammonia nitrogen (– ve) and nitrate nitrogen (+ ve) to be the best predictor variables associated with the immature abundance, nearly always consistent in their effects within and between seasons. Application of synthetic nitrogenous fertilizers to the rice fields was followed by a rise in concentration of ammonia nitrogen and a subsequent increase in nitrate nitrogen level in the rice field water, during which an increase in the density of larval instars was observed.

As mentioned above JE, one of the important mosquito-borne diseases in Nepal is now expanding its prevalence up to a thousand cases per year. The disease regularly occurs during the rainy season, corresponding with high densities of vector populations. Several studies on JE vectors in Thailand (Gould *et al*., 1974; Mori *et al.*, 1983; Leake *et al*., 1986; Gingrich *et al*., 1992) revealed that, besides *Cx tritaeniorhynchus* Giles, *Cx gelidus* Theobald, *Cx fuscocephala* Theobald, and *Cx vishnui* Theobald may be involved in the transmission of JE virus.



JE, at present the disease is endemic in 24 districts namely Jhapa, Morang, Sunasari, Saptari, Siraha, Udayapur, Dhanusa, Mahottari, Sarlahi, Sindhuli, Rautahat, Bara, Parsa, Makwanpur, Chitwan, Nawalparasi, Rupandehi, Kapilvastu, Palpa, Dang, Banke, Bardiya, Kailali and Kanchanpur.Among them, 10 districts namely Jhapa, Morang, Sunsari, Parsa, Rupandehi, Dang, Banke, Bardiya, Kailali and Kanchanpur are affected most. The mosquito borne mode of JE transmission was elucidated with the isolation of JE virus in 1983 and subsequently in other field studies that also established the role of aquatic birds and pigs in the viral enzootic cycle. (Bista and Shrestha, 2005).

Patterns of disease transmission are influenced by the vector abundance. In order to understand adequately the dynamics of vector-borne disease, one must understand how and why vector populations change over time and environmental factors. It is often hypothesized that the abundance of mosquitoes in a house is associated with the distribution of livestock and humans. However, no information is available regarding the abundance of the major JE vectors, *Cx tritaeniorhynchus* and *Cx gelidus* in Nepal. The genus *Culex* belongs to the sub family culicinae among blood sucking group of mosquitoes. Genus *Culex* includes subgenuses *Culex, Culiciomyia*, *Lophoceraomyia*, *Lutzia* and *Eumelanomyia* from Nepal. (Darsie and Pradhan,1990). The most important vectors of JE are *Cx tritaeniorhynchus* Giles, *Cx. pseudovishnui* Colless and *Cx vishnui* Theobald, *Cx gelidus* Theobald and *Cx fuscocephala* Theobald that may be involved in the transmission of JE virus.

Male mosquitoes feed primarily on flower nectars while the female require blood meal to produce to produce some viable eggs. Species that prefer feeding human blood is known as anthrophilous and those feeding on animals are called zoophilous, (Harris *et al.*, 1969; Harwood and James, 1979). Females typical feed every 3-5 days and in a single feeding a female usually anchors more than its own weight of blood. Some species prefer to feed at dusk, twilight or dusk while some prefer feeding at day. The principal vector of JE is *Cx tritaeniorhynchus*. Females of *Cx tritaeniorhynchus* are infective 9–10 days after having taken the viraemic blood meal, having undergone three gonotropic cycles (Gajanana *et al.,* 1997). Other Culicine mosquitoes that can transmit JE include *Cx bitaeniorhyncus*, *Cx epidesmus*, *Cx fuscocephala*, *Cx gelidus*, *Cx pseudovishnui*, *Cx sitiens*, *Cx vishnui* and *Cx whitmorei* (Sehgal and Dutta, 2003). In Australia, *Cx annulirostris* was found to be the major JE vector species (Hanna *et al.*, 1996). In Kerala, South India, JE was isolated from *Mansonia indiana* (Arunachalam *et al.*, 2004). Although JE vectors are able to breed in ground water habitats, sunlit pools, roadside ditches, tidal marshes of low salinity, or manmade containers, one of their major preferred larval habitats are rice fields (Mogi, 1984). The ecology of *Culex* spp. in rice fields has been studied and reviewed in great detail (Lacey and Lacey, 1990). Since very high densities of JE vector species were found consistently in rice fields, it was concluded that the impact of these man-made breeding sites is much more important than that of natural breeding places. For example, a significant increase in the abundance of *Cx tritaeniorhynchus* and increased human vector contacts have been noted following completion of the large rice-irrigation scheme in the Mahaweli project, Sri Lanka (Amerasinghe and Ariyasena, 1991). JE vector abundance is closely related to agroclimatic features (Phukan *et al.,* 2004), most notably temperature and monthly rainfall (Solomon *et al.,* 2000. However, the most important causative factors of JE is the management of paddy water, and the peak periods of mosquito abundance are associated with cycles in local agricultural practices. In Thailand, the highest numbers of larvae and pupae of JE vectors were collected when the rice fields were ploughed with the water in the fields (also termed puddling). The vector population decreased after transplanting when the fields were flooded, and stayed low until harvesting In Malaysia, small plots in the rice fields, which are common before planting and contain vegetation, were found to be conductive factors to facilitate enhanced breeding of JE vectors; up to 40 pupae were collected per m2 (Heathcote, 1970). The practice of paddy cultivation, proximity of houses to water bodies and suitable climatic factors were the most important environmental factors associated with several recent JE outbreaks in Northeast India (Phukan *et al.,* 2004).

The presence of pigs and marsh birds is crucial in the etiology of JE, as the virus is carried by birds and amplified by pigs (Broom *et al.*, 2003). The latter are the most important natural host for transmission of JE to humans. Pigs have high and prolonged viraemias, are often common in endemic countries, and are generally reared in open and unroofed pigpens, which are located near houses (Mishra *et al.,* 1984; Solomon et al., 2000). Humans, goats, cattle and horses are considered dead-end hosts (Reuben *et al.,* 1992). For example, in the Thanjavur district, India, an area with extensive rice agriculture, a very low JE incidence has been reported, which has been explained by a high cattle to pig ratio (400:1) (Vijayarani and Gajanana, 2000). The important role of birds was demonstrated in Indian villages with or without herons in close proximity; in rice-growing villages without herons, seroconversion rates in children aged 0–5 and 6–15 years were 0% and 5%, respectively. In ecologically-similar villages with herons, the corresponding rates were 50% and 56%, respectively (Mani *et al*., 1991a). The second category of contextual determinants comprises vaccination and transmission interruption strategies.

Since JE is endemic in 24 districts of Nepal and continued in 40 residents of Kathmandu valley, the investigation of the abundance of *Cx tritaeniorhynchus* and *Cx gelidus,* coupled densities relevant human hosts known to mediate host seeking behavior will foster the development of better mosquito control strategies in Kathmandu valley also. This attempt, when coupled with numerous other strategies such as integrated vector control measures that are being considered to combat JE, may dramatically reduce the immense human suffering and financial burden that currently is shouldered by developing countries like Nepal and regions which are suffering from this deadly disease. In other words, a deeper understanding of the mosquito abundance may facilitate the development of methods that can interfere with the interaction of insect vectors with their host organisms. The increasing concern regarding insecticide resistance strongly argues for the development of new insecticides as well as novel control strategies. In this light, the ability to modulate integrated vector control measures will provide a potentially important opportunity to reduce disease transmission by targeting vectorial capacity.

1.2 Objectives:-

General:

- To report observation on the abundance of *Culex tritaeniorhynchus* and *Culex gelidus* in Kathmandu valley.

Specific:

- To study the monthly distribution of *Culex tritaeniorhynchus* and *Culex gelidus* in Kathmandu, Lalitpur and Bhaktapur districts.
- To observe the sitewise and monthwise variation of *Culex tritaeniorhynchus* and *Culex gelidus*
- To report the abdominal condition of *Culex tritaeniorhynchus* and *Culex gelidus* mosquitoes collected from different habitats.

CHAPTER - II

LITERATURE REVIEW

Japanese Encephalitis is a mosquito transmitting disease of vertebrate animals and its transmissitable to man through the bites of vector mosquitoes. It is primarily a zoonotic disease infecting mainly animals. Man involves as an accidental host and plays no role in perpetuating the virus (Pradhan *et al.*, 1991).

Japanese Encephalitis Virus was first isolated in 1933 in Japan and was initially called Japanese 'B' encephalitis (Hayashi, 1934 in Kuwayama *et al.*, 2005). It is a taxon originally known as arthropod borne group 'B' virus (Cosals and Brown, 1954). The occurrence of JEV had been documented for more than four decades in Taiwan, Thailand, Malaysia, Japan, Korea, Indonesia and India.

The annual incidence and mortality estimates for JE are 30,000–50,000 and 10,000, respectively (Solomon, 2004). However, there is considered to be severe under-reporting of JE and one study estimated the annual incidence at 175,000 per year (Tsai, 2000). JE outbreaks occur in cycles that may be linked to climatic patterns and the immune status of the populations. The great majority of cases and death occur in World Health Organization regions of South-East Asia and the Western Pacific. In 2002, the estimated global burden of JE was 709,000 DALYs (WHO, 2004). At present there are no established antiviral treatments against JE. Interferon alpha was the most promising drug in small open-label trials, but it failed to affect the outcome in children with JE (Solomon *et al.*, 2003).

The transmission has been observed in other parts of South-East Asia and the Western Pacific, most likely due to an expansion of irrigated agriculture and pig husbandry, as well as changing climatic factors. Water resource development and management, in particular flooded rice production systems, are considered among the chief causes for several JE outbreaks (Amerasinghe and Ariyasena, 1991). Conversely, the occurrence of the disease has changed considerably over the past time Siberia and the Republic of Korea in the North, to most parts of China and the Philippines in the East, Papua New Guinea in the South, and India and Nepal to the

West (Broom *et al.,* 2003). Some outbreaks of JE have been reported Southward in Australia, and westward in Pakistan (Solomon *et al.,* 2000). Currently, approximately 90% of the world's rice 56% is produced in Asia. In most of the countries, where JE outbreaks have been reported, rice is not only a staple food, but rice growing also is a major economic activity and key source of employment and income generation (Consultative Group on International Agricultural Research *et al.,* 1998). Hence, there is considerable concern in public health circles, as the intensification of rice production systems as well as the extension of the flooded surface area, particularly in semi-arid areas, contributes greatly to increased frequencies and intensities of JE outbreaks.

JE was first found in plain terai of western region of Nepal in 1978 with the outbreak of encephalitis in Rupandehi district. Although in Nepal, clinical cases were reported before 1975 and an epidemic of JE reported before 1975 and an epidemic of JE was recognized for the first time in Rupandehi district of the western development region in 1978 (EDCD, 2000). The disease was then though to be imported from Gorakhpur and surrounding areas of Uttar Pradesh of India, where a JE epidemic occurred in previous year (Takashi, 1996).

The mosquito borne mode of JE transmission was elucidated with the isolation of JE virus in 1983 (Khatri *et al*., 1983) and subsequently in other field studies that also established the role of aquatic birds and pigs in the viral enzootic cycle. (Bista and Shrestha,2005). Zimmerman *et al.* (1997) reported the first proven outbreak of JE in the Kathmandu Valley. At present the disease is endemic in 24 districts (Singh and Gurung, 2002) namely Jhapa, Morang, Sunasari, Saptari, Siraha, Udayapur, Dhanusa, Mahottari, Sarlahi, Sindhuli, Rautahat, Bara, Parsa, Makwanpur, Chitwan, Nawalparasi, Rupandehi, Kapilvastu, Palpa, Dang, Banke, Bardiya, Kailali and Kanchanpur.Among them, 10 districts namely Jhapa, Morang, Sunsari, Parsa, Rupandehi, Dang, Banke, Bardiya, Kailali and Kanchanpur are affected most (Bista and Shrestha, 2005).

The Haemagglutination inhibition test was done on human and animal serum during 1978 to 1980 in Nepal (Bista and Shrestha, 2000). In Nepal serological diagnosis has shown that pigs and ducks are main reservoir hosts. During the 1985-86 epidemics, JEV was isolated from human brain/ CSF, sentinel pigs and pools of female mosquitoes. A serological survey conducted in mid-western region of Nepal found 62 percent significant antibody titvas to JE virus (Bajracharya *et al.,* 2001).

An epidemiological study of JE was carried out in all epidemic districts of Nepal during the year 1989. It reported 868 total JE cases out of which 227 died. All ages and both sex groups were affected from the disease (Parajuli *et al*. 1992).

An epidemiological survey of JE was carried in all endemic areas of Nepal through NZFHRC) from 1990 to 1993. The CFR were 36.0%, 38.0%, 35% and 31.7% in 1990, 1991, 1992 and 1993, respectively. (Joshi *et al.,* 1995).

In 2006 1,481 AES cases were reported nationwide through the national surveillance network. Out of 1,291 clinical case with serum of CSF samples, 292 (23%) were confirmed as JE at the NPHL of at the BPKIHS. The 959 cases with samples but no avoidance of anti- JE IgM antibody and 190 cases with no sample were classified as AES-unknown. The 292 JE positive cases were distributed across 42 districts including 3 districts in the Kathmandu valley. The laboratory analysis confirmed JE in the terai and Inner terai as 1.6/ 100,000. A total of 48 cases were reported from Kathmandu valleys, among them 40 cases were verified from the residents of Kathmandu valley and 30 had no history of travel outside the valley during the 30 days before the onset of illness. The overall incidence of JE in the Kathmandu valley was 2.1/ 100,000 (Patridge *et al*., 2007).

Peters and Dewar (1956) were the first to record certain Culicine species including *Culex vishnui*, *Cx fuscocephala*, *Cx gelidus*, *Cx bitaeniorhynchus* and *Cx barraudi* the suspected vectors of JE in Nepal.

Joshi *et al*. (1965) made a major contribution by reporting 59 species of Culicine including 28 new country records. the new records included the *Cx tritaeniorhynchus*; the principal vector of J.E. and *Cx whitmorei* the other suspected vector of JE.

Darsie and Pradhan (1994) reported 168 species and subspecies in 16 genera of Mosquitoes from Nepal. Their publications continued the record of *Cx* *tritaeniorhynchus*, the principal vector and other suspected vectors of JE from different parts of the country.

Neupane *et al.*, (2009) carried out a study on 10 villages of Chitwan district of Nepal during August-September 2007 to March - April, 2008. The study reported 12 species including *Cx quinquefasciatus*, *Cx fucocephala*, *Cx tritaeniorhynchus*, *Cx gelidus*, *Cx bitaeniorhynchus*, *Cx whitmorie* and *Cx sinensis*. *Cx Quinquefasciatus* was most abundant both in pre-monsoon(18.7%) and post monsoon(26.90%). Highest resting density (human) of 9.33 was in post monsoon at 25.750c in Champanagar VDC of Chitwan.

In Japan large numbers of human cases were reported until routine JE vaccination was introduced in 1968. Most recent small outbreak reported from Chugoku district in 2002. In Bangladesh most human cases were reported from May to October. One outbreak of human disease reported from Tangail District in 1977. In Burma most human cases were reported from May to October. Outbreaks of the disease were documented in shan shate. JEV antibodies documented in animals and human in other areas. In China human cases were reported from all provinces except Xizang (Tibet), Zinjang and Qinghai. The cases were reported from April to October in which southwest and south central provinces reported the higher rates. In India most human cases were reported from May to October from all states except Dadra, Daman, Gujarat, Himachal, Jammu, Kashmir, Lakshadweep, Meghalaya, Nagar Havali, Punjab, Rajasthan and Sikkim. States like Andhra Pradesh, Assam, Bihar, Goa, Haryana, Karnataka, Kerala, Tamil Nadu, Uttar Pradesh and West Bengal reported the higher rates. In Srilanka the disease was recorded endemic countrywide expect in mountainous areas. Highest rates of human disease were reported from Anoradhapura, Gampaha, Kurunegala, Polonaruwa and Puttalam distrcits. In Pakistan human cases were reported from around Karachi between May to October (CDE, 2010)

According to the study conducted by Joshi et al., 2004 from 1998 to 2003 in Nepal a total number if 8874 cases and 1264 deaths were recorded with an average case fatality rate of 14.2 percent in aggregate since 1998. During six years period of time the highest number of cases were reported in 1999 (2924 cases) and the second highest were in 2001 (1888 cases). the lowest number of cases (330 cases) with highest mortality (CFR 20.9 percent) was reported during the year 2003. The overall mortality of JE varies from 9.77 percent during the year 2000 to 20.9 percent during the year 2003. Comparative assessment of disease in different regions showed that Far- western and Mid-Western Development regions have reported the highest number of JE cases during the years 1998 to 2003. The reporting districts based on the abundance of number of cases per 100,000 populations were in the order of Banke, Kailali, Kanchanpur, Parsa, Rupandehi and Morang.

The mosquito *Cx tritaeniorhynchus* has been shown to be the principal vector of JE in Japan and other south eastern countries. Through experimental and field studies by a number of workers in the past considerable amount of information has been accumulated on its biology in relation to the mode of transmission of the disease. An excellent review was made by Sujuki and coworkers in 1967 on literature of Japanese encephalitis referring to biology and epidemiology of *Cx tritaeniorhynchus* in Japan. In addition, different workers has been carried out studies on its role and bionomics in different countries such as in Thailand (Gloud *et al.*, 1974), Pakisthan (Reisen *et al.*,1978), India (Reuben, 1971), Korea, Taiwan and Japan (Kono and Kim, 1977), Ryukyu Island, Sarawak (Heathcole ,1970).

Cx gelidus is also believed to be the suspected vector of Japanese Encephalitis and was the most abundant mosquito in sunsari district during September/October 1985. It is associated closely with man and his domestic animals. Their immature live in puddles, pools, rice fields and marshy depressions having abundant vegetation. Their preferred hosts are bovines and swine but they also readily attack man. (Darsie and Pradhan, 1990)

Multiple feeding was reported in field populations of vectors of malaria, eastern equine encephalitis, St. Louis encephalitis, and western equine encephalitis (Burkot *et al.,* 1988; Mahmood and Crans, 1997; Amerasinghe and Amerasinghe, 1999).

Studies on the line were followed by Imatomi *et al.,* 1955-56 (in Silver 2008) using the light traps for ten years. A series of field investigations were made on bionomics and behavior of *Cx tritaeniorhynchus summorosus* and associated mosquito species in Okayama in 1967 (Sasa *et al.*, 1968). The seasonal age composition of the species was also studied. In those studies many newly emerged males and females and some gravid females resting on grasses on near breeding places such as rice plants were collected.

Cx tritaeniorhynchus was reported breeding in a variety of habitats such as ponds, irrigation ditches, paddy field, and to a lesser extent in containers (La Casse & Yamaguchi, 1955). Rice field, ground pools, fish ponds and ditches were also reported breeding habitats of *Cx tritaeniorhynchus* by Macdonald *et al.*,(1965) and Hill *et al.*,(1969). In India its larvae was reported from different water collections such as paddy field, field with rice (paddy) plants and follow field flooded with water and ploughed but not yet planted with rice plants , were the major source of larvae of *Cx tritaeniorhynchus.* In Japan, La Casse & Yamaguchi, (1955) found *Cx tritaeniorhynchus breeding* chiefly in ground pools, ponds, ditches and paddy fields. Both Macdonald *et al.,* (1965), and Hill *et al.,* (1969) found *Cx tritaeniorhynchus to* be the dominant mosquito in rice growing areas in Sarawak. Hill *et al.,* (1969) found that *Cx tritaeniorhynchus* and *Cx pseudovishnui* were abundant in shallow pools and in fallow rice fields but that recently plant rice fields yielded no *Cx tritaeniorhynchus*.

Reisen and Milby, (1986) revealed that outdoor resting collections recovered comparable proportion of such reproductive class, however, indoor resting collections were significantly bias ($P= 0.05$) for blood fed females and biting collection for unfed female. *Cx tritaeniorhynchus* females were found typically resting outdoors. Only a small proportion entered shelter after taking a blood meal. Most blood fed female regressed from indoor resting sites in the evening following entry and did not return, as relatively few gravid females were collected resting indoor. Mostly newly emerged females were collected resting indoors.

Reisen *et al*., 1986 collected more newly emerged females resting indoors in some areas where no suitable outdoor resting places were available. 88% of bitting of females dissected were of stage I ovarian condition, Slightly more than 98% were inseminated. The 10% at ovarian stage II had imbibed an incomplete blood meal initially and were refeeding. The seasonal dynamics of *Culex tritaeniorhynchus* reproductive activity and age structure had been studied in Japan (Kawai, 1969; Oda and Wada 1973; Yajima *et al.,* 1971).

Reisen *et al.*, (1978, 1980 b) noted that *Cx tritaeniorhynchus* is short lived. During the study authors recorded more than 80 % in their dilatation classes. Nulliparous females were found to be 99 %. However, accuracy decreased to 80 % to nulliparous and 61 % for oviparous females. The unfed female mosquitoes recorded were about 90 % and gravid recorded were only 69 %.

A series of field investigations were made by Sasa *et al.*, (1968) on bionomics and behavior of *Cx tritaeniorhynchus summorosus* and associated mosquito species in Okayama in 1967 . The authors emphasized on quantitative studies to their biting and resting habit in the areas outside of a small agricultural village of Utoma by using different methods for the survey of mosquito densities. The study on seasonal changes in the population density was carried out using dry ice trap. In their study *Cx tritaeniorhynchus* showed a sharp peak in the mid-summer but reduced towards autumn.

The biting rhythm of *Cx tritaeniorhynchus* was observed by the density on pig and on man showed nocturnal bimodal i.e. two peaked curve, one in dusk and one in dawn peaks (Sasa et al., 1968; Reisen and Aslamkhan, 1979). Diurnal pattern were also observed on the number mosquitoes resting on plywood plates and adhesive rape sets near a block of dry ice. Both showed two prominent peaks at about three hours before and after midnight. It was estimated that about 50 % of the total number of all-night catches in a dry ice trap can be collected 2 or 3 hoursafter sunset (Sasa *et al.,* 1968).

The resting behaviors in relation to the time of a day and gonotrophic cycle were investigated both by direct observations and by quantitative collections on bushes with the frame net traps. Many newly emerged males and females and some gravid females resting on grasses near breeding places such as rice plants were collected. The main day time resting places for both sexes and for females of all gonotrophic stages were low on thick vegetation with wide leaves, such as sweet potatoes and strawberry fields. They however, mostly flew away from these shelters after the sunset. Both unengorged and newly engorged females made frequent stop on the trees, grasses and walls near pig-stay during the night time but these temporary resting places were usually evacuated in the morning after the sunrise (Sasa *et al.,* 1968).

Rice field, ground pools, fish ponds and ditches were found to be preferable breeding places of *Cx tritaeniorhynchus (*Macdonald *et al.*, 1965. Hill *et al.*, 1969).

In Singapore, Colless, (1957) found the member of the group *Cx vishnui*, breeding in permanent and transient fresh water, especially hyacinth ponds. It was his opinion that they were originally fresh water swamp dwellers and moved out into the man-made hyacinth ponds, which in many ways resembled the swamp. It is interesting to note that cultivated rice *Oryza sativa* is supposed to evolve from a marsh grass (Zaheer, 1966 in Reisan and Aslamkhan, 1978) and that paddy field resemble swamp in many respects.

In Japan, La Casse & Yamaguti, (1955) found that, *Cx tritaeniorhynchus breed* chiefly in ground pools, ponds, ditches and paddy fields. But they did not find large numbers of larvae in paddy fields. They concluded that, Paddy fields were not the most important breeding sources for the species. Both Macdonald *et al.*,(1965) and Hill *et al.*,(1969) found *Cx tritaeniorhynchus to* be the dominant mosquito in rice growing areas in Sarawak. It was also commonly found in other types of ground water in the absence of paddy fields. 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.

Cx tritaeniorhynchus and *Cx pseudovishnui* were abundant in shallow pools and in fallow rice fields as reported by Hill *et al.,* 1969. Plant rice fields yielded no *Cx tritaeniorhynchus* in their conclusion. They concluded that heavy rainfall might have been detrimental to the larvae at the time when their surveys were conducted.

A study was made to identify the host range of *Cx tritaeniorhynchus* Giles in Okinawa of Ryukyu Island by Pennigton et al., 1968. Engorged mosquitoes were collected in light traps located in areas pre-selected to provide various types of animal habitats. When blood meals collected from mosquitoes were analyzed, the blood of chickens and rabbits to the blood of pigs, cows, goats, horses, dogs and human serum were found. The study revealed that *Cx tritaeniorhynchus* generally feed on larger domestic animals such as cows and pigs. Only 0.02 % *Cx tritaeniorhynchus* was found to be fed on man. (Pannigton *et al*., 1968).

A study was conducted to explore the overwintering habit of C*x tritaeniorhynchus* in Korea from 1969 to 1974 during the months of November to February. All possible kinds of hibernating places were investigated using various techniques like plastic tents and dry ice. The study failed to capture even a single specimen of C*x tritaeniorhynchus* during the winter season. Thus the study concluded that the species C*x tritaeniorhynchus* overwinters in extremly in small number. (Ree *et al.*,1976).

The studies on the host preference of Japanese encephalitis vectors in Chiangmai, North Thailand reported that, there was no difference in the proportions of fed females of C*x tritaeniorhynchys* and *Cx vishnui* between the cowshed and pigsty collections and *Cx gelidus* fed significantly more on the cows than pigs. (Mwandawiro *et al.*1999).

The study on long term vector abundance and seasonal prevalence in relation to the occurrence of Japanese encephalitis in Gorakhpur district, India reported that C*x tritaeniorhynchus* together with other known vector species remained more active during the period of paddy cultivation (Kanojia *et al.*, 2003).

The average vector abundance per man hour for C*x tritaeniorhynchus* was found 324.5 per month for the period June 1998 – May 200 in south Indian villages. The average minimum infection rate per month for same mosquito species was 1.4 (range 0.0 - 5.6). Sero conversions were recorded in 14 goats (70%) in the first year and 23 goats (74%) in the second year (Rajendra *et al.*, 2003).

The study on the prevalence and seasonal abundance of the dominant mosquito species in a large marsh near coast of uslan reported that, *Anopheles sinensis* was most abundant (53.4%) in species ratio) followed by C*x tritaeniorhynchus* (43.0%), *Cx inatomii* (1.6%) *Ochleratatus dorsalis* (1.3%) and C*x pipiens pallens*(0.5%). A total of 3,663.3 females of malaria vector *Anopheles sinensis* and 31,425 females of JE vector *Cx tritaeniorhynchus* were collected per trap night from June to September for the years respectively. According to the bi-weekly population changes at the area, *Cx inatomii* was the most abundant in the early July during 1999-2001 (Jeong *et al*., 2003).

The observation on the multiple feeding behavior of C*x tritaeniorhynchus* in Kerala, southern India reported that C*x tritaeniorhynchus* fed mainly on cattles (56.6%). Pig feeding accounted 6.3% of total samples. Some samples (n = 980, 38.3%) were of serologic mixed origin. Mixed blood meals were mostly (96.7%) from cattles and goats (Arunachalam *et al.*, 2005)

Summarizing, this dissertation includes studies on the entomological aspects of vectors of JE; *Cx tritaeniorhynchus* and *Cx gelidus*. Combining vector abundance data to estimate a vector population can provide a comprehensive tool to assess disease risk.

CHAPTER-III
MATERIALS AND METHODS

4.1 Study Area

Kathmandu valley is roughly elliptical in shape, 25km along its east-west axis and has maximum width of 19 kilometers. It lies in lesser Himalayas of Central Nepal. The valley lies at a mean elevation of about 1350m above the sea level. Mountains on all sides surround the bowl shaped topography of the valley. Some of the surrounding hills having heights of more than 2000m are Shivapuri in the North, Nagarkot in the east, Phulchoki in the south and Chandragiri ridge in the west. The valley comprises three administrative districts namely Kathmandu, Bhaktapur and Lalitpur. Its population was 1,645,091 inhabitants was according to Census 2001.

The climate of the valley is subtropical, continental and semi-humid. The average temperature the valley experience is 18.3^{o}C while the main annual rainfall is 1439.7mm. The main city; Kathmandu is located in the northwestern part of the valley. The city covers an area of 50.67 square kilometers (19.56 sq. miles). The city is bounded by Lalitpur Sub-metropolitan city in its south, Kirtipur municipality in south west, Madhyapur Thimi Municipality in east and different Village Development Committees of Kathmandu in north, west and north east. Eight Rivers that flow through the city are the Bagmati, Bishnumati, Dhobikhola, Manahara, Hanumant and Tukucha Rivers. The mountains from where these rivers originate are in the elevation range of 1,500 - 3000 meters and have passes, which provide access to and from Kathmandu and its valley.

As Kathmandu is densely populated most part of the valley covers the urban area including ring road, subways, government offices, market area, Tribhuvan international airport, stadium, education institutions, hospital area, culturally important durbar squares and large number of residential area. A small portion of farmland including traditional Newar villages surrounds the valley. Also the Bramins of Gothatar V.D.C. are especially famous for their cowsheds from the ancient time. The agricultural commodities like rice, wheat, potato, maize and some vegetables are grown by the people of Kathmandu valley. The local rice; 'Tieching' and its beaten rice produced by the Newar community is specially famous in Kathmandu. The local curd; 'Juju Dhau' of Bhaktapur is also famous in Kathmandu. In spite some of the farmlands in Kathmandu are occupied by the people outside the valley for cattle farming and other agricultural purpose. Most of the cowsheds and piggery farms of such lands are temporary and their residence is also temporary. The farmers have taken the land in rent from the landowner and get their settlements for income generating purpose.

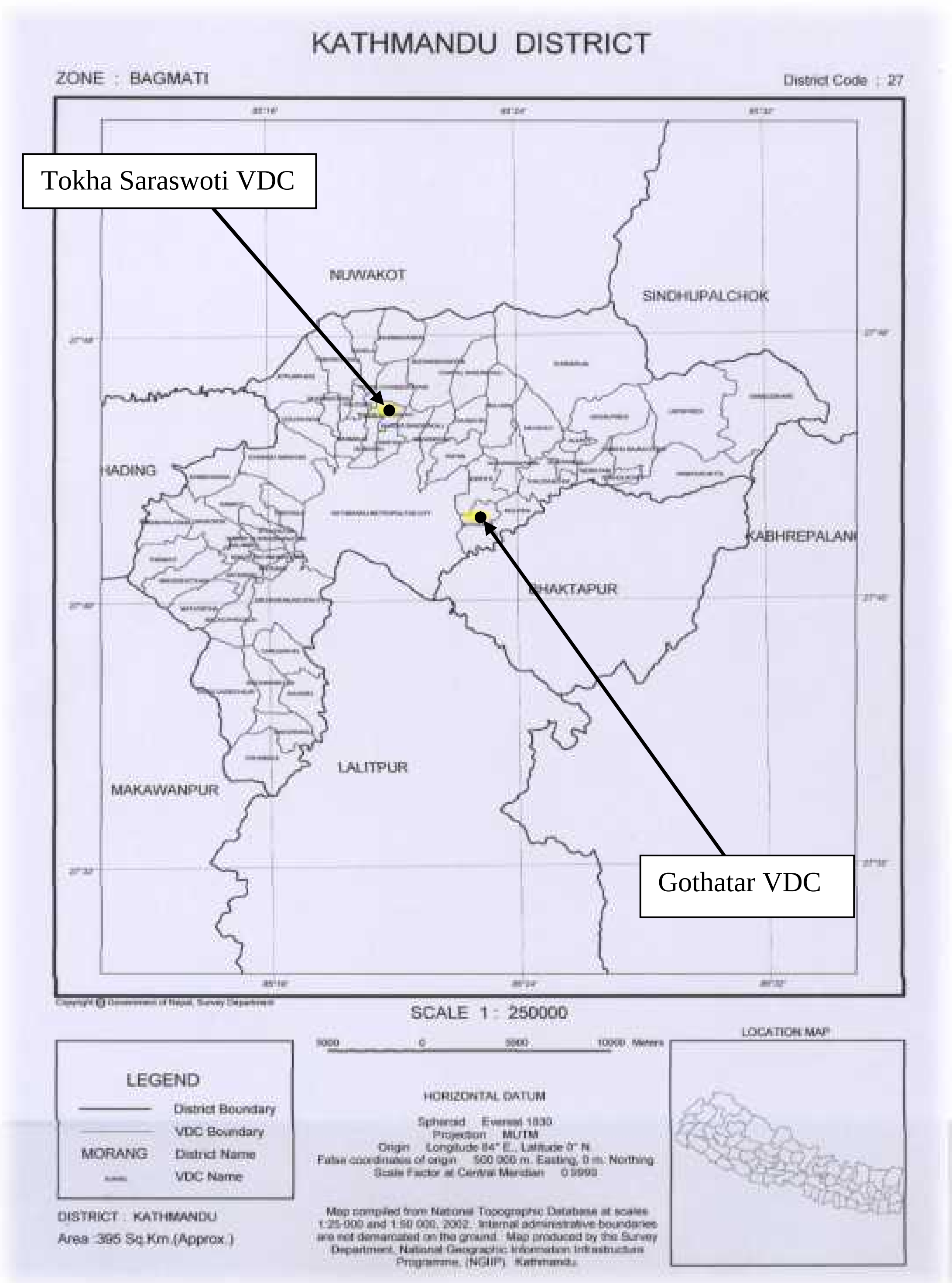
Study sites

Four sites were selected in Kathmandu, Lalitpur and Bhaktapur district for this study to record the distribution of the JE vectors, *Cx tritaeniorhynchus* and *Cx gelidus*.

Kathmandu district (Tokha and Gothatar area)

The Tokha area of Kathmandu district lies towards the north of the Kathmandu valley. The area can be reached by 45 minutes bus ride from the ring road point Samakhusi. The area covers a rough transect of 2- km including ward number 4 and ward number 2 of Tokha Sarasawoti V.D.C. In this area traditional cattle sheds were most common. Although the modern cattle farming structures were also present. A small portion of farmland is present among the human dwelling area.

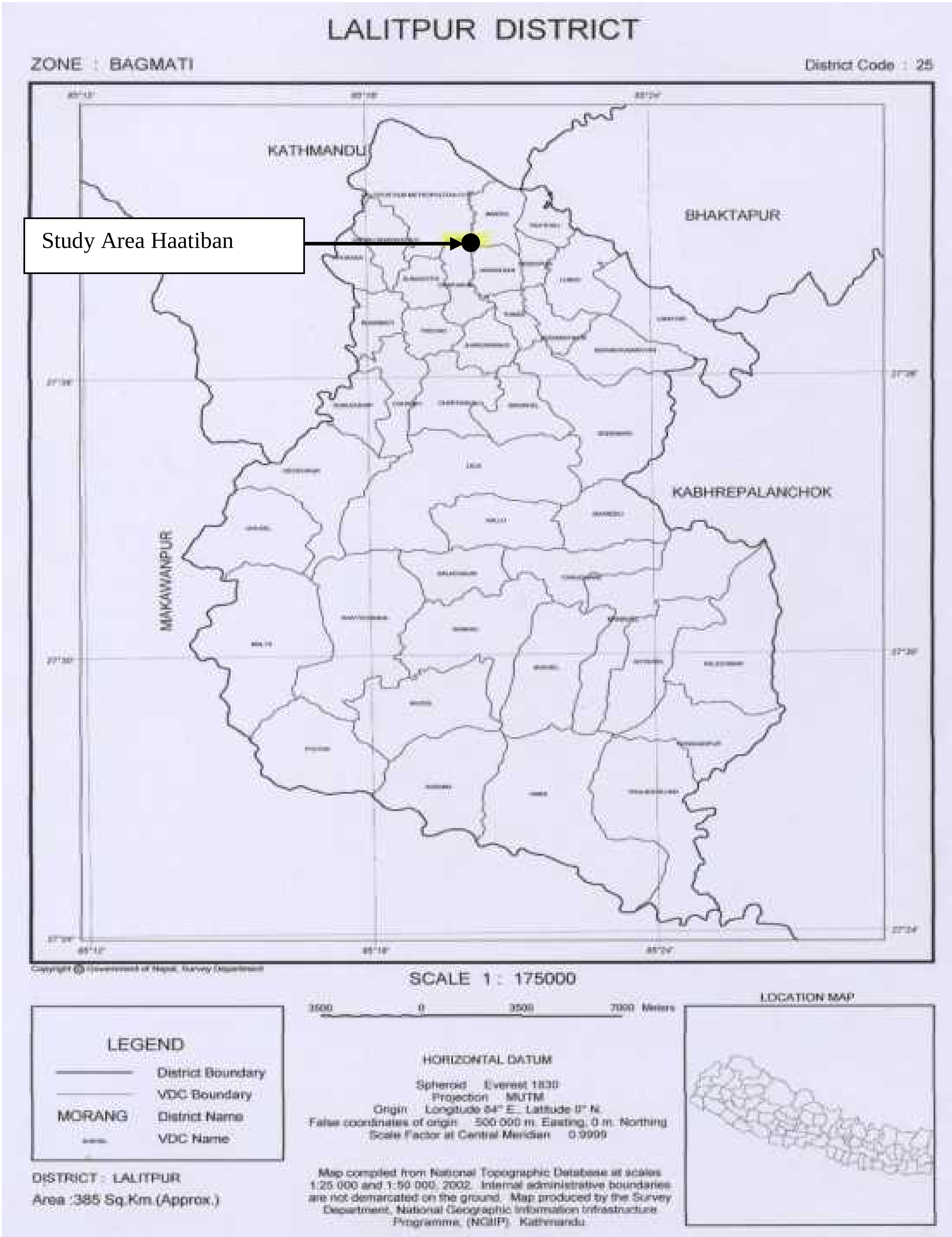
The Gothatar area of Kathmandu district lies towards the east of Kathmandu valley. The area can be reached by 30 minutes bus ride from the ring road point Koteshwor. It lies at a distance of 3 kilometers from Koteshwor. The area covers a rough transect of 2 kilometers including ward number 2 and ward number 4 of Gothatar V.D.C. Traditional cowsheds were most common in ward no 2 area while piggeries were common in ward number 4 area. The river Bagmati passes by the side of the piggery area.



Map showing Study area of Kathmandu District

Lalitur district (Hattiban area)

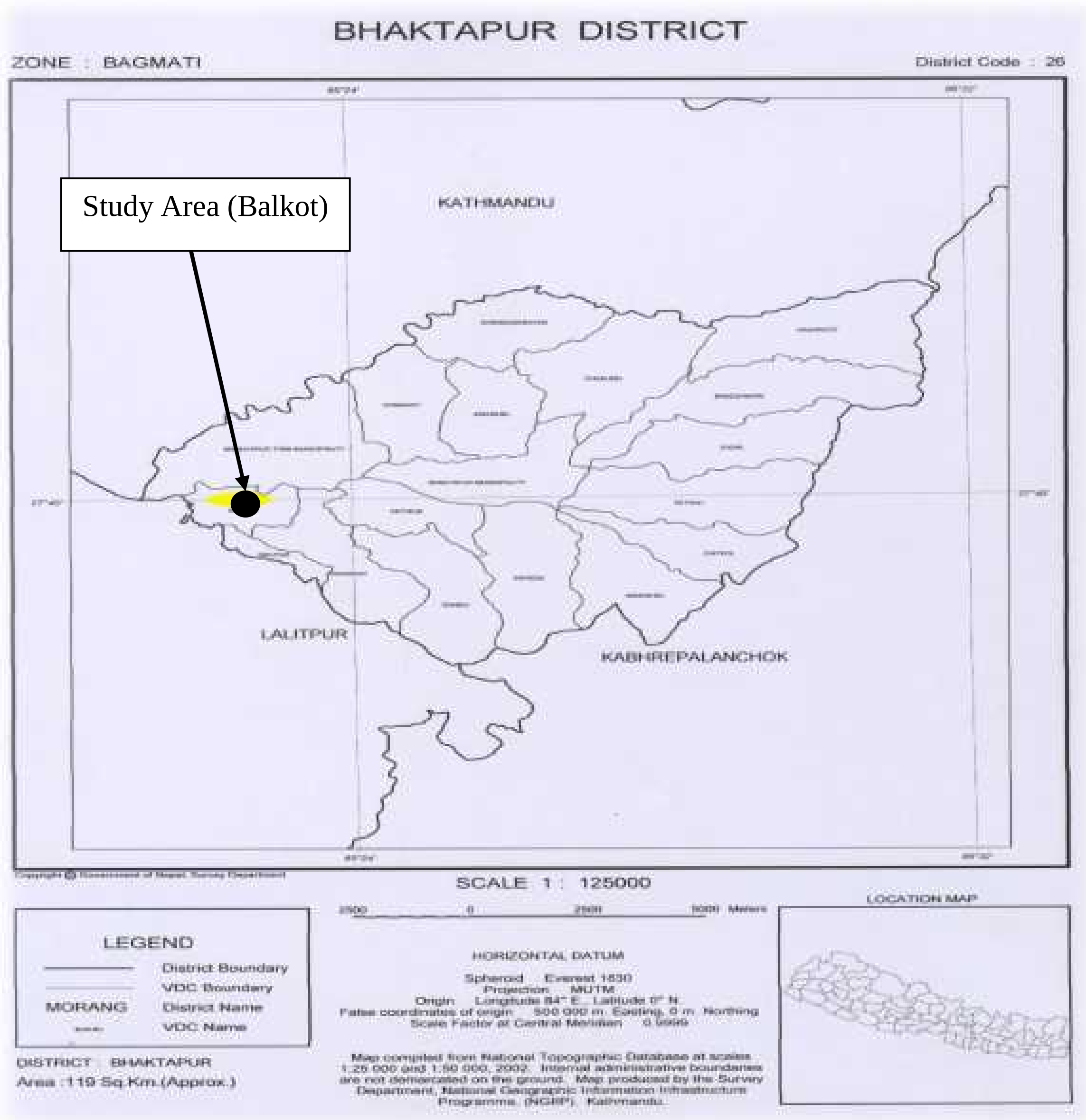
The Hattiban area lies towards the south-west part of the Kathmandu valley. The area can be reached by walking 15 minutes westwards from the ring road point, Saatdobato. The area covers a rough transect of nearly 2-3 kilometers including 15, 7 and 8 wards of Lalitpur sub-metropolitan city, ward number 3 of Imadol V.D.C. and ward number 1 of Dhapakhel V.D.C. In these area cattle sheds and thatched roofed houses were most common among the rice-cultivated field. Although few piggeries were also present.



Map showing study area of Lalitpur District

Bhaktapur district (Balkot area)

The Balkot area of Bhaktapur district lies towards the part of Kathmandu valley. It can be reached by walking 20 minutes from Gatthaghar point of Bhaktapur road. The area covers a rough transect of 2 km including ward number 2 and ward number 3 of Balkot V.D.C. In this area traditional cowsheds and rice field were most common including 2 large piggery farms.



Map showing study area of Bhaktapur district

4.2 Study Design

The study was conducted from April 2009 to September 2009. Emphasis was given to select the villages located on the river side, agro-field ecosystem areas and presence of cattle on the areas. Besides that areas were selected on the basis of accessibility as well. In each sample site 4 households were surveyed in sequence monthly along the block from the starting point household between 6:00 am to 8:00 am. The houses where resident did not provide permission for the survey, were not sampled and large bunglows, schools, office buildings were also omitted. Preference was given to traditional houses with farmlands and cattlesheds or piggery farms at their periphery. Both indoor and outdoor collection was carried out between 6:00 am - 8:00 am in the morning. For indoor collection each collector spent 15 minutes in each household. After completing indoor collection outdoor collection was made by 2 collectors in 4 households jointly spending 30 minutes by each collector. The animal baited net-trap was set up on the second evening and the collection was made on the next morning. The indoor and outdoor collection was made for 2 days and animal baited net trap collection was made on the third morning. Both fieldwork and labwork was repeated on each month in each site. Immediately after termination of the first survey the sampling procedure were repeated. All the mosquitoes were also recorded and brought in the laboratory to identify adequately.

The methodology applied in this dissertation work is according to WHO (1975). Mannual on Practical Entomology in Malaria, PartII, Methods and Techniques. WHO offset publications, No.13, Geneva.

4.3 Consent

The households with farmlands, bushes, cattle sheds and piggeries were preferred for sample collection. Consent was obtained from community leaders and the household owners before starting the collection especially for indoor hand collection and animal baited net trap collection. The participants were informed in simple and clear language about the objectives, study protocol, and advantages and inconveniences. Villagers had complete liberty to accept or refuse to give permission. Indoor hand collection (human) was carried out in the rooms of human dwelling when permitted. Outdoor hand collection was made at the house premises near cattle sheds, piggery or farmland. Animal Baited Net trap was set up near the cattle shed or farmland inside the house premises.

4.4 Entomological Surveys

Different collection tools were used to collect *Cx tritaeniorhynchus* and *Cx gelidus*, *the* vectors of JEV. The different methods used are as stated below:

1. Indoor hand collection:

Indoor hand collections were carried out inside different shelters in the morning time starting from 6.00- 8.00 AM in human (4), mixed (2) and animal (2) dwelling spending 15 minutes in each house by one collector. Two collectors spent 4 man hours in 16 houses in each village on the selected site of the valley in the month of April to September, 2009. All the mosquito samples were brought in the laboratory for identification and enumeration.

2. Outdoor hand collection

Outdoor collections was similarly attempted outside the house from outside walls, under leaves, vegetation and bushes around cattle sheds and piggeries, and in and around outdoor stored materials etc., for two hours by each collector and transported to the laboratory for identification and enumeration.

3. Animal baited net trap collection

One animal baited net trap, the bed net measuring 5m x 5m x 5m was used to collect mosquitoes. And, in the trap one animal bait was kept whole night. In the morning the mosquitoes trapped in the net trap were collected using aspirator and brought in the laboratory.

4.5 Collection Technique

The specimens were collected by the help of simple hand aspirator and flash light. Specimens were then transferred to plastic cups. Each empty plastic cups were covered with pieces of nets with a hole in the center and hole covered with a piece of cotton.

4.6 Killing Method

The mosquito samples into the plastic cup were killed using chloroform. A small piece of cotton was soaked with two/three drops of chloroform and placed at the top of the cup and made airtight. The adult mosquitoes were transferred from the cup by means of aspirator through this hole.

4.7 Mounting Method

Cx tritaeniorhynchus and *Cx gelidus* mosquitoes were separated from each sample after identification. Ivory papers were cut into the small triangular form. A tiny drop of colorless nail polish was placed at the slightly downward bended tip of triangular card. Each specimen was fixed from the thoracic pleuron with the help of colourless nail polish. The other end of the card was picked with an entomological pin. Those species were placed in box to prevent the damage of wings, legs, maxillary palpi, proboscis and abdomen giving the code numbers. Naphthalene balls were kept inside the box.

4.8 Materials

S.N.	Materials	Use
1.	Writing and Marking pen (1+1)	Noting places and marking the collected samples
2.	Hand lens (10 X) –1	Identification work
3.	Aspirator – 1	For adult collection
4.	Torch light – 1	For indoor collection
5.	Net pieces –1	Copping the samples
6.	Paper cup (acc. To need)	– Keeping sample
7.	Cotton	– Closing the aspirator hole of sample paper cup.
8.	Data sheet	– Recording the data (no, stage, sex of sample)
9.	Chloroform -500 ml	– As killing agent.
10.	Transparent nail polish -1	– For staging mosquitoes
11.	Cards with labels	– For carding and labelling.
12.	Entomological pin (Number 3)	– For pinning mosquitoes
13.	Bed Net Trap	– For adult collection
14.	Napthalene ball	– As preservative.

4.9 Identification

Each and every specimen were identified in lab to species level by using the key by Darsie and Pradhan (1990)

4.10 Specimen deposition

The collected specimens were preserved and deposited in T.U Natural history Museum, Swoyambhu and Museum, Central Department of Zoology,T.U, Kirtipur

4.11 Data analysis

Entomological data were recorded on the entomology collection sheet. Mosquito (only females) abundance were calculated as number collected per human-hour.

The man hour-density was calculated as

$$\text{Man hour density} = \frac{\text{Number of Mosq.collected}}{\text{Total man hrs.spent}}$$

4.11.1 Tools for Analysis and Presentation

The collected specimens of both *Cx triteniorhynchus* and *Cx gelidus* were separated from each sample and counted, recorded in data sheet. Only female mosquito abundance were calculated as number collected per human-hour. The data was analyzed by the help of tables, bar diagrams, line graphs and ANOVA Table, has done the analysis. The abdominal condition of each specimen was noted as unfed, full fed, half gravid and gravid.

4.11.2 Analytical Framework

The variation in the number of vectors between different study sites and month wise survey was analyzed by using two-way ANOVA-table.

4.11.3 Analysis of Distribution of Vectors Using ANOVA Table

Formulation of Hypothesis

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

1. between four study sites

i. Null Hypothesis: H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4$

I.e. there is no significant different in the distribution of mosquitoes between four study sites.

Alternative Hypothesis

H_1: $\mu_1 \neq \mu_2 \neq \mu_3$ i.e. there is significant difference in the distribution of mosquitoes between four study sites.

2. between Six months

ii. Null Hypothesis: H_0: $\mu_0 = \mu_1 = \mu_2 = \mu_3 = \mu_4$

I.e. there is no significant different in the distribution of mosquitoes between six months.

Alternative Hypothesis

H_1 : $\mu_1 \neq \mu_2 \neq \mu_3$ i.e. there is significant difference in the distribution of mosquitoes between six months.

4.12 Limitation of the study

This study describes the adult abundance of JE vectors in four study areas of Kathmandu valley. As a result of visits to limited numbers of localities and period (six months), in each district, the result does not reflect a complete figure regarding the one year seasonal abundance of the vector species.

CHAPTER - IV

RESULTS

Out of 30,602 mosquitoes collected during the six months survey only 459 vectors (404 *Cx tritaeniorhynchus* and 55 *Cx gelidus*) were recorded. In Hattiban area (Lalitpur district), a total of 1066 from indoor (human), 640 from outdoor and 6,564 *Cx* mosquitoes were recorded from animal baited net trap. In Balkot area (Bhaktapur district), a total of 793 from indoor (human), 329 from outdoor and 7,184 *Cx* mosquitoes were recorded from animal baited net trap. In Gothatar area (Kathmandu district), a total of 441 from indoor (human), 535 from outdoor and 6,015 *Cx* mosquitoes were recorded from animal baited net trap. Similarly in Tokha area(Kathmandu district), a total of 946 from indoor(human), 608 from outdoor and 5,481 *Cx* mosquitoes were recorded from animal baited net trap, Altogether 3,246 from indoor (human), 2,112 from outdoor and 25,244 *Culex* mosquitoes were recorded from animal baited net trap as mentioned in table 1. Total 192 man hours (72 days) were spent in this study.

Out of 404 adult *Cx tritaeniorhynchus* and 55 *Cx gelidus* collected during the six months survey 175 were *Cx tritaeniorhynchus* and 15 were *Cx gelidus* recorded from indoor hand collection. A total of 162 *Cx tritaeniorhynchus* and 33 *Cx gelidus* were recorded from outdoor habitats and a total of 67 *Cx. tritaeniorhynchus* and 7 *Cx gelidus* were recorded from Animal Baited Net trap.

Table. 1 Total Number of Culex Mosquitoes Collected From Different Areas

Site / Habitat	Hattiban	Balkot	Gothatar	Tokha	Total Number
In door (Human)	1066	793	441	946	3246
Outdoor	640	329	535	608	2112
ABNT	6564	7184	6015	5481	25244
Total	8270	8306	6991	7035	30602

5.1 Indoor human collection

Of the 30,602 mosquitoes collected 45 *Cx tritaeniohynchus* and only two *Cx gelidus* mosquitoes were recorded from indoor (human) habitats of Hattiban area. A total of 41 *Cx tritaeniohynchus* and two *Cx gelidus* were recorded from Balkot area. A total of 28 *Cx tritaeniohynchus* and eight *Cx gelidus* were recorded from similar habitats of Gothatar area. Similarly a total of 61 *Cx tritaeniohynchus* and three *Cx gelidus* were recorded resting inside the houses in indoor hand collection from Tokha area as mentioned in table 2.

The maximum number of 38 *Cx tritaeniohynchus* was recorded from Tokha in August followed by 23 at Haatiban in September which was followed by 21 at Balkot in August. Similarly the maximum number of three *Cx gelidus* was recorded from Gothatar in July and August followed by 3 at Tokha in August as (tables 3-6).

Table. 2 Total Number of vector species Collected From Different Areas

Habitat	Hattiban		Balkot		Gothatar		Tokha	
	Culex tritaeniorhynhus	*Culex gelidus*	*Culex tritaeniorhynhus*	*Culex gelidus*	*Culex tritaeniorhynhus*	*Culex gelidus*	*Culex tritaeniorhynhus*	*Culex gelidus*
Indoor (human)	45	2	41	2	28	8	61	3
Outdoor	28	5	31	7	37	13	66	8
ABNT	19	2	11	5	10	0	27	0

5.2 Outdoor Collection

Of the 30,602 mosquitoes collected 28 *Cx tritaeniohynchus* and five *Cx gelidus* mosquitoes were recorded from outdoor habitats of Hattiban . A total of 31 *Cx tritaeniohynchus* and seven *Cx gelidus* were recorded from outdoor habitats of Balkot area. A total of 37 *Cx tritaeniohynchus* and 13 *Cx gelidus* were recorded from similar habitats of Gothatar area. Similarly 66 *Cx tritaeniohynchus* and eight *Cx gelidus* were recorded from Tokha area during the six months study period (table 2).

The maximum number of *Cx tritaeniohynchus* (44) was recorded from Tokha in September followed by 25 from Gothatar in September and 18 at Hattiban in

September in outdoor collection. Similarly the maximum number of eight *Culex gelidus* was recorded from Tokha in August followed by six at Gothatar in September and five in July month of Gothatar (table number 5 and 6).

Table 3. Month wise collection of mosquitoes in indoor(human) and outdoor from Hattiban area of Lalitpur district in April-September 2009.

Month of Year	*Culex tritaeniorhynchus*				*Culex gelidus*			
	Indoor (human)		Outdoor		Indoor (human)		Outdoor	
	No. of Mosq.	Density	No.of Mosq.	Density	No. of Mosq.	Density	No. of Mosq.	Density
April	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0
July	8	2	2	1	0	0	0	0
August	14	7	8	4	0	0	0	0
September	23	5.57	18	9	2	0.5	5	2.5

Table 4. Monthwise collection of mosquitoes in indoor (human) and outdoor from Balkot area of Bhaktapur district in April-September 2009.

Month of Year	*Culex tritaeniorhynchus*				*Culex gelidus*			
	Indoor(human)		Outdoor		Indoor (human)		Outdoor	
	No. of Mosq.	Density	No. of Mosq.	Density	No. of Mosq.	Density	No. of Mosq.	Density
April	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0
July	8	2	10	5	0	0	0	0
August	21	5.25	6	3	0	0	2	1
September	12	3	15	7.5	2	0.5	5	2.5

Table 5. Monthwise collection of mosquitoes in indoor (human) and outdoor from Gothatar area of Kathmandu district in April-September 2009.

Month of Year	*Culex tritaeniorhynchus*				*Culex gelidus*			
	Indoor (human)		Outdoor		Indoor (human)		Outdoor	
	No.of Mosq.	Density	No.of Mosq.	Density	No.of Mosq.	Density	No. of Mosq.	Density
April	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0
July	0	0	0	0	3	0.75	5	2.5
August	9	2.25	12	6	3	0.75	2	1
September	19	4.75	25	12.5	2	0.5	6	3

Table 6. Monthwise collection of mosquitoes in indoor (human) and outdoor from Tokha area of Kathmandu district in April-September 2009.

Month of Year	*Culex tritaeniorhynchus*				*Culex gelidus*			
	Indoor (human)		Outdoor		Indoor (human)		Outdoor	
	No.of Mosq.	Density	No.of Mosq.	Density	No.of Mosq.	Density	No. of Mosq.	Density
April	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0
July	0	0	0	0	0	0	0	0
August	38	9.5	22	11	3	0.75	8	4
September	23	5.57	44	22	0	0	0	0

In August, 2009 the man hour density of Cx *tritaeniorhynchus* was found maximum (9.4) in Tokha Area in total indoor hand collection. Similarly the maximum man hour density in outdoor collection was recorded 22 per man hour from the same area in August, 2009 (table 5). For *Cx gelidus* maximum man hour density was 0.75 per man hour in indoor collection carried out in July and August in Gothatar and in Tokha it was also 0.75 in August. Similarly maximum man hour density was four per man hour in outdoor collection made in August in Tokha(table 5 and 6).

5.3 Animal Baited Net Trap Collection

A total of 19 *Cx tritaeniorhynchus* and two *Cx gelidus* mosquitoes were recorded from animal baited net trap set up in Hattiban area during the six months (table 7). Similarly a total of 11 *Cx tritaeniorhynchus* and five *Cx gelidus* mosquitoes were recorded from Balkot area (table 8). A total of 10 *Cx tritaeniorhynchus* were recorded from the similar trap fitted at Gothatar area during the six months period (table 9). Similarly, a total of 27 *Cx tritaeniorhynchus* and not a single Cx *gelidus* were recorded from Tokha area (table 10).

From total Samples of Animal Baited Net Trap the maximum number of *Cx tritaeniohynchus*(27) was recorded from Tokha in September followed by nine from Hattiban in August and nine from Balkot in September. The maximum number of *Cx gelidus* (three) was recorded from Balkot in July followed by two from Haatiban in August and two from Balkot in September.

Table 7. Monthwise collection of mosquitoes from Animal Baited Net Trap Fitted at Haatiban area of Lalitpur district in April-September 2009.

Month of Year	*Culex tritaeniorhynchus*	*Culex gelidus*
April	0	0
May	0	0
June	0	0
July	7	0
August	9	2
September	3	0

Table 8. Monthwise collection of mosquitoes from Animal Baited Net Trap fitted in Balkot Area of Bhaktapur district in April-September 2009.

Month of Year	*Culex tritaeniorhynchus*	*Culex gelidus*
April	0	0
May	0	0
June	0	0
July	2	3
August	0	0
September	9	2

Table 9. Monthwise collection of mosquitoes from Animal Baited Net Trap fitted at Gothatar Area of Kathmandu district in April-September 2009.

Month of year	*Culex tritaeniorhynchus*	*Culex gelidus*
April	0	0
May	0	0
June	0	0
July	0	0
August	4	0
September	6	0

Table 10. Monthwise collection of mosquitoes from Animal Baited Net Trap fitted at Tokha Area of Kathmandu district in April-September 2009.

Month of Year	*Culex tritaeniorhynchus*	*Culex gelidus*
April	0	0
May	0	0
June	0	0
July	0	0
August	0	0
September	27	0

Figure. 1 Number of *Culex tritaeniorhynchus* Collected from Hattiban, Lalitpur

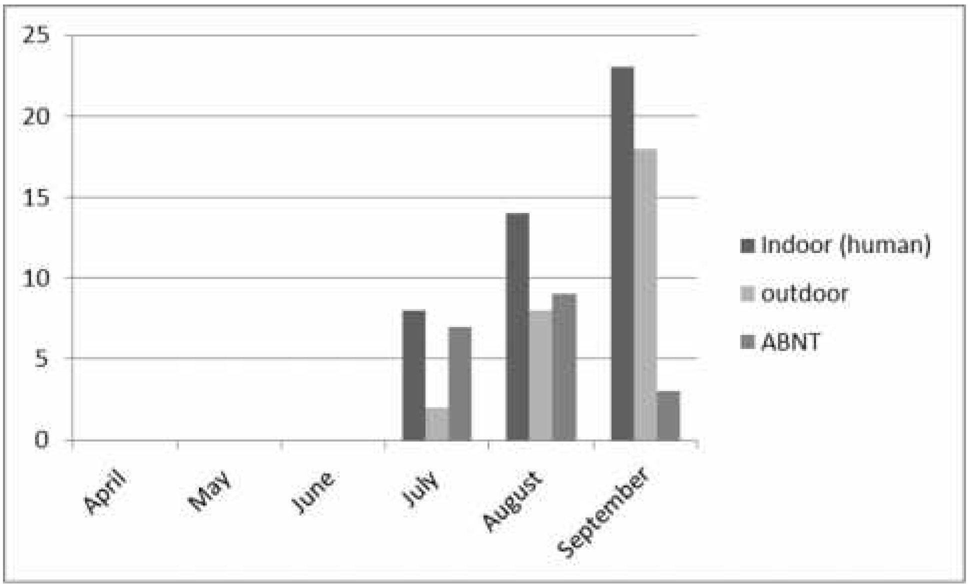


Figure. 2 Number of *Culex gelidus* collected from Hattiban, Lalitpur.

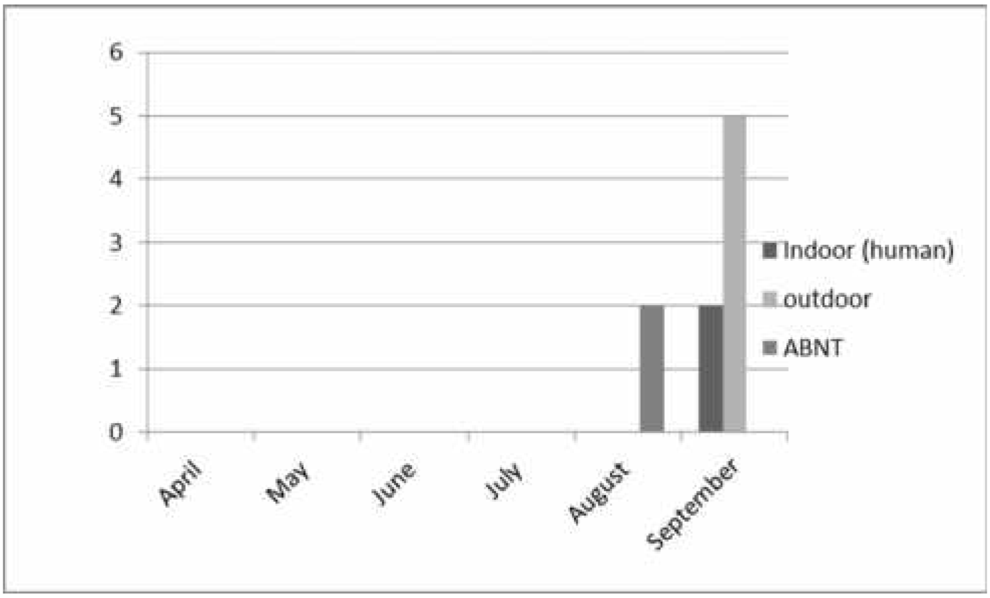


Figure. 3 Number of *Culex tritaeniorhynchus* Collected from Balkot, Bhaktapur

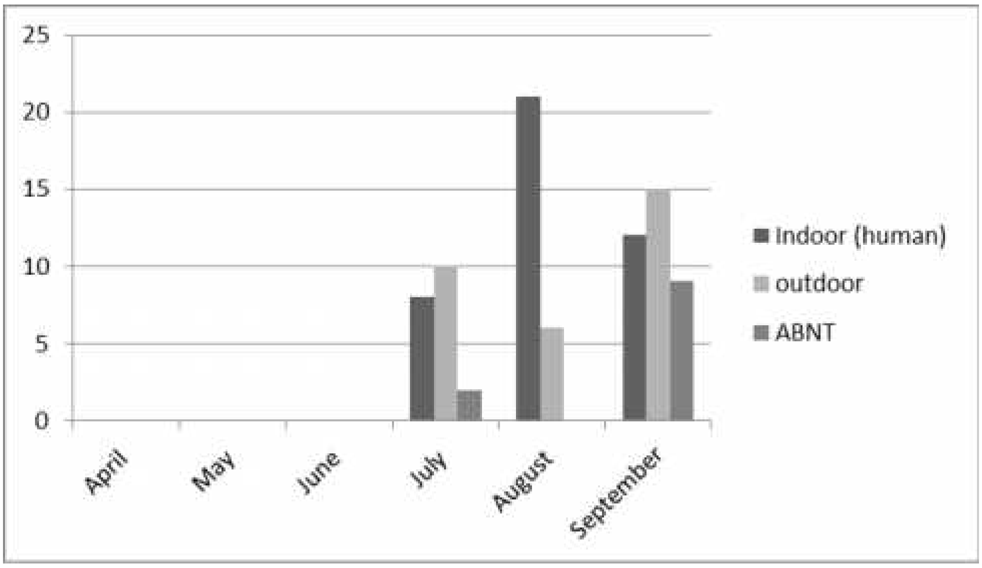


Figure. 4 Number of *Culex gelidus* collected from Balkot, Bhaktapur

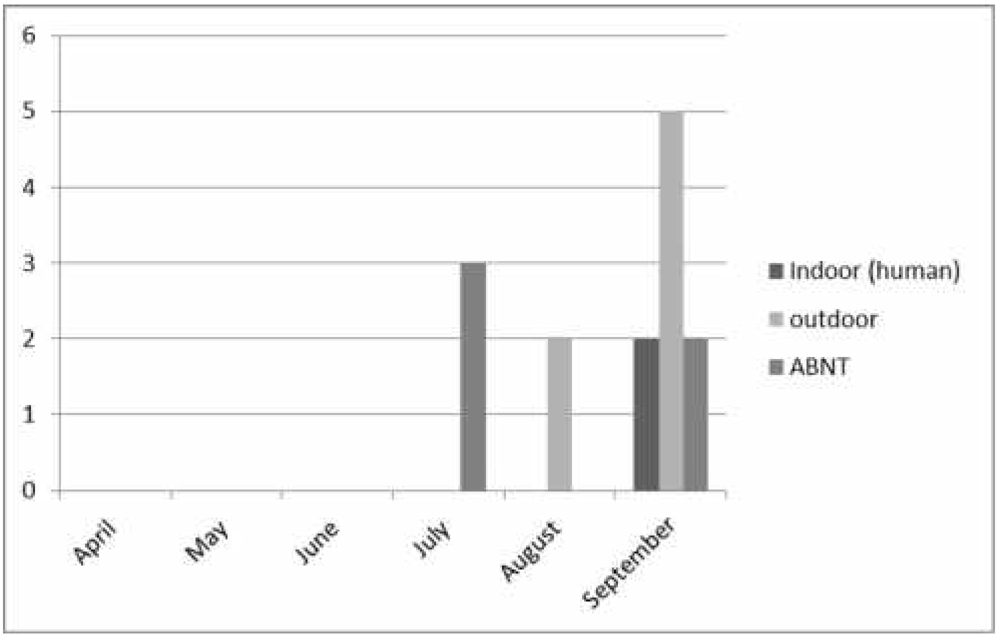


Figure. 5 Number of *Culex tritaeniorhynchus* Collected from Gothatar, Kathmandu.

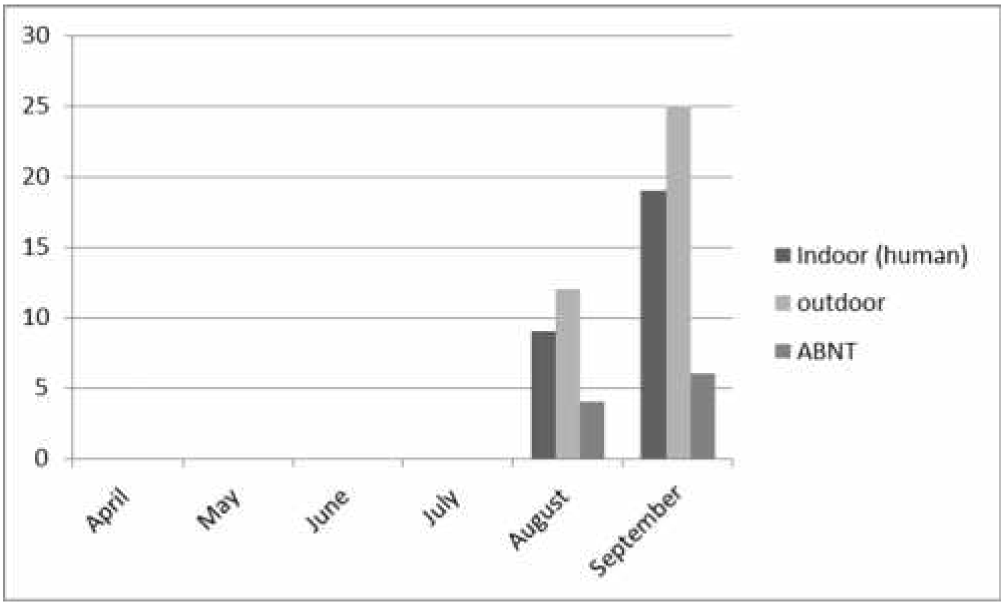


Figure. 6 Number of *Culex gelidus* collected from Gothatar, Kathmandu.

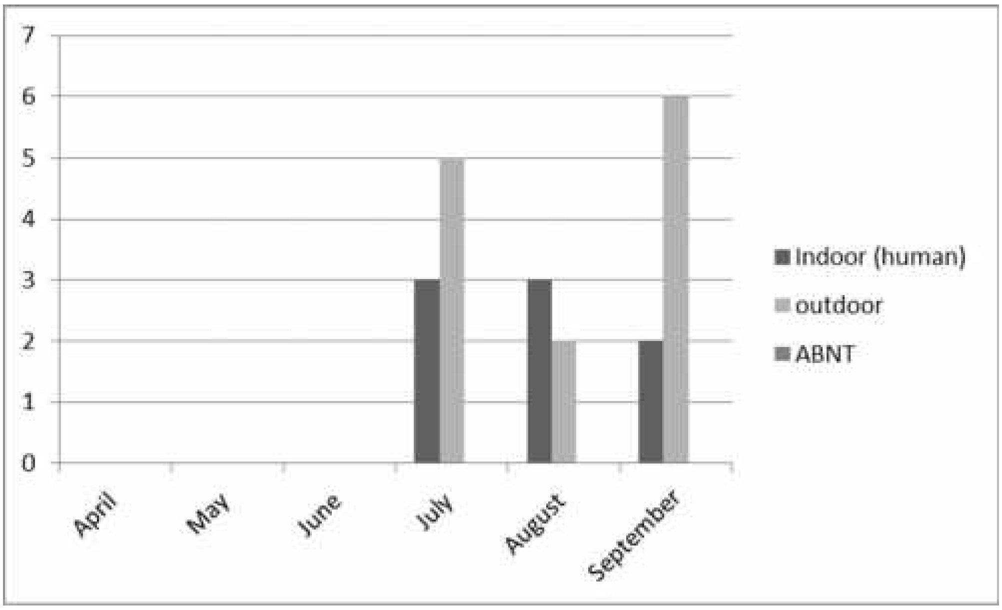


Figure. 7 Number of *Culex tritaeniorhynchus* Collected from Tokha, Kathmandu

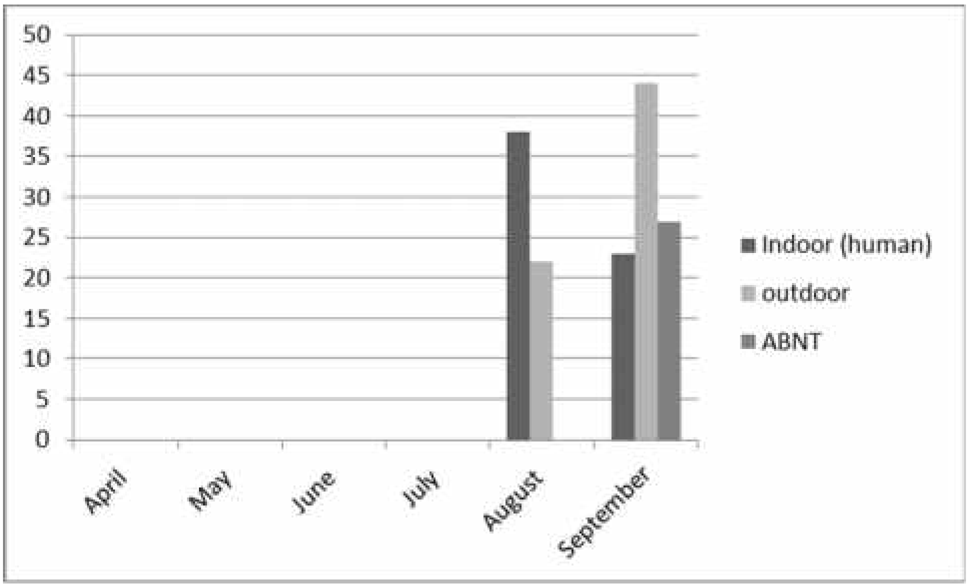
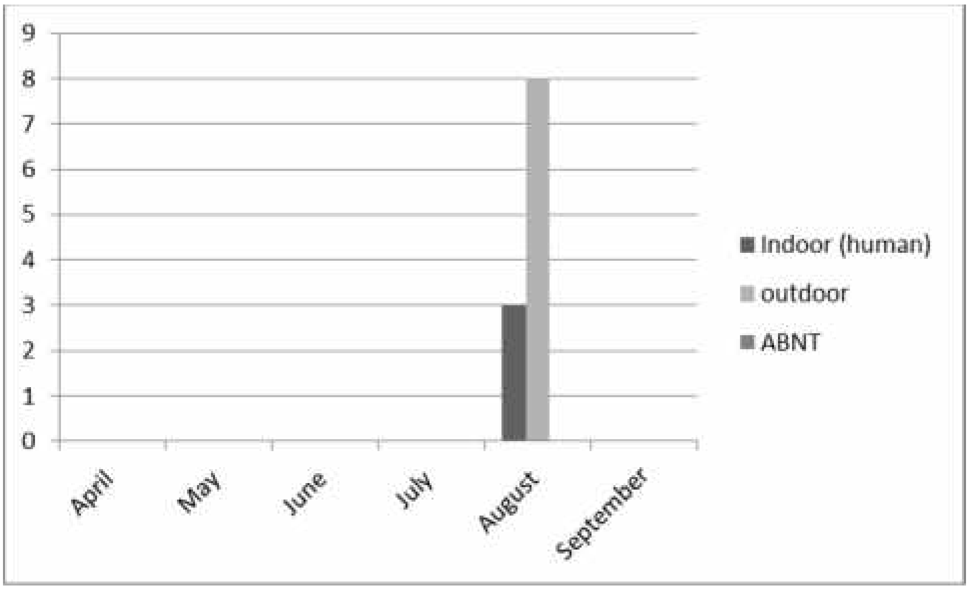


Figure. 8 Number of *Culex gelidus* collected from Tokha, Kathmandu



5.4 Vector Abundance

The vector abundance of the mosquitoes is calculated as,

$$\text{Vector abundance} = \frac{\text{Number of Mosq.of def.sps.}}{\text{Total collected Mosquitoes}} \times 100$$

(For Vector Abundance)

The vector abundance in each area is tabulated and figured in tables 11-14 and figures 9-12. In the present study *Cx tritaeniorhynchus* was found to be more abundant than *Cx gelidus*. As shown in table 11 and figure 9 the percentage abundance of *Cx tritaeniorhynchus* was 0.025% in July, 0.374% in August and 0.532 % in September, 2009, while it was 0 in July in case of *Cx gelidus* but 0.024% in August and 0.084% in September, 2009 in Haatiban area of Lalitpur district. The abundance of *Cx tritaeniorhychus* was maximum; 1.336% in september, 2009 in Tokha area of Kathmandu district. The abundance was found minimum in April, May and June, 2009 in all areas surveyed. The abundance of *Cx gelidus* was recorded maximum (0.151%) in August, 2009 from Tokha area of Kathmandu district. In comparison to other study areas Tokha area was found to be with higher abundance of vectors.

Table 11. Abundance of Vectors in Hattiban Area, Lalitpur

Month of Year	*Culex tritaeniorhynchus*		*Culex gelidus*	
	Number of Mosq.	% Abundance	Number of Mosq.	% Abundance
April	0	0	0	0
May	0	0	0	0
June	0	0	0	0
July	17	0.25	0	0
August	31	0.374	2	0.024
September	44	0.532	7	0.084

Total Number of Mosquitoes Collected from Hattiban. = 8,270

Table 12. Abundance of Vectors in Balkot Area, Bhaktapur

Month of Year	*Culex tritaeniorhynchus*		*Culex gelidus*	
	Number of Mosq.	% Abundance	Number of Mosquitoes	% Abundance
April	0	0	0	0
May	0	0	0	0
June	0	0	0	0
July	20	0.240	3	0.036
August	27	0.325	2	0.024
September	36	0.433	9	0.108

Total Number of Mosquitoes collected from Balkot= 8,306.

Table 13. Abundance of Vectors in Gothatar Area, Kathmandu

Month of Year	*Culex tritaeniorhynchus*		*Culex gelidus*	
	Number of Mosq.	% Abundance	Number of Mosquitoes	% Abundance
April	0	0	0	0
May	0	0	0	0
June	0	0	0	0
July	0	0	8	0.114
August	25	0.357	5	0.071
September	50	0.715	8	0.114

Total Number of Mosquitoes collected from Gothatar = 9661.

Table 14. Abundance of Vectors in Tokha Area, Kathmandu.

Month of Year	*Culex tritaeniorhynchus*		*Culex gelidus*	
	Number of Mosq.	% Abundance	Number of Mosquitoes	% Abundance
April	0	0	0	0
May	0	0	0	0
June	0	0	0	0
July	0	0	0	0
August	60	0.823	11	0.151
September	94	1.336	0	0

Total Number of Mosquito collected from Tokha = 7,035.

Graphical Representation of the Vector Abundance of JE

Figure. 9 Abundance of JE Vectors in Hattiban, Lalitpur

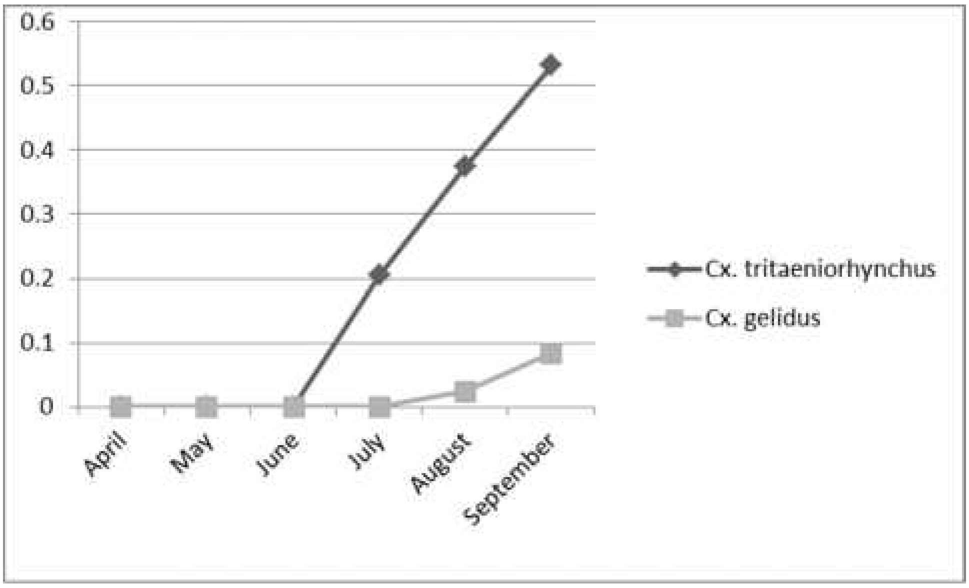


Figure. 10 Abundance of JE Vectors in Balkot, Bhaktapur

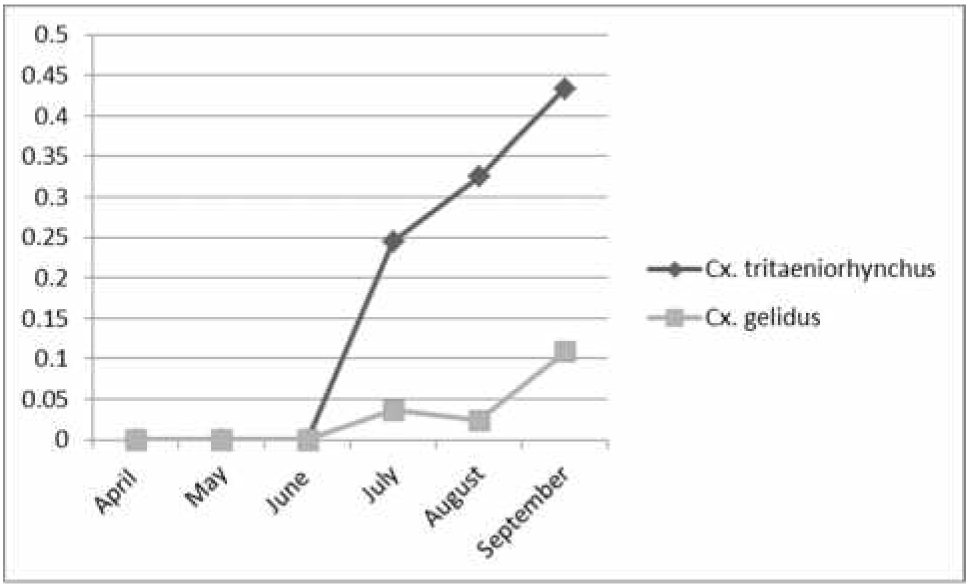


Figure. 11 Abundance of JE Vectors in Gothatar, Kathmandu.

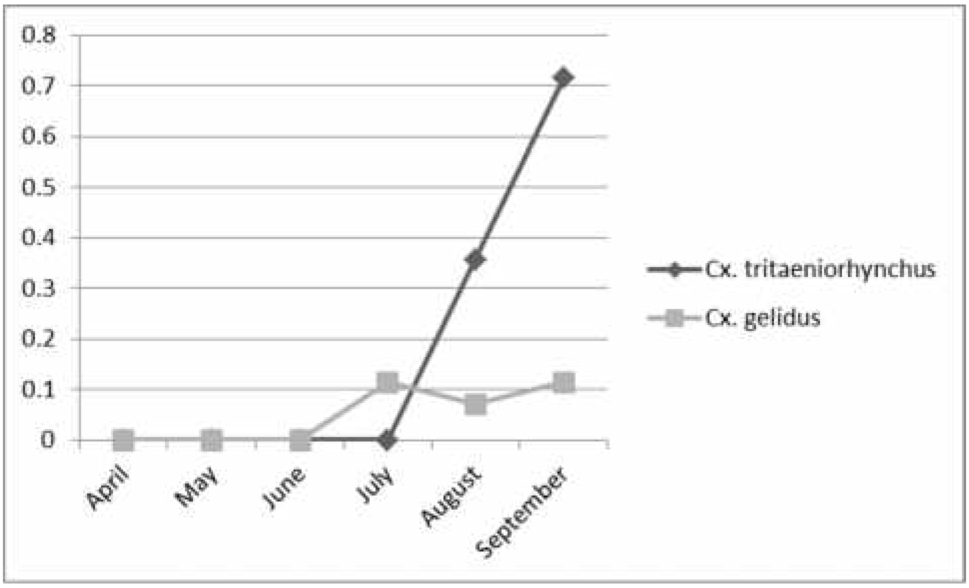
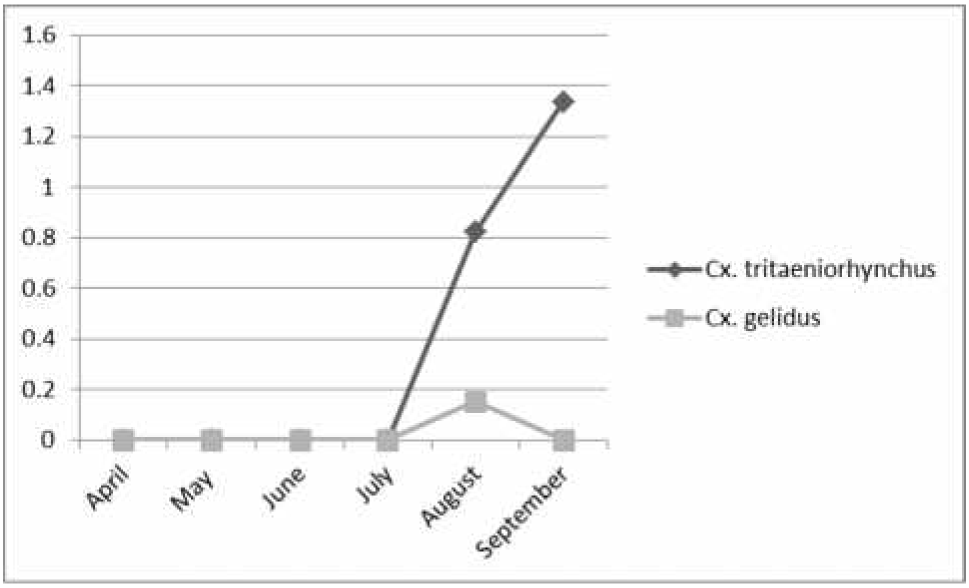


Figure. 12 Abundance of JE Vectors in Tokha, Kathmandu.



5.5 Resting Habit

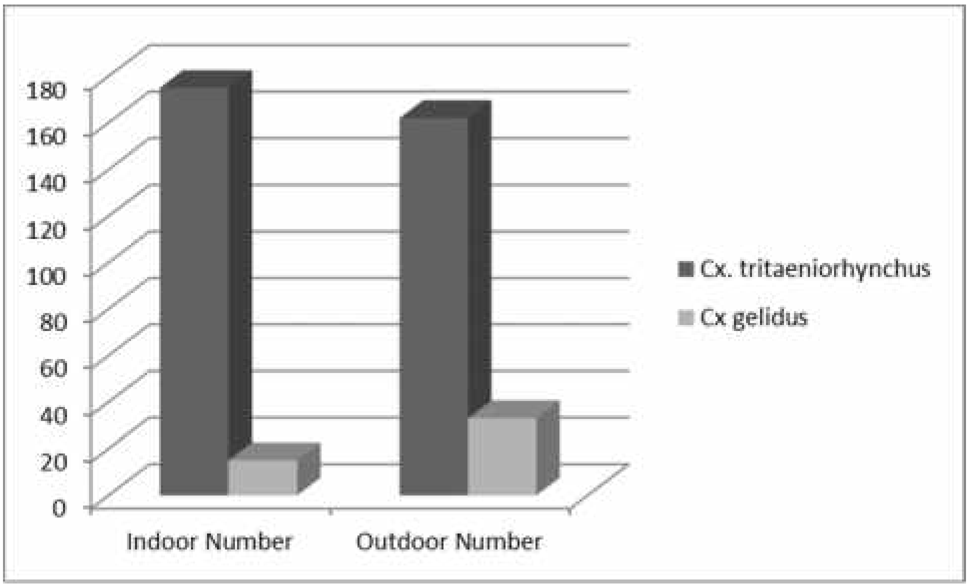
The resting habit of vectors is the ratio of number of mosquitoes of each species in indoor collection and number of mosquitoes of each species in outdoor collection.

Table 15. Resting Habit of JE vectors

Vector species	Indoor Number	Outdoor Number	Resting Habit
Culex tritaeniorhynchus	175	162	1.080
Culex gelidus	15	33	0.454

In the present study, *Cx tritaeniohynchus* was found to be predominant in indoor human dwellings near traditional cattle sheds and rice fields. *Cx tritaeniorhynchus* showed greater resting habitat at indoor. Similarly *Cx gelidus* showed greater resting habitat at outdoor.

Figure. 13 Number of JE vectors recorded at indoor and outdoor.



5.6 Abdominal condition of the vectors Collected during the study period

The abdominal condition of both vector species *Cx tritaeniorhynchus* and *Cx gelidus* were observed and recorded. In overall the gravid conditions of the vectors were most common, while unfed, half gravid and full fed conditions follow the order. In case of *Cx tritaeniorhynchus*, 23 gravid female were recorded from indoor collections of Hattiban area in September, 2009(table 16). The highest numbers of gravid females were recorded in the following trend. 38 gravid *Cx tritaeniorhynchus* were recorded from indoor collections of Tokha in September, 2009(table 19). Similarly 29 gravid females were recorded from Animal Baited Net Trap fitted at Tokha in September, 2009(table 27). In case of *Cx gelidus* three gravid females were recorded from indoor collection of Tokha area in August 2009(table 19). A total of 24 full fed females were recorded from outdoor collections of Balkot in July, 2009(table 21) and four Gravid females were recorded from outdoor collections of Tokha area in August, 2009(table 23). Similarly four full fed and 3 gravid females were recorded from Animal Baited Net Trap fitted at Tokha area on September, 2009(table 27).

5.6.1 Indoor Collection

Table 16. Abdominal Condition of vector mosquitoes collected from indoor (human) in Hattiban Area of Lalitpur district.

Month of Year	*Culex tritaeniorhynchus*					*Culex gelidus*				
	UF	FF	1/2 Gr.	Gr.	Total	UF	FF	1/2 Gr	Gr.	Total
April	0	0	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0	0	0
July	2	0	0	0	0	0	0	0	0	0
August	2	0	1	11	14	0	0	0	0	0
September	0	0	0	23	23	2	0	0	0	2

Out of total Samples of *Cx tritaeniorhynchus* Collected from indoor (human) in Hattiban, Lalitpur two unfed females were recorded in July, and two unfed females in August 2009. A single half gravid female was recorded in August, 2009. But 11 gravid females were recorded in August and 23 gravid females were recorded in September, 2009. In case of *Cx gelidus* two unfed females were recorded in September, 2009 while rest other months had a zero record.

Table 17. Abdominal Condition of vector mosquitoes collected from indoor (human) in Balkot Area of Bhaktapur district

Month of Year	*Culex tritaeniorhynchus*					*Culex gelidus*				
	UF	FF	1/2 Gr.	Gr.	Total	UF	FF	1/2 Gr	Gr.	Total
April	0	0	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0	0	0
July	0	0	0	0	0	1	2	0	0	3
August	3	0	3	15	21	0	0	0	0	0
September	2	0	0	10	12	2	0	0	0	2

The total Samples of Vector mosquitoes collected from Balkot area included three unfed female mosquitoes in August, two unfed in September, three half gravid in August, 15 gravid in August and 10 gravid in September,2009 among Culex tritaeniorhynchus. Similarly, in the samples of *Cx gelidus* one unfed in July, two unfed in September, and two full fed were collected in July, 2009.

Table 18. Abdominal Condition of vector mosquitoes collected from indoor (human) in Gothatar Area of Kathmandu district.

Month of Year	*Culex tritaeniorhynchus*					*Culex gelidus*				
	UF	FF	1/2 Gr.	Gr.	Total	UF	FF	1/2 Gr	Gr.	Total
April	0	0	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0	0	0
July	0	0	0	0	0	0	3	0	0	3
August	0	0	0	9	9	0	0	1	2	3
September	0	0	0	0	0	0	0	0	0	0

In Gothatar area one gravid female of *Cx tritaeniorhynchus* were collected from indoor (human), in August, 2009. Not a single Mosquito was recorded in other Months. Three full fed females of *Cx gelidus* were collected in July, 2009 Which was followed by two gravid and one half gravid female in August, 2009.

Table 19. Abdominal Condition of vector mosquitoes collected from indoor (human) in Tokha area of Kathmandu district.

Month of Year	*Culex tritaeniorhynchus*					*Culex gelidus*				
	UF	FF	1/2 Gr.	Gr.	Total	UF	FF	1/2 Gr	Gr.	Total
April	0	0	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0	0	0
July	0	0	0	0	0	0	0	0	0	0
August	2	0	4	32	38	0	0	0	3	3
September	0	0	0	38	38	0	0	0	0	0

In Tokha area 32 gravid, four half gravid and two unfed females of *Cx tritaeniorhynchus* were collected from indoor (human) in August 2009. This was followed Maximum number (38) of gravid *Cx tritaeniorhynchus* in September, 2009.

But three gravid females of *Cx gelidus* were collected in August, 2009. Not a single vector was recorded in rest of the months.

5.6.2 Outdoor collection

Table 20. Abdominal Condition of vector mosquitoes collected from outdoor in Hattiban Area of Lalitpur district.

Month of Year	*Culex tritaeniorhynchus*					*Culex gelidus*				
	UF	FF	1/2 Gr.	Gr.	Total	UF	FF	1/2 Gr	Gr.	Total
April	0	0	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0	0	0
July	2	0	0	0	9	0	0	0	0	0
August	5	0	1	3	9	0	0	0	0	0
September	5	0	0	13	18	3	0	0	2	5

In Hattiban area five unfed, one half gravid and three gravid females of *Cx tritaeniorhynchus* were collected from outdoor in August, 2009. This was followed by five unfed and 13 gravid *Cx tritaeniorhynchus* in September, 2009. But three unfed and two gravid females of *Cx gelidus* were collected in September, 2009. Rest of the months had no Vector records.

Table 21. Abdominal Condition of vector mosquitoes collected from outdoor in Balkot Area of Bhaktapur district.

Month of Year	*Culex tritaeniorhynchus*					*Culex gelidus*				
	UF	FF	1/2 Gr.	Gr.	Total	UF	FF	1/2 Gr	Gr.	Total
April	0	0	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0	0	0
July	7	0	0	3	10	0	24	0	0	24
August	6	0	0	0	6	2	0	0	0	2
September	7	0	0	0	7	0	0	0	0	0

In Balkot area seven unfed and three gravid females of *Cx tritaeniorhynchus* were collected from outdoor in July, 2009. This was followed by six unfed females in August, 2009 and seven unfed females in September, 2009. In case of *Cx gelidus* 24 fullfed female were collected in July, 2009. In addition two unfed females were collected in August, 2009.

Table 22. Abdominal Condition of vector mosquitoes collected from outdoor in Gothatar Area of Kathmandu district.

Month of Year	*Culex tritaeniorhynchus*					*Culex gelidus*				
	UF	FF	1/2 Gr.	Gr.	Total	UF	FF	1/2 Gr	Gr.	Total
April	0	0	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0	0	0
July	0	0	0	0	0	3	0	0	2	5
August	7	0	0	5	12	2	0	0	0	2
September	11	0	0	14	25	2	0	0	4	6

In Gothatar area seven unfed and five gravid females of *Cx triaeniorhynchus* were collected from outdoor in August, 2009. This was followed by 11 unfed and 14 gravid females in September, 2009. But three unfed and two gravid females of *Cx gelidus* were collected in July, 2009 which was followed by two unfed females in August and two unfed and four gravid females in September, 2009.

Table 23. Abdominal Condition of vector mosquitoes collected from outdoor in Tokha Area of Kathmandu district.

Month of Year	*Culex tritaeniorhynchus*					*Culex gelidus*				
	UF	FF	1/2 Gr.	Gr.	Total	UF	FF	1/2 Gr	Gr.	Total
April	0	0	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0	0	0
July	0	0	0	0	0	0	0	0	0	0
August	6	0	0	16	22	4	0	0	4	8
September	14	0	0	8	22	0	0	0	0	0

In Tokha area six unfed and 16 gravid females of *Cx tritaeniorhynchus* were collected from outdoor in August, 2009. This was followed by 14 unfed and eight gravid females in September, 2009. But four unfed and four gravid females of *Cx gelidus* were collected in August, 2009. There was no vector in rest of the months.

5.6.3 Animal Baited Net Trap collection

Table 24. Abdominal Condition of vector mosquitoes collected using Animal Baited Net Trap in Haatiban Area of Lalitpur district.

Month of Year	*Culex tritaeniorhynchus*					*Culex gelidus*				
	UF	FF	1/2 Gr.	Gr.	Total	UF	FF	1/2 Gr	Gr.	Total
April	0	0	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0	0	0
July	0	7	1	0	8	0	0	0	0	0
August	2	6	1	0	9	0	0	2	0	2
September	3	0	0	0	3	0	0	0	0	0

In Hattiban area seven full fed and one half gravid females of *Cx tritaeniorhynchus* were collected from Animal Baited Net Trap in July, 2009. This was followed by two unfed, six full fed and half gravid in August and three unfed females in September. But two half gravid females of *Cx gelidus* were collected in August, 2009. There were no vector records in rest of the months.

Table 25. Abdominal Condition of vector mosquitoes collected using Animal Baited Net Trap in Balkot Area of Bhaktapur district.

Month of Year	*Culex tritaeniorhynchus*					*Culex gelidus*				
	UF	FF	1/2 Gr.	Gr.	Total	UF	FF	1/2 Gr	Gr.	Total
April	0	0	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0	0	0
July	2	0	0	0	2	3	2	0	0	5
August	0	0	0	0	0	0	0	0	0	0
September	7	2	0	0	9	2	0	0	0	2

In Balkot area two unfed females and of *Cx tritaeniorhynchus* were collected from Animal Baited Net Trap in July, 2009. This was followed by seven unfed, two full fed females in September. Similarly, three unfed and two females of *Cx gelidus* were collected in July, 2009 which was followed by two unfed females in September, 2009.

Table 26. Abdominal Condition of vector mosquitoes collected using Animal Baited Net Trap in Gothatar area of Kathmandu district.

Month of Year	*Culex tritaeniorhynchus*					*Culex gelidus*				
	UF	FF	1/2 Gr.	Gr.	Total	UF	FF	1/2 Gr	Gr.	Total
April	0	0	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0	0	0
July	0	0	0	0	0	0	0	0	0	0
August	4	0	0	0	4	0	0	0	0	2
September	4	2	0	0	6	0	0	0	0	0

In Gothatar area four unfed females of *Cx tritaeniorhynchus* were collected from Animal Baited Net Trap in August, 2009. This was followed by four unfed, two full fed females in September. But not a single *Cx gelidus* was recorded throughout the six months.

Table 27. Abdominal Condition of vector mosquitoes collected using Animal Baited Net Trap in Tokha Area of Kathmandu district.

Month of Year	*Culex tritaeniorhynchus*					*Culex gelidus*				
	UF	FF	1/2 Gr.	Gr.	Total	UF	FF	1/2 Gr	Gr.	Total
April	0	0	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0	0	0
July	0	0	0	0	0	0	0	0	0	0
August	0	0	0	0	0	0	0	0	0	0
September	0	6	12	9	27	0	4	0	3	7

In Tokha six fullfed 12 half gravid and nine gravid females of *Culex tritaeniorhynchus* were collected from Animal Baited Net Trap in September, 2009. But four fullfed and three gravid females of *Culex gelidus* were collected in September, 2009. There were no vector records from Animal Baited not Trap in rest of months.

CHAPTER - V

DISCUSSIONS

The present study was conducted from April, 2009 to September, 2009 in the Kathmandu, Lalitpur and Bhaktapur districts. This study has reported the distribution and abundance of *Cx tritaeniorhynchus* and *Cx gelidus;* the vector of JE. Besides the monthly variation in man hour density and vector abundance were also evaluated. A total of 30,602 culex mosquitoes were collected during the study period. Out of the total collection 459 vector species including 404 *Cx tritaeniorhynchus* and 55 *Cx gelidus* were recorded. Not a single *Cx tritaeniorhynchus* and *Cx gelidus* was recorded in the earlier three months; April, May, June, Although other Culex Mosquitos like *Cx quinquifasciatus* was recorded in these months. The highest man hour density for *Cx tritaeniorhynchus* (9.5) was recorded from the area with traditional cattle sheds in August, 2009 from indoor human dwellings of Tokha area. The area with artificial cattle sheds recorded the least man hour density (2.0) in Balkot and Hattiban area for *Cx tritaeniorhynchus* in July, 2009 from indoor human dwellings. The result was similar for outdoor collection also. The lesser Number of vector species were recorded in the Animal Baited Net Trap fitted in the respective sample sites. The abundance of *Cx tritaeniorhynchus* (max 1.336%) was greater than the *Cx gelidus (max* 0.151%). The abundance was zero in April-June months (hot and dry season) and it was higher in July-September months (wet and rainy season).

Cx tritaeniorhynchus was found predominant in outdoors in Warangal and Karim Nagar district of Andhra Pradesh, India (Das *et al.,* 2004). In contrary present study the predominance of *Cx tritaeniorhynnchus* was recorded at indoor habitats including human dewellings near traditional cattle sheds.

Mosquito's population was found at peak during March-April and September with very high incidence in some of taluks with less or no irrigation system (Geevaryhese *et al.,* 1994). In Kathmandu valley the mosquito population was found at peak during September and was least in April as revealed from the present study.

The vector abundance was found to be high in monsoon (May-October), moderate in transition (March-April and November-December) and low in dry

(January- February) seasons in Bangkok (Gingrich *et al.*, 1992). In the present study the vector abundance was high in late monsoon(August and September) while not a single vector was recorded during the summer(April, May, June) Similarly, the vector abundance was found to be lowest in the hot and dry season (April-June) and highest in cool and wet season (October-December) in south Arwt district in Tamil Nadu, India (Gajamama *et al.*,1997).

Cx tritaeniohynchus, the primary vector of Japanese Encephalitis together with other vector species remained more active during the period of paddy cultivation in Gorakhpur district (Kanojia *et al.,* 2003). The present study has also recorded the higher value of vector abundance during the paddy cultivation period (July-September). So the vector's activeness was observed in this time.

A number of mosquitoes (n = 20,996) of *Cx tritaeniorhynchus* were found resting indoor than in outdoor vegetations (n=383) in Bellary district, India, despite the availability of outdoor resting sites. This is due to indoor residual insecticide which provided an effective control method in this area (Kanojia *et al.,* 2007). In present study, 175 *Cx tritaeniorhyhcus* found resting indoors and 142 resting outdoors were found out of total vectors collected in indoor and outdoor.

Self *et al*., (1973) studied the seasonal abundance of *Cx tritaeniorhynchus* from Sintaein, Pusan and Seoul areas of Republic of Korea. The overall adult densities were found lowest at rice growing site where agricultural pesticides were extensively used. A short period of man-vector contact occurred at each study site at low densities when the natural population was at its peak. In this study, the adult densities of *Cx tritaeniorhynchus* and *Cx gelidus* were highest in Tokha and Gothatar areas. Both Tokha and Gothatar areas consist of higher number of cattle sheds and some number of open farmlands. At Tokha there were no piggery farms and the paddy cultivation area was relatively few. The densities of both *Cx tritaeniorhynchus* and *Cx gelidus* were relatively low in the Balkot and Hattiban area. These areas represent the areas with large proportion of rice cultivated field.

The comparable number of vectors was recorded from both indoor and outdoor habitats. Some indoor habitats were found closely connected with cattle sheds. The maximum number of *Cx tritaeniorhynchus* (n = 38) was recorded from

Tokha in August 2009 from the overall indoor habitats. This area includes the mixed dewellings of human and cattle. The maximum number of *Cx tritaeniorhynchus* (n=44) was recorded from outdoor habitats of Tokha in September 2009. The number of vector was relatively less on outdoor habitats. Similarly the number of vector were recorded relatively few in case of Animal Baited Net Trap collections. The maximum number of 27 *Cx tritaeniorhynchus* was recorded in September 2009 from the Animal Baited Net Trap collections of Tokha area. Similarly the maximum number of *Cx gelidus* (n = 3) was recorded from Gothatar (July and August) and Tokha in August, 2009 from the overall indoor habitats. The maximum number of *Cx gelidus* (n=8) was recorded from outdoor habitats of Tokha in August, 2009. The maximum number of *Cx gelidus* was recorded in July, 2009 from the Animal baited Net trap collections of Balkot area.

Neupane *et al*., (2009) reported 12 Species including *Culex quinquefasciqtus*, *Cx Fuscocaphala, Cx tritaeniorhynchus, Cx gelidus, Cx bitaeniorhynchus, Cx Whiteimorie and Cx sinensis* in 10 villages of Chitwan district. *Cx quinquefasciatus* was most abundant both in premonsoon (18.7%) and post Monsoon (26.90%).

In present study the abundance of *Cx tritaeniorhynchus* was higher than *Cx gelidus* in all study sites. There were no records of both vectors from April, May and June. In the following three months the maximum density of *Cx tritaeniorhynchus* than *Cx gelidus* was recorded. The vector abundance was maximum (1.336%) in September, 2009 as recorded from Tokha area, similarly the maximum abundance (0.151%) of *Cx gelidus* was recorded in August, 2009 from the same area. Among the total collected samples the numberof gravid females were highest. The maximum number (38) gravid females of *Cx tritaeniorhynchus* were recorded from indoor collection of Tokha area in August 2009. This number was followed by unfed, half fed and full fed conditions respectively of both Japanese Encephalitis vectors. From the statistical analysis it was found that both *Cx tritaeniorhynchus* and *Cx gelidus* were equally distributed throughout the Kathmandu valley. But *Cx tritaeniorhynchus* showed variable distribution among different Six months in indoor (human) and outdoor. No significant variation was found in distribution of *Cx gelidus* both at indoor (human) and outdoor in six different months. *Cx tritaeniorhynchus* was more abundant in August and September months.

CHAPTER VI

CONCLUSION AND RECOMMENDATIONS

Conclusion

This study reports observation of fluctuation in densities, resting preferences and age grading of *Cx tritaeniorhynchus* and *Cx gelidus* which would be useful for possible inclusion of the Kathmandu valley in the National Japanese Encephalitis Prevention and Control Programme. The highest density of Japanese Encephalitis vectors were recorded from Tokha and Gothatar area of Kathmandu district, although the hypothesis testing results showed no significance difference between all four sites. The resting habit of vectors was higher at indoor habitats in all prevalent months. The abundance of vectors was higher in August and September as revealed by the results of hypothesis testing. The abundance of *Cx tritaeniorhynchus* was higher than that of *Cx gelidus.* On the whole the abundance of *Cx tritaeniorhynchus* was maximum in September, 2009 as recorded from outdoor collections at Tokha area.

The present study concludes that both *Cx tritaeniorhynchus* and *Cx gelidus* are equally abundant on indoor and outdoor habitats. The distribution of both *Culex tritaeniorhynchus* and *Cx gelidus* was rare in April, May and June. The vector *Cx tritaeniorhynchus* rised to its peak on September in all study sites. This can prove the higher possibility of JE transmission in September after a sudden outbreak in any area. So, in order to control the future outbreak and rapid transmission of the disease vector control measures should be applied in both indoor and outdoor areas. Further outdoor breeding habitats and indoor resting habitats must be focussed for effective control.

Recommendations

1. Avoidance of mosquito bites by the use of insecticide impregnated mosquito nets and use of long sleeved clothes while of work in farm area or cattle sheds is strongly suggested.
2. Improvement of traditional cattle sheds removal of cracked or old houses and construction of well ventilated rooms reduces the probable mosquito habitats.
3. Construction of cattle sheds and piggery farms away from the settlement area and clearing off of thick bushes around houses makes the mosquito contact low.
4. Knowledge to the general public regarding the Japanese Encephalitis disease causative agent, mode of transmission, vector habitats and efficient control method should be given.
5. Time to time blood sample collection should be initiated to predict the pre-epidemic condition and prevention of Japanese Encephalitis disease.
6. Better maintenance of the amplifying hosts like pigs, managing a proper drainage from their farms or sheds, proper sanitation and personal hygiene of the workers involved in piggery farm is strongly recommended.

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ANNEX

Hypothesis testing

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

1. Between four study sites

i. Null Hypothesis: H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4$

i.e. there is no significant different in the distribution of mosquitoes between four study sites.

Alternative Hypothesis

$H_1 : \mu_1 \neq \mu_2 \neq \mu_3$ i.e. there is significant difference in the distribution of mosquitoes between four study sites.

2. Between Six months

ii. Null Hypothesis: H_0: $\mu_0 = \mu_1 = \mu_2 = \mu_3 = \mu_4$

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

Alternative Hypothesis

$H_1 : \mu_1 \neq \mu_2 \neq \mu_3$ i.e. there is significant difference in the distribution of mosquitoes between six months.

Analysis of *Culex tritaeniorhynchus*:

Table a. Number of *Culex tritaeniorhynchus* in indoor

	Hattiban	Balkot	Gothatar	Tokha	Total
April	0	0	0	0	0
May	0	0	0	0	0
June	0	0	0	0	0
July	8	8	0	0	16
August	14	21	9	38	82
September	23	12	19	23	77
Total	45	41	28	61	175

Two way ANOVA Table

Source of Variation	Sum of Square	d.f.	Mean sum	F-ratio
Between sites (SSC)	92.459	4-1= 3	30.82	$F_1 = \frac{30.82}{120.64} = 0.25$
Between months (SSR)	1950.959	6-1=5	390.19	$F_2 = \frac{390.19}{120.64} = 3.23$
Residuals	1809.582	15	120.64	

Table b. Number of *Culex tritaeniorhynchus* in outdoor

Month	Hattiban	Balkot	Gothatar	Tokha	Total
April	0	0	0	0	0
May	0	0	0	0	0
June	0	0	0	0	0
July	2	10	0	0	12
August	8	6	12	22	48
September	18	15	25	44	102
Total	28	31	37	66	162

Two way ANOVA Table

Source of Variation	Sum of Square	d.f.	Mean sum	F-ratio
Between sites (SSC)	151.5	4-1 = 3	50.5	$F_1 = \frac{50.5}{111.4} = 0.453$
Between months (SSR)	2119.5	6-1=5	423.9	$F_2 = \frac{423.9}{111.4} = 3.805$
Residuals	1671	15	111.4	

Table c. Number of *Culex tritaeniorhynchus* in Animal Baited Net Trap

Month	Hattiban	Balkot	Gothatar	Tukha	Total
April	0	0	0	0	0
May	0	0	0	0	0
June	0	0	0	0	0
July	7	2	0	0	9
August	9	0	4	0	13
September	3	9	6	27	45
Total	19	11	10	27	67

Two Way ANOVA Table

Source of Variation	Sum of Square	d.f.	Mean sum	F-ratio
Between sites (SSC)	31.46	4-1=3	10.486	$F_1 = \frac{10.486}{52.094} = 0.201$
Between months (SSR)	192.12	6-1=5	38.424	$F_2 = \frac{38.424}{52.094} = 0.737$
Residuals	781.42	15	52.094	

Analysis of *Culex gelidus:*

Table d. Number of *Culex gelidus* in Indoor

Month	Hattiban	Balkot	Gothatar	Tukha	Total
April	0	0	0	0	0
May	0	0	0	0	0
June	0	0	0	0	0
July	0	0	0	0	3
August	0	0	3	3	6
September	2	2	2	0	6
Total	2	2	8	3	15

Two Way ANOVA Table

Source of Variation	Sum of Square	d.f.	Mean sum	F-ratio
Between sites (SSC)	4.125	4-1=3	1.375	$F_1=\frac{1.375}{1.6}=0.859$
Between months (SSR)	10.875	6-1=5	2.175	$F_2=\frac{2.175}{1.6}=1.359$
Residuals	24	15	1.6	

Table e. Number of *Culex gelidus* in Outdoor

Month	Hattiban	Balkot	Gothatar	Tokha	Total
April	0	0	0	0	0
May	0	0	0	0	0
June	0	0	0	0	0
July	0	0	5	0	5
August	0	0	2	8	12
September	5	5	6	0	16
Total	5	7	13	8	33

Two Way ANOVA Table

Source of Variation	Sum of Square	d.f.	Mean sum	F-ratio
Between sites (SSC)	5.79	4-1=3	1.93	$F_1=\frac{1.93}{7.75}=0.249$
Between months (SSR)	60.88	6-1=5	12.176	$F_2=\frac{12.176}{7.754}=1.57$
Residuals	116.33	15	7.75	

Table f. Number of *Culex gelidus* obtained from ABNT

	Hattiban	Balkot	Gothatar	Tokha	Total
April	0	0	0	0	0
May	0	0	0	0	0
June	0	0	0	0	0
July	0	3	0	0	3
August	2	0	0	0	2
September	0	2	0	0	2
Total	2	5	0	0	7

Two Way ANOVA Table

Source of Variation	Sum of Square	d.f.	Mean sum	F-ratio
Between sites (SSC)	2.789	4-1=3	0.929	$F_1=\frac{0.929}{0.800}=1.161$
Between months (SSR)	2.209	6-1=5	0.442	$F_2=\frac{0.442}{0.800}=0.5525$
Residuals	12.002	15	0.800	

Table g. Calculated Value of F1 and F2

Culex tritaeniorhynchus		
Habitat	F-ratio	
	Between Sites(F1)	Between Months(F2)
Indoor	0.25	3.23
Outdoor	0.453	3.805
ABNT	0.201	0.737
Culex gelidus		
Indoor	0.859	1.359
Outdoor	0.249	1.57
ABNT	1.161	0.5525

Tabulated Value of F1 at 5% level of significance for d.f 3 and 15=3.29

Tabulated value of F2 at 5% level of significance for 5 and 15 d.f. = 2.90

Decision

Variation/Distribution between Study Sites

The calculated value of F1 is smaller than tabulated value for Indoor, Outdoor and ABNT collections of both *Cx tritaeniorhynchus* and *Cx gelidus*. Hence, there is no significant difference in the distribution of *Cx tritaeniorhynchus* and *Cx gelidus* between study sites.

Variation/Distribution between Six Months

The calculated value of F2 is greater than the tabulated value for Indoor and Outdoor collections of *Cx tritaeniorhynchus*. Hence there is significant difference in the monthly distribution of *Cx tritaeniorhynchus* in indoor and outdoor habitats. But the calculated value is less in case of Animal Baited Net Trap of *Cx tritaeniorhynchus* and Indoor, Outdoor and Animal Baited Net Trap of *Cx gelidus*. Hence there is no significant difference in distribution of *Cx gelidus* in Indoor and Outdoor habitats.

Daily field collection sheet
Adult collection

Routine/general ……………………
Region …………………District …………… VDC   Village
Collection starting time   …………………… Collection finishing time
Date:

S.N.	Type of station	Insecticide	Station		Station +ve for mosquito			Time in minutes	Total mosquito collected	Remarks
			Indoor	Outdoor	Mosqito	*Cx.tritaeniorhunchus*	*Cx. gelidus*			

Type of station:
Human Hu
Mixed Mx
Animals: An

Daily field collection sheet

Animal baited net trap collection

Routine/general …………………… Date:

Region ……………….District …………VDC ……………Village

Collection starting time ……………………Collection finishing time

S.N.	Type of station	Time of collection		Station of baits +ve for mosquito		Time	Total mosquito collected	Remarks
				Cx. tritaeniorhunchus	*Cx. gelidus*			

Daily field collection sheet
Daily laboratory performance

Routine/general ……………………………………………… **Date:**
Region …………………District ………………… VDC …………illage ………………………..
Collection starting time ………………………………Collection finishing time ……………………

S.N.	Name			Type of Survey	Collection type	species	Abdominal condition			
	VDC	Ward No.	Village				Unfed	Full fed	Semi-gravid	Gravid