Chapter 1

Introduction & Review of literature
Most of the Investigations on transmissible diseases were done during 19th and early 20th centuries. Koch’s postulates proved that various microorganisms were the causes of diseases. Now it is clear that what kind of agents decimated both human and animal health, and effective control techniques are instituted. Quarantine is practiced to prevent the spread of diseases that are transmitted from one person to another. Vaccinations have been developed for typhoid fever, diphtheria and small pox that reduced both morbidity and mortality. Antibiotics and other chemotherapeutic agents are improved to suppress many serious diseases. Despite this success, failures lurked in the background. Many vector borne diseases, which had complex epidemiologies and reservoirs in various animals other than humans are flared up unpredictably. The vector borne agents include viruses, rickettsia, bacteria, protists and helminthes. Except for the blood flukes (*Schistosoma* spp.), most of the disease agents were found to be transmitted by arthropods: mosquitoes, black flies, midges, sand flies, ticks, lice, bugs and mites.

Out of all the blood-feeding groups of insects, the mosquitoes are the most important group (Order: Diptera, Family: Culicidae) responsible for transmission of diseases and causing of human mortality and morbidity. Mosquitoes act as vectors of pathogens and parasites causing Yellow fever, Malaria, Japanese encephalitis, Dengue, Filariosis, Chikungunya and several other diseases to
mankind. In India, among all mosquito borne diseases, Japanese encephalitis is known to be highly epidemic in many states.

1.1 Japanese encephalitis

Japanese encephalitis (JE) is an arboviral disease, spread to humans by infected mosquitoes. JE disease can affect central nervous system (CNS) and cause severe complications even leading to death. JE basically is a rural disease as the major vectors of JE breed in rice fields. It is estimated that around 1.9 billion people live in rural JE-prone areas of the world (Arunachalam et al., 2009). Japanese encephalitis virus (JEV) is one of the most important arboviral childhood viral encephalitis in Asia, causing at least 50,000 clinical cases and 10,000 deaths every year (WHO 2005). JE virus belongs to the family Flaviviridae and genus Flavivirus.

1.1.1 Outbreaks of JE disease

JE outbreaks are attributed to Japanese B encephalitis virus (JBEV) first reported in Japan in 1870s. The term type B encephalitis is used to distinguish the summer epidemics from von Economo’s encephalitis lethargica (sleeping sickness) which is known as type A encephalitis. Only after 25 years of its recognition, the mode of transmission of JE virus by mosquito vectors was elucidated. In 14 Asian countries, JE outbreaks take place frequently with about 3060 million people at risk of infection (Sabesan, 2003). JE epidemic takes place in entire South East Asia regularly every year (Umenai et al., 1985; Tiroumourougane et al., 2002; Solomon and Vaughn, 2002) and
also extended its geographical range to previously untouched areas of Asia (China, Indonesia, India, Cambodia, Japan, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Republic of Korea, Sri Lanka, Thailand, Vietnam and the South Eastern Russian federation). Apart from South-East Asia, JE cases were also reported in other non-Asian countries like, Torres Strait of Australia mainland (Hanna et al., 1999). Nearly 3 billion people (about 60% of the world’s population) live in JE endemic regions (Halstead and Tsai, 2004).

In India, Japanese Encephalitis was clinically diagnosed for the first time in 1955 at Vellore in North Arcot District Tamil Nadu (Webb and Pereira, 1956). Many JE outbreaks of varying intensity were reported from different parts of India (Rodrigues, 1984). In 1973, the first major JE epidemic from India was reported in the Bankura and Burdwan districts of West Bengal where more than 700 cases and 300 deaths occurred. Subsequently, the second outbreak occurred in 1976 where number of death cases was reported. Since then, JE outbreaks have been reported from these regions, generally during the post monsoon season of a year. Major outbreaks of JE were reported in rural areas of India (Reuben and Gajanana, 1997). A study showed that nearly 36.36% of cases were reported from urban areas whereas, 63.63% from rural areas of Lucknow (Roy et al., 2006). The spread of JE infection is found in many states of India and it spreads mostly during monsoon or post monsoon period.
The Andhra Pradesh (AP) state has suffered from a series of epidemics of JE from the late seventies onwards and JE appears to have become endemic in many districts. JE is one of the most important public health problems in AP and the death toll among children due to JE is reported to be increasing year after year (Murty et al., 2000). A series of severe epidemics were reported during 1981, 1986, 1993 and 1999. In 1981, 439 deaths were recorded out of 1273 cases. In 1986, 1993 and 1999 i.e. after an interval of every 5 to 7 years JE cases were noticed to have increased considerably. 2,048 cases with 640 deaths were reported in 1986. Similarly in 1993 and 1999 more than 1000 JE cases were reported in AP. During 1999, the worst affected districts in Andhra Pradesh were Kurnool followed by Prakasam, Ananthapur and Cuddapah. The incidence of JE was noticed almost every year in AP in the districts of Kurnool, Ananthapur, Prakasam, Warangal (Murty et al., 2000). In the year 2000, 343 cases with 72 deaths and in 2003, 329 cases with 183 deaths were reported.

Entomological assessment indicates that Cx. tritaeniorhynchus is the primary vector in Kurnool district, AP based on relative abundance, and more number of virus isolations (Arunachalam et al., 2009). Minimum infection rate (MIR) and maximum likelihood estimates of JE Virus infections of Cx. gelidus are lower when compared with Cx. tritaeniorhynchus, which indicates that the primary role played by Cx. tritaeniorhynchus (Arunachalam et al., 2009). Cx.
gelidus is highly zoophagic and poorly anthropagic; therefore, it may have an important role in amplifying JEV transmission (Reuben et al., 1992; Geevarghese et al., 2003).

1.1.2 Life cycle of JE

Life cycle of Japanese encephalitis is very complex that involves pigs as amplifying hosts, ardeid birds as reservoirs and mosquitoes as vectors (Fig. 1.1). JE virus is maintained by transovarial transmission (vertical transmission) in vector mosquitoes (Arunachalam et al., 2002; Thenmozhi et al., 2001; Soman et al., 1986). JE virus has been isolated from 19 mosquito species in different parts of India and the most important vectors are Culex vishnui Theobald and Culex tritaeniorhynchus Giles from which the largest number of isolations have been made (Geevarghese et al., 2003). These mosquitoes are mainly pig and cattle blood feeders and humans are the dead end host (Self et al., 1973).

People get infected inadvertently when they encroach on this cycle, but they are considered as “dead-end” hosts because normally they do not have adequately high or prolonged viraemia to transmit the virus further (Solomon, 2004). Cattle were also considered as dead end hosts as they do not develop enough viraemia to infect mosquitoes (Carey et al., 1969). After infected mosquito bites the host, the virus thought to amplify peripherally, causing a transient viraemia before invading the CNS in the host. Studies with a hamster model of St. Louis encephalitis virus, the olfactory route has shown to play an
important route for the virus transmission to the CNS in the host (Monath et al., 1983).

Fig. 1.1: Life cycle of Japanese encephalitis.

Immunohistochemical staining of human postmortem material had shown diffuse infection throughout the brain, indicating a haematogenous route of entry (Desai et al., 1995; Johnson et al., 1985). It has suggested that head trauma during the transient viraemia could facilitate viral entry into the CNS (Shiraki, 1970). Electron microscopic studies of the brains of infected mice have shown that the virus replicates in the rough endoplasmic reticulum and golgi apparatus. Hypertrophy of the endoplasmic reticulum and degeneration into cystic structures caused extensive dysfunction (Hase et al., 1990).
1.1.3 Prevention of Japanese encephalitis

Japanese encephalitis is a vaccine-preventable disease and vaccination against JE should be routinely practiced in all areas of Asia where the virus is responsible for human disease (Monath, 2002). An essential component of a surveillance system should be formed by the monitoring of JEV infection in vector mosquitoes (Arunachalm et al., 2008). For surveillance of Japanese encephalitis virus (JEV) activity, existing infrastructure with the health department can be effectively used in endemic areas (Tewari et al., 2008).

Vector control is a challenge for JE control because of exophagic and exophilic behaviour of JE vectors, which limits the effectiveness of conventional vector control methods like Indoor Residual Spray (IRS). Therefore IRS is not recommended for prevention of JE. Still, in areas where vector is endophilic like *Mansonia annulifera*, IRS should be considered for vector control in high-risk pockets. Commercial mosquito repellants like cream, mats and coils are widely practiced to repel mosquitoes. Pyrethroid impregnated bed nets and curtains have shown to reduce man-mosquito contact. Two feasible methodologies, water management system with intermittent irrigation system and incorporation of neem products in the soil, have been demonstrated to control breeding of mosquitoes in rice fields. Using neem products as fertilizer in rice fields will not only enhance the grain production but also suppress the breeding of Culicine vector of JE (ICMR bulletin, 1992).
In JE endemic areas, pigs are associated with human habitations. Pig rearing is very common among the weaker section of the communities in Andhra Pradesh (AP) and there were 570 million pigs available as per the 2003 animal census, which is quite high compared with other states of India (Arunachalam et al., 2009). Pig immunization or slaughtering should be practiced as a controlling method. JE vectors prefer to feed on cattle than on the pig (Mwandawiro et al., 1999; Arunachalam et al., 2005). Cattle do not develop enough viraemia to infect mosquitoes (Ilkal et al., 1988). Hence, increasing the availability of cattle would help to reduce the spread of JE by diverting the mosquito vectors from pigs to cows as the initial tendency of the vector species is to feed on the cattle than on pigs.

1.1.4 Vectors of Japanese encephalitis

The Cx. vishnui subgroups of mosquitoes are the major vectors and play an important role for JE epidemiological outbreaks in India (Mishra, 1984). The other species of Cx. vishnui sub-group include Cx. tritaeniorhynchus Giles, Cx. vishnui Theobald and Cx. pseudovishnui Colless. These species are extremely common, widespread and breed mainly in paddy fields, sunlit pools and roadside ditches (Mogi, 1984; Sucharit et al., 1989). The important JE vectors in Asian and South-East Asian countries, Cx. tritaeniorhynchus, Cx. gelidus and Cx. vishnui, have shown to feed mainly on cows in some places and pigs in other places depending on host availability (Reuban et al., 1992).
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Cx. tritaeniorhynchus and Cx. gelidus are major vectors in Kurnool District, Andhra Pradesh, South India. Cx. tritaeniorhynchus breed mostly in fresh waters and the densities that are closely associated with rain fall and agricultural practices. Cx. tritaeniorhynchus population is more abundant during the monsoon (July - October) and immediate post-monsoon seasons due to the availability of paddy fields (only rural) and rainwater pools (Arunachalam et al., 2009). Large polluted water bodies with aquatic weeds are the preferred breeding habitats for Cx. gelidus. Cx. gelidus is the predominant species in periurban areas of Kurnool, and no seasonal pattern on population density is observed, unlike for Cx. tritaeniorhynchus due to availability of perennial larval habitats such as riverbed pools created by rain, semi permanent pools, which persist throughout the year because of urban effluents (Arunachalam et al., 2009).

1.2 Aerodynamic classification of insects

Insects are aerodynamically classified into neurogenic or myogenic, based on the oscillatory behaviour of flight muscles as synchronous or asynchronous respectively. The muscles are called as synchronous when each contraction of the flight muscle is produced by the arrival of a nerve impulse. The flight muscles of Lepidoptera, Orthoptera and Odonata are synchronous. In fibrillar muscles possessing insects, the wingbeat frequency is in excess of 100 Hz. In fibrillar muscles, several contractions follow the arrival of each nerve
impulse. The muscles in which the ratio of contractions to stimuli differs from the normal 1:1 ratio are called as asynchronous. Asynchronous muscles can contract and relax at high frequencies, but high frequency stimulation causes them to contract tetanically. A nerve impulse is necessary for the initiation of contractions, but subsequent contractions are the products of the muscles themselves and are said to be myogenic. Myogenic contractions occur only in an oscillating system such as the thorax.

Generally natural fliers consist of four classes of flight muscles. Indirect muscles, Direct muscles, Accessory indirect muscles and Accessory dorso-ventral muscles. The indirect and direct muscles undergo contraction and relaxation by the sliding effect of proteins (actin and myosin) as a result of which the wing undergoes upstroke and downstroke. Indirect muscles are nothing but power producing muscles. Normally flight is initiated by the discharge of impulses in the nerves to the indirect flight muscles. Direct muscles are nothing but power drawn muscles and are directly connected to wings. In locust, changes in the angle of attack of the wings during the stroke are produced by the timing and force of contraction of the direct muscles (Wilson and Weis-Fogh, 1962; Wilson, 1962). Accessory indirect muscles are tonically contacting group that change the structural characteristics of the thoracic box (Bonhög, 1949). Accessory dorso-ventral muscle is the main power producing upstroke muscle, part of it differentiates into the close packed tergo-
trochanteral muscle in many Dipterans, where the rest of the muscle is fibrillar.

The mechanism of insects fly includes the wings and their articulations with the body, integument, which constitutes the complex underlying skeletal framework, the muscles with storage of energy furnishing the motive force; the respiratory exchange, which brings O$_2$ to the working tissues and removes CO$_2$ from them at high rates demanded by their intense performance; the circulation, which assists in prolonging flight through the mobilization of energy reserves from other depots in the body and it may be, through removal of the waste products of metabolism, and finally, the nerves activating the motor system and integrating the functions of its intricately related parts.

The internal arrangement of the muscle cells varies in different muscles, particularly in muscles moving the wings. Shortening of the muscles involves the filaments sliding between each other. The muscles are stimulated to contract by the arrival of nerve impulses which cause local changes in the electrical properties of the muscle membrane and induce chemical changes within the cell. The output of power by the flight muscle is high and the associated metabolic rate is higher than in any other tissue. To retain such a high level of metabolism, the supply of O$_2$ and of fuel must be adequate and insects are adapted anatomically, physiologically and bio-chemically to maintain such metabolism.
1.3 Wing beat frequency of Mosquito

In a flier (insect, bird or bat), the wing beat frequency is an important parameter not only from the point of view of aerodynamics and bio-energetics, but also from the point of view of ecological relationships. Mosquito is the smallest insect and has high wing beat frequency. This wing beat frequency of mosquito plays a main role in spreading the disease. Small insect move through air at low speed, but if the wing beat frequency is high, the movement involves high acceleration. The vector mosquito has the capability of spreading the disease from one place to other due to its wing beat frequency.

Many attempts have been made theoretically and experimentally to study the wing beat frequency of insects, birds and bats. Several empirical and semi-empirical relations have been reported for the computation of wing beat frequency of insects and birds, among which the “mechanical oscillation” theory proposed by Greenewalt (1960) is noteworthy. It has been reported that the wing beat frequency of any flier in the hovering state of flight could be determined from knowledge of the rate of mass flow of air induced downward by the wing disc (Puranik et al., 1977). The effect of body phenomenon and environmental influences on wing beat frequency of flight of insects was studied by Adeel Ahmed (1982). Radha Krishna (1995) studied the frequency of wing vibration in *Aedes aegypti*, the principal vector of dengue fever.
1.4 Design of flight surface (wing) of natural fliers

Flight surface (wing) is the basic and most important flight apparatus of a natural flier. Flight surface is not same in different fliers with respect to the structure and design. Successful flight apparatus have been developed by Insects, birds and bats. Though there are differences in their phylogeny, anatomy and physiological adaptations of these fliers, each group has to face the same aerodynamic problems associated with the flight (Adeel Ahmed, 1984). The proximal part of the flight surface is responsible for the generation of the lift and the distal part for the thrust (Puranik et al., 1976). The flight of insects is characterized by relatively high wing beat frequency due to the presence of resilin (rubber like structure) at the wing base. Birds and bats have low wing beat frequency due to lack of resilin. In *Tessaratoma javanica* there is a hook mechanism between fore and hind wings and the wings vibrate as a whole. In dragonflies, the fore and hind wings vibrate separately. The surface density of the flight surface of fliers (insects or birds) shows an exponential decrease with wing length. This can meet the aerodynamic requirement for flight maneuverability by providing the necessary rigidity at the wing joint and flexibility at the appropriate pints along the flight surface.

The only invertebrates which are magnificently characterized by the possession of wings are insects. Excluding the Apterygota and few secondarily wingless pterygotes, all modern insects bear laterally a pair of wings on each meso and meta-thorax and are commonly called
as the fore and hind wings respectively. Both pairs of wings perform the flight in majority of insects or one pair of wings may modify into altogether different structures like the halters found in Diptera and Strepsiptera and the elytra or tegmina in Dermaptera, Orthoptera and Coleoptera. The wing bears a group of sclerites at its base and a complex of longitudinal and cross veins throughout the wing body. The wing also bears various types of sense organs and pigments.

1.5 Aerodynamic parameters of Mosquito

Aerodynamic parameters are useful in understanding flight behaviour, flight energetics and power economy of a natural flier (insect, bird or a bat).

Flow chart 1.1: Air induced downwards by the combination of flight muscles and flight surface
When a flier is in the hovering state, it is said to be in the dynamical equilibrium which is achieved by the flier by generating the air induced downwards due to wing beat in turn develops a reacting force, just to balance its body weight. The flier and induced air put together is considered as a “system”. Thus the system constitutes an “action-reaction pair” in the hovering flight which helps the flier to be airborne. Radha Krishna (1995) studied the static and dynamic parameters of the system (flier + air) and aerodynamic parameters of Aedes aegypti and Anopheles stephensi. Aerodynamic parameters of Cx. quinquefasciatus were studied by Murty (1993). Siad riaz ahmed (2001) studied the aerodynamic parameters of Apis dorsata. Sugnana Kumari (2003) studied aerodynamic parameters of Exorista sorbillans.

1.6 Lift and other forces

In an idealized horizontal wing, the Lift acts at right angles to the direction of the airstream flowing over the wing due to static pressure differential. For an airplane in a horizontal flight the relative wind is of course horizontal and this wing lift is directed vertically, counteracting gravity. However in the flapping flight of a bird, the situation is more complex. In steady flapping, the wings are angled differently at each instant during the cyclic wing strokes. There are also local air currents. Thus in discussing bird flights, we shall frequently need to refer to the average lift generated by the wings during a stroke of a maneuver. Though lift provides the upward force against gravity, in some cases the direction of aerodynamic lift
generated by bird’s wings must have some forward components in steady flight, if not there would be no thrust to propel, the bird overcome other factors such as viscous resistance and stagnation at leading edges and other properties of airstream which tend to resist such motion (they contribute to drag).

Fig. 1.2 explains forces acting on the bird, with the help of these diagram, we find the resultant upward force due to drag, thrust, lift and gravity acting on the bird. If the resultant is positive the bird climbs. If the upward force is equal to the gravitational force and the thrust is equal to drag, forward flight is maintained.

![Diagram of forces acting on the bird](image)

**Fig. 1.2: Forces acting on the airfoil.**

The drag acting on a flier can be described in terms of several physical effects as follows;

- Surface friction drag is the friction between the entire surface of the body and the air streaming past it.
- Pressure or form drag is the size of the surface presented to the relative wing, the leading edges of the wing and the front end of the body.
• Profile drag is experienced by the beating wings in moving forward and is independent of the lift generated.

• Induced drag is brought about by compensatory airflow around the tip of the wing. This depends upon the lift.

The wings in a level flight produce a mean aerodynamic force that balances two force vectors: Weight, which is vertical, and body drag, which is horizontal. This mean force is conventionally resolved into the orthogonal components, lift and thrust. The beating wings generate these forces by changing the momentum of the air in their vicinity. Thrust is generated as the wings accelerate air backwards, and lift is generated as air is accelerated downwards (Tucker, 1973).

1.7 Speed of Flight in Insects

The speed of flight of a number of insects is given by Wingglesworth (1942). The speed of flight varies from 0.6 (Chrysopa) to 15.0 (Spingids) meters per second. Speed of flight for the bee vary from 2.5 to 6.0 meters per second and for Diptera from 2.0 (Musca) to 14.0 (Tabanus). In many cases such estimates of speeds have been arrived at by observation alone. Estimation of the cruising speed of the unfed female Aedes aegypti has given values of 0.5 to 1.0 meter per second when seen clearly in a beam of light or against a suitable background. However, accurate determination of speed by such method is difficult. In general, male has more rapid flight than the female.
Kennedy (1940) showed that the flight of the female is largely controlled by visual response to the background. In a wind tunnel, over a background of moving shadow stripes the mosquitoes flew in the same direction as the movement, often faster but never more slowly than the stripes. In a gentle wind of 40 cm/sec, over a stationary background the mosquitoes made headway and were never carried backwards. Few individuals temporarily swept in the direction of the wind sometimes turned in mid air and flew up in the wind again. When disturbed, the mosquitoes (females) flew directly against a 40 cm/sec. As the wind grew stronger, more and more mosquitoes alighted, those still flying in 100 cm/sec wind maintaining their position until they suddenly alighted on the floor without being carried backwards. With winds over 150 cm/sec they were made to alight and could not take off. Afridi and Abdul Majid (1938) found that Cx. fatigans refused to take off in wind of 10 miles an hour (450 cm/sec). These results indicate an average speed of about 50 cm/sec and a maximum speed of 150 cm/sec.

Mosquitoes dispense by random flights towards sites for satisfying the biological needs such as feeding, resting, mating and oviposition (Provost, 1952). Mosquitoes are able to fly for 3 km and more in uninhabited areas (Adridi and Majid, 1938). In Anopheles gambiae, the maximal flight distances were 9 km when sugar-fed and 10 km when blood-fed, while in starved females it was below 3 km and the average speed was around 1 km/h. Maximal flight distances of An.
atroparvus were 10-12 km when sugar-fed, 4.5 km when blood-fed, and below 3.5 km when starved, with an average speed of 1.3 km/h (Kaufmann and Briegel, 2004).

The mechanism of flight of mosquito is very similar as seen in majority of insects. The main action is the up and down movements of the wing brought about through the powerful indirect wing muscles in the thorax acting indirectly on the wing by leverage. To ensure forward progress, the wing is also rotated to some degree on its long axis and so composed with a thickened anterior and more flexible posterior portion that result some degree of propeller like action. The wings of mosquito in flight require, the main up and down movement, some rotation of the wing in its long axis or other adaption required for progressive flight, and extension and flexion with adjustment of the wings to the resting position.

1.8 Power requirements of Insect flight

Flight apparatus of a natural flier consists of two components – flight muscles to meet the energy requirements of the flight and flight surface (wings) to develop required aerodynamic forces. Power requirement studies on bio-energetics of fliers is useful in understanding the “mechanical efficiency” of flight muscles, “dynamic efficiency” of flight surfaces and “aerodynamic efficiency” of fliers during their flights.

Power output is defined as the rate at which mechanical work is done, while the corresponding “power input” is defined as the rate
at which the chemical energy is consumed. Thus, the power input is calculated as the power output divided by the efficiency of the power producing system, plus the basal metabolic rate.

\[ P_i = \left( \frac{P_o}{E} \right) + B \]

Where,  
- \( P_i \) is the power input  
- \( P_o \) is the power output  
- \( E \) is efficiency  
- \( B \) is the basal metabolic rate.

The term “basal Metabolic rate” or “basal metabolism” refers to the heat production of a resting animal in a thermally neutral environment and in the fasting or post absorptive state. Pennycuick (1975) proposed a theoretical model to calculate the metabolic rate of a bird during the flight which was later modified by Tucker (1975).

The power released from the oxidation of fuel (glycogen, proteins and lipids) is divided into two parts, one goes to the flight muscles (\( P_{i,w} \)) and the other is diverted towards maintenance, circulation and respiration. The power going to the flight muscles is larger in magnitude but only a fraction of it appears as the rate at which work is done on the air. The aerodynamic work rate is the rate at which muscles do mechanical work, since negligible part of the mechanical work rate is used to overcome internal friction of joints and moving tissues.

The power output of the flight muscles comprises of three parts; one is used to support the body weight of the filer (\( P_m \)), second is used
Flow chart 1.2: Analysis of power for flight

to overcome the drag of the body (P_a) and the third is used to move the wings through air (P_i). Pennycuick (1968 and 1975) and Tucker (1973) gave the methods for estimating the components of the power output. A large amount of data has been reported on metabolic rate, aerodynamic power and dynamic efficiency of many insects by weis-Fogh (1952, 1964, 1972 and 1973), Sotavalta (1952) and Adeel Ahmed (1978).

The total mechanical power output required by a flier can be estimated as the sum of Basal metabolism, Parasite power, Profile
power, Induced power and inertial power which can be evaluated separately. Basal metabolism is the metabolic rate on the basis of body weight. Parasite power is the power required to overcome the skin friction and from drag of the body. Profile power is the power needed to overcome the profile drag of the wings.

Induced power is the power needed to impart downward momentum to the air, adequately and rapidly to produce a reaction balancing the flier's weight. Thus, the induced power is defined as the power required by a flier to support its weight in the air which is different for a flier at different states of flight. The weight of the flier must be supported by the upward reaction on the wings in a hovering flight. It means that the rate at which the downward momentum is imparted to the air is equal to the body weight. The rate of change of momentum is the product of the downward induced velocity and the mass of air to which this velocity is imparted in unit time.

The work done in imparting angular kinetic energy to the wing at the beginning of each down stroke and each upstroke is been considered in the power calculation. However, the energy is recoverable as the wing can be slowed by aerodynamic forces at the end of the stroke, and its kinetic energy can be transferred to the air. The inertial power is the power required to accelerate the wings at each stroke. Aerodynamic power is the power required for the horizontal motion of the flier in air. Dynamic efficiency is an important aerodynamic parameter of a flight surface and is defined as the ratio
of induced power \( (P_{in}) \) to the sum of the induced power \( (P_{in}) \) and the inertial power \( (P_w) \). The present study is confined to induced power and inertial power, Aerodynamic power and Dynamic efficiency.

### 1.9 Energy reserves for Flight of Insects

Energy expend while flying, running, and swimming requires the mobilization of metabolites in insects, and the storage and release of carbohydrates, lipids, proteins, and amino acids are strongly under endocrine control (Gäde, 2004). Insect stores energy reserves in the form of glycogen and triglycerides in the adipocytes, the main fat body cell. The fat body of insect plays a vital role in energy storage and utilization. The fat body is the central storage depot for excess nutrients, and is an organ of great biosynthetic and metabolic activity (Law et al., 1989).

Insects have to use energy constantly, and if they are not feeding, they must live on reserves accumulated in periods of food abundance. Glycogen and triglyceride are the energy reserves in animal cells. Glucose is stored in a polymeric form, glycogen, which can be readily degraded on demand to be used as a glycolytic fuel (Steele, 1982). Fatty acids stored as triglyceride could be used for energy production through β-oxidation (Athenstaedt et al., 2006). Glycogen is stored in a bulky hydrated form, whereas triglyceride is stored in an anhydrous form. Triglycerides also have a higher caloric content per unit of weight than glycogen, and provide a useful source of water upon oxidation, yielding almost two times more
metabolic water than glycogen. These considerations have direct implications on energy metabolism of insects (Elliott et al., 1984).

Fat reserves are the important reserve used by insects to meet their energy demand during diapause (Hahn et al., 2007), to provide energy for the developing embryo (Ziegler et al., 2006), and to fuel prolonged periods of flight (Beenakkers et al., 1984). Adult mosquitoes have specialized feeding habits. Their energy requirements meet in 3 distinct ways. They may depend on reserves already present at emergence, or they may acquire a blood meal from a vertebrate host or a sugar meal from plants. Energy reserves accumulated during the feeding stage of the last larval instar, although diminished during the non-feeding pupal stage, are usually sufficient to maintain the adults for several days (Van Handel, 1984).

The flight energy of mosquitoes is derived from the polysaccharide glycogen stored in fat body and flight muscle, and the disaccharide sucrose (or its components glucose and fructose) obtained from nectar and fruit juices and stored in the crop (Van Handel, 1985). The caloric reserves of the mosquito are synthesized in the fat body, which is group of cells attached to the wall of the abdomen. Fat body is not equivalent to the vertebrate fat cells (adipose tissue), but more to the vertebrate liver. The fat body synthesizes the hemolymph sugar trehalose and the yolk that will be stored in the developing eggs. In addition, it also synthesizes and
stores carbohydrate (glycogen) and fat (triglycerides) (Van Handel, 1984).

In nature, plant juices, particularly flowers (nectar), are the principal food of male mosquitoes and of species not known to suck blood. The female mosquito is sustained by the sugar meal until it finds its host and allows an infected mosquito to live long enough to oviposit, to bite repeatedly, and to become infective. Hence, feeding on floral and extrafloral nectar, fruits and other plant juices therefore plays an important, indirect role in disease transmission (Van Handel, 1984). The way in which the female metabolizes sugar and blood affects its longevity, flight range and vectorial capacity. In contrast to the male mosquito, the female supported by stored fat can survive a long time, resting on the ground or under dense foliage, in spite of unfavorable nutritional and climatic conditions.

In female *Aedes aegypti*, 50% of glucose incorporated with the diet was used for the synthesis of lipids, and 35% was used for glycogen synthesis (Zhou *et al.*, 2004). Incorporation of glucose in the last instar larval fat body of the silkworm has shown that lipogenesis predominates in the first half of the stage, whereas glycogen synthesis becomes more active at the late stage (Inagaki *et al.*, 1986).

Glycogen utilization depends on the activity of glycogen phosphorylase, which provides glucosyl residues for trehalose synthesis (Steele, 1982; Thompson, 2003). The trehalose is used for maintenance of energy metabolism during fasting or non-feeding
periods, and also as a substrate for insect flight (Thompson, 2003). Long-term flyers, such as locusts (Van der Horst et al., 1980) and mosquitoes, subjected to several hours of flight (Kaufmann and Briegel, 2004; Kaufmann and Brown, 2008) start flying using trehalose and after some time switch to lipids. Studies using half-thorax preparations of Locusta migratoria showed the ability of electrically stimulated flight muscles to metabolize lipids and carbohydrates (Robinson and Goldsworthy, 1977).

Mobilization of fat body lipids during flight is shown in many insects (Beenakkers et al., 1985; Canavoso et al., 2003; Gade and Auerswald, 2002; Kaufmann and Briegel, 2004; Ziegler and Schulz, 1986). Fat body lipids are commonly secreted into the hemolymph as diacylglycerol, which is transported to the tissues by the insect lipoprotein, lipophorin (Soulages and Wells, 1994; Van der Horst et al., 2002). The utilization of lipids in insects has been reviewed on several occasions (Arrese et al., 2001; Beenakkers et al., 1985; Canavoso et al., 2001; Downer and Matthews, 1976). Short-term flyers such as the cockroach Periplaneta americana (Elliott et al., 1984) use mostly trehalose. Bees exclusively utilize carbohydrates to power flight (Suarez et al., 2005).

For Anopheles gambiae, an average dispersal of only a few hundred meters was reported (Sabatinelli et al., 1986; Costantini et al., 1996). Despite their different sizes and environmental conditions, females of the two species achieved similar flight potentials, although
following different metabolic strategies. Study on the energy substrate during flight of *Ae. taeniorhynchus* and *Ae. sollicitans*, revealed glycogen and sugar as the flight substrates, but not lipids (Nayar and Sauerman, 1973). In *Aedes vexans*, Briegel *et al.*, (2001b) found evidence for some lipid to be used during flight. The discrepancies concerning lipid utilization might be caused by short flight trials (Nayar and Van Handel, 1971) *versus* long flights (Briegel *et al.*, 2001a, b).

Proline oxidation during flight of *Aedes aegypti* by Scaraffia and Wells (2003) explains the disappearance of lipids during lasting flight activities, along similar lines as shown for *Glossina* by Bursell *et al.*, (1974). The flight potential and the possible role of lipids as a flight substrate for *An. gambiae* were demonstrated by Kaufmann and Hans Briegel (2004). Flight performance of *Aedes aegypti* and its quantitative relationships to the glycogen and lipid reserves presented at selected times of life by Briegel *et al.*, (2001).

Male mosquitoes indirectly play a role in spread of disease by participating in mating and migration (Murty *et al.*, 2000; Hight *et al.*, 2003). Different mosquito species have variable potency of vector carrying capacity. This Vector carrying capacity depends directly or indirectly on various biophysical and biochemical parameters of mosquito. Hence, the present study was carried out with an aim to analyze the biophysical and biochemical parameters of JE vectors, *Culex tritaeniorynchus* and *Cx. gelidus*. 
1.10 AIM OF THE STUDY

Aim of the present investigation is to study the biophysical and biochemical parameters of Japanese encephalitis disease vectors, *Cx. tritaeniorhynchus* and *Cx. gelidus* in Andhra Pradesh.

The following specific objectives were pursued to achieve this goal.

- Calculation of wing beat frequency in *Cx. tritaeniorhynchus* and *Cx. gelidus*.
- Design of flight surface and compute the Aerodynamic parameters of *Cx. tritaeniorhynchus* and *Cx. gelidus*.
- Determine the speed of flight in *Cx. tritaeniorhynchus* and *Cx. gelidus*.
- Calculation of power requirements for the flight of *Cx. tritaeniorhynchus* and *Cx. gelidus*.
- Comparative biochemical analysis in *Cx. tritaeniorhynchus* and *Cx. gelidus*. 