RESEARCH ARTICLE

ENTOMOLOGICAL INVESTIGATIONS OF AN OUTBREAK OF JAPANESE ENCEPHALITIS IN THREE DISTRICTS OF CHHATTISGARH, INDIA IN THE YEAR 2017

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Abstract
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Background: Japanese encephalitis (JE) is a serious Zoonotic Vector Borne infection of the brain caused by the Japanese encephalitis virus (JEV) of family Flaviviridae .Many outbreaks have been occurred in many parts of Southeast Asia including India. An outbreak of JE was occurred in three districts of Chhattisgarh during the year 2017.

Objectives: The purpose of this study To review and assess the situation of dengue outbreak in Durg district and conduct and entomological survey in all the the JE affected areas.

Methods: The Entomological study was carried out during December 2017three districts namely Dantewada, Bastar and Sukma in Gorakhpur of Chhattisgarh. A total of 12 JE cases repoted from these districts. Larval collection was done and per dip density was calculated and for adult collection PMHD was calculated.

Results: During the outbreak investigation 9 different types of mosquito breeding were detected in all the affected areas. Highest breeding was detected in Fallow fields (36.59 %) followed by Drains (32.14 %), Percent river bed pool (28.57%), paddy fields (19.35 %), Mud pools (6.25 %), Hoof prints (3.5%). The various Culex species collected by hand catch method included, Cx. quinquefasciatus Cx. vishnui Cx. pseudovishnui Cx. Tritaeniorhynchus.

Interpretation & Conclusion: The findings showed that scattered cases of JE and most of the cases under age of 0-15 years old, facilitated by different species of Culex in the affected areas. JE is a vaccine-preventable disease, so an immunization may be done and an appropriate vector control strategy and application of standard hygiene practices in these affected areas could be a result in reduction in morbidity and mortality due to JE virus.

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Introduction:-
Japanese encephalitis (JE) is a serious Zoonotic Vector Borne infection of the brain caused by the Japanese encephalitis virus (JEV) of family Flaviviridae (Soloman 2004).It is a single stranded RNA virus.Different types genotypes of this virus have Geographically distribution but all belong to the same serotype. Virulence and host

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preference are similar in all the major genotypes of JE virus. The first isolation of JEV in the Republic of Korea (ROK) was made during an autopsy of an American soldier at Incheon in 1946 (Sabin *et al*. 1947). JE, has emerged as a major public health problem in India since 1955 from Vellore, north Arcot district with a very high mortality and morbidity (Work and Shah 1956). It is most prevalent in rural and peri-urban areas. It is the main cause of viral encephalitis in many countries of Asia spreading over three million populations (Ghosh and Basu 2009).The first JEV was isolated based on serological surveys in 1955, in Tamil Nadu, India (Namachivayam and Umayal 1982).It is one of the major encephalitis in the tropical regions of Asia, including India, China, Japan, Taiwan, Korea and Philippines (Solomon 1997). In India Since 1955, many major outbreaks have been occurred in different parts of the country including Andhra Pradesh, Assam, Bihar, Goa, Karnataka, Manipur, Tamil Nadu, Uttar Pradesh and West Bengal. Outbreaks of JE usually coincide with monsoons and post-monsoon period when the vector density is high (Sudhanshu *et. al.* 2018).

The JE case-fatality rate varies across ranging from 10 to 30% among infected patients (Libraty *et al.* 2008). Among those with disease symptoms; 20-30% of those who survive suffer permanent neuropsychiatric sequelae Most of the cases having very little or no symptoms of the disease, sometimes inflammation of the brain occurs. Symptoms may be included meningism, like neck rigidity, photophobia, nausea, headache, vomiting, fever, confusion, and seizures and altered sensorium. Abnormal movements like coarse tremors, convolutions are seen in children. Neurological sign develops like ataxia, abnormal reflexes, paralysis, delirium, prolonged confusion with poor prognosis. Incubation period of the JEV may be 5 to 15 days after infection. There are paralysis and mental retardation in 30-70 percent survivors. In areas where the JE virus is common, encephalitis occurs mainly in young children because older children and adults have already been infected and are immune. Japanese encephalitis virus (JEV) is transmitted through mosquito and maintained in a zoonotic cycle, which can be both enzootic and epizootic. JE has been isolated from 16 species of mosquitoes belonging to the genera of Culex (10), Anopheles (3) and Mansonia (3) (Philip Samuel *et al.* 2000). The *Culex vishnui* subgroup of mosquitoes consisting of *Cx. tritaeniorhynchus, Cx. vishnui and Cx. pseudovishnui* have been implicated as major vectors of JE in different parts of India (NVBDCP 2018). Human is the accidental host (Fig.2). Human is also the dead-end host. Pigs are the major reservoir/amplifying host for JE virus and water birds such as Heronetc.are the carriers for JEV. There is no human to human transmission reported till date. Environmental factors such as temperature rain fall humidity etc., plays an important role in transmission of JE virus in population. JE Virus transmission is seasonal in temperate areas of Asia, Human disease usually peaks in the summer and fall. Transmission pattern in the subtropics and tropics, can occur year-round, often with a peak during the rainy season. Several factors like temperature pattern, distribution of vector population, agriculture practices (wetland rice crop) swine rearing and their proximity to human populations are responsible for JE outbreaks in India (Lin & Lu 1995, Gingrich *et al.*1992)

 Specific treatment for Japanese encephalitis is not available and treatment can be supportive with assistance given for feeding, breathing or seizure control as required. Raised intracranial pressure may be managed with mannitol. Japanese encephalitis (JE) vaccine is the only JE vaccine is available. JE vaccine is recommended for long time travelers who are plan to visit for long time (More than One month) in endemic areas during the JE virus transmission season. Pregnant women are on high risk who must travel to an area where risk for infection is high should be vaccinated with when the risk of immunization is significant than by the risk of infection (CDC 2015). Prevention is always safe and effective with the Japanese encephalitis vaccine. Other measures including avoidmosquito bites with the use of personal protection measures as mosquito repellent, use of bed net etc, (WHO 2015). JE vector species of mosquitoes are known to be zoophilic, and mostly preferring cattle for feeding over man (Carey et al 1969). In India a large number of Cattle- pig ratio is predominant. JE vectors are primarily zoophilic and exophilic in nature, however due to their high density, particularly during outbreaks/ epidemicsman- vector contact may reach at significant level even indoors.

Three districts of Chhattisgarh are recently affected with an outbreak of JE disease with a total of 13 cases and 2 deaths. Outbreak started from 28.11.2017 from Dondapal block of Sukma district. it spreads in 3 nearby districts namely Dantewada, Bastar and Bijapur. All the affected patients age ranges from 1-5 years' age group. Females were more affected than males. In some patients mixed infection was also reported. Some patients were affected with Dengue, Malaria & Japanese encephalitis (JE). One patient was died of mixed infection of Dengue, Malaria & JE. All the patients tested with IgM E Elisa in Jagdalpur Hospital. In view of the severe outbreaks with life losses and on the request of the Health department, Govt. of Chhattisgarh, an entomological investigation was carried out in all the affected villages during the outbreak period to assess the prevalence of JE vectors, their infection status, risk factors for transmission of JE and to suggest appropriate vector control intervention measures for

implementation by the state/district Health Department. A team from NCDC, Delhi visited in the JE affected areas for a period of 20th December 2017 to 1st January 2018 and an entomological survey was carried out to identify the vectors involved in the transmission of this disease. Survey was carried out in all the Japanese encephalitis affected villages.

Materials And Methods:-

Study area:

Chhattisgarh is located in the center-east of the country. It is the tenth-largest state in India,it covers an area of 135,191 km^2 ,with 25.5 million population (Census 2011). About 44% of the total area of Chhattisgarh is covered by

forest. The climate of the state is mainly dry and hot. Chhattisgarh receives a good amount of rainfall. The state receives rainfall mainly from the South West monsoon. Temperatures in Summer Chhattisgarh can reach 45 °C. The rainy season is from June to October but in winter it is very in morning and evening. Chhattisgarh receives an average of 1,292 millimeters (50.9 in) of rain per year. Three districts of Chhattisgarh namely, Bastar (1,302,253), Sukma (249,841) and Dantewada (719,065) were affected with JE cases in the month of October and November 2017 (Winter season). Total population of these three districts is 2,271,159 according to census 2011 and covers

.Fig.1. Map showing study villages in Japanese encephalitis (JE) affected community health centers (CHCs) of Chhattisgarh.
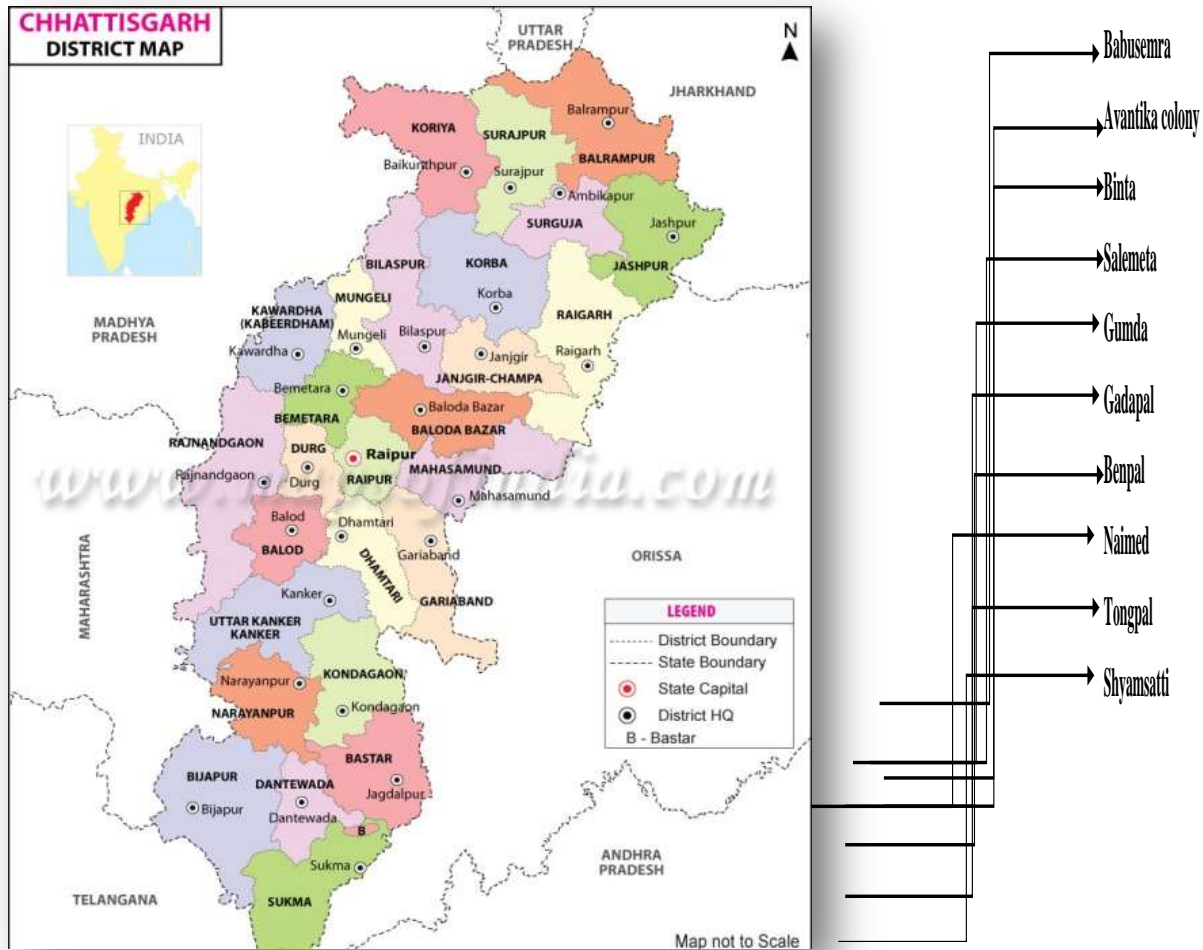
13077 sq. km area of Chhattisgarh. Population density Dantewada is 211 inhabitants per square km followed by Bastar (87) and Sukma (46). These all three districts surrounded by forest. Chhattisgarh is a basically tribal state with majority of population residing in rural areas. Chhattisgarh is boarded by Madhya Pradesh on its north western part, Maharashtra on the west and Andhra Pradesh on its south. Main crop of these district is paddy.

Epidemiological Data collection:
Epidemiological investigations included discussion with the District authorities and to know the background information of the affected areas, genesis of outbreak, investigations carried out so far and control measures undertaken; visit to affected areas. Data for the last 3 years of VBD's of all four districts was obtained to assess the current situation of outbreak from District authorities. Besides this Descriptive analysis on the basis of time, place and person was carried out. Collected data was analyzed and plane of investigation made accordingly.

Entomological investigations:
Entomological investigation was carried out as per the guidelines of NVBDCP, Delhi. Various entomological parameters were observed during outbreak investigation. Following parameters were used:

Ecological Condition: Ecological condition of the JE affected areas was assessed to check whether pigsty is near to human dwellings or not, present of water birds like Water Herons, Egret bird etc. Presence of water bodies in which vector species of mosquito can breed.

Mosquito collection:
Adult Collection:
Adult mosquito collection was done normally early in the morning 7.00-9.00 AM, with the help of Torch and aspirator to know the vector density from both human dwellings and cattle shed. This method is also used to know the indoor resting density. Per Man Hour density was calculated with the help of following formula:
PMHD = No. of mosquito collected / Time spent in hours

Pyrethrum Sheet Collection:
Pyrethrum spray sheet collection was done in all the affected areas to know the fauna vector species. One dark room in which one or two persons slept in the previous night was selected and all the food stuffs and other material were covered with the help of white bed sheet and pyrethrum was sprayed in all the room and left for 30 minutes. After 30 minutes all the mosquito collected in vials with the help of forceps. PRD (Per Room Density) was calculated with the help of following formula:
PRD = No. of mosquito collected / no of rooms

Survey of Immature stages:
Various breeding habitats i.e. pits, pools and paddy fields, drains, etc have been searched for mosquito breeding in all the affected areas by dipping dipper in water bodies. The required dips were obtained depending upon the size of various habitats. Sequential sampling was done at the gap of 2 meters on either side Drain or canal. Collected different stages of larvae and pupae were kept for adult emergence and identified up to species level. Per Dip Density was calculated with the help of following formula:
Per Dip Density = No. of Larvae collected / Total no. of Dip

Results:-
Epidemiological Investigations:
Epidemiological trend of JE and other vector borne diseases in 3 Districts of Chhattisgarhshows that Malaria is a major public health problem in these three districts. More cases reported from Dantewada district (17331) followed by Bastar and Sukuma during the year 2016 and maximum number of deaths were reported from Bastar (32) followed by Dantewada (06) and Sukuma (04). In the year 2017, maximum numbers of malaria cases were reported from Dantewada (15017) followed byBastar (11048) and Sukuma (10061). The details of VBDs reported from these four districts are given in Table 1.

Dengue is the second important public health problem in these four districts. Maximum number of dengue cases reported from Bastar district (57) followed by Sukuma (41) during the year 2016. A total 18 cases reported from Bastar district and 11 cases from Dantewada and no dengue cases reported fromSukumadistricts during the year 2017. In all affected districts no death was reported during the year 2016 and 2017.

JE cases and deaths were reported from all these threedistricts since 2016 except from Sukuma district. District Sukuma reported a total of 7 JE cases and 3 deaths during the year 2016. During 2017, JE cases and deaths were reported from all these three districts. More cases reported from Dantewada (6 Cases and 2 deaths) followed by Bastar (4) and Sukuma (2) with nil death. The epidemiological trend of VBD's in all these four districts are given in Table 1.

Table 1:- Epidemiological trend of VBD's in 3 Districts of Chhattisgarh.

District/Block	Year	JE		Malaria		Dengue	
		Cases	Death	Cases	Death	Cases	Death
Bastar	2016	Nil	Nil	14662	32	57	Nil
	2017	04	Nil	11048	10	18	Nil
Dantewada	2016	Nil	Nil	17331	06	Nil	Nil
	2017	06	03	15017	03	11	Nil
Sukma	2016	07	03	14109	04	41	Nil
	2017	02	Nil	10061	01	Nil	Nil

Ecological Condition:
Pigs are the reservoir host for JE virus and in most of the visited JE affected areas egret bird found near pond and other water bodies. Various types of breeding place were detected near the affected areas. Pigsty was also observed in every JE affected village. Pig and Egret bird are the reservoir host of the JE virus.

Entomological investigations:
Mosquito collection:
Survey of Immature stages:
During the outbreak investigation 9 different types of mosquito breeding were detected in all the affected areas (Table. 2.). Highest breeding was detected in Fallow fields (36.59 %) followed by Drains (32.14 %), Percent river bed pool (28.57%), paddy fields (19.35 %), Mud pools (6.25 %), Hoof prints (3.5%). Minimum breeding was detected in earthen pots (2.94%), while no breeding was detected in Irrigation channel and Ponds.All emerged mosquitoes were identified as *Culex quinquefasciatus, Cx. tritaeniorhynchus, Cx. vishnui, Cx.fuscocephala, Anopheles stephensi, An. varuna, An. subpictus ,An. Annularis An. tesselatus, An. fluviatales, An. jeporensis, An. Maculates, An. barbirostris,* and *Armigeres* sps (Table 2.)

Table.2:- Number of breeding habitats positive for mosquitoes in all affected areas of Chhattisgarh.

		Number of breeding habitats positive for mosquitoes								
S N		**Bastar**		**Dantewada**		**Sukuma**		**Total searched**	**Total +ve**	**Positivity Percent**
	Habitats	**No. surveyed**	**+ve**	**No. surveyed**	**+ve**	**No. surveyed**	**+ve**			
1	Paddy field	15	3	9	2	7	1	31	6	19.35
2	Irrigation channel	1	0	0	0	0	0	1	0	0.00
3	River bed pool	5	2	2	0	0	0	7	2	28.57
4	Pond	2	0	1	0	0	0	3	0	0.00
5	Fallowfield	40	20	23	7	19	3	82	30	36.59
6	Earthen pot	14	1	11	0	9	0	34	1	2.94
7	Drain	15	5	8	3	5	1	28	9	32.14
8	Hoof print	20	2	15	0	11	0	46	2	4.35
9	Mud pool	5	1	6	0	5	0	16	1	6.25

Adult Collection:
Adult mosquito collections:
Different species of adult mosquito collected through various methods are shown in fig. 2, 3 &4. Maximum number of mosquitoes collected from Indoor collection followed by outdoor. 19 species belonging to two genera collected by Indoor collection in which Culexwas the most predominant followed by Anopheles. Highest Per men hour

density was observed   for Cx. quinquefasciatusin Indoor collection in comparison of Outdoor and dusk collection from Bastar district. `
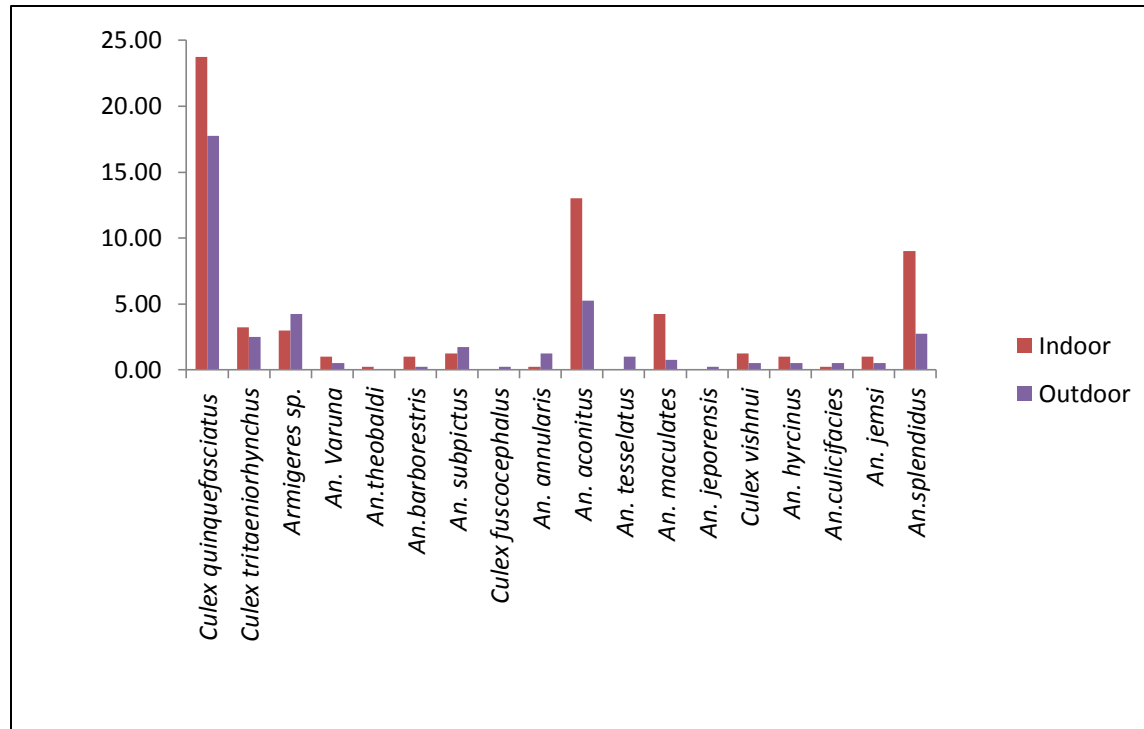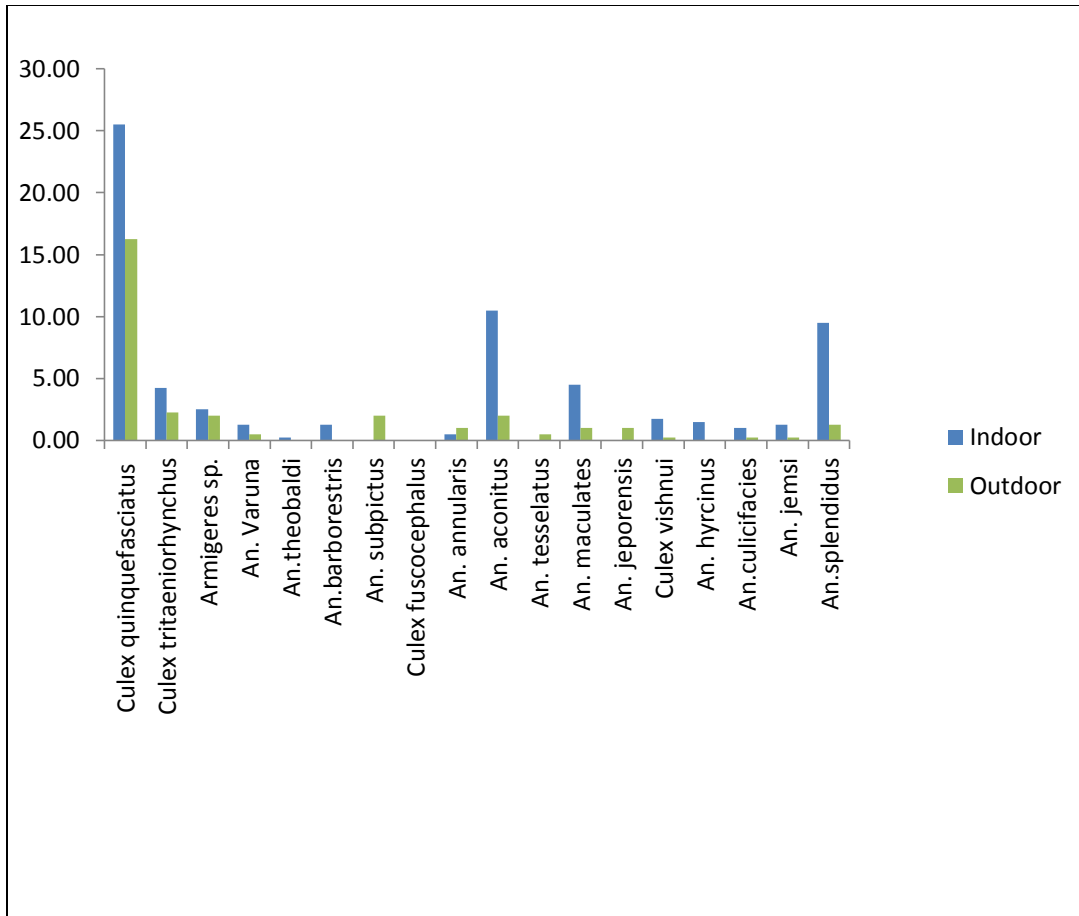

Fig. 2:- Hand collection done at Batar.

Based on Indoor collection done in Bastar district 4 culex species were collected in which, Cx. quinquefasciatus (PMHD-23.75) was found predominant followed by Cx. tritaeniorhynchus (3.25), Cx. vishnui (1.25). Of the 14 Anopheline species An. acconitus (PMHD-13.0) was found most prevalent followed by An. splendidus (9.0), An. Maculates (4.25), Ansubpictus (1.25), Anhyrcinus (1.0), Anjemsi. (1.0), Anvaruna (1.0), An. barborestris (1.0), An. culicifacies (0.25), An. Annularis (0.25) and An. theobaldi (0.25).   High density of Cx. quinquefasciatus (PMHD-17.75) was also recorded in outdoor collection followed by Cx. tritaeniorhynchus (4.25), Cx. vishnui (0.50) and Cx. fuscocephalus (0.25) and Armiger species (4.25). Among Anopheline species, An. acconitus (5.00) was found most prevalent followed by An. Splendidus (2.50), An subpictus (1.75), An. Annularis, An. Maculates (0.75), An tesseletus (0.50), An. culicifacies (0.50), An hyrcinus (0.50), An jemsi (0.50), An varuna (0.50), An. Barborestris (0.25), and An. jeporensis (0.25).

Indoor mosquitoes obtained from Dantewada comprised 3 Culex species and 11 Anopheles species and Armigers species. Among Culex species, Cx. quinquefasciatus (PMHD-25.55) was found most predominant followed by Cx. Tritaeniorhynchus (4.25) and Cx. vishnui (1.25). Out of 11 Anopheles species, An. Aconitus (10.50), An. splendidus (9.50), An. maculates (4.50), Anhyrcinus (1.50) Anjemsi (1.25), An. Barborestris (1.25), Anvaruna, (1.25) An. culicifacies (1.0), An. theobaldi (0.25), An. Annularis (0.50) and An. theobaldi (0.25). Density of Armigers species was 2.50 in Indoor collection and 2.0 in outdoor collection Fig.2.

Mosquitoes collected from Dantewada in Outdoor collection (Fig.2.) shows that only two species of culex and 10 species of anopheles were collected. PMHD for Cx. quinquefasciatus was 16.25 and for Cx. tritaeniorhynchusit was 2.25. Among anopheles species An subpictus (2.00), An. Aconitus (2.00) An. splendidus (1.00), An. maculates (1.00), An. Annularis (1.00), An. Jeporensis (1.00), An varuna (0.50), An tesseletus (0.50), An hyrcinus (1.0), An jemsi. (0.25), An. culicifacies (0.25).

F ig. 3:- Hand collection done at Dantewada.

Mosquitoes collected from Sukma district during indoor collection shows in fig.4. During indoor collection highest PMHD was recorded for Cx.quinquefasciatus (26.50) followed by Cx. Tritaeniorhynchus (2.00) and Cx. vishnui (1.50). Among anopheline species An. Aconitus (15.00) was most prevalentfollowed by An. Splendidus (8.50), An. Maculates (3.25), An. Subpictus (1.25), An. Barborestris (1.50), An hyrcinus (0.50), An varuna (0.50), An.culicifacies (0.25). Similar results were obtained in outdoor collection. Culex quinquefasciatus (10.50) shows high PMHD followed by Cx. Tritaeniorhynchus (1.25) Cx. vishnui (0.50) and Cx. fuscocephalus (0.25). Among anopheline species An. aconitus (2.75) was most prevalent followed by An. splendidus (2.25), An. culicifacies (0.50), An hyrcinus (0.50), An. jemsi (0.50), An. Jeporensis (0.50) An. subpictus (0.50), An. maculates (0.25), An. barborestris (0.25).
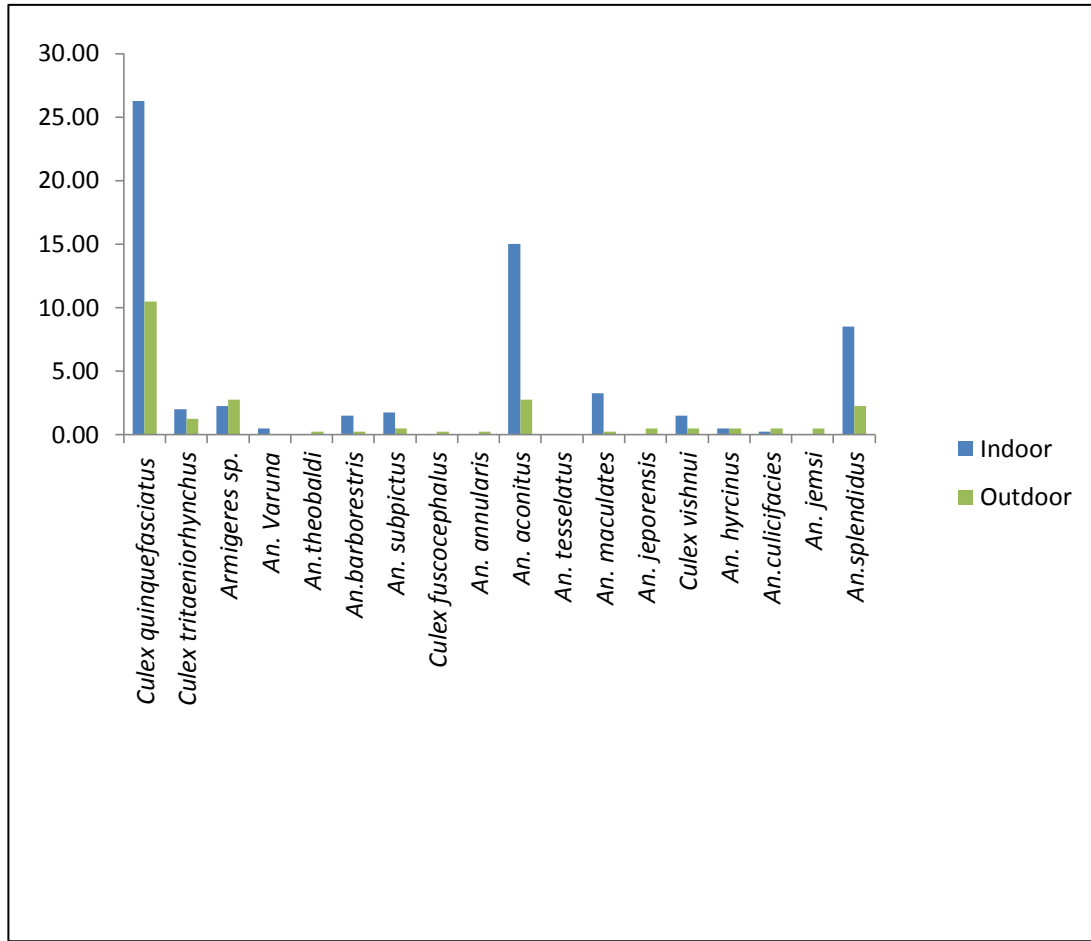
Fig. 4:- Hand Collection done at Sukma.

Adult density by Pyrethrum Spray sheet collection:
Pyrethrum spray sheet collection method was also done in every JE affected village. High Per Room density of Culex quinquifasciatus was recorded in Bastar followed by Sukma and Dantewada. Various Anopheles species were also collected. Only one JE vector species was collected from all the affected areas. PRD for Cx. tritaeniorhynchus was recorded In Dantewada PRD for Anopheles fluviatilis was recorded high followed by Bastar and Sukma districts. An. Annularis only collected from Basar district and An. culicifacies was collected only from Dantewada. Less or more equal PRD was recorded for armigeres sp. From all the affected districts. (Fig.5.).
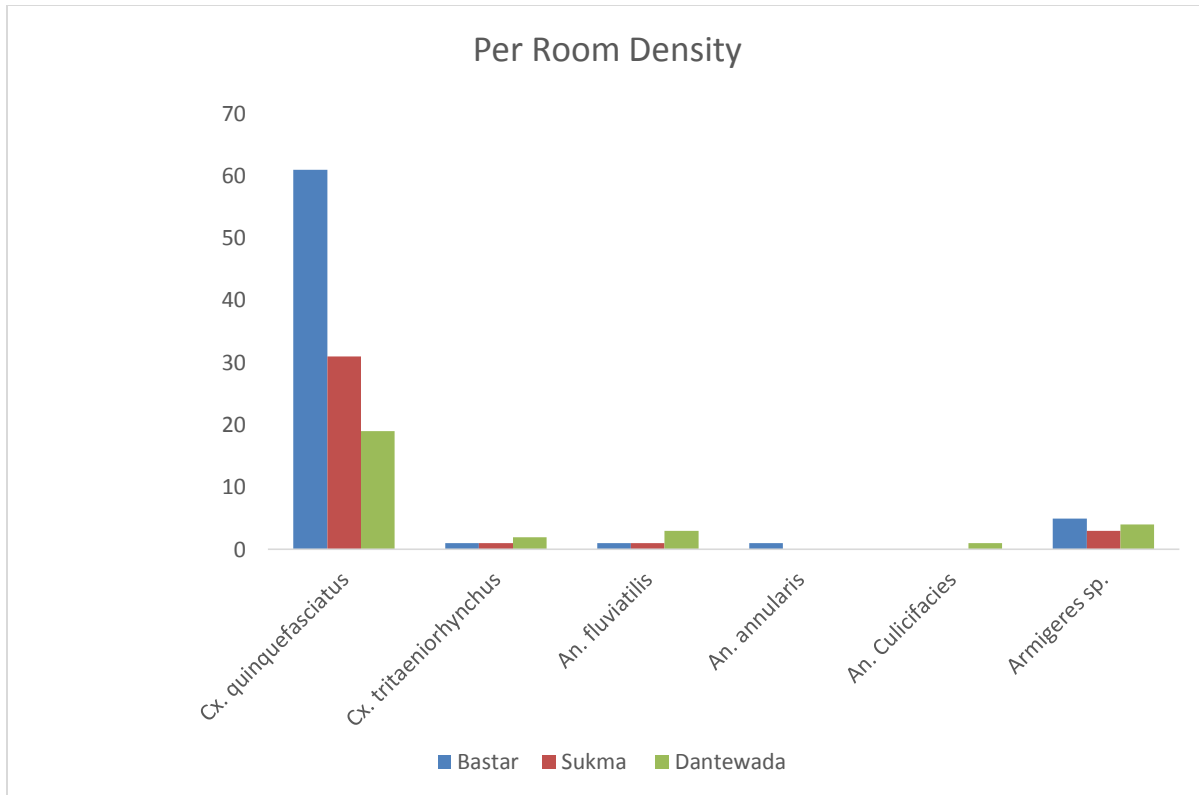
Fig.5:- Per Room Density in All the JE affected areas.

Discussion: -

JE is a mosquito borne disease. Scattered JE cases were reported from these three districts of Chhattisgarh namely Bastar, Sukma and Dantewada. Previously there were no case of JE in Bastar and Dantewada but in Sukma in the year 2017, 7 cases of JE with 3 deaths. JE virus is naturally circulating between ardeid birds and pigs by Cx. vishnui subgroup of mosquito species, comprising of Cx. tritaeniorhynchus, Cx. vishnui and Cx. fuscocephalus etc. in India (Ruben et al. 1992. Survey conducted in three districts of Chhattisgarh; the larval collection indicated that the various habitats contributing different degree of breeding percent.

Data obtained from entomological collection done in all the affected villages' shows that the density of Culex mosquitoes was found to be high. This is because of human dwelling and cattle shed are found to be very near to each other (Low et al 2012). Pigsties were found in all the affected village near human dwelling, this is risky (Bhowmik et al. 2012). Most of the affected patients were children. JE cases first time reported from Bastar and Dantewada but in the year Sukma reported 7cases with 4 deaths. JE disease is mostly occurred in the area where rice cultivation is done near human population where vector mosquito can breed in proximity with pigs, birds and ducks.

The current study was conducted in post rainy seasons. Due to post rainy season most of the JE vector breeding was detected from fallow fields. Paddy fields are the main breeding source for vishnui group (Medhi et al. 2017). Most of the cases were scattered reported with symptoms of vomiting, dizziness and loss of appetite when they were admitted to the hospital. This led the doctors to suspect that they were suffering from JE/AES. Entomological investigations showed that the presence of three vector species of JE in the study villages, including the two major vector species viz., Cx. vishnui and Cx. tritaeniorhynchus. Very less PMHD were recorded for the of vector species in all the affected villages where in some villages fogging was already done. The limitation of the study was that, density for the vector species of JE could not done before and after fogging, because fogging was already in done by state health authority, the present study being an entomological outbreak investigation of JE/AES, the selection of study villages for entomological. Based on the collected information regarding availability of preferred breeding place as paddy filed, irrigation channels, fallow fields and adult collection done by aspirator and torch method and spray sheet collection, appropriate vector control measures were recommended to local general/health administration and they mounted a well-funded campaign to control the outbreak. Vector control interventions such as fogging

indoors and outdoors with 5% malathion and 5% cyphenothrin, larvicidal (Bti) spray in temporary breeding habitats found inside the villages has been started and LLINs/non-impregnated nets were also distributed in the affected villages. Pigs are the reservoir host for JE virus, In most of visited villages pigsties were found near human dwellings so it was suggested to community to keep the pigs in pig pens constructed in the isolated places 2.5 km away from the villages. Community awareness programme of health education and social mobilisation has been started by state health authority in all the JE affected areas.

Conclusion:-

JEV is emerging as a serious threat to human in most of the country first case was reported in the year 2017 in these affected areas of Chhattisgarh.The study has identified the potential or actual larval habitats of mosquitoes and adult vector density of Japense encephalitis in all the affected areas. As Pigs are the reservoir host of JEV, Pigisties are are observed in all the affected ares near the human dwelling. The abundance of JE vector and incidence of JEV cases in both rural and urban areas necessitate intensified surveillance and control of mosquito during high temperature and rainfall seasons. Culex tritaeniorhynchus, the main vector of JE was collected during hand collection both from human dwelling in Babusemra village, Avantika Colony, Binta and Salemeta villages of district Bastar.hich may act as an effective strategy for controlling the burden of JE fever. Fogging was done in some areas. No regular anti larval operations were carried out in all the affected areas. Lack of IEC was observed in the affected community about vector borne disease.

Recommendation:-

Regular anti larval operations are required in all these affected areas. Paddy fields are potential breeding place throughout the year so regular anti larval measures should be ensured there. Piggeries may be kept away from human dwellings. Regular entomological surveillance should be carried out in affected areas as well as in adjacent areas to determine vector density for proper control measures. Reorientation training of field staff is needed and should be done on priority basis. IRS may be carried out in piggeries on regular interval. LLIN may be distribute in all affected areas to reduce man mosquito contact.

Acknowlegement:-

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