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RESEARCH ARTICLE

ENTOMO-ECOLOGICAL STUDIES ON VECTOR POTENTIAL IN AES AFFECTED AREAS OF MUZAFFARPUR DISTRICT, BIHAR (INDIA) WITH REFERENCE TO PREVALENCE OF RODENT AND THEIR ECTOPARASITES

Kaushal Kumar and *Abhay Kumar Sharma

Centre for Medical Entomology and Vector Management, National Centre for Disease Control, 22-Sham Nath Marg, Delhi-110054, India

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ABSTRACT

Acute encephalitis syndrome (AES) is a major public health problem in India. Seasonal outbreaks of AES occurring regularity in Muzaffarpur district, Bihar. Present investigation was carried out to explore the possible entomological causative factors for sudden onset of the disease. A total of fifty two rodents belonging to four different species viz. *Mus musculus*, *Bendicota benglensis*, *Rattus rattus*, and *Suncus murinus* were collected. In commensal rodents overall infestation rate of ectoprasites was recorded as 77.5% and in wild rodents 33.3%. Mites were the predominant ectoparisite retrieved from rodents. The oriental rat fleas (*Xenopsila cheopis*) were retrieved giving an overall flea index as 0.33 and vector larval trombiculid mite chiggers (*Leptotrombidium deliense*) were collected from commensal rodents with chigger index 7.5. Total 533 mosquitoes belong to *Culex quinquefasciatus* (86.9%) followed by *Armigeres* subalbatus (12.9%) and *An. subpictus* (0.19%). Maximum number of *Cx. quinquefasciatus* females collected from Bhikhanpur village with per man hour density (PMHD) of 31.5. Larvae and pupae were collected from the various water sources and belongs to *Cx. quinquefasciatus* (77.3%) and *Ar.* subalbatus (22.7%). Present investigations do not support any vector borne diseases in AES affected areas but ecology and environment highly support the propagation and multiplication of rodents and their ectoparasites. Present investigation suggests further deep study to correlate entomological findings with other clinical and epidemiological studies.

INTRODUCTION

In India, Muzaffarpur district (Bihar) suffers repeated epidemics of acute encephalopathy in children for the past 16-17 years. A major outbreak of this mystery disease, with high case fatality (63.3%) was reported in children in June 2011. Thereafter varying number of cases and deaths are repeated every year from Muzaffarpur and adjoin districts. Children affected from diseases are from age group of 2-10 years belonging to low socioeconomic background. Almost all cases are from rural area and clinical presentation indicates acute encephalitis syndrome. Teams from National Institute of Virology (NIV) in Pune and National Centre for Disease Control (NCDC) in Delhi visited affected areas -Muzaffarpur, West Champaran, East Champaran, Samastipur, Vaishali, Shivhar and Chintamani and collected clinical samples for virological testing. All clinical samples were negative for known virus causing acute encephalitis like *Japanese Encephalitis* (JE), Nipah, West Nile and Chandipura virus. Some specimens were processed for the discovery of novel agents. However, no agent has been found which can be attributed to the cause of the mystery disease in Muzaffarpur (NIV, 2011, Sahni, 2012). The cases are occurring routinely in months of May-June every year in the area since 1995 (Table 1). This occurrence of cases coincides with large scale production of Lychee (*Litchi chinensis*), a very famous seasonal fruit grown in part of the state with a high demand of export worldwide. Some studies reported positive correlation between numbers of AES suspected cases and amount of lychee harvest (Sahni 2013, Paireau *et al*., 2012, John and Das, 2014). In order to ascertain the potential of various vector borne diseases including tick and mite borne diseases a study was undertaken in district Muzaffarpur (Bihar) by a team from the National Centre for Disease Control (NCDC) in May, 2013 with the following objectives:

i. To know the prevalence/distribution and potential of mosquitoes/sandfly and other arthropod vectors in the study villages
ii. To know the prevalence and density of rodent species in the study villages
iii. To know the prevalence of ectoparasites on these rodents and their role in transmission of AES study villages, if any

Corresponding author: Abhay Kumar Sharma,
Centre for Medical Entomology and Vector Management, National Centre for Disease Control, 22-Sham Nath Marg, Delhi-110054, India

MATERIALS AND METHODS

Study area

Muzaffarpur is located at 26°07′N 85°24′E and situated in northern part of Bihar and is bounded by the districts of Sitamarhi and East Champaran on its northern side, Darbhanga and Samastipur districts on eastern side, Vaishali and Saran districts on southern side and district Saran and Gopalganj on its western side. The district has a population of 3.7 million as per 2001 census, of which about 7.3 per cent is urban based. The population density of the district is 1180 per Sq Km. and the overall literacy rate is 48.15%. The district has 1811 villages, 16 block primary health centres (PHC) and 59 APHCs, 478 sub-centres, one district hospital and a medical college. Besides paddy, wheat, paddy, pulses, oilseeds, lychee and mango fruits are the major agricultural crops of this area. During the visiting period, most of the agriculture fields were barren and lychee fruits crop was in harvesting stage. Houses are mostly mixed dwellings; human and cattle live together. The main occupation of the people in Muzaffarpur is agriculture, fisheries and daily wage labour. Between April and June, the climate of Muzaffarpur is extremely hot and humid (28-40°C, 90% humidity) and most epidemics occurred at the height of temperature (38-40°C) and humidity (70%-80%).The number of cases suddenly decreases with the onset of rain and sudden drop in temperature.

Village selected

Five villages in Mushahari Primary Health Centre (PHC) and one village in Ahiyapur PHC were surveyed and selected for trapping rodent and other entomological investigations. Following villages were selected:

i. Gangapur (PHC-Mushahari)
ii. Manika Harikesh (PHC-Mushahari)
iii. Bhikhanpur (PHC-Mushahari)
iv. Jhapah (PHC-Ahiyapur)
v. Behtolia (PHC-Mushahari)
vi. Gopalpur Tarora (PHC-Mushahari)

Mosquitoes and sandfly collection

During the investigation, the mosquito and sandfly were collected by torch light and suction tube method from six villages of two primary health centres to know density and prevalence of vector species. Most of the houses in the village have mixed dwelling. Mosquitoes and sandfly resting in houses were collected after dusk from sixteen houses in each village and spent ten minute per house. Attempt was also made to collect mosquitoes resting on vegetation and bushes around houses. In agriculture field BPD hop cage was used to collect mosquitoes in each village. Hop cages were used ten times while collecting day-resting mosquitoes and the mosquito density was measured as average number of female mosquitoes collected per ten hop cages (PTHC) by the following formula:

Mosquito density/ten hope cage collection (PTHC) = Total numbers of female mosquitoes collected / Total numbers of hopping attempts made on vegetation × 10

Larval stages were collected from all the water source near houses using standard methodology and their density was calculated. Collected material was transported to the laboratory for identification and identification was followed by Barraud (1934), Reuben *et al*. (1994) and Rueda (2004) with the help of light microscope.

Rodent trapping, processing and examination of ecto-parasites

Rodents were collected using live traps (wire cage and wonder traps). The traps were baited with fried eatables smeared with butter and laid in the evening at pre-selected sites. Traps were collected on the next morning and brought back to the laboratory. Wild rodents were also collected from peri-domestic areas with the help of professional rodent catcher (A particular community called Mushar eat rodents after digging rat burrow in agriculture fields). Rodents collected were anaesthetized and identified after recording their different morphological characteristics. The ectoparasites were recovered by combing the rodents against the fur of rodents over a white tin pan. The snout, ears, limbs and axillary region of individual rodents were combed and ectoparasite were collected and preserved in 70% alcohol for further identification. The contents of the enamel tray were examined carefully with a hand lens and any ectoparasites seen were recovered. All preserved ectoparasites were later mounted using clearing, dehydration and mounting procedure for identification using the standard method described earlier by Kumar *et al*. (1997).

Table 1. Year wise cases of AES & JE reported during 2002-2011 and age wise distribution of encephalitis cases reported in 2011 from Muzaffarpur district, Bihar

Year	Cases	Deaths
2002	0	0
2003	0	0
2004	52	24
2005	105	42
2006	0	0
2007	NA	NA
2008	0	0
2009	0	0
2010	74	21
Up to 23-06-2011	92	32
Age wise cases, Muzaffarpur district, Bihar, 2011		
Age group (yrs.)	No. of cases	No. of deaths
0-1	3	-
2-3	32	11
4-5	33	13
6-7	15	4
8-9	5	2
10 & above	2	1
Not Known	2	1
Total	92	32
70.6% cases were between 2-5 years of age and had a higher mortality 37 %		

Table 2. Results of rodents and their ectoparasite from different villages in AES affected area of district Muzaffarpur, Bihar during May 2013

Villages	Trap laid	Trap found +ve	Rodent Species (Per cent)					Ectoparasites Collected (No. of Infested Rodent)					
			BB	RR	SM	MM	T	LD	LN	XC	Lice	Tick	T
Commensal rodents collected													
Gangapur	40	7		2-M	2-M,3-F		7	10(SM-1M), 8(SM-1F), 40(RR-2M)			6(SM-2M)	5(SM-1F)*	69 (6)
Manika Harkeshpur	40	8		1-M,4-F	3-F		8	8(RR-1M), 36(RR-4F) 31(SM-3F)					75 (8)
Bhikanpur	40	7	1-F	1-M,1-F	2-M,2-F		7	3(BB-1F), 63(RR-1M), 5(SM-1F)	1(SM-1M)	2(SM-1M)	1(SM-1M)	1(RR-1M)**	76 (5)
Jhapah	40	6		1-M,3-F	2-F		6	25(RR-1M), 8(RR-2F)		7(RR-2F)	14(SM-1F)		54 (4)
Bihtolia	40	7		4-M	2-F	1-F	7	4(SM-1F), 25(RR-2M), 2(MM-1F)		2(SM-1F)			33 (5)
Gopalpur Tarora	40	5		1-M,1-F	3-M		5	5(SM-1M), 22(RR-1F), 5(RR-1M)	1(RR-1M)	2(SM-1M)		3(SM-1M)**	38 (3)
Total	240	40	1 (2.5)	19 (47.5)	19 (47.5)	1 (2.5)	40	300 (25)	2 (2)	13 (5)	21 (4)	9 (3)	345 (31)
Wild rodents collected from peri-domestic areas of following village													
Gangapur			3-M,1F				4			1(BB-1M)			1(1)
Manika Harkeshpur			4-M				4	9(BB-2M)					9(2)
Bhikanpur			2-M,2-F				4		1(BB-1F)		1(BB-1F)	15(BB- 1F)***	17(1)
Total			12 (100)				12	9(2)	1(1)	1(1)	1(1)	15(1)	27(4)

T-Total, M-Male, F-Female, BB-*Bendicota benglensis*, RR-*Rattus rattus*, SM-*Suncus murinus*, MM-*Mus musculus*, LD- Leptotrombidium *delienes*, LN- *Lealaps nuttalli*, XC- Xenopsylla *cheopis*, * *Rhipicephalus spp., ** Haemoptysis spp. ***Rhipicephalus spp.(10) & Hyalomma* spp.(5)

Table 3. Larvae and adult mosquitoes collected from different villages in AES affected area of district Muzaffarpur, Bihar during May 2013

Villages	Mosquito Species	Hand Catch Method			Larvae Collected			BPD Hop Cage Method		
		No. Collected		Density PMH	Number collected	Pupae	Density per dip	No. Collected		Per Ten Hop Cage Density
		Female	Male					Female	Male	
Gangapur	*Cx. quinquefasciatus*	57	26	21.4	235	8	31	-	-	-
	Ar. subalbatus	2	-	0.75	75			1	1	1.0
Manika Harkeshpur	*Cx quinquefasciatus*	40	18	15.0	157	12	20.9	-	-	-
	Ar. subalbatus	5	2	1.88	52			-	-	-
Bhikhanpur	*Cx. quinquefasciatus*	84	18	31.5	144	5	19.7	-	-	-
	Ar. subalbatus	28	4	10.5	53			5	-	5.0
Jhapah	*Cx. quinquefasciatus*	68	10	25.5	134	18	17.2	-	-	-
	Ar. subalbatus	14	2	5.25	38			4	-	4.0
	An. subpictus	1	-	0.38	-				-	-
Behtolia	*Cx. quinquefasciatus*	44	8	16.5	136	25	27.2	-	-	-
	Ar. subalbatus	7	1	2.63	-			-	-	-
Gopalpur	*Cx. quinquefasciatus*	82	8	30.75	160	12	22.6	-	-	-
Tarora	*Ar.* subalbatus	4	-	1.5	66			-	-	-

RESULTS

Rodent and their ectoparasites

During the investigation rodents were collected from domestic and wild habitats. A total 240 rodent trap were laid in houses in different villages to collect domestic rodents. The overall traps positivity rate was recorded as 16.7 per cent. A total of 40 rodents in order of their prevalence were *M. musculus* Linnaeus, 1758 (19%), *B. benglensis Gray*, 1835 (19%), *R. rattus* Linnaeus, 1758 (1%) and *S. murinus* Linnaeus, 1766 (1%) were collected. The overall infestation rate of ectoprasites was recorded 77.5% (Table 2). Total of twelve wild rodents were collected with the help of professional rodent catchers by digging rodent burrows and only one rodent species viz. *B. benglensis* was collected with 33.3% overall infestation rate of ectoparasites. Village wise number of traps laid, rodent collected and ectoparasites retrieved are shown in table-2. Ectoparasites (fleas, mite, and lice) retrieved from the trapped rodents were preserved in 70% alcohol for identification and further processing. As a result of combing of the domestic rodents mites (87.5%) were the predominant ectoparisite retrieved from all the villages followed by lice, fleas and tick. Over all rodent ectoparasite index was 8.6 per rat. Total 300 vector larval trombiculid mite chiggers (*L. deliense*) were collected from the rodents (Table 2). Chigger infestation rate was found to be 12.0 per rat. The chigger infestation was found on all the four rodent species collected. The chigger index was calculated as 7.5. During the combing of these rodents 13 fleas were retrieved giving an overall flea index as 0.33. The maximum fleas were collected from village Jhapah. The flea species collected from rodents was *X. cheopis.* Similarly among the wild rodent ticks (55.6%) were the dominant ectoparasite followed by mite (37%), flea (3.7%) and lice (3.7%). Rodent infestation rate was 33.3%. Chigger infestation rate was calculated as 4.5 per rat. The chigger infestation was found mainly on two *B. benglensis* (male) collected.

Mosquitoes collection

Total 533 mosquitoes (Female-81.8%, Male-18.2%) belong to three genera viz. *Culex*, *Armigeres* and *Anopheles* was collected with help of oral aspirator and flash torch. Maximum number of mosquito belongs to *Cx. quinquefasciatus* with 86.9 per cent followed by *Ar.* subalbatus (12.9%) and *An. subpictus* (0.19%) were collected. No sandfly could be collected during the present surveillance (Table 3). Maximum number of *Cx. quinquefasciatus* females collected from Bhikhanpur village with per man hour density (PMHD) of 31.5 and *Ar. subalbatus* with PMHD of 10.5. While using BPD hop cage method only eleven *Ar.* subalbatus could be collected. Density of *Ar.* subalbatus *was 1.0 (village Gangapur), 5.0 (village Bhikhanpur) and 4.0 (village Jhapah) per ten hop cages (PTHC).* A total 1,250 larvae and 80 pupae were collected from the various water sources around house. Collected larvae and pupae were identified after emergence as *Cx. quinquefasciatus* (77.3%) and *Ar.* subalbatus (22.7%).

DISCUSSION

During survey environmental condition were very hot and dry (Temperature 24-36^{0}C and RH 54%) not supportive of mosquito/sandfly vectors. No large water stagnant could be seen around houses/villages. Resulting which density of malaria/JE vector was found to be nil or not significant. The density of kala-azar vector Phlebotomus argentipes *was also found to be very low. The observations were also substantially by the findings of adult mosquito catch by BPD hop cage method and mosquito/larval collection.*

Culex quinquefasciatus was the predominant specie collected by hand catch method from village Bhikhanpur with PMHD of 31.5 followed by village Gopalpur Tarora PMHD of 30.75 and village Japah PMHD of 25.5. *Ar.* subalbatus, vector of parasites for many human diseases like malaria, Japanese encephalitis, filariasis etc. *was another important species collected from Bhikhanpur PMHD of 10.5, Japah PMHD of 5.25 and Behtalia PMHD of 2.63. Last year observations of* Dinesh *et al.* (2013) and *Samuel* et al. *(2013) also support the present finding wherein same mosquito species were collected from different villages of Muzaffarpur district. Samuel* et al. *also tested Cx. quinquefasciatus* (8 pools), *Ar.* subalbatus *(8 pools) and* An. subpictus *(5 pools) to detect the JE antigen in mosquitoes and no pools was found positive for JEV (Samuel* et al. *(2013).* There is no documented survey of ectoparasites on rodents in AES affected areas of Muzaffarpur district, Bihar. So, present study was done to obtain data on the distribution of rodents and their ectoparasites to know the causative factors and mechanism of mysterious disease and possibilities of zoonotic diseases in Muzaffarpur district.

In domestic areas four different species of rodents were trapped and *R. rattus* and *S. murinus* were the main species rodents collected. Maximum number of rodents was collected from village Manika Harkeshpur. In the peri-domestic areas only one species of rodent (*B. benglensis*) was collected from Gangapur, Manika Harkeshpur and Bhikanpur while no rodents could be collected from Jhapah, Bihtolia and Gopalpur Tarora village. The presence of four different rodent species shows the diversity of rodent prevalence in the area with their ectoparasite (Table 2). These rodents are an important vector for zoonosis and can act as reservoirs and involved in the transmission of more than sixty infectious diseases to humans. Some examples of such diseases are plague, leptospirosis, salmonellosis, rat-bite fever, leishmaniasis, Chagas' disease, Omsk hemorrhagic fever, Murine typhus and Lassa fever (Bell *et al*., 1988), Crimean Congo Hemorrhagic fever (Inokuma *et al*., 2001, 2003). In domestic areas thirty one (77.5%) rodents were found to be infested with at least one species of ectoparasite with 41.9% male and 58.1% female.

No association between the sex of rodents to the infestation rate was noticed. Earlier similar observation was revealed by Paramasvaran *et al*. (2009) in Kuala Lumpur, Selangor, Negeri and Sembilan States of Malaysia. In peri-domestic areas 33.3% rodents were found to be infested with at least one species of ectoparasite. Domestic rodent were collected from all the village except Gangapur and Manika Harkeshpur and these rodents were found to be infested with vector of plague i.e. *X. cheopis* (Rat flea) with flea index 0.33 per rat. In peri-domestic areas only one *B. benglensis* collected from Gangapur was found to be infested with rat flea (flea index-0.08 per rat). In both the habitat flea index is used to estimate human and

epizootic risk for plague (Moore and Gage), a flea index >1 represents an increase plague risk in human (Dennis *et al*. 1999). The flea index found in the present study was 0.33 and 0.08, less than the threshold for plague transmission. Trombiculid mite chigger *(L. deliensis*) is an established scrub typhus vector recovered from all the rodent species collected from houses in all villages while in peri-domestic rodents two *B. benglensis* recovered from village Manika Harkeshpur was found infested with chigger mites. These chigger mites are habitat specific and found in abundance with forested terrain with long grasses (Saxena 1989). In the present study the chigger index found to be 7.5 (domestic rodent) and 0.75 (peri-domestic rodents) per rodent, which was above the critical level of chigger load i.e. 0.69 per rodent (Oleson and Bourgeosis 1982). Earlier during an outbreak investigation in Himachal Pradesh, Kumar *et al*. (2004) calculated the chigger index as 2.46. Mesostigmatid mites *Laelaps nuttalli* was also recovered from *R. rattus, S. murinus* and *B. benglensis* collected from Bhikanpur and Gopalpur Tarora village of Mushahari PHC. Present investigation does not support any vector borne disease but ecology and environment in AES affected areas of Muzaffarpur and nearby areas highly support the propagation and multiplication of different rodent species, rat flea (vector of plague), chigger mite (vector of scrub typhus), ticks and lice and requires further deep study to correlate entomological findings with other clinical and epidemiological studies.

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