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Entomological Studies Vector Abundance and other related aspects of vector ecology of *Phlebotomus argentipes* in highly VL Priority blocks in Madhepura, Bihar, India

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ABSTRACT

Kala-azar is caused by Protozoan *Leishmania donovani* and transmitted by the sandfly vector *Phlebotomus argentipes*. Some ecological and environmental conditions of the local areas are responsible for the endemicity of the disease. Madhepura district is one of the endemic districts for Kala-azar is Bihar. The highly VL affected blocks Gamharia, Ghailar and Udakishunganj were selected for the study. The man hour density of the Kala-azar vector *P. argentipes* was found 2.5 and 6.2 in insecticide sprayed village and 1.9 to 9.1 in control village. The bionomics of the established vector *P. argentipes* was studies. Presence of vector species in these highly endemic villages depicts the possibility of continuous transmission throughout the year. The finding will be helpful in making the effective control measures to control sandfly density in endemic area of Kala-azar.

Key Words - Protozoan, *Leishmania donovani, Phlebotomus argentipes,* endemic

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INTRODUCTION

Kala-azar is caused by a Protozoan *Leishmania-donovani* and transmitted by the sand fly vector *Phlebotomus argentipes* (Diptera; Psychodidae) Swaminath *et al.* (1947), Dinesh *et al.* (2000). The parasite primarily infects reticuloendothelial system and may be found in abundance in bone marrow, spleen and in liver. Sand fly of genus *Phlebotomus argentipes* are the only known vector of kala-azar in India. Indian kala-azar has a unique epidemio logical feature as it is anthroponotic and human is the only known reservoir of disease.

Kala-Azar Global Scenario

The *Leishmaniasis* is endemic in ninety-seven countries and territories and 4 countries have previously reported cases. Over 616 million population lives in endemic areas at risk of infection. An estimated 147 million people in 119 districts in 4 countries namely, Bangladesh, Bhutan, India and Nepal are at risk with an estimated 1,00,000 new cases each year.

India

Kala-azar is endemic in 54 districts in the India country of which 33 districts of Bihar, 4 district of Jharkhand, 11 district of West Bengal, besides occurrence of cases in 6 districts of eastern Uttar Pradesh. Sporadic cases are also reported from Assam, Himachal Pradesh, Kerala, Madhya Pradesh, Sikkim and Uttrakhand. Imported cases have been reported from Delhi, Gujarat and Punjab.

Bihar

The state of Bihar out of 38 districts 33 are affected. The population at risk is 99 million in approximately 12000 villages spared over 458 blocks. 88% of these blocks have achieved elimination target by end of 2020. 10 districts out of 38 affected districts 200 or more cases annually and contributed to about

50% cases of the state. Kala-azar cases are spared across from highly endemic district which are located in the flood plains of North Bihar.

Area of study

Madhepura has remained one of the endemic districts of Bihar. The district is occupied an area of 1787 km^2 surrounded by Araria and Supaul district in the north, Bhagalpur in the south, Purnia in the east and Saharsa district in the west. Madhepura is situated in the plains of River Koshi and located in the north eastern part of Bihar at longitude between 25^0.34' to 26^0.07' and latitude between 86^0.34' to 87^0.07' with 95.4%. Rural population affected by flood, famines and drought.

Map of Bihar

Figure - District Map Madehpura (Bihar)

The average rain fall, climate, air temperature and humidity data of month wise are as :-

Monthly mean highest and lowest rain fall (No. of rainy days) along with standerd deviation (SDJ and Co-efficient of variation (CV) of the Madhepura district.

Name of the month	Lowest Temp.	Highest Temp.	Mean Temp.	SD (Standard deviation)	Co-efficient of variation (CV)
January	0.0(0)	72.9(6)	15.9(1.1)	18.4(1.5)	116(125)
February	0.0(0)	40.0(4)	11.4(1.1)	13.1(1.3)	115(119)
March	0.0(0)	91.2(6)	20.5(1.5)	29.1(1.8)	142(121)
April	0.0(0)	110.3(7)	38.7(2.8)	32.2(2.1)	83(76)
May	0.0(0)	246.4(20)	119.6(8.1)	38.4(4.7)	57(57)
June	0.0(0)	512.3(25)	241.8(13.2)	124.7(5.5)	52(41)
July	140.7(10)	735.3(27)	386.5(17.8)	149.4(4.9)	39(27)
August	160.6(8)	592.3(25)	330.6(17.4)	137.0(4.7)	41(27)
September	103.3(5)	1074.0(29)	266.2(13.7)	193.4(5.4)	73(40)
October	0.0(0)	366.4(17)	912.0(5.2)	85.2(3.2)	93(62)
November	0.0(0)	147.7(9)	12.0(0.9)	30.5(1.8)	253(205)
December	0.0(0)	73.8(13)	11.8(1.3)	17.3(2.5)	146(189)

The maximum temperature of the district ranges from 35^{0}C to 45^{0}C and the minimum temperature varies from 7^{0}C to 9^{0}C.

Climate & Weather average chart of the district

Name of the month	Lowest Temp.	Highest Temp.	Mean Temp.	Precipitation (m.m)	Humidity (%)
January	09^{0}C	22^{0}C	15^{0}C	7.3	73
February	13^{0}C	27^{0}C	20^{0}C	11.2	64
March	17^{0}C	33^{0}C	25^{0}C	10.0	50
April	22^{0}C	37^{0}C	30^{0}C	11.6	43
May	25^{0}C	38^{0}C	32^{0}C	41.8	53
June	27^{0}C	37^{0}C	32^{0}C	105.9	65
July	27^{0}C	34^{0}C	30^{0}C	185.9	78
August	27^{0}C	34^{0}C	30^{0}C	185.3	79
September	26^{0}C	33^{0}C	30^{0}C	118.8	78
October	22^{0}C	32^{0}C	30^{0}C	41.1	72
November	16^{0}C	29^{0}C	27^{0}C	1.1	69
December	11^{0}C	24^{0}C	22^{0}C	0.3	74

- **Major drainages -** The major drainages are Koshi dhar
- **Major Soil type -** Sandy, loamy, loam, silty loam

District - Yearly Kala-Azar Table

Table-1: Year wise cases of Kala-azar in Madhepura district:

Year	2013	2014	2015	2016	2017	2018	2019	2020
Cases of Kala-azar	497	299	222	151	117	66	31	17

The study areas were highly endemic PHCs like Gamharia, Ghailar and Udakishanganj. Cases of Kala-azar were also declining in these PHCs from 2015-2020 (Table-2)

Table-2: Year wise cases of Kala-azar PHC of study area

PHC	2015	2016	2017	2018	2019	2020
1. Gamharia	16	11	10	03	06	02
2. Ghailar	17	10	11	07	05	02
3. Udakishunganj	15	15	22	06	03	02

Year wise cases of Kala-azar and prevalence of sandflies Kala-azar cases were recorded and was found drastic declination in the number of cases from 2015 to 2019 and reached to zero level during 2020. It is good indication towards elimination of Kala-azar (Table-3).

Three villages were selected for test and one as control based on insecticide spray for control of sand flies like (1) Jogbani (2) Bhatrandha (3) Nayanagar (4) Jajhat. The study was conducted during years 2018 to 2020.

Table-3: Year wise cases of Kala-azar in study villages

Village	PHC	2015	2016	2017	2018	2019	2020
1. Jogbani	Gamharia	01	03	03	02	02	0
2. Bhatrandha	Ghailar	08	06	03	03	0	0
3. Nayanagar	Udakishunganj	00	00	11	03	0	0

MATERIAL AND METHODS

Resting sandflies were collected from indoor houses and cattle shed using mouth aspiration flashlight early in the morning each month. Sandflies were brought in the PG Department of Zoology, BNM University, Madhepura) and scrutinized under microscope. Sandflies were identified using the key of Kalra and Bang (1988). Data of *P. argentipes* were recorded and the specimens were preserved in 70% alcohol inside micro tubes (200 ml) for further studies. A portion of live female *P. argentipes* was confined in the sandfly rearing pots for further study on their developmental studies. All developmental stages were observed closely for the maintenance of the colony on optimal temperature and humidity i.e., 26-28°C and relative humidity more than >72%. The case history of Kala-azar was collected from respective PHCs of the village and verified with house-to-house search in the study villages.

RESULTS

Kala-azar is under elimination target, hence with implementation of effective control measures, the decline trend of cases of Kala-azar was found (2015-2020) Table (1).

The average seasonal density from 2018-2020 indicated high number of vector density (per man hour density) in summer season in comparison to winter and rainy season. Low density of sandflies was found in winter season (Table-4). The density of sandflies was found higher in control village in comparison to insecticide sprayed villages. The insecticide (alphacypermethrin) was used as indoor residual spray for control of sandflies.

Table 4- Sandfly Collection Status

Sl. No	Name of the PHC	Name of Village	Male		Female		Total P. argentipes (M+F)	Total sergetimeniys	Total Sandfly
			Fed	Unfed	Fed	Gravid			
1	Gamharia	Jogbani	0	0	1	0	2	9	12
			1	0	0	0	1	0	2
2	Ghailar	Bhatrandha	0	0	0	0	0	0	0
			1	0	0	0	1	4	6
3	Udakishunganj	Nayanagar	1	0	3	0	4	0	8
			0	0	0	0	0	0	0
		Total	3	0	4	0	8	13	28

Table 5- Average season wise density of *P. argentipes* (Man Hour Density) in study and control villages in year 2018 to 2020

Village	Summer	Winter	Rainy
1. Jogbani	6.2	3.1	4.5
2. Bhatrandha	5.9	2.3	3.6
3. Nayanagar	8	2.5	7.1
4. Jajhat	9.1	1.9	7.5

Seasonal Prevalence

As already mentioned, high relative humidity, warm temperature, high sub-soil water and abundance of vegetation favors proliferation of *P.argentipes* and accordingly, depending on throughout the year in majority of areas of prevalence with complete absence in winter months with humidity, the density increases till June with sudden decline due to high temperature. It is again followed by increasing trend reaching the maximum during and just after the monsoon rain. A similar trend has been reported in Bihar with a minor peak in March/April and a major peak in August/September in densities of *P.agrentipes*.

DISCUSSION

The density of sand flies depends on microclimatic and ecological conditions at particularly where moisture contains are high and soils are alluvial. Such conditions are prevalent in the villages of Bihar established on the bank of rivers like Ganges, Koshi etc. Madhepura is one of them situated on the bank of Koshi and having alluvial soil i.e., suitable for moisture contains as it was found in Bihar and in some parts of Jharkhand, India. Bihar is expended in total area of 94,163.00 sq. kms and the population is 8,28,78,796 situated in the lower and middle

Gangetic region. The annual temperature varies from 6°C to 43°C. Normal rainfall is 1,205 mm.

The study was made keeping in view finding out the presence or absence of vector species in the villages where indoor residual spray of alpha cypermethrin was continuing in comparison with the unsprayed village to see the sandfly density, bionomics and its associated factors like their density, species composition, ecology of house, nature of soil and association of human and cattle. Sand flies prefer animal blood, dark and humid places. The house is generally made of mud with tiled roof. The rooms inside are very congenial to sand flies hence the density was found very high. In Bihar animals are being kept mostly in small huts or in open sky or partial shed and sometimes inside room that are very suitable environment for breeding to sand flies. Sand flies prefer cattle blood either from domestic or peridomestic situations. For the resting and breeding sites of sand flies three types of soils are essential which is found in all endemic areas i.e., alluvial, swamp and terai having pore capacity to hold water molecule for longer period i.e., prime requirement for the development of larvae and resting of adult sand flies. Hence, the disease transmission is frequent in these particular areas since more than century. Sand flies of two genus of *Phlebotomus* sp (*P. argentipes and Sergentomyia* (*Sergentomyia* sps.) were also collected.

The high density of sand flies was found in endemic area of Bihar in comparison to epidemic zone due to high contact rate with sand fly which provide protection by inoculating saliva protein in human body (Kumar *et al.,* 2009, Valenzuela *et al.,* 2001, Siva *et al.,* 2005, Clement *et al.,* 2009). Houses were made of mud and tiled roof sharing with cattle in separate room inside same premises. Sometimes cattle are in the same premises or outside the house. The house was made of brick with mud and tiled roof, a few muds with tiled roof and huts. The rooms were ill ventilated, larger and dark at Jharkhand in comparison to Bihar. It seems that presence of sand flies is not mare dependent on the nature of soil. It may present in any endemic or nonendemic area under congenial microclimatic conditions in association with cattle. The nature of house depends on socioeconomic conditions of the population. In a study among low socio-economic groups. Bihar the houses were found made of soil and bamboo sticks; some houses were made of bricks without cement plastering. However, with few exceptions, Kala-azar cases also occurred in concrete houses in north and south Bihar. Both areas have some soft stem vegetation, such as plant of *Musa sapientum*, creepers etc. in the peridomestic region that resembles with the study as risk factor for Kala-azar transmission (Ranjan *et al.,* 2005). The potential indoor breeding sites like cracks and crevices of wall can be sealed by using brick chimney fly ash and lime to get environmental control of sandfly population (Dinesh *et al.,* 2017).

The study proves that *P. argentipes* requires the micro- climatic conditioned irrespective of geographical distribution. The diversity is quite distinct in the environmental factors, ecological conditions and sand fly status in both states. There may be sibling species of among *P. argentipes* which might be different due to different geographical distribution or morphologically different that can be assessed to know the factors associated with transmission of the disease. (Dinesh *et al.*, 2005)

This study will lead for extensive study in finding the potentiality of *P. argentipes* of both regions in transmission of the disease. The programme should be aware for presence/absence of vector species of Kala-azar in endemic and nonendemic zone for implementation of effective control measures.

CONCLUSION

The presence of critical density of sandfly vector in the locality is also a major factor in the transmission of the disease. The Man Hour Density (MHD) of the Kala-azar vector; *Phlebotomus argentipes* was found 6.2 and 2.5 in insecticide sprayed villages in comparison to unsprayed village was 1.9-9.1. Factors responsible for the transmission of the disease were present in endemic zone of Kala-azar in Madhepura district of Bihar, even the cases are reducing 497 (2013) to 17 (2020). Presence of vector may increase the number of cases in future.

Hence vector surveillance and control strategies need to be strengthening. The study on seasonal abundance of vector population is required for implementation of effective vector control measures in due time. The effectiveness of IRS may be reviewed in terms of vector density Pre and Post.

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