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Bionomics of *Phlebotomus argentipes* (Diptera: Psychodidae) in High Endemic Madhepura District of Bihar, India

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A B S T R A C T

Visceral Leishmaniasis is a major health problem of Bihar, India. It is under elimination mode. However, the transmission of the disease is continuing. Some ecological and environmental conditions of the local areas are responsible for the endemicity of the disease. Madhepura district was one of the highly endemic districts for Kala-azar in Bihar. The highly affected blocks Gamharia, Gaihar and Udakishanganj were selected for the study. The cases of Kala-azar declined from 2013 (n=497) to 2020(n=17).The presence of critical sandfly density vector in the locality was also one of the major factors in the transmission of the disease. The Man Hour Density (MHD) of the Kala-azar vector; *Phlebotomus argentipes* was found 2.5 and 6.2 in insecticide sprayed villages and 1.9 to 9.1 in control village during 2018-20. The bionomics of the established vector *P.argentipes* was studied in detail with the developmental stages by rearing in the laboratory. The life cycle was completed within one month time at optimal temperature, 26-28^0 C and humidity, >72%. Sand flies were found scattered in side dwellings in whole areas of endemic regions and confined in dark mud house having cattle sharing the room of human dwelling. All factors responsible for the transmission of the disease like the presence of infected host, ability of vector in the region to pick up parasite, proper development of parasite in the gut of vector and its transmission in fresh host under suitable environmental conditions were present. The findings will be helpful in making the effective control measures to control sandfly density in endemic areas of Kala-azar.

Introduction

Visceral leishmaniasis (VL) / Kala-azar is a neglected tropical disease prevalent in more than one-third of the 200 countries or territories reporting VL cases to WHO, including 45% of countries in the South-east Asia region (WHO, 2018). Kala-azar is caused by a Protozoan parasite *Leishmania donovani* and transmitted by the sand fly vector *Phlebotomus argentipes* (Diptera; Psychodidae) (Swaminath *et al.*, 1947; Dinesh *et al.*, 2000). The Parasite Primarily infect reticuloendothelial system and may be found in abundance in bone marrow, Spleen and in liver. Sand fly of genus *P. argentipes*

is the only established vector for Kala-azar in India and its subcontinents. Indian Kala-azar is anthroponotic and human is the only known reservoir of the disease. In India, the four states of Bihar, Jharkhand, Uttar Pradesh and West Bengal are endemic to VL, while some sporadic cases are reported in other areas. Between 2015 and 2018, 4380–8500 new VL cases including post-Kala-azar dermal leishmaniasis (PKDL) cases were reported in India (NVBDCP). In 2019, 633 administrative blocks in 54 districts were endemically reporting a total of 4072 cases comprising of 3128 VL cases, 817 VL-PKDL cases and 127 VL- human immunodeficiency virus cases (WHO, 2020). VL was targeted to be eliminated in India by 2015 (WHO, 2005), but despite substantial progress made is still prevalent in many districts in the country. A new global road map for the elimination of 20 NTDs, including VL, aims to eliminate VL by 2030 (WHO, 2020).

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

The population at risk in Bihar are 99 million in approximately 12000 villages spared over 458 blocks. 88% of these blocks have achieved elimination target by end of 2020 but not achieved as yet by 2021.

10 districts out of 38 affected districts are highly affected and shares 200 or more cases annually which are 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.

Study area

Madhepura District (Map): This is one of the high 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. 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.

Materials 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 district hospital (Malaria office/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. 26-28^0 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 and Discussion

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 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)

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).

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 were 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.

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*.

Feeding behavior

All species of sandflies feed on plant sugar and the female soften feed on vertebrate host including man. The females of genus Phlebotomus feed on mammals. Though *P. argentipes* are commonly known as zoophilic, it has been observed by various workers that the anthropohilic index whereas the samples collected from cowshed in the same are showed only 21.6% anthropophily. The same was true in the case of bovid blood index which was 96% in the cowshed and 48% in the human dwellings. This indicates clearly that *P. argentipes* are primarily indiscriminate (opportunistic) feeder and the type of blood meal largely depends on availability of host in its immediate vicinity.

Flight range

Sandflies are not capable of flying very long distances. Their usual mode of flight pattern is a series of short erratic hopping in which the fly usually covers a distance less than ½ meters. Further, the dependence of most of the vectors of humidity for their survival limits their movements.

Resting Sites

The most favored resting sites for sand flies include soil cracks and crevices, burrows (rodent burrows), tree holes, termite hills, caves, bird tunnel, in earthen mounds, understone and foliage, etc. In eastern parts of India like Bihar & West Bengal, *P. argentipes* prefers to rest indoors, about 9-10 times higher in cattle dwellings than the human dwellings. In western India sand flies rest outdoor also in considerable numbers. As could be seen, all these resting places provide them dark and damp shelter where the microclimatic humidity is very high. They usually leave these shelters at dusk and are active in open in the evening and night. Usually sand flies remain active throughout the night but they are sensitive to decreasing temperature and air currents. Even a gently breeze of 1.5-2 metre/second may greatly reduce the inactivity.

Longevity

In India, *P. argentipes* have been reported to undergo 5 geotropic cycles under laboratory conditions, duration each cycle being 4 to 5 days at 26+ 2^0 C i.e. a minimum longevity up to 23-27 days under laboratory conditions. Field studies conducted on party have also shown presence of triparouses and "fourparous" females in nature indicating probability of longevity under field conditions to be upto 16-20 days in a proportion of natural population. However, longevity in field is dependent on ecological factors.

Immature stages

Egg

The freshly laid eggs are creamy white in colour which later becomes dark. The eggs are usually deposited in cracks and crevices with high organic content, humidity and darkness. Sometimes eggs are also found in loose soil.

The eggs are glued to the surface through flattened while the convex side faces upwards. The eggshell has sculptors, and their size varies from 0.336 - 0.432 mm x 0.096 - 0.160 mm. A wide range has been observed for total number of eggs laid per female (5-68). The eggs hatch in 3-4 days at $26+2^0$ C in laboratory.

Larva

The creamy white larva with distinct head, thorax and abdomen has numerous hairs on its body. The larva feeds on organic matter available in the soil. There are four larval stages:

Instar -I: The delicate larva is whitish with a brown head capsule lacking eyes. A pair of black caudal bristles and presence of egg breaker on the posterior portion of head are the characteristic features of I instar. The average life is 2-4 days.

Instar-II: Presence of 2 caudal bristles, round 3rd antennal segment and absence of egg breaker are the features of II instar which lives for about 2-5 days.

Instar-III: Presence of 2 caudal bristles on completely dark last abdominal segment, practically elongated 3rd antennal segment and yellowish body hairs on a well developed larva help in identifying III instar which lasts for about 3-4 days.

Instar-IV: It is a well developed brown larva with dark brown head capsule, elongated, oval 3rd antennal segment with a pointed seta. Two pairs of spiracles and two pairs of well developed caudal bristles are conspicuous. The stage lasts for 4-7 days and transforms in to pupa. The total larval period may vary from 11-29 days.

Pupa

The elongated comma shaped pupa is milkly white in the beginning and turns brown. It is a non feeding stage lasting for about 6-10 days. The sexes are differentiated in this stage. The total life cycle from egg to adult is reported to take about 20-36 days with average 26.75 days in *Ph. argentipes* in laboratory.

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 Gangase, 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 6^0 C to 43^0 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 alphacypermethrin 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.

Fig.1 Map of Bihar

Fig.2 Map of Madhepura

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

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

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

Table.4 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

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 P. papatasi*) 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 mud 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.

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 strengthen.

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References

Clements, M F, Gidwani K, Kumar R, Hostomska J, Dinesh D S, Kumar V, Das P, Muller I, Hamilton G, Volfova V, Boelaert M, Das M L, Rijal S, Picado A, Volf P, Sundar S, Davies C Rogers ME. Measurement of recent exposure to *Phlebotomus argenti-opes*, the vector of Indian Visceral Leishmaniasis, by using human antibody responses to sand fly saliva Am J Trop Med Hyg, 2010; 82(5): 801-07.

Dinesh D S, Kar S K, Kishore K, Palit A, Verma N, Gupta A K, Chauhan D S, Singh D, Sharma V D & Katoch VM (2000). Screening sand flies for natural infection with *Leishmania donovani*, using a non-radioactive probe based on the total DNA of the parasite. Annals Trop Med Parasitol, 94: 447-451.

Dinesh D S, Kishore K, Singh V P, Bhattacharya S K. Morphological variations in Phlebotomus argentipes Annandale & Brunetti (Diptera: Psychodidae). J Commun Dis., 2005; 37(1): 35-8.

Dinesh D S, Kumari S, Hassan F, Kumar V, Singh V P and Das P. Efficacy and evaluation of environ-mental management system to control sandfly vector of Kala-azar, J Environ Manage, 2017; 201: 366-368.

Kalra N L and Bang Y H (1988) Manual on entomology in visceral leishmaniasis, World Health Organisation SEA/VBC/35

Kumar V, Kesari S, Kumar A J, Dinesh D S, Ranjan A, Prasad M *et al.,* Vector density and the

control of kala-azar in Bihar, India. Mem Inst Oswaldo Cruz, 2009; 104: 1019-22. http://dx.doi.org/10.1590/S0074-02762009000700014.

National Vector Borne Disease Control Programme. Kala-azar situation in India [Internet]. Delhi: National Vector Borne Disease Control Programme (Available from: https:// nvbdcp.gov.in/index4.php?lang=1&level=0 &linkid= 467&lid=3750).

Ranjan A, Sur Dipika, Sing V P, Siddique N A, Manna B, Lal C S, Sinha P K, Kishore K, Bhattacharya S K. Risk factors for India kala-azar. Am J Trop Med Hyg, 2005; 73: 74-78.

Silva F, Gomes R, Prates D, Miranda J C Andrade B, Barral-Netto M, Barral A. Inflammatory cell infiltra- tion and high antibody production in BALB/c mice caused by natural exposure to *Lutzomyia longipalpis* bites. Am J Trop Med Hyg, 2005; 72: 94-8.

Swaminath C S, Shortt H E, Anderson L A. Transmis-sion of Indian Kala-azar to man by bites of *Phlebotomus argentipes* Annandale & Brunnetti. In-dian J Med Res., 1942; 30: 343-47.

Valenzuela G J, Belkaid Y, Garfeild M K, Mendez S, Kamhawi S, Rowton E D, Sacks D L, Ribeiro J M. Toward a defined anti-leishmania vaccine targeting vector antigens: characterization of a protective sali-vary protein. J Exp Med, 2001; 194: 331-42.

World Health Organization. Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030. World Health Organization: Geneva, 2020. (Available from: https://apps.who.int/iris/ha ndle/10665/338565).

World Health Organization. Independent assessment of kala-azar elimination programme India [Internet]. World Health Organization, Regional Office for South-East Asia: New Delhi; 2020.

World Health Organization. Regional strategic framework for elimination of kala-azar from the South-East Asia region (2005–2015). World Health Organization: New Delhi, 2005.

World Health Organization. Surveillance of leishmaniasis in the WHO European Region, 2016 and global leishmaniasis surveillance update, 1998–2016. WklyEpidemiol Rec 2018:40:521–540

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