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Seasonal distribution of phlebotomine sand flies-vector of visceral leishmaniasis

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The results of entomological studies, mainly seasonal distribution of Phlebotomine sand flies-vector of visceral leishmaniasis (kala-azar), carried out in Dohar village of Dhanusha district in the year 1983 and in Bansbari of Morang in 1990/91 are presented. It is noted that although the high resting density of Ph. papatasi with two peaks - one before and other after monsoon were observed in both the places, the man biting rate of Ph. argentipes, the implicated vector of kala-azar in India, was high with only one peak starting from April extending upto November in Bansbari. The cases of kala-azar as recorded by Hospitals and District Public Health Offices in Nepal for the years from 1981 to 1990 are presented. The district wise case records from 1985 to 1990 are also given, which shows the spreading of cases towards hill and west. Lastly, it is strongly recommended that before starting the programme of prevention and control of the disease a well planned epidemiological study including of various entomological aspects should be carried out to avoid ill fate already faced by other global eradication programme.

INTRODUCTION

The Visceral Leishmaniasis, which is also known as *Kala-azar (the black sickness)*, is a disease caused by protozoal parasite-*Leishmania Donovani* and transmission is maintained through the bites of infected sand flies. This disease has been a public health problem and plagued mankind since very long time.

The disease had long been confused with malaria and not clearly identified in ancient Hindu medical writings (Jaggi, 1979). The first outbreak, which epidemiologists and medical historians are of concensus that it was kala-azar had its epicenter in Jessore in 1824 where took some 75,000 souls. The epidemic progressed and reached Burdwan in 1862. From 1872 to 1882 it progressed towards North Bengal, Bihar and Assam. In all those epidemics the mortality was so high that the dead were left in their houses or thrown into beels or rivers. Thus the vast region, the Gangetic Plain was (and is) the landscape epidemiology of Indian kala-azar although there have been outliers of discrete foci in Tamil Nadu, Andhra Pradesh and Orissa (Desowitz, 1988). The parasite was only discovered in 1900 by William Leishman in

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spleen smears of a soldier who died from a fever contracted at Dum-Dum, India known as Dum-Dum fever or kala-azar. This observation was published in 1903. In this very year Charles Donovan found the same parasite in smears made from splenic puncture. Patton in 1907 proved that the leishmania stage could be found in wandering histiocytes in the peripheral blood and that leptomonas stage forms occurred in the intestine of insects fed upon kala-azar patients. The actual transmission of *L. Donovani* by the sand fly *Phlebotomus argentipes* was demonstrated by the Indian Kala-azar Commission (1931-1934). Recently Goverdhan et al (1976) reported isolation of Phlebotomus fever virus from sandflies and humans during the same seasons in Aurangabad, Maharastra, India.

For Nepal no reliable record is available. Raghvan (1953) after a survey in 1949 concluded that kala-azar was endemic in the entire Nepal Terai.

However this disease ceased to be a problem in almost all the areas of its distribution, whenever antimalaria operations with insecticide spraying were started, including in Nepal in 1959. As the malaria eradication programme progressed the insecticide spraying was withdrawn from many parts in India as well as in Nepal. This is probably the reason the disease assumed epidemic in North Bihar 1975-76, the maximum being in 1977.

The senior author first saw a case of kala-azar in Dhanusha district while investigating malaria in the year 1981. In the year 1982 five cases of kala-azar were reported by Janakpur Zonal Hospital, Dhanusha.

This arose the interest to study about the disease in the country. First such study was carried out in Dohar, Dhanusha in 1983, while he was still working for Malaria Eradication Programme (NMEO). Similar such studies was again conducted in Bansbari village of Morang district in 1990/91 with the financial support from TDR.

Epidemiological study, although is very important, was not included in the present studies.

Situation of Kala-azar in Nepal

The record is not available which might show the magnitude of the problem before the initiation of the DDT spraying by NMEO in 1959. The first ever published report on health and health administration in Nepal by Directorate of Health Services in 1969 did not even mention about kala-azar or visceral leishmaniasis. However, the practitioners in terai region recall seeing kala-azar cases in the course of their practices.

During the present study period i.e. 1990/91 the efforts were made to gather the record of kala-azar cases from hospitals and district public health offices as far back as possible. Desowitz (1988) stated that there were 557 cases with 47 deaths during the period from 1981-85. He also stated that the status of kala-azar in Nepal is even less clear than in Bangladesh. Recently, again Division of Epidemiology published the record for the period from 1980 to 1992. Given below are the record from those two sources:

Table No. 1: Showing the status of kala-azar in Nepal from 1980 to 1990.

Year	Number of cases	Number of deaths
1980	51	2
1981	133	1
1982	266	35
1983	60	4
1984	94	5
1985	60	0
1986	110	6
1987	127	8
1988	443	1
1989	283	5
1990	429	4

Some descrepancy in record published by division of Epidemiology was noted about 446 in 1990, but our record shows only 429.

The district wise distribution of cases in Nepal is presented below for the period from 1985 to 1990.

Table No. 2 : Showing district wise distribution of kala-azar 1985-90.

Districts	Broader topography of districts	Number of recorded cases in the years					
		1985	1986	1987	1988	1989	1990
Jhapa	Terai	0	0	0	1	2	0
Morang	Terai	16	36	27	212	153	85
Sunsari	Terai	1	0	0	0	0	11
Saptari	Terai	4	4	13	20	52	29
Siraha	Terai	21	53	65	178	26	73
Dhanusha	Terai	9	10	13	28	37	120
Mahottari	Terai	1	0	3	0	1	16
Sarlahi	Terai	2	0	0	0	0	64
Rautahat	Terai	1	0	0	0	0	11
Bara	Terai	0	0	0	4	3	6
Banke	Terai	3	4	0	0	0	0
Makawanpur	Valley	0	0	0	0	2	0
Surkhet	Valley	0	0	1	0	0	0
Sankhuwasabha	Hill	1	2	0	0	0	0
Dhankutta	Hill	0	0	1	0	0	0
Solukhumbu	Hill	1	1	2	0	0	0
Okhaldhunga	Hill	0	0	2	0	7	14
Total		60	110	127	443	283	429

From the tables 1 and above it is apparant that there does not seem to have any definite trend or pattern of case occurrences. This might probably be due to non-existence of the case detection mechanism or the programme responsible coupled with some remedial measures, which were taken irregularly in time and spaces under political pressures only. It also shows that the problem is spreading westward and hills. At least five cases in Okhaldhunga were found to be autochthonous occuring at an elevation of about 1200 - 1225 meter above MSL.

Entomological Study

As in any vector borne diseases the study of vector, parasite and human beings are necessary to understand properly the epidemiology of kala-azar for proper planning and control of the disease. In this respect the disease kala-azar is the one least studied. The reason is that the disease remained in dormant state during mass campaign against malaria and also the priority given to other diseases like Japanese viral encephalitis. Now the disease came back in many countries and the studies are underway.

But in Nepal no systematic study towards prevention and control of the disease is yet planned. Some entomological studies were undertaken and the results are presented herein. First entomological study was conducted in Dohar village of Dhanusha district and second such study was carried out in Bansbari village of Morang district. Unfortunately enough on both occasions seasonal distribution studies could only be carried out. In Bansbari night landing catches were also included.

The considerations for selecting those villages were (a) workable density of flies, (b) accessibility throughout the year, and (c) recent cases of kala-azar (VL) in the case of Bansbari, Morang.

Description of Study Villages

The village Dohar is a small one with only 38 houses and 40 other structures, which were used as animal sheds and barns. The total population of the village were 230 in the beginning of 1983. All the houses were mud-walled with thatch roofs. There was a pond of about 3000 sq. M in the middle of the village. This pond was used for fish farming and makhan cultivation. Besides there were lotus, eichornia sp., and pistis sp. There is also a small stream flowing north south which was rich of aquatic vegetation.

The village Bansbari is comparatively large with 175 houses and only 33 other structures used as animal sheds and barns. The total population of the village was 960 as per census taken during the work. The houses were varying sizes and made from different materials. There were a few well constructed cemented houses but most of the houses were mud-walled with thatch roofs. There were five ponds of varying sizes. There is also a long irrigation canal. All these water bodies were rich of aquatic vegetation.

Both the villages, Dohar and Bansbari, are within 15 km. from Nepal-India border and bordering with Sitamarhi and Jogbani districts of Bihar state of India respectively.

The main crop of both of those districts are paddy. So far occupation is concerned the people of Dohar largely depend upon agriculture, whereas of Bansbari about 30 percent of the adult population work in factory and other merchandise.

METHODOLOGY

Daytime resting collections:

This involved the collections of flies from inside premises with the help of aspiration tubes and flash lights in the early morning hours from 0600 - 0900. In each day of collections the captures from 4 human dwellings, 2 animal sheds and 2 mixed dwellings were made by each collectors. Such collectors were only two in Dohar one is the present senior author with one other Entomologist. Whereas in Bansbari 8 collectors were employed part time to work. In Dohar only one morning collections were made every month and in Bansbari three morning collections and 2 night landing collections were made every month.

The collections were kept separate for each dwellings in test tubes and plugged with cotton wool. They were thus transferred to the laboratory at Janakpurdham in Dhanusha and Biratnagar in Morang.

Night landing catches:

The night landing catches were carried out only in Bansbari, Morang. For this the collectors were divided into 4 teams of 2 collectors each. Two such teams were grouped together under one group leader (Assistant Entomologist) each. In each group 2 bait collectors were kept inside and 2 outside of houses and they were exchanged at midnight. The collections were carried out from dusk to dawn next morning (i.e. 18.00-06.00 hours). Two such collections were carried out every month. The collections were kept separate for each type of sheds and hours of collections in test tubes. They were transferred to the laboratory at Biratnagar for further processing.

Processing of collected samples:

In the laboratory flies from one dwelling only were anaesthetised at a time with chloroform. Then they were examined for recumbent hairs (Genus Sergentomyia) or erect hairs (Genus Phlebotomus) in the thorax and abdomen under sterioscopic microscope. Phlebotomus flies were further identified to the species using identification key proposed by Lewis, 1978. When felt necessary other literature were also consulted. Sergentomyia flies were kept in 10 dr. vials and examined at later dates in Kathmandu.

DNA probing for Leishmania donovani

Only in Bansbari Ph. argentipes were crushed onto DNA hybridization membrane with proper record. The membrane were received from Dr. Richard Lane and he was also kind enough to process by DNA probing in London School of Hygiene and Tropical Medicine, London for examination of leishmania infection.

RESULTS AND DISCUSSIONS

Daytime resting collections:

In Dohar the collections were carried out every month from January to December 1983 except in March, when collections could not be carried out due to unavoidable circumstance. In Bansbari they were carried out from May 1990 to April 1991, but arranged below in tables from January to December to ease caparison. The Dohar data is included here as they are not yet published and so to keep in record. Further it may be noted that the unidentifiable specimens from Dohar were turned out to be *Ph. papatasi*. This is so happened because of the techniques used in making permanent slides.

The collection record of *Phlebotomus* flies only are presented below in table no. 3.

From the collections given above in table no. 3 the per man hour density of Phlebotomus papatasi and Ph. argentipes were calculated and tabulated below in table no. 4.

Table No. 3: Showing collections of female Phlebotomine sand flies in Dohar, Dhanusha (Jan.–Dec. 83) and Bansbari, Morang (May. 90–Apr. 91.

Months	Dohar, Dhanusha					Bansbari, Morang			
	Man hours spent	Number collected				Man hours spent	Number collected		
		Total	pap.	arg.	ser.		Total	pap.	arg.
Jan.	16	6	3	0	3	26.00	1	1	0
Feb.	8	2	2	0	0	24.75	0	0	0
Mar.	--	--	--	--	--	24.00	10	9	1
Apr.	4	97	82	10	5	24.50	316	297	19
May	8	147	135	5	7	25.07	121	112	9
Jun.	4	91	88	3	0	27.07	202	168	34
Jul.	4	76	72	3	1	28.78	294	257	37
Aug.	4	61	61	0	0	28.14	184	147	37
Sep.	4	122	117	2	3	26.45	222	179	43
Oct.	4	38	37	1	0	28.50	247	219	28
Nov.	4	48	46	0	2	28.66	204	183	21
Dec.	6	56	55	1	0	25.40	39	30	9
Total	66	744	698	25	21	317.32	1840	1602	238

pap. = *Ph. papatasi* arg. = *Ph.* argentipes and ser. = *Ph.* sergenti

Table No. 4: Showing per man hour density of *Phlebotomus papatasi and Ph. argentipes* in Dohar, Dhanusa and Bansbari, Morang.

Months	Per man hour density of Phlebotomus					
	Dohar, Dhanusha			Bansbari, Morang		
	Total	papatasi	argentipes	Total	papatasi	argentipes
Jan.	0.37	0.19	0	0.04	0.04	0
Feb.	0.25	0.25	0	0	0	0
Mar.	–	–	–	0.41	0.37	0.04
Apr.	24.25	20.50	2.50	12.90	12.12	0.78
May	18.50	16.87	0.62	4.83	4.47	0.36
Jun.	22.75	22.00	0.75	7.46	6.21	1.26
Jul.	19.00	18.00	0.75	10.22	8.93	1.29
Aug.	15.25	15.25	0	6.54	5.22	1.31
Sep.	30.50	29.25	0.50	8.39	6.77	1.63
Oct.	9.50	9.25	0.25	8.67	7.68	0.98
Nov.	12.00	11.50	0	7.12	6.39	0.73
Dec.	9.33	9.17	0.17	1.54	1.18	0.35
Total	11.29	10.54	0.38	5.80	5.05	0.75

It must be noted first that 95 percent of the total collections were from human dwellings. This might probably for the reason that other buildings like animal sheds and mixed dwellings were wide open with broad daylight thus rendering unsuitable for the sandflies to rest at day time. Otherwise it was noted that 90 percent of Ph. argentipes were collected from animal sheds in the hills of Okhaldhunga, where animal sheds were closed at least on two sides, while investigating case occurrence in the area.

Table no. 3 above shows that during 11 months of 1983 a total of 66 man hours were spent in Dohar and 317 in Bansbari in 12 months of 1990/91, searching for phlebotomine sand flies resting inside premises in the early morning hours. Thus a total of 1356 and 4000 flies were collected from Dohar and Bansbari respectively. Of them 266 flies of Dohar and 1367 of Bansbari were belonging to the Genus Sergentomyia. The flies from Dohar were *Sergentomyia babu, geoffrey and minutus* and from Bansbari were *Se. babu* and *kauli*. The deatils will be reported elsewhere at later date. Of the remaining 1090 flies of Dohar and 793 of 2633 from Bansbari were males. All these are excluded in the table no. 3 above.

Of the remaining 744 flies belonging to the Genus *Phlebotomus* 698 (93.80%) were *Ph. papatasi*, 25 (3.36%) *Ph. argentipes* and 21 (2.84%) *Ph. sergenti* for Dohar. Similarly of the 1840 females from Bansbari 1602 (87.06%) were *Ph. papatasi* and 238 (12.94%) *Ph. argentipes*. The details are presented in table no. 4 above.

From the table no. 4 it is also apparent that *Ph. papatasi* has two peaks of its density - one before other after monsoon. The building of the population starts from April and reach its first high in June, (Per Man Hour Density, PMMD 22.00) and second highest peak being in September (PMHD 29.25), where as *Ph. argentipes* has only one peak highest being in April (PMHD 2.50) for Dohar in 1983. Similarly for Bansbari *Ph. papatasi* showed also two peaks first one in April (PMHD 12.12) and second one in October (PMHD 7.68), where as of *Ph. argentipes* there was only one peak starting from June (PMHD 1.26) and extending upto September (PMHD 1.63).

This difference in peaking pattern of *Ph. papatasi* and *Ph. argentipes* might be due to the time of the study. It is noteworthy to mention here that the study in Dohar was carried out just at the beginning of the building up of the sand fly population in the area and with a few case occurrence in the whole district. Recent study in the same district showed comparatively good density of *Ph. argentipes*. While the study in Bansbari was conducted when already 7 cases were reported for the village of 960 population in the year 1989.

However, similar phenomenon of two peaks of *Ph. papatasi* and one peak of *Ph. argentipes* were also reported by Hati, 1983 for Hargovindpur, West Bengal state of India.

Of the three species described above *Ph. argentipes* was the only one reported by Lewis (1978). Other species he reported quoting different sources were *Ph. teshi, Ph. major, Ph. comatus* and *Ph. hindustanicus*. The localities he mentioned for those species all lie in hills. Zahar (1980) quoting Kaul, Wattal and Sanyal (1979) reported that during the period from June 1976 and November 1979 in 31 districts of Bihar, India recorded 19 species. Of them four *Phlebotomus* species were *papatasi, argentipes, colobaensis* and *shantoni*. The highest density found was of *Ph. papatasi*.

Night landing catches:

To understand the epidemiology of the vector borne diseases it is very essential to know the degree of man-vector contact. Thus man-biting rate (MBR) becomes one of the important parameter required for determining Vectorial Capacity of the vector(s) involved in the transmission of the disease. For this reason the attempt was made to collect flies alighted to bite bait collectors at night in Bansbari, Morang from May 1990 to April 1991. The results of the collections is tabulated below in table no. 5.

From the table it becomes apparant that a total of 73 flies were collected of which 58 (79.45%) were *Ph. argentipes*. This shows that although the density of *Ph. papatasi* was high resting inside premises, but the man biting of *Ph. argentipes* was much higher. This also indicates that the species *argentipes* must be the one responsible for the transmission of kala-azar in the area.

Table No. 5 : Showing monthly man-biting rate (MBR) of *Ph. papatasi* and *Ph. argentipes* in Bansbari, Morang 1990/91.

Months	Man-biting rate of Phlebotomus flies (No. collected)					
	Total		Indoor		Outdoor	
	papatasi	argentipes	papatasi	argentipes	papatasi	argentipes
Jan.	0	0	0	0	0	0
Feb.	0	0	0	0	0	0
Mar.	0	0.12(2)	0	0.12(1)	0	0.12(1)
Apr.	0.25(4)	0.56(9)	0.50(4)	1.12(9)	0	0
May	0.12(2)	0.19(3)	0.25(2)	0.12(1)	0	0.25(2)
Jun.	0	0.69(11)	0	0.75(6)	0	0.62(5)
Jul.	0.19(3)	0.25(4)	0	0.25(2)	0.37(3)	0.25(2)
Aug.	0.06(1)	0.44(7)	0	0.50(4)	0.12(1)	0.37(3)
Sep.	0.19(3)	0.25(4)	0.37(3)	0.25(2)	0	0.25(2)
Oct.	0	0.37(6)	0	0.50(4)	0	0.25(2)
Nov.	0.12(2)	0.56(9)	0.25(2)	0.75(6)	0	0.37(3)
Dec.	0	0.19(3)	0	0.37(3)	0	0
Total	0.08(15)	0.30(58)	0.11(11)	0.40(38)	0.04(4)	0.31(20)

The monthly biting density was nil for two months of January and February and the highest being in the month of June. Almost equal number of argentipes flies were collected landing on bait-collectors inside(6) and outside (5). Comparatively high number of flies (9) were also collected in the months of April and November (MBR=0.56). This shows that the transmissible is possible from April upto November and the outdoor transmission seems to be possible during warm months when the people have the habit of sleeping outside in the porch.

Efforts were also made to time the peak biting activity of the flies. Given below in table no. 6 is the hourly distribution of night landing flies.

papatasi does not seem to important in the transmission of kala-azar unless unusual situation occurs.

The mention is required here that males were also collected along with females. They were probably following females either after copulation or for the same after meals.

DNA probing for *Leishmania Donovani:*

A total of 271 *Ph.argentipes* were crushed onto hybridization membrane for DNA probing. They were submitted to Dr. Richard Lane in London School of Hygiene and Tropical Medicine and found all to be negative for leishmania infection. This may probably be due to poor sampling or faulty technique of crushing.

Table No. 6 : Showing hourly distribution of night landing *Ph. papatasi* and *Ph. argentipes* in Bansbari - 1990/91.

Months	Percentage of collections of Phlebotomus flies in different hours of night (number collected)					
	Total		Indoor		Outdoor	
	papatasi	argentipes	papatasi	argentipes	papatasi	argentipes
1800-1900	6.67(1)	0	9.09(1)	0	0	0
1900–2000	6.67(1)	0	9.09(1)	0	0	0
2000-2100	13.33(2)	12.06(7)	9.09(1)	13.16(5)	25.00(1)	10.00(2)
2100-2200	6.67(1)	10.34(6)	9.09(1)	7.89(3)	0	15.00(3)
2200-2300	26.66(4)	25.86(15)	9.09(1)	34.22(13)	75.00(3)	10.0(2)
2300-2400	6.67(1)	13.80(8)	9.90(1)	7.89(3)	0	25.00(5)
2400-0100	13.33(2)	17.25(8)	18.18(2)	10.53(4)	0	30.00(6)
0100-0200	0	5.17(3)	0	7.89(3)	0	0
0200-0300	6.67(1)	8.62(5)	9.09(1)	13.16(5)	0	0
0300-0400	13.33(2)	6.90(4)	18.19(2)	5.26(2)	0	10.00(2)
0400-0500	0	0	0	0	0	0
0500-0600	0	0	0	0	0	0
Total	100(15)	100(58)	100(11)	100(38)	100(4)	100(2)

From the table above it is clear that the biting of *Ph. argentipes* starts at 2000 hours when usually children in villages are about to go to bed. The peak of the activity were from 2200 to 0100 hours. It should also be noted that more than 60 percent of the collected population of *Ph. argentipes* completed taking their blood meals before midnight. The human biting activity of *Ph.*

RECOMMENDATIONS

The reports presented herein should be taken as preliminary observation only and a step towards epidemiological and entomological study of the transmission of the disease kala-azar. Before embarking upon the programme of prevention and control of the disease a well planned systematic study

should be undertaken. Such study should include the entomological study in different topo-ecological areas with a view to ilucidate the following aspects, so that the vectorial capacity could be estimated for different areas. This will help in planning proper and cheaper remedial measures in the areas when needed.

a) Determination of vector(s)

b) Seasonal fluctuations of vector(s)

c) Gonotrophic cycle of vector(s) in different areas and seasons.

d) Estimation of longevity of vector(s) in different areas and seasons.

e) Probable resting places inside and outside houses.

f) Flight range.

g) Susceptibility status to different insecticides presently in use in the country and already cleared by WHO for public health programmes.

h) Socio-econmic conditions in relation to disease transmission.

The study, if conducted properly, might save from headache of spraying all the sprayable surfaces and thus limiting remedial measures. Otherwise the remedial measure could be very expensive specially for poorer countries like Nepal, which has to depend upon donors for insecticides. Further, the programme if started without proper studies and planning might lead to the same fate which global eradication programme faced.

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