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Development status of *Wuchereria bancrofti* in experimentally infected *Culex quinquefasciatus* with seasonal fluctuations: A study in slums of Burdwan

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Abstract
Changing seasons have effect on development of *Wuchereria* larvae. The effect of seasonal fluctuations on the development were assessed by experimental infection to *Culex quinquefasciatus* from a human volunteer (microfilaria (*mf*) carrier). Study indicates that the rainy season provides optimal conditions for transmission of *Wuchereria* in terms of minimum time span for the development of *mf* into infective L_3 larvae without any apparent loss of parasitic load during the process of development. There exists a robust relationship between ingestion of *mf*, production of L_3 and *mf* density in human blood which is a crucial determinant within the transmission dynamics of filaria.

Keywords: *Wuchereria*, development, fluctuation, season

1. Introduction
Lymphatic filariasis (LF) generally attacks lymphatic system and results in chronic illness. 856 million in 52 countries remain threatened by this diseases. DEC in conjunction with albendazole is recomended as treatment against LF [1]. Stability of transmission of *Wuchereria* depends upon many factors [2, 6] as well as variation within the density of *mf* within the blood and parasite [7, 8]. *Culex quinquefasciatus* is taken under consideration as primary vector of *Wuchereria bancrofti* [9, 10]. High humidity and optimum temperature plays important role in survivality of vector mosquito also as development of *Wuchereria* [11]. Microfilariae (*mf*) required a minimum of 16-17 days within the mosquito to succed within the infective stage [12]. When the temperature was above 37°C and humidity below 65% no transmission was recorded in Khurda district of Orissa [13]. Many existing literatures are there which aren't sufficient to elucidate the effect of seasonal variation on parasitic development inside the vector species [12, 14, 15, 16].
The present study was designed to assess the effect of differences due to the season on the devlopment of *Wuchereria bancrofti* from *mf* to infective stage (L_3 stage) in *Culex quinquefasciatus* in order that proper strategy even be adopted to manage vector population especially season and it's getting to produce cost-effective results around the year on the highest of things operations.

2. Materials and Methods
2.1 Source, maintenance and Identification of experimental mosquito
Adult blood fed mosquitoes were collected from slum (Hatgobindapur (23.25°C N, 87.97°C E), Pandaveswar (23.70°C N, 87.27°C E), Jamuria (23.70°C N, 87.07°C E) and Memari (23.17°C N, 88.10°C E) of Burdwan during the year March 2019 to February 2020. Adult mosquitoes were then introduced in mosquito cage together with a clear polysterene 250 ml cup partially filled with distilled water overnight. Female lay egg one by one arranging them into head down array that sticks together to form the egg raft. Larvae were reared in plastic trays (30 cm × 25 cm × 5 cm) and were fed with Brewer yeast, dog biscuits and algae in a ratio of 3:1:1 respectively [17]. The water was changed on alternate day. The last instars larvae on transforming to pupae were manually collected, transferred to a beaker containing tap water and kept inside a mosquito cage for adult emergence. The emerged adults were put in adult-holding cages and fed with 5% sucrose solution. Key provided by Christophers [18], Barraud [19] and Chandra [20] were used to identify mosquito.

2.2 The Experiment
2.1.1 Volunteers selection
Microfilariae carrier volunteer (sex-male, age- above 18, health status- no clinical signs of filariasis, medicines/treatment taken before- no treatment) from slums of Burdwan were selected at random as hosts for blood meal. Written consent was taken from them after describing them the nature of study. Densities of microfilariae in the blood done by the protocol of Chularerk and Desowitz [21]. At the end of the study they were treated by recommended dose of DEC by WHO [22].

2.2.2 Infection of mosquito
Laboratory reared adult female mosquito (nearly 100) of day 4 age were kept separtely in mosquito cage and subjected to starvation for one full day. One hand of human volunteer carrier of *mf* (*Wuchererеia bancrofti*) was inserted into the mosquito cage at 1900 hour and allowed the mosquito to imbibe blood. Within one hour interval, nearly about 70% mosquitoes were found to be blood fed. Then the hand of volunteer was withdrawn and glucose solution (5%) was supplied in soaked cotton in the cage.

2.2.3 Volume of the blood ingested by mosquito

The amount of ingested blood was determined by weighing 25 unfed female mosquito before the blood meal and 25 female immediately after the blood meal. The volume of the blood consumed was estimated by dividing the weight differences by 1055 mg/ml (approx. density of human blood).

2.2.4 Number of ingested microfilariae

The expected uptake of *mf* was calculated based on Bryan and Southgate [23]. By dividing the observed *mf* intake by the expected *mf* ingested we estimate the concentration factor. Number of *mf* ingested by mosquito was calculated by mutiplying the microfilariaemia of each volunteer by the mean volume of ingested blood. The actual number of ingested *mf* were calculated by dissecting out the gut of the insect. Adult mosquitoes were anasthesized in a test tube by applying few drops of chloroform in a cotton which is used to plug the test tube. Within few minutes mosquitoes were anasthesized. They were placed in a clean glass slide. Legs and wings were removed. Smear was made of the contained blood, fixed in methanol, stained with Giemsa, and the number of ingested *mf* was determined.

2.2.5 Collection of blood from human volunteers to measure mf density

Blood from veins of volunteers were collected using vacuntainers containing EDTA (1 mg/ml) as an anticoagulant. *Mf* density was estmated by counting the number in 60 mm^3 of blood (*mf*/ml).

2.2.6 Development of *Wuchereria bancrofti* larvae in vector mosquito and collection of meterological data

Ten mosquitoes were dissected on day 0, 3, 6, 9, 12, 15, 18 and 21 after each blood meal. The head, thorax and abdomen were examined to find whether it was infected by *Wuchereria* larvae. In case of infected mosquitoes, the number of *mf*, 1st, 2nd or 3rd stage larvae were counted in the locations (head, thorax, and abdomen). The experiments were repeated thrice (n=3) in three seasons in a year (summer, rainy and winter). In rainy and summer the experiments were continued up to 15th day of blood meal but in case of winter season the experiments were continued up to 21st day after blood meal. We have also collected data from meteorological department regarding maximum and minimum temperature, rainfall and humidity.

2.2.7 Statistical Analysis

Stastical analysis was done using Z test in order to test the significance difference between average load of *mf*/mosquito on day 0 and average load of L_3/ mosquito on day 15 for summer, rainy and winter season in four different slums of Burdwan under study.

3. Results

3.1 Volume of ingested blood

The mean volume of ingested blood was 4.24±0.53 ml (Table 1).

Table 1: Estimated weight and volume of ingested blood by female *Culex quinquefasciatus*

Mean weight of mosquitoes (mg)		Mean weight of blood ingested by mosquitoes (mg)	Mean volume of blood ingested by mosquitoes (ml)
Unfed	Blood fed		
1.67±0.16	6.15±0.52	4.47±0.56	4.24±0.53

3.2 Number of ingested *mf*

Mean no. *mf* /infected mosquito varies from 5.50 to 8.00 and the concentration factor ranged from 1.16 to 1.89 (Table 2).

Table 2: Uptake and concentration of *Wuchereria bancrofti* by *Culex quinquefasciatus* immediately after feeding on blood meal with different microfilarial densities

mf density in blood meal (mf/ml)	No. of dissected mosquito	Mean no. *mf* /infected mosquito	No. of *mf* expected	CF (Concentration Factor)
800	20	6.43	3.39	1.89
1000	22	5.50	4.24	1.29
1250	20	6.20	5.30	1.16
1500	21	8.00	6.36	1.25

3.3 Measurement of *mf* density in human blood

The mf density ranges between 800-1500 *mf*/ml (Table 2)

3.4 Development of *Wuchereria bancrofti* larvae in vector mosquito

Altogether 180, 180 and 240 mosquitoes were dissected and examined in three sets of experiments during rainy, summer and winter season (as developmental period is delayed) respectively. Total number of *Wuchereria* larvae obtained in three sets of experiments in three different seasons is presented in Table 3.

Table 3: Total number of *Wuchereria* larvae obtained in three sets of experiments for each season and four slums

Name of Slums	Dissected on day	Total number of *Wuchereria* larvae detected																	
		mf						1st stage			2nd stage			3rd stage					
		Abdomen			Thorax			Thorax			Thorax			Thorax			Proboscis		
		S	R	W	S	R	W	S	R	W	S	R	W	S	R	W	S	R	W
H	0	190	160	169	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P		220	180	190	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
J		205	170	192	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M		170	150	156	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H	3	30*	-	42*	25*	158	101	89	154	-	-	-	-	-	-	-	-	-	-

P		40*	-	64*	31*	193	120	115	193	-	-	-	-	-	-	-	-	-	-
J		35*	-	49*	29*	171	111	103	177	-	-	-	-	-	-	-	-	-	-
M		20*	-	30*	10*	145	93	76	143	-	-	-	-	-	-	-	-	-	-
H	6	-	-	-	-	-	-	32*	-	89	63	159	-	-	-	-	-	-	-
P		-	-	-	-	-	-	40*	-	110	101	197	-	-	-	-	-	-	-
J		-	-	-	-	-	-	38*	-	101	86	183	-	-	-	-	-	-	-
M		-	-	-	-	-	-	16*	-	76	54	149	-	-	-	-	-	-	-
H	9	-	-	-	-	-	-	-	-	22	13**	-	52	61	131	-	-	22	-
P		-	-	-	-	-	-	-	-	40	20**	-	90	90	153	-	-	42	-
J		-	-	-	-	-	-	-	-	35	18**	-	80	81	140	-	-	35	-
M		-	-	-	-	-	-	-	-	21	9**	-	46	43	89	-	-	11	-
H	12	-	-	-	-	-	-	-	-	-	-	-	57	14**	-	-	51	151	-
P		-	-	-	-	-	-	-	-	-	-	-	95	23**	-	-	72	180	-
J		-	-	-	-	-	-	-	-	-	-	-	86	18**	-	-	62	165	-
M		-	-	-	-	-	-	-	-	-	-	-	51	9**	-	-	40	131	-
H	15	-	-	-	-	-	-	-	-	-	-	-	9	-	-	66	40	161	-
P		-	-	-	-	-	-	-	-	-	-	-	12	-	-	79	65	190	-
J		-	-	-	-	-	-	-	-	-	-	-	8	-	-	61	56	171	-
M		-	-	-	-	-	-	-	-	-	-	-	1	-	-	49	31	139	-
H	18	NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	6	NA	NA	31
P		NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	10	NA	NA	62
J		NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	9	NA	NA	50
M		NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	2	NA	NA	31
H	21	NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	29
P		NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	52
J		NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	36
M		NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	-	NA	NA	11
H	Average load/ Mosquito	6.33	5.33	5.63													1.33	5.36	0.96
P		7.33	6.00	6.33													2.16	6.33	1.73
J		6.83	5.66	6.40													1.86	5.70	1.20
M		5.66	5.00	5.20													1.03	4.63	0.36

Number of mosquito dissected on each day=10 and three replicates (10 ×3 =30), S=summer, R= Rainy, W=winter; H=Hatgobindapur, P=Pandaveswar, J=Jamuria, M=Memari*= Degenerating, **=Deformed or Degenerating

Table 4: Z test of four selected slums of Burdwan district in three different seasons (summer, rainy, winter)

Slums	Statistical parameters	Seasons		
		Summer	Rainy	Winter
Hatgobindapur	Difference	139	138	138
	Z observed	37.60	46.87	55.57
	Z critical value	1.96	1.96	1.96
	P value (Two tailed)	<0.0001	<0.0001	<0.0001
	Alpha	0.05	0.05	0.05
Pandaveswar	Difference	148	138	128
	Z observed	93.60	27.23	48.37
	Z critical value	1.96	1.96	1.96
	P value (Two tailed)	<0.0001	<0.0001	<0.0001
	Alpha	0.05	0.05	0.05
Jamuria	Difference	143	135	142
	Z observed	42.16	41.66	21.86
	Z critical value	1.96	1.96	1.96
	P value (Two tailed)	<0.0001	<0.0001	<0.0001
	Alpha	0.05	0.05	0.05
Memari	Difference	130	139	125
	Z observed	37.27	152.26	24.13
	Z critical value	1.96	1.96	1.96
	P value (Two tailed)	<0.0001	<0.0001	<0.0001
	Alpha	0.05	0.05	0.05

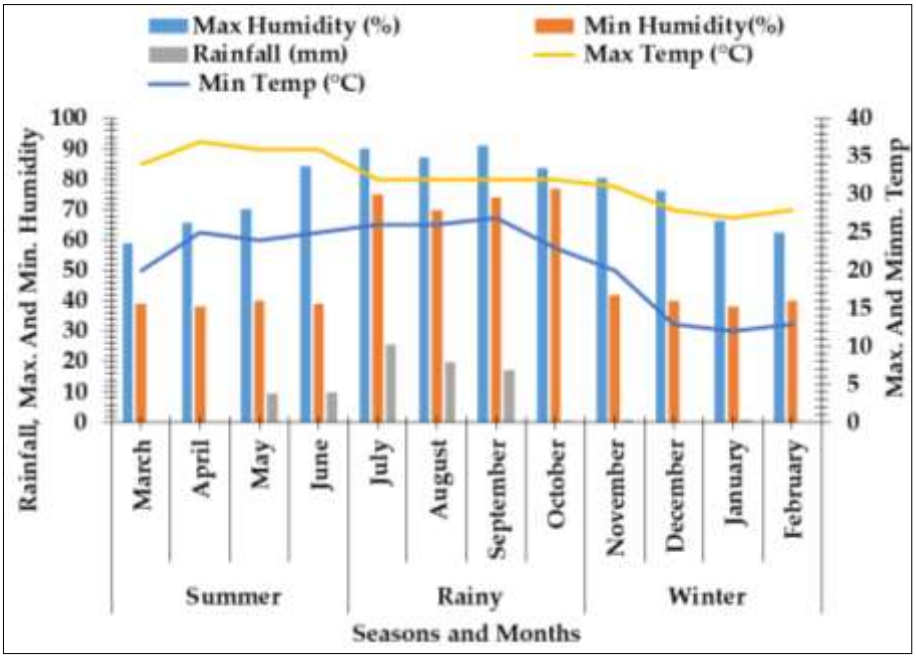

Fig 1: Maximum and minimum temperature, rainfall, maximum and minimum humidity (March 2019 to February 2020)

3.5 Meterological data
Maximum and minimum temperature, rainfall, max. And min. humidity (March 2019 to February 2020) collected is plotted in Figure 1.

3.6 Statistical Analysis
The results of statistical analysis of four slums is presented in Table 4. In all cases (in all seasons and slums) the P value is lower than than the significant level (alpha level=0.05), therefore, we should safely reject the null hypothesis, and accept alternative hypothesis (H0=the difference between the mean is 0; Ha= the difference between the mean is different from 0). It indicates that significant difference existed between the average load of *mf*/mosquito on day 0 and average load of L_3/mosquito on day 15.

4. Discussions
From past it has been recognized that there are many intrinsic difference in the dynamic relationship between *W. bancrofti* and its vectors necessitates the performance of detailed studies in each endemic area [24, 27]. Our data confirm the positive correlation between *mf* density in the donor blood and the number of *mf* ingested by mosquitoes that has been described by others [28, 25, 23, 29, 30].

During rainy season, *mf* of *Wuchereria bancrofti* required minimum time (9 days) to attain the 3rd infective stage larvae. A certain number of 3rd stage larvae also migrated from thorax to proboscis within 9 days. No deformed or degenerating larvae was detected in the dissected mosquitoes during rainy season.

During summer, *mf* arrived at infective stage within 9 days but none of them migrated to the proboscis of the vectors. Besides, a certain percentages of the larvae were found either deformed or degenerated at different stages of devlopment due to high temperature and less humidity [31].

During winter, *mf* reached the infective stage on day 15 of development migrated to proboscis on day 18. The process of devlopment was delayed due to extremely low temperature and humidity. It was also noted that all the *mf* failed to escape from the midgut of the mosquitoes due to low temperature as also found by Hu [32] and Chandra [33]. Though no degenerating 3rd stage larvae was observed in the thorax of the mosquitoes, it can be assumed that from the results that all the 3rd stage larvae could not pass from the thorax to proboscis probably due to effect of weather.

In the rainy season, *mf* of W. *bancrofti* reached the proboscis of the vector after devloping into 3rd stage very rapidly and average load of the parasites remained more or less unchanged during the course of devlopment in the vector population.

During summer and winter, parasitic devlopment was slow and the average load of parasites in the vector population gradually or sharply decreased with the advancement of parasitic devlopment. It indicates that temperature and humidity are two significant limiting factors involved in filarial transmission. The rainy season is considered to be the high time for transmission of the disease as this season provides optimum conditions to reach the Vector Efficiency Index (VEI) to its peak (which is based on parasitic devlopment, proper nursing and low parasitic damage or death). Therefore personal protection from mosquito bite specifically during rainy season should be an essential and effective step in any filariasis control programme.

5. Conflict of Interest
We declare that we have no conflict of interest

6. Ethical approval
This article is not under consideration or published elsewhere. Ethical clearance for the study was obtained from IAEC, Approval No. 23/IAEC (06)/RNLKWC/2020, dated 08.02.2020.

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University, Raja Narendra Lal Khan Women's College (Autonomous).

8. Authors Contribution:

IB- Data curation, Writing Original Draft, Statistical analysis

BM- Reviewing, Editing

PPC- Designing, Monitoring, Reviewing, Communication

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