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SURVIVAL RATES OF *ANOPHELES CULICIFACIES S.L.* AND *ANOPHELES PULCHERRIMUS* IN SPRAYED AND UNSPRAYED VILLAGES IN GHASSREGHAND DISTRICT, BALUCHISTAN, IRAN, 1991[1]

M. ZAIM, A. H. ZAHIRNIA AND A. V. MANOUCHEHRI

Department of Medical Entomology, School of Public Health, P. O. Box 6446, Teheran 144155 Iran

ABSTRACT. Survival rates, expectation of life, and expectation of infective life of *Anopheles culicifacies* and *Anopheles pulcherrimus* were estimated in sprayed and unsprayed villages in the highly malarious Ghassreghand District, Iran, during the 2 peak malaria transmission seasons in 1991. The daily survival rate of *An. culicifacies* was estimated as 0.84 and 0.78 during May and 0.89 and nil during September–October in the unsprayed and sprayed villages, respectively. For *An. pulcherrimus* the daily survival rate was estimated as 0.80 and 0.78 in May and 0.83 and 0.78 in September–October in the unsprayed and sprayed villages, respectively. The impact of indoor residual spraying during the first peak of transmission on *An. culicifacies* and *An. pulcherrimus* (expressed as the product of the degree of reduction of the expectation of infective life and the degree of reduction of expectation of life) was calculated as 3.5 and 1.5 times, respectively, that in the absence of control. The impact of indoor residual spraying on *An. culicifacies* during the second peak of transmission was almost complete, whereas that for *An. pulcherrimus* was 3.4 times than that in the absence of control.

INTRODUCTION

Malaria is the most important health problem in southeast Iran. Ghassreghand District in Baluchistan, with an annual parasite incidence of about 60 per 1,000 population, is one of the highly endemic areas in this region. *Anopheles culicifacies* Giles and *Anopheles pulcherrimus* Theobald are the 2 most common anopheline species, with the former species regarded as the main and the latter as a probable vector of malaria (Zaim et al. 1993a).

Any attempt at malaria control in a given locality should be preceded by an evaluation of the incidence of malaria and conditions for transmission of the disease. One of the basic elements in such a malaria survey is the determination of the vectorial capacity of the suspected vector. Vectorial capacity, defined as the average number of inoculations with a specific parasite originating from one case of disease in unit time that a vector population would distribute to its host if all the vectors biting the case became infective (Garrett-Jones 1964), combines biting density per host, host preference, and the expectation of infective life. The average daily survival rate of the vector is the most sensitive parameter that would directly influence the vectorial capacity. Small variations in this parameter have a large effect on the potential vectorial capacity of vector species.

The main objectives of the present investigation were to determine the average daily survival rate of *An. culicifacies* and *An. pulcherrimus* in the sprayed and unsprayed villages of Ghassreghand, to estimate the expectation of life and expectation of infective life, and to determine the impact of indoor residual spraying on the population of the 2 species, during the 2 main malaria transmission seasons.

MATERIALS AND METHODS

The investigation was carried out in 1991 in the villages of Bogan (sprayed) and Zeineddini (unsprayed) in the Ghassreghand District during the 2 peak malaria transmission seasons (i.e., May and September–October). Both villages were almost identical as regards anopheline species as well as types and extent of the mosquito breeding sites (mainly rice fields, stream and streambed pools, and palm irrigation plots). The village of Bogan was sprayed with malathion (2 g/m^2) on February 1 and with primiphos-methyl (2 g/m^2) on September 17, 1991.

From May 9 through May 23, 1991, the indoor-resting populations of *An. culicifacies* and *An. pulcherrimus* were sampled for 15 consecutive days from 4 fixed human and animal shelters located in different parts of the 2 villages. A team of 2 collectors was assigned to each village. Sampling started at 0600 h each day, in each village, when the same 2 technicians caught mosquitoes with aspirators for 15 min in each place. The same procedure was followed in the second round of study from September 30 through October 14, 1991, except that hand catch of the resting pop-

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Table 1. Hand catches of indoor resting *Anopheles culicifacies* and *Anopheles pulcherrimus* in the unsprayed (Zeineddini) and sprayed (Bogan) villages of Ghassreghand District, Baluchistan, Iran, May 9–23, 1991.

	Unsprayed				Sprayed			
	An. culicifacies		*An. pulcherrimus*		*An. culicifacies*		*An. pulcherrimus*	
Day	Total	Parous (%)	Total	Parous (%)	Total	Parous (%)	Total	Parous (%)
1	76	46	13	100	149	26	2	0
2	107	35	23	50	94	28	4	0
3	120	40	14	57	121	27	0	
4	62	35	3	100	21	50	0	
5	83	55	8	80	49	47	0	
6	91	56	8	50	32	52	3	33
7	80	56	5	50	78	48	0	
8	39	65	7	60	68	38	2	0
9	58	68	19	50	48	43	3	50
10	79	70	7	40	101	34	3	100
11	32	50	18	50	34	37	2	100
12	66	32	25	42	131	44	8	17
13	64	63	8	50	68	37	12	80
14	59	62	8	20	111	56	8	29
15	24	50	7	83	74	38	5	100
Total	1,040		173		1,179		52	

ulations of the 2 species was only possible in the unsprayed village. In the sprayed village (Bogan), pyrethrum space-spray catches had to be performed because of the very low number of indoor-resting mosquitoes. *Anopheles culicifacies* and *An. pulcherrimus* females were classified as unfed, newly fed, semigravid, or gravid, and the first 2 categories were dissected and scored as nulliparous or parous on the basis of the tracheolar skeins of their ovaries.

Estimates of the survival rate of both species were obtained either through the time-series analysis of parous rate data (Mutero and Birley 1987) or the parous rate formula of Davidson (1954). Samples of mosquitoes collected freshly fed in the unsprayed village of Zeineddini were kept alive in cages in animal shelters for up to 48 h to observe the state of their ovarian development. This was not only used for calculating the duration of the gonotrophic cycles, but those that failed to develop eggs and hence reverted to the unfed state were recorded as pregravid. This latter information was used in the time-series analysis of parous data (Mutero and Birley 1987). The pregravid rate was estimated for nulliparous individuals based upon the later dissection of ovaries.

The total impact of insecticide spraying, expressed as the product of the degree of reduction of the expectation of infective life and that of expectation of life, was calculated, as described by Garrett-Jones and Grab (1964). This is given as the product of 2 ratios: 1) the ratio of the unsprayed to the sprayed values of the expectation of infective life, and 2) the ratio of the unsprayed and sprayed values of the expectation of life, for each species.

RESULTS AND DISCUSSION

Table 1 summarizes the hand catches of indoor-resting *An. culicifacies* and *An. pulcherrimus* in the unsprayed (Zeineddini) and sprayed (Bogan) villages from May 9 to May 23, 1991. The estimates of the daily survival rate, expectation of life, and the expectation of the infective life for each species in the 2 villages are summarized in Table 2.

In the unsprayed village of Zeineddini, *An. culicifacies* and *An. pulcherrimus* constituted 81.3 and 13.5% of the indoor-resting anophelines captured, respectively. About 54% of *An. culicifacies* and 55% of *An. pulcherrimus* were unfed or newly fed individuals. The direct observation of the samples of freshly fed *An. culicifacies* and *An. pulcherrimus* females revealed gonotrophic cycles of 2–3 days at a mean daily temperature of 31.5°C and pregravid ratios of 20 and 0% for the 2 species, respectively. The time-series analysis of parous data for *An. culicifacies* revealed high correlation for the data ($r = 0.97$ at time delay $u = 0$ and $r = 0.6$–0.87 at time delays $u = 1$–10)

Table 2. Daily survival rate and other population indices of *Anopheles culicifacies* and *Anopheles pulcherrimus* in the sprayed and unsprayed villages of Ghassreghand District, Baluchistan, Iran, May 9–23, 1991.

Species	Village treatment	Daily survival rate	E[1]	Ratio of E A/B	EIL[2]	Ratio of EIL A/B	Product of ratios
An. culicifacies	(A) Unsprayed	0.84	5.7	1.4	1.60	2.5	3.5
	(B) Sprayed	0.78	4.0		0.65		
An. pulcherrimus	(A) Unsprayed	0.80	4.5	1.1	0.90	1.3	1.5
	(B) Sprayed	0.78	4.0		0.67		

[1] E = expectation of life (days).
[2] EIL = expectation of infective life (calculated for *Plasmodium falciparum*).

and presented a daily survival rate of 0.84 (gonotrophic cycle of 3 days) as the best estimate of this parameter. The daily survival rate of *An. pulcherrimus* was estimated as 0.80 (gonotrophic cycle of 3 days), however, the correlation index of the data was not as high as that for *An. culicifacies*. The mean parous rate formula also gave the same daily survival rate estimate for *An. pulcherrimus* in Zeineddini.

In the sprayed village of Bogan, *An. culicifacies* and *An. pulcherrimus* constituted 93 and 4% of the indoor-resting anopheline mosquitoes collected. About 67% of *An. culicifacies* and 64% of *An. pulcherrimus* were unfed or newly fed individuals. The time-series analysis of parous data showed a daily survival rate of 0.78 for *An. culicifacies*, but with clear peaks of correlation at 2-day intervals (= gonotrophic cycles of 2 days). The correlation was 0.95 at time delay $u = 0$ and ranged from 0.29 to 0.80 at time delays $u = 1$–10. The clear difference between the estimates of the gonotrophic cycles between these 2 relatively close villages may have been due to the analysis of the data with the assumption of a pregravid rate of 20%, equal to that of the nonsprayed village. The very low number of *An. pulcherrimus* females captured in the sprayed village (52 females) (Table 1), precluded a time-series analysis of the data. The daily survival rate of 0.78 was estimated for this latter species, using the mean parous formula.

The total impact of insecticide spraying (Table 2) on *An. culicifacies* (mainly endophilic) (Zaim et al. 1993b) and *An. pulcherrimus* (mainly exophilic) (Zaim et al. 1992) 3 months after the indoor residual spraying program, but at the time of the peak malaria transmission, was calculated as 3.5 and 1.5 times that in the absence of control for the 2 species, respectively.

The results of the hand collecting of indoor-resting *An. culicifacies* and *An. pulcherrimus* in the unsprayed village of Zeineddini from September 30 to October 14, 1991, are presented in Table 3. However, in the sprayed village of Bogan, the very low number of indoor-resting anophelines did not allow hand collecting of mosquitoes and a pyrethrum space-spray catch was performed.

In the unsprayed village, *An. pulcherrimus* and *An. culicifacies* comprised 80 and 13.8% of the total anophelines captured. About 72% of *An. pulcherrimus* and 82% of *An. culicifacies* collected were unfed or freshly fed individuals. The direct observation of the samples of freshly fed *An. culicifacies* and *An. pulcherrimus* females captured and held in the cattle sheds in Zeineddini for 48 h revealed gonotrophic cycles of 2–3 days at a mean daily temperature of 28.6°C and pregravid ratios of 30 and 0% for the 2 species,

Table 3. Hand catches of indoor resting *Anopheles culicifacies* and *Anopheles pulcherrimus* in the unsprayed (Zeineddini) and sprayed (Bogan) villages of Ghassreghand District, Baluchistan, Iran, September 30–October 14, 1991.

	An. culicifacies		*An. pulcherrimus*	
Day	Total	Parous (%)	Total	Parous (%)
1	5	75	33	42
2	4	67	26	83
3	4	75	34	68
4	3	50	50	83
5	2	100	25	50
6	4	100	29	68
7	3	0	29	64
8	1	100	23	45
9	6	67	51	64
10	5	80	40	68
11	12	55	21	75
12	5	100	25	65
13	12	64	25	53
14	9	83	27	75
15	1	0	11	83
Total	76		441	

Table 4. Daily survival rate and other population indices of *Anopheles culicifacies* and *Anopheles pulcherrimus* in the sprayed and unsprayed villages of Ghassreghand, Baluchistan, Iran, September 30–October 14, 1991.

Species	Village treatment	Daily survival rate	E[1]	Ratio of E A/B	EIL[2]	Ratio of EIL A/B	Product of ratios
An. culicifacies	(A) Unsprayed	0.89	8.6		3.0		
	(B) Sprayed	—[3]	—		—		
An. pulcherrimus	(A)Unsprayed	0.83	5.4	1.4	1.0	2.5	3.4
	(B) Sprayed	0.78	4.0		0.4		

[1] E = expectation of life (days).
[2] EIL = expectation of infective life (calculated for *Plasmodium falciparum*).
[3] Very low density of *An. culicifacies*.

respectively. The time-series analysis of the parous data of *An. pulcherrimus* in this village showed a high correlation of the data ($r = 0.96$ at time delay $u = 0$ and $r = 0.69$–0.94 at $u = 1$–10), and the daily survival rate was estimated as 0.83 (Table 4). The catch size of *An. culicifacies* was relatively small during this period (76 females, Table 3) and the mean parous rate formula of Davidson (1954) was used to estimate the daily survival rate (0.89) of this species (Table 4).

Pyrethrum space-spray catches in human and animal shelters in the sprayed village of Bogan recovered 24 anophelines of which 79.2% were *An. pulcherrimus*. No *An. culicifacies* was collected in Bogan during October 1991. The mean parous rate formula showed a daily survival rate for *An. pulcherrimus* of 0.78 (Table 4).

The total impact of indoor residual spraying (primiphos-methyl, 2 g/m^2) on the mostly exophilic species, *An. pulcherrimus*, one month after the program, was estimated to be 3.4 times than in the absence of the control. The impact of this program on the mainly endophilic species, *An. culicifacies*, however, was almost perfect and the mean indoor resting density of this species in the sprayed villages was zero.

The mean indoor resting density of 23.2 for *An. culicifacies* in the Ghassreghand District during May 1991, 3 months after spray, obtained through monthly pyrethrum space-spray collections of human and animal shelters (Zaim et al. 1993b), along with the relatively high daily survival rate of 0.78 observed in the sprayed area in this study, is consistent with occurrence of malaria cases during the same time period (Zaim et al. 1993a, Fig. 1) and shows the wrong timing of the spray program. The second round of indoor residual spraying in early September reduced the indoor resting density of *An. culicifacies* to almost zero in the sprayed village, when the mean indoor resting density and the daily survival of this species in the unsprayed area in October were 4.1 and 0.89, respectively. Nevertheless the exophilic habits of *An. pulcherrimus* made this species less vulnerable to indoor residual spraying (Table 4) and this seems to have been mainly responsible for the number of cases of malaria reported in early autumn in the sprayed zone. An immunoradiometric assay performed on *An. pulcherrimus* in this area during the same period of time showed this species to be a potential vector of malaria in southeast Iran (Zaim et al. 1993a).

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