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Impact of diethylcarbamazine on vector competence of *Culex pipiens* L. to *Wuchereria bancrofti* Cobbold

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ABSTRACT

Filariasis control aims to interrupt transmission by reducing human microfilaraemia through chemotherapy, rather then controlling the vector. We investigated whether the drug diethylcarbamazine (DEC) mediates sustained microfilaria reductions and decreases mosquito infection. A prospective study was designed to determine the potential of DEC-treated *Wuchereria bancrofti* carriers (6mg DEC/kg body weight) to infect *Culex pipiens* at 2, 3, 6, 9 and 12 months intervals post-treatment. Microfilaraemia of six treated patients vanished but did not clear, even in three subjects that received a second dose, 8 months after initial treatment. Blood microfilaraemia or the presence of filariae in dissected mosquitoes indicated that 3 months post-treatment, two patients were still microfilaraemic. Likewise, from 6-12 months post-treatment, all patients were microfilaraemic. After having received one dose of DEC, all patients were infectious to mosquitoes, 2 to 12 months post treatment. However, infective worms were recovered from mosquitoes that have fed on 2 of them. Mosquito ingestion and infectivity rates ranged from 2.9-14.2 and 2.4-14.1%, respectively. The infectivity of mosquitoes membrane-fed infected blood containing 0.06 mg DEC/ml was reduced by 55% compared to control. The parasite burden decreased by 26%. DEC (0.06 mg/ml) in a sugar solution 3.5 to 6.5 d after an infected blood meal had no effect on filaria infection, but caused a decline when ingested 8.5 to 13.5 d post-blood meal (29 and 36% decline, respectively). Presumably, the drug was released from the crop with the sugar at these last time periods. We concluded that an annual single dose of DEC has great potential to mediate sustained microfilaria reductions, thereby reducing, but not eliminating, transmission. In addition, DEC kills filarial parasites within the mosquito.

KEYWORDS: Filariasis, *Wuchereria bancrofti*, *Culex pipiens*, diethylcarbamazine

INTRODUCTION

Human lymphatic filariasis, resulting primarily from *Wuchereria bancrofti* infections, affects more than 120 million people. In Egypt, a remarkable resurgence of filarial infection has been recorded (Harb *et al.* 1993). Recently, the WHO has identified lymphatic filariasis as a public health problem, and has targeted it for elimination (Ottesen *et al.* 1997). Control of filariasis aims to interrupt transmission by reducing either microfilaria prevalence rates or vector density. Because vector control alone was ineffective in filaria endemic areas of the world, reduction of microfilariae in humans through chemotherapy has been proposed as an alternative, to limit or break transmission of the parasite (Ottesen & Ramachandran 1995; Bockarie *et al.* 1998).

Over the past 50 years, diethylcarbamazine citrate (DEC) has been the drug of choice for the control of W. bancrofti (WHO 1992). DEC activity significantly reduces microfilaraemia. Its mode of action is thought to kill microfilariae directly (Ottesen 1985), or to temporarily affect the fecundity of adult worms (Kazura *et al.* 1993). DEC activity may extend to 18 months post-treatment and, in general, repeated annual doses are necessary to interrupt filaria transmission in communities (Kazura *et al.* 1993).

We carried out a prospective study to determine the potential of DEC-treated *W.*

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bancrofti carriers to infect *Cx. pipiens*. In addition, we investigated the impact of DEC on parasite development within its vector mosquito.

MATERIALS AND METHODS

Selection of DEC-treated microfilaraemic hosts Within the scope of epidemiological studies carried out by Ain Shams University (ICIDR research grant) in a filaria endemic village, microfilaraemic *W. bancrofti* infected subjects have received a single dose of 6 mg DEC/kg body weight. To follow-up the potential of DEC-treated microfilaraemic patients to infect the filaria vector over 12 months post-treatment, informed and consenting treated subjects were selected for mosquito competence studies. Pre-treatment microfilaraemia of selected patients was determined by filtration of 1 ml venous blood collected between 22:00 and 00:00.

Mosquito exposure to DEC-treated *W. bancrofi* carriers: *Culex pipiens* larvae were collected in a highly polluted drainage canal in Greater Cairo (Qaliubiya Governorate) and reared to maturity at 27±2°C, 70-80% R.H., and 16L:8D. Emerging adults were supplied with a 10% sugar solution till shortly before being exposed to selected subjects. Three to 5 d old females were allowed to feed on the forearms of volunteers for 20 min between 22:00 and 02:00. To determine microfilaraemia at the time of mosquito feeding, thick blood films were prepared from 50 µl finger prick blood samples (WHO 1987). Aliquots of fully engorged mosquitoes were cold-killed immediately after feeding, and dissected out for the presence of *W. bancrofti* microfilariae. Mosquito ingestion rate was the percentage of females with one or more microfilariae. Remaining mosquitoes were maintained on a sugar solution until the extrinsic incubation period (EIP) of the parasite (Gad *et al.* 1988). Mosquitoes surviving the EIP were killed and dissected out for the presence of *W. bancrofti* infective larvae (L3). Infectivity rate was the percentage of surviving mosquitoes that contained one or more L3 larvae. The parasite burden was the mean number of filaria worms a female surviving till dissection contained. Mosquitoes were exposed to treated subjects at 3-month intervals, for one calendar year post-treatment.

Impact of DEC on parasite development in *Cx. pipiens*: To investigate the filaricidal effect of DEC ingested by *Cx. pipiens* that engorged with infected blood, two series of experiments were carried out. In the first series, microfilaraemic venous blood was collected from consenting volunteers and microfilariae counts determined in 50 µl Giemsa-stained thick smears. One ml blood, to which was added 0.06 mg DEC was offered to sugar-starved 3- to 5-day-old female *Cx. pipiens* through a chick membrane, fitted to a glass feeder. The blood was warmed up to 37°C by connecting the membrane feeder to a water circulator (Isotemp Immersion Circulators, P/N U00438, Fisher Scientific Inc., Pittsburgh, PA, USA). A second group of mosquitoes served as control, by being simultaneously exposed to a second feeder containing 1 ml blood, with no DEC added, from the same volunteer. Blood-engorged mosquitoes were maintained on a sugar solution until the EIP of the parasite had elapsed, and were dissected out for the presence and number of infective larvae. The parasite burden was the mean number of larvae contained in infective mosquitoes.

In the second series, an attempt was made to simulate a situation in which *W. bancrofti* infected mosquitoes would obtain a subsequent blood meal from DEC treated filaria patients, and therefore, to investigate the impact of DEC on the parasite stages within the mosquito. For this, 3- to 5-day-old sugar-starved females were allowed to feed between 22:00 to 02:00 on an informed and consenting microfilaraemic human volunteer. Microfilaraemia was determined in two thick-smeared 50-µl fingerprick blood samples taken immediately before feeding. Blood engorged mosquitoes were separated into two aliquots, each subdivided into 4 groups. In one aliquot, each group was offered from 10:00 to noon a

DEC/sugar/dye diet at one of 4 intervals: 3.5, 5.5, 8.5, or 12.5 d post-infected blood meal. The concentration of DEC (0.06 mg/ml of 10% sugar solution) was the maximum expected in a freshly treated patient. To identify sugar-fed females, carbohydrate solution was tinted using Carmoisine E 122 (5 µg/ml of 10% sugar solution). Groups in the second aliquot served as control, by simultaneously offering the females a sugar/dye diet at one of the intervals. To assess potential filaria development to the next stage, sugar-fed mosquitoes from either aliquot were dissected out at intervals post-infected blood meal. The presence, number, and maturity of parasitic larvae were recorded.

Statistical analysis: Difference between sample proportions was evaluated by the Chi-square test. Difference between two group means was evaluated by the Mann-Whitney test for non-parametric data. Analysis was carried out on a personal computer, using a statistical package (SPSS Inc. © Version 8.00, 1989-1997).

RESULTS

Infection rates of *Cx. pipiens* fed blood from six microfilaraemic human volunteers treated with a single dose of DEC were determined at intervals, for one calendar year (Table 1). Three months post-treatment, one subject (pre-treatment 121 mf/ml blood) had microfilariae in his blood. However, microfilariae smears of this and other hosts, were negative, 6 months post-treatment. Because three patients (pre-treatment 9, 127 and 218 mf/ml blood, respectively) had positive smears 8 months after initial treatment, they received a second dose of DEC. Two of these patients (pre-treatment 9 and 127 mf/ml blood) had positive smears (0-1 mf/50 µl) one and 4 months, respectively, after they were given the second dose of DEC, but were not infectious to mosquitoes (Table 1). In contrast, females ingested microfilariae when fed blood from the third patient (pre-treatment 218 mf/ml), 4 months after the second dose of DEC. Nevertheless, ingested microfilariae did not develop to the L3 stage. Two to 12 months after having received one dose of DEC, all patients could infect mosquitoes. Mosquito ingestion and infectivity rates were generally low, ranging between about 2.5 and 14% (Table 1). The parasite burden of infective mosquitoes did not exceed 1.

The potential filaricidal effect of DEC ingested by *Cx. pipiens* with *W. bancrofti* infected blood (microfilariae = 12-41 mf/50 µl blood, and 62 mf/50 µl blood) was tested twice. Because data from both replicates were consistent, results were combined and analyzed as one entity. The infectivity rate of mosquitoes that had ingested DEC-treated infected blood was reduced by 55% (25.4%, n = 122) compared to that of the control group (56.0%, n = 100) ($X^2 = 21.6$, df = 1, $p < 0.001$). The parasite burden of infective mosquitoes (2.06±2.05, n = 31) that had ingested DEC treated blood, was significantly reduced compared to that of infective control females (2.79 ± 2.32, n = 56) (U = 660, p = 0.046).

To investigate the impact of DEC on the parasite stages within *Cx. pipiens*, females were offered DEC in a sugar solution, at intervals determined according to the temporal development of the filaria parasite, then dissected at set time periods (Table 2). The infection rates of mosquitoes offered a sugar/DEC diet 3.5 to 6.5 days post-infected blood meal were not significantly lower compared to control ones ($X^2 = 1.4$, df = 1, p = 0.239 and (2 = 0.7, df = 1, p = 0.388, respectively). The intensity of infection did not vary between DEC-treated and untreated females as well (U = 259, p = 0.097 and U = 155.5, p = 0.318, respectively). In both mosquito groups, 4.5 days post-blood meal, most microfilariae have grown to L1 (67/74 and 67/72, respectively). The infection rates of females offered a sugar/DEC diet 8.5 or 12.5 d post-infected blood meal declined by 29 and 36%, respectively, compared to that of mosquitoes in the control group ($X^2 = 4.5$, df = 1, p = 0.033 and ($X^2 = 5.8$, df =1, p = 0.016, respectively). However, the parasite burden of infective mosquitoes that had ingested DEC 8.5 d post-infective blood meal did not differ significantly from that of control ones (U =

912.5, p = 0.395). In contrast, 12.5 d post-infective blood meal, the parasite burden of infective DEC-treated females was significantly reduced compared to that of untreated ones (U = 151, p = 0.001).

Table 1: Prospective *W. bancrofti* infection of *Cx. pipiens* fed on single dose DEC treated filaria patients

Patients	Pre-treatment Mf/ml blood	Post-treatment					
				Mosquito infection			
				Ingestion [b]		Infectivity [c]	
		Months	Mf/50ul blood	N dissected	%	N dissected	%
A[a]	9	2	0-0	23	0.0	36	0.0
		3	0-0	32	0.0	91	0.0
		6	0-0	31	3.2	121	0.0
		9	0-1	21	0.0	29	0.0
		12	0-0	26	0.0	126	0.0
B	18	2	0-0	29	0.0	35	0.0
		3	0-0	34	0.0	49	0.0
		6	0-0	19	0.0	90	0.0
		9	0-0	17	0.0	31	0.0
		12	0-0	22	4.6	136	0.0
C	81	3	0-0	34	0.0	127	0.0
		6	0-0	37	0.0	82	2.4
		9	0-0	33	0.0	54	0.0
		12	0-0	30	0.0	343	0.0
D	121	3	3-6	30	0.0	46	0.0
		6	0-0	35	2.9	77	0.0
		9	0-0	34	0.0	84	0.0
		12	0-0	33	0.0	161	0.0
E[a]	127	3	0-0	30	0.0	50	0.0
		6	0-0	32	3.1	73	0.0
		9	0-0	28	0.0	80	0.0
		12	0-1	25	0.0	136	0.0
F[a]	218	2	0-0	21	14.2	71	14.1
		3	0-0	33	0.0	88	0.0
		6	0-0	21	4.8	39	0.0
		9	0-0	28	0.0	36	0.0
		12	0-0	26	3.9	130	0.0

[a]Patients administered a second dose of DEC, 8 months after initial treatment.
[b]Percent females ingesting mf with the blood meal.
[c]Percent females developing infective worms.

Table 2: Impact of DEC on development of *W. bancrofti* in *Cx. pipiens*

Sugar feeding post-infected blood meal (days)	Dissection post-blood meal (days)	DEC + Sugar					Sugar				
		Mosquito		Parasite			Mosquito		Parasite		
		N	% infection	Stage	Mean ±SD	Range	N	% infection	Stage	Mean ±SD	Range
3.5	4.5	121	19.8	L1	3.1±2.1	1-8	110	26.4	L1	2.5±2.2	1-9
5.5-6.5	8.5	81	19.8	L2	2.0±1.9	1-10	91	25.3	L1/L2	1.9±1.9	1-8
8.5-9.5	12.5	82	36.6	L3	2.8±5.3	1-29	130	51.5	L2/L3	1.8±1.7	1-10
12.5-13.5	13.5-14.5	43	46.5	L3	1.1±0.5	1-4	40	72.5	L3	5.9±9.4	1-39

DISCUSSION

The microfilaraemia of patients treated with a single dose of DEC diminished but did not clear. Moreover, it persisted in two patients that were treated twice. Three months post-treatment, one patient (D) was still microfilaraemic and mosquitoes developed L3 larvae from another patient (F), although microfilariae were not demonstrated in his blood. Six to 12 months post-treatment, the presence of the filarial parasite in blood or in mosquitoes fed on tested hosts indicated that all six patients were microfilaraemic. Studies have demonstrated that the sensitivity of fingerprick blood samples is relatively low (Desowitz & Southgate 1973; Eberhard *et al.* 1988) and that this screening method misses most low microfilaria carriers (Ramzy *et al.* 1991, 1995). Furthermore, mosquitoes are able to concentrate microfilariae in low microfilaraemic blood (Hammad *et al.* 1998), and therefore can be used to assess the importance of residual microfilaraemia after treatment with DEC. Although single-doses of DEC are capable of mediating sustained reductions by 90% for 12 (Ottesen *et al.* 1997) or 18 months after treatment (Kazura *et al.* 1993), high-count patients are much more likely to harbour residual *W. bancrofti* microfilariae than are persons with low counts (Eberhard *et al.* 1988). This, however, does not follow a strict pattern, as presently observed, because some high-count carriers may become microfilariae-negative and, conversely, many low-count patients may remain microfilariae-positive after the administration of DEC (McCarthy *et al.* 1995). Immunological differences may explain such variable responses to DEC (Eberhard *et al.* 1990). Persistent microfilaraemia was also attributed to the presence of a class of resistant infections which do not clear even after 2 or 3 repeated full therapeutic DEC courses (Desowitz & Southgate 1973). These, and the present data, suggest that repeated doses of DEC are necessary to generate a significant decrease in filaria transmission.

Infectivity of *Cx. pipiens* membrane fed infected blood containing DEC, was reduced by 55% and parasite burden decreased by 26% demonstrating that the drug had a significant, although limited, filaricidal effect on *W. bancrofti.* DEC is, however, known to be eliminated from the blood within 24 hours following treatment (G Weil, personal communication). This suggests that the prolonged reduction of microfilaraemia after treatment cannot be explained solely by the killing of microfilariae. The exact mode of action of DEC on filarial worms in humans is difficult, if not impossible, to determine. Based on microfilariae reduction in blood, microfilaricidal and partial macrofilaricidal activity have been reported (Kazura *et al.* 1993; Ottesen & Ramachandran 1995; Cao *et al.* 1997). DEC is thought to induce the temporary sterility of adult worms, thereby affecting microfilarial production (Weil *et al.* 1988). The present data suggested that microfilariae reduction may result from a combination of these effects.

Ingestion of a DEC/sugar diet of mosquitoes that had engorged an infectious blood meal 3.5 to 6.5 d earlier, had no effect on filaria infection, in contrast to that observed for mosquitoes that ingested the drug 8.5 to 13.5 d post-blood meal. The drug reduced but did not eliminate the infection and infectivity of such mosquitoes. Most blood-fed mosquitoes do not sugar feed for 3 d post-blood meal (unpublished data), suggesting that they do not need a daily source of carbohydrates. Carbohydrates ingested by mosquitoes are stored in their crop, and released whenever needed to provide energy. It may be that the DEC/sugar solution ingested at early intervals was retained in the crop, or not released in sufficient quantity to affect filaria parasites. The solution may have been released to provide the mosquito with sufficient energy for laying eggs a few days later, thereby explaining the observed delayed effect of DEC. This hypothesis, however, needs further verification. It is interesting to note that although the infection rate of mosquitoes that had ingested DEC 8.5-9.5 d post-blood meal decreased, the parasite burden was not affected, in contrast to that observed for females that have ingested DEC12.5-13.5 d post-blood meal. This last observation confirms data

obtained by the membrane blood feeding experiment.

We concluded that treatment of filariasis with a single dose of DEC reduces the potential of mosquitoes for transmission of filariasis, without eliminating it. Besides reducing microfilaraemia of treated individuals, it is capable of killing filarial parasites within the vector mosquito.

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الملخص العربى

تأثير عقار داى ايثيل كاربامازين على كفاءة بعوض *كيولكس ببينز* الناقل لطفيل *فوشيريريا بانكروفتى*

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تم دراسة ما إذا كان عقار داى ايثيل كاربامازين (**DEC**) له القدرة المستمرة على إنقاص المستوى العددي ليرقات الميكروفيلاريا في دم الأفراد المصابة وبالتالي انخفاض نسبة إصابة البعوض بالعدوى وذلك عن طريق دراسة إمكانية نقل طفيل الميكروفيلاريا بواسطة بعوض *كيولكس ببينز* من ٦ أفراد مصابة وتم علاجها بعقار **DEC** (٦ مليجرام /كجم من وزن الجسم) وتكملة دورة حياته في البعوض وقد تم متابعة هذه الدراسة لمدة سنة كاملة على فترات ربع سنوية. أوضحت النتائج انخفاض المستوى العددي ليرقات الميكروفيلاريا في دم الأفراد المعالجة انخفاضا ملحوظا على الرغم من عدم اختفائه تماما حتى في الثلاث أفراد الذين تم إعطائهم جرعة ثانية من عقار **DEC** بعد ٨ شهور من الجرعة الأولى. أوضحت النتائج أيضا أنه في فترة الثلاث شهور الأولى من العلاج ما زال ٢ فرد من الأفراد المعالجة حاملا طفيل الميكروفيلاريا وذلك عن طريق الكشف عن وجود يرقات الميكروفيلاريا في الدم أو وجود الطفيل في البعوض المغتذى في هذه الفترة. وبالمثل، في الفترة من ٦-١٢ شهر بعد العلاج كان جميع الأفراد المعالجة حاملي طفيل المرض (عدد البعوض الذي تم تشريحه = ٢٣٢٦). أما في الفترة من ٢-١٢ شهر بعد العلاج فوجد أن جميع الأفراد بعد الجرعة الأولى (الأفراد المعالجة بجرعة واحدة أو المعالجين بجرعتين و لكن في الفترة قبل الجرعة الثانية) كان لهم القدرة على إصابة البعوض بالعدوى. أما الطور المعدي لطفيل الفيلاريا فقد وجد في البعوض المغتذى على ٢ فرد منهم فقط. وقد تراوحت معدلات الإصابة والعدوى في البعوض بين ٢.٩ -١٤.٢ % و ٢.٤ -١٤.١% على الترتيب. وفي دراسة معملية مقارنة لبعوض *كيولكس ببينز* المغتذى تغذية صناعية من خلال غشاء على وجبة دم معدية ممزوجة بجرعة من **DEC** (٠,٠٦ مليجرام /مل دم) فقد انخفض معدل العدوى في البعوض الناقل انخفاضا ملحوظا بمقدار ٥٤.٦ % و كذلك انخفض متوسط الحمل الطفيلي داخل البعوض بمقدار ٢٦.٢ % مقارنة بالمجموعة الضابطة من البعوض المغتذى على وجبة الدم المعدية فقط. ولدراسة تأثير عقار **DEC** على نمو طفيل الفيلاريا داخل البعوضة فقد تمت تغذية مجموعات من إناث بعوض *كيولكس ببينز* على وجبة دم معدية ثم على جرعة من عقار **DEC** (٠,٠٦ مليجرام /مل) في محلول سكري على فترات مختلفة. أوضحت النتائج أن عقار **DEC** الذي تم ابتلاعه في فترات ٣.٥- ٦.٥ يوم بعد وجبة الدم لم يكن له تأثير ملحوظ على معدل إصابة البعوض بالعدوى على العكس منها في فترات ٥, ٨ – ١٣.٥ يوم حيث كانت نسبة الانخفاض تمثل ٢٨.٩ و ٣٥,٩ % على الترتيب. حيث المفترض أن جرعة **DEC** تم تحريرها مع السكر من الحوصلة في تلك الفترات الأخيرة. ومن هنا يمكن أن نستنتج أن جرعة واحدة سنوية من عقار **DEC** لها تأثير فعال ومستمر في خفض المستوى العددي ليرقات الميكروفيلاريا في الدم للأفراد المصابة وبالتالي الحد من انتشار المرض ولكن ليس القضاء عليه نهائيا. وبالمثل له القدرة على قتل طفيل الفيلاريا داخل البعوض الناقل.

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Host-feeding patterns of *Culex pipiens* (Diptera - Culicidae) in El-Abtal village, Ismailia Governorate, Egypt.

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ABSTRACT

Host selection patterns of *Culex pipiens* were determined over the mosquito's seasonal activity period (May to November) during the year 1999 at El-Abtal village, representing new settlements in Ismailia Governorate. A total of 224 blood meals were analyzed by an enzyme-linked immune-sorbing assay. Almost half of the indoor tested mosquitoes were found to feed on human 50.7% (n=142). The outdoor collected samples had fed predominately on mammals other than humans 65.8% (n=82). Mixed meals were recognized 14.1% (n=142) and 9.8% (n=82) from total engorged female *Cx. pipiens* from both indoor and outdoor respectively. The occurrence of mixed meals of the blood of humans and other mammals were calculated in order to evaluate the potentials of this species as a vector of human and zoonotic diseases in new settlements.

KEYWORDS: *Culex pipiens*, feeding, ELISA.

INTRODUCTION

Mosquito host-feeding patterns are important in the epidemiology of mosquito-borne pathogens. Diseases of public health significance in Egypt are commonly caused by mosquito-borne pathogens, such as RVF viruses (kenawy *et al.* 1987; Gad *et al.* 1987, 1995) and filariasis (Soliman 1990, 1995)

A series of studies on mosquito feeding in Egypt was carried out on mosquitoes from Gharbiya Governorate by Zimmerman *et al.* (1985), West Desert Oases (Kenawy *et al.* 1986), Aswan Governorate (Kenawy *et al.* 1987; Gad *et al.*1999) and Sharqyia Governorate (Gad *et al.* 1995).

The present study was conducted in El-Abtal village, an area representing new settlements in Ismailia Governorate. The objective of this study is to estimate the potential of *Culex. pipiens* in the transmission of mosquito-borne diseases by the determination of host feeding preferences.

MATERIALS AND METHODS

The study site: El-Abtal village is located at the East of lakes area at Ismailia Governorate, which represents a new settlement surrounded by newly reclaimed lands. It is inhabited with immigrants mostly from the Nile valley. The surrounding lands are irrigated during the whole year round by El-Salam canal, where crops of alfalfa, wheat and rice are rotated in the fields. Citrus, mango, grapes, pomegranate, apricot, fig and banana orchards are presents in some areas. Wild vegetation is scattered near the canals.

Mosquito sampling: Engorged mosquitoes were collected from outdoor areas and inside houses over the mosquito activity period (May to November) through the year 1999. A battery-powered aspirator (D.vac) (Nasci 1981) was used to collect engorged mosquitoes, which were resting outdoors. Mosquito were sampled in a series of 5-min collections in vegetation surrounding houses, along irrigation canals and in the fields within 250 metres of houses during late afternoon. Samples inside houses were done by spraying pyrethroids

insecticides with a hand pump and collecting mosquito as they felt onto a 1-m^2 index cloth with two handles. The cloth was moved around the walls and also under furniture and other objects. Collected specimens were identified according to the key of Gad (1963) and Harbach (1985). Blood samples were sorted according to species and location, each group was placed in a labeled vial and stored at -70^{0}C until tested.

Human population and domestic animals density were estimated in the village. Over 80% of the households contained at least one domestic large animal. Most of the houses are consisted of more than one room and the villagers there used to keep the animals with them indoors. Free ranging dogs and cats were numerous in the village, but the census underestimated them. Other potential hosts such as birds and rodents were not surveyed.

Blood meals were identified using the direct enzyme immunoassay (ELISA) developed by Beier *et al.* (1988) with slight modification by Bahgat (1997). The forage ratio was determined according to Hess *et al.* (1968). This ratio was calculated for each host category as the percentage of positive blood meals divided by the percentage of available hosts, with ratios >1 indicating preference, ratios <1 indicating avoidance and ratios approaching 1 indicating little preference or avoidance. The probability of interrupted feeding was determined according to the method described by Burkot *et al.* (1988). Data was statistically analyzed using statistical package "Microstate".

RESULTS

Mosquitoes collected from indoors and outdoors were exclusively *Cx. pipiens*. Based on the total number of mosquitoes collected during the study period, blood fed *Cx. pipiens* were almost twice abundant in indoors as compared to the outdoor ones (Table 1).

Table 1: Host blood meal sources of *Cx. pipiens* female mosquitoes collected from indoors and outdoors in El-Abtal village, Ismailia Governorate.

Hosts	Indoor collected (% total)	Outdoor collected (% total)
Human	50.7	14.6
Bovine	4.9	12.2
Ovine	1.4	4.8
Equine	13.4	39
Dog	—	--
Cat	1.4	9.8
Rat	—	--
Unknown	14.1	9.8
Mixed meals	6.5	9.8
Total	142	82
	χ^2= 201.57 (P = 0.000)	χ^2= 37.11 (P = 0.00004)

The host-feeding pattern of *Cx. pipiens* differed between indoors and outdoors. Engorged mosquitoes collected from indoors (65.8%, n=142) (χ^2 = 201.57, 4 degree of freedom, P = 0.000) were Significantly feed on human , whereas about 56.1% (n=82) out of the total engorged *Cx. pipiens* collected from outdoors fed predominantly on mammals other than humans. Equine group represented the most significant blood source for outdoor *Cx. pipiens* (39%, n=82) followed by bovine group (12.2%, n=82) (χ^2 = 37.11, 3 degree of freedom P =0.00004).

Monthly estimates of available human and domestic animal hosts in selected households during the study period were done and the average ratios were used to calculate the forage ratios among engorged females of *Cx. pipiens* for each host (human, cows, buffaloes, sheep, goat and donkeys). Highest forage ratio (indoors and outdoors) was for Equine and bovine, the high forage ratio for ovoid was recorded among engorged *Cx. pipiens* collected from

outdoors. The engorged *Cx. pipiens* collected from both indoors and outdoors showed a high degree of avoidance towards humans blood, forage ratio <1 (Table2).

Table 2: Forage ratio for *Cx pipiens* female mosquitoes collected from indoors and outdoors in El-Abtal village, Ismailia Governorate

	Human	Bovine	Ovine	Equine
Animal census	0.92	0.04	0.02	0.02
Indoor forage ratio	0.55	1.23	0.7	6.7
Outdoor Forage ratio	0.158	3.05	2.4	19.5

Mixed meals were recognized at ratios of 14.1% (n =142) and 9.8% (n =82) out of the total engorged females collected from both indoors and outdoors respectively (Table 1).

Out of 20 indoor mixed meals about 60% were on humans and other mammals such as equines and bovines and about 40% were on mammals rather than human such as, bovines equines and cats. Outdoor mixed meals were on the blood from bovines and equines (50%), or bovines and ovines (50%) (Table 3).

Over all, the predicted probability of a blood meal being interrupted was 0.02. The proportion of mixed meals estimated the probability of interruption of *Cx. pipiens* meals is shown in Table 4.

Table (3) Multiple blood meal sources of *Cx. pipiens* mosquitoes in El-Abtal village, Ismailia Governorate.

	Mosquito blood host %	
Blood meals	Indoor	Outdoor
Bovine/ Equine/ Cat	10	—
Equine/ Cat/ Human /Bovine	5	—
Human /Equine	30	—
Human/ Bovine	20	—
Bovine/Equine	10	50
Bovine/ Ovine	15	50
Cat / Ovine	5	
Human / Ovine	5	
Total No.	20	8

Table (4) Probability of *Cx. pipiens* being interrupted during blood feeding.

	No. human Meals	No. mixed human meals	Total meals	Proportion of mixed human Meals	Predicted Q	Predicted I_H
Indoor	86	14	142	0.01	0.512	0.02
Outdoor	12	-	-	-	-	-

Q = Proportion of meals with only human blood + (proportion of mixed human meals/2)
IH = Probability of interruption of human blood meal.
IN = Probability of interruption of non human blood meals
Assuming IH =IN= Proportion of mixed meals / Q (1-Q).

DISCUSSION

Culex pipiens was the most common mosquito species in the study area, an observation which is in accordance with most previous studies which have shown that *Cx. pipiens* is the most abundant species in Egypt (Hurlbut & Weitz 1956; Hoogstral *et al.* 1979; Kenawy *et al.* 1987). It appeared to be fairly endophagic as most of the engorged *Cx. pipiens* female mosquitoes were collected from indoors and less partially from outdoors.

The indoor-engorged *Cx. pipiens* fed predominantly on human and other large mammals mainly equine and bovine. This may attribute to the movement of mosquitoes from room to room within houses searching for proper hosts (animals slept inside most of the

surveyed houses). Outdoor collections provided less biased samples of engorged mosquitoes than indoor ones, the outdoor human feeding rate was 14.6%. Earlier studies reported that *Cx. pipiens* from the Aswan (kenawy *et al.* 1987) showed marked anthropophagi. Zoophagy was reported for female *Cx. pipiens* from Gharbiya Governorate (Zimmerman *et al.* 1985).

Much of the geographic variations in host by mosquito species in Egypt can be attributed to relative host abundance, which is largely a reflection of ecological conditions and human customs (Zimmerman *et al.* 1985; Beier *et al.* 1987). Forage ratios for indoor and outdoor collected mosquitoes based on the proportion of humans and three other classes of large mammals, especially equines, bovines and ovines. Earlier studies in Fayium Oasis reported similar results where this species exhibited an elevated forage ratio for bovines and ovines (Beier *et al.* 1986).

Although the abundance and relative proportion of large mammals was estimated monthly the census may be not accurate enough and underestimated the wild animals as a risk factor for the transmission of a human parasitic diseases such as *W. bancrofti* as the infective mosquito which moves from a human host to a non human host would loose part of its filarial larvae (L_3) (De Meillion *et al.* 1967). In the present study, no data were collected concerning mixed human feeds (human / human) such feeds are designated as cryptic mixed meals by Boreham & Garrett Jones (1973) and differentiated on the basis of their blood groups. Accordingly the opportunistic feeding habits of *Cx. pipiens* and its ability to switch from human to non-human vertebrate hosts probably affect its efficiency as a human filariases vector. This is counter balanced by its tremendous abundance in Egypt.

The proportion of mixed feeds including human and other mammals represented 9.9% of all feeds and the probability value for any of *Cx. pipiens* blood feed to be interrupted was 0.02. The effect of interrupted feeding is to increase the number of total feeds taken by a mosquito with consequent epidemiological implications (Burkot *et al.* 1982). It may be that the increased number of feeds taken increase the vectorial capacity of the population by increasing the chances acquiring and transmitting the agents of zoonotic diseases. It is not known, however, if this stands for RVF virus infection if is introduced to such new settlements. This shows that multiple blood feeding by mosquito vectors still needs more studies to evaluate the significance of interrupted feeding in both human and zoonotic diseases.

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الملخص العربى

السلوك الأغتذائى لبعوض كيولكس بيبنز (رتبة ثنائية الأجنحة – كيولسيدى) فـــي قريـــة الأبطـــال محافظة الاسماعيليه – مصر

إيمان محمد بهجت

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تمت دراسة التفضيل الغذائي لبعوضة *كيولكس بيبنز* أثناء فترة نشاطها الموسمي (مايو–نوفمبر) خلال عام ١٩٩٩ فـي قرية الأبطال آلتي تمثل مناطق الاستصلاح الحديثة في محافظة الاسماعيليه٠ خلال هذه الدراسة تم تحليـــل ٢٢٤ مـــن البعوض المتغذي على وجبة دم باستخدام الطريقة المباشرة لقياس الادمصاص المناعي الانزيمى٠ وقد شكل الاغتـــداء على دم الإنسان نسبة ٧ر٥٠ % من عدد ٤٢ من البعوض المجمع من داخل المنازل ٠أما عن البعوض المجمـــع مـــن خارج المنازل فقد اظهر تفضيل لدم الثدييات الأخرى دون الإنسان حيث شكل الاغتذاء على الثدييات نســـبة ٨ر٦٥ % من مجموع العينات آلتي تم تجميعها وتحليلها (٨٢ بعوضة) ٠ وبالنسبه للوجبات المركبة شكلت نسبة ١ر١٤ % (مـن ١٤٢بعوضه) و ٨ر٩ % (من ٨٢ بعوضه) وذلك بالنسبة لإناث بعوض *كيولكس بيبنز* المجموع مـــن داخـــل وخـــارج المنازل على التوالى٠وتعتبر دراسة نسبة الوجبات المركبة من دم الإنسان والثدييات الأخرى والناتجـــة عـــن إزعـــاج البعوض أثناء اعتذائه وكذلك قدرة معدل الاغتذاء على كل عائل منسوباً آلي النسبة آلتي يشكلها من التعداد الكلى للعوائل المختلفة بغرض تقييم كفاءة هذا النوع من البعوض (*كيولكس بيبنز*) كعائل وناقل لبعض الأمراض الخاصة بالإنســـان وكذلك الأمراض المشتركة بين الإنسان والعوائل الحيوانية الاخرى٠