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Life table analysis of *Cimex hemipterus* F. (Hemiptera: Cimicidae) reared on different types of human blood

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ABSTRACT

Objective: To determine the development time of each immature stage and total development time of *Cimex hemipterus* reared on human blood type A, B, O and AB, and to compare survivorship and fecundity of *Cimex hemipterus* reared on blood type A and AB.
Methods: Bed bugs were reared on human blood type A, B, O and AB, fed through an artificial feeding system. The number of live and dead individuals, number of nymphs that molted, and number of eggs laid were observed and recorded daily.
Results: Statistical analysis showed that no significant difference was observed for the development from egg to adult emergence. Bed bugs reared on blood type A and AB had a life expectancy of 88 and 105 days respectively from the egg stage. The net reproductive rate (R_0), mean generation time (T), intrinsic rate of increase (r_m), finite rate of increase (λ), and doubling time (DT) for bed bugs reared on blood type A were 12.24, 67.84, 0.037, 1.038 and 18.73, respectively. On the other hand, the same parameters calculated for bed bugs reared on blood type AB produced R_0 = 12.58, T = 83.36, r_m = 0.030, λ = 1.030 and DT = 23.10.
Conclusions: Different blood sources may have different effects on the development time and life characteristics of bed bugs.

1. Introduction

Bed bug is a hematophagous ectoparasite that feeds primarily and exclusively on blood where all instar stages required blood meals to molt to the next life stages and the adults needed blood meals for reproduction[1-4]. Although bed bugs are not known to vector any human pathogen[3,5], feeding these bugs on human or animal may cause discomfort to the host as their bites have been reported to cause rashes and itchy skin, and repeated exposure to external allergens leads to skin reactions[2,3,5,6].

Montes *et al.* developed an artificial feeding system that could feed all the five nymphal instars and the adults[7]. They found that heparinized blood was the most suitable blood meal for feeding bed bugs using an artificial feeding system. Chin-Heady *et al.* used rabbit blood with sodium citrate acted as anticoagulant in their experiment to compare two artificial feeding systems, water bath method and Petri dish method[8]. Previous studies also showed that bed bugs can be reared with blood of vertebrates including human, mice, rabbit, guinea pig, bird and chicken[3,9-12]. Various information on the life history of an organism can be derived from a life table constructed from the organism's survivorship data. This information is useful in aiding the development of more sophisticated pest control measures. Age-specific life table is normally used for organisms with shorter life spans, particularly those completed within a year such as bed bug and other insects[13-15].

Generally, the life cycle of bed bug from the first hatch of egg to the next offspring is approximately 6 weeks under natural conditions[3,4]. Adult bed bugs emerged on Week 7 when they were fed using water bath method and on Week 8 when they were fed using the Petri dish method[8]. Both methods were using rabbit blood that contains 2% sodium citrate as their blood meals. Barbarin *et al.* reported that the development from egg to adult of *Cimex lectularius* (*C. lectularius*) reared on human blood was significantly shorter than those reared on rabbit blood[11]. They also reported that the life expectancy of *C. lectularius* fed on human blood is longer than the ones fed on rabbit blood. Therefore, the objectives of this study were to determine the development time of each immature stage and total development time of tropical bed bug, *Cimex hemipterus* (*C. hemipterus*), reared on different types of human blood, A, B, O and AB, and to compare survivorship and fecundity of *C. hemipterus* reared on blood type A and AB. Bed bugs were fed on recently expired whole blood by using an artificial feeding system.

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2. Materials and methods

2.1. Bed bugs

The tropical bed bugs, *C. hemipterus*, were collected from Kuala Lumpur International Airport, Malaysia. Collected samples were reared in 200 mL plastic container (8 cm in height, 8 cm in diameter) containing folded A4 paper strips (Double A, Chachoengsao, Thailand) as their harborage. The paper strips were placed perpendicular to the bottom of the container to provide a surface for the bed bugs to walk and deposit eggs. Plastic containers were covered with a piece of fine net cloth (13 cm × 13 cm) with a pore size of ~1 mm for ventilation and a rubber band to hold it in place. The bugs were reared according to their developmental stages, first instar to adult stages. The tropical bed bugs colonies were cultured under standard environmental condition in an incubator with temperature at (26 ± 1) °C, (65 ± 5)% relative humidity and 12:12 (light: dark) photoperiod[12,14,16]. Environmental conditions were selected to ensure maximum survival and fecundity. Tropical bed bug colonies were fed on whole blood via an artificial feeding system once a week[7,8,10].

2.2. Blood meals

Recently, expired human bloods were collected from Blood Transfusion Unit, Penang Hospital. Whole blood was used in this study and the bed bugs were fed through an artificial feeding system. The whole blood sachets collected were ensured to be within a week of its expiration date at the time of collecting. Although blood meals can last up to three months, new sachets of whole blood were collected every month to assure that the bed bugs colonies were fed with viable blood meals. The blood meals were transferred to Schott Duran bottles for easy handling and stored in a refrigerator with the temperature maintained at 2–6 °C. Gloves and other appropriate attires were worn when handling the blood meals as the blood may harbor noxious agents.

2.3. Egg culture

Life table parameters were built starting with newly oviposited eggs. Fifth-instar tropical bed bug nymphs were removed from the main colony and placed into separate plastic containers. Bed bugs were allowed to feed on whole blood according to the blood type via an artificial feeding system and were followed to adulthood, and later labelled accordingly. Ninety eggs were randomly selected for three replicates (30 eggs per replicate) for each blood type. Paper strips with thirty eggs were collected from the adults' containers according to the blood type and placed in a new plastic container.

2.4. Bed bug development from egg to adult

Once the fifth instars molted to adults, 15 males and 15 females were randomly selected, and fed on the blood by using an artificial feeding system[7,8,10] according to the blood type. The adults were allowed to mate in a new plastic container that contained folded A4 paper and left to produce eggs. Over a period of seven days, eggs production was observed and counted. Once 30 eggs were produced, the folded A4 paper were removed and placed in a new plastic container. Hatched first instar nymphs were recorded daily. The first instar nymphs were fed once all the eggs hatched or after 10 days if the number of nymphs that hatched was less than 30. The next instar nymphs, second to fifth instars, were fed once all the previous instar nymphs had molted. If the nymphs that molted were less than 30, feeding was conducted on the third day after the last nymphs molted. All instar nymphs were fed on their respective blood type by using an artificial feeding system. Bed bugs were observed daily from egg to the emergence of adults to record the development of the immature stages of bed bugs. Observations consisted of counts of the number of live and dead individuals, number of nymphs that molted, and the number of adults produced (male and female). Molting was signified by the presence of exoskeletons in the containers[16].

2.5. Bed bug survivorship and fecundity fed on human blood type A and AB

Adult bed bugs have an average life span of 6–12 months[2-4] and it is logistically challenging to feed and observe a large number of individuals from egg to the end of the adult stage[14]. Therefore, bed bugs that were reared on blood type A and AB were elected to be monitored for the survivorship and fecundity characteristics, based on the availability of blood sources and the time constraint. All three replicates of bed bugs that reared on blood type A and AB were placed in new plastic containers in which folded red A4 paper provided harborages and followed through the adulthood. The number of live and dead individuals was observed and recorded daily until the death of the last individuals. The number of eggs laid was recorded daily until egg laying ceased. The adults were fed approximately every 10 days following cessation of egg laying and later placed in a new plastic container. Containers which contained the eggs were observed daily and the number of the first instar nymphs that hatched was recorded. Colored A4 paper facilitated in counting the number of eggs laid by the tropical bed bugs.

2.6. Life table construction

Data on development of the immature stages from bed bugs reared on blood type A, B, O and AB were used to construct survivorship life tables through adulthood, while the data on survival and fecundity of bed bugs reared on blood type A and AB were used to derive a complete life table. Fecundity (m_x) generally refers to the ability to reproduce. In demography, fecundity is the potential reproductive capacity of an individual or population, usually measured as female offspring per female of age *x*. Other life table parameters, namely, gross reproductive rate (GRR), net reproductive rate (R_0), intrinsic rate of natural increase (r_m), finite rate of increase (λ), mean generation time (T), and doubling time (DT) were subsequently calculated for tropical bed bugs reared on blood type A and AB, respectively.

Several assumptions were made in developing the life tables. All of the eggs laid were assumed fertile and that failure to hatch was due to natural mortality and not accidental damage during handling. The mortality rates were assumed equally applicable to males and females for the eggs and immature stages since the sex of individual bed bugs could not be determined in those stages. Finally, an assumption of a 1:1 sex ratio of male and female was made in keeping with previous observations of laboratory-reared bed bugs by Polanco *et al.*[14]. The parameters and formulae (Table 1) for constructing and analyzing the life tables were found in Molla *et al.*, Manikandan *et al.*, Sowilem *et al.*, Patil *et al.*, Tan *et al.*, and Henderson and Southwood[15,17-21].

2.7. Statistical analysis

Data on development of immature stages were analyzed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Data were checked for normality prior to analysis. Since the data were not normally distributed, data were transformed and later subjected to One-way ANOVA and means were separated using Tukey's honest significant difference. Both data on development of each immature stage and total development from egg to the emergence of adults were analyzed using One-way ANOVA to determine the significant difference between immature stages and evaluate the effects of human blood type on bed bug development time.

Table 1

Symbols and formulae used to construct life table.

Symbols	Description	Formulae
n	Duration in days	
x	Age interval in days	
N_x	The number surviving at the beginning of age x	
d_x	The number dying during the age interval x	
l_x	The probability of being alive or surviving to age x	N_x/N_0
${}_np_x$	The probability of surviving during the age interval	N_{x+1}/N_x
${}_nq_x$	The probability of dying during the age interval	d_x/N_x
${}_nd_x$	The fraction of the original cohort that die between the interval	d_x/N_0
${}_nL_x$	The fraction of surviving during the interval per individual	$(l_x - {}_nd_x/2)(n)$
T_x	The total number of days lived beyond age x	$\sum L_x$
e_x	The life expectancy of an individual alive at age x	T_x/l_x
m_x	The eggs produced per surviving female	Eggs/2
GRR	Gross reproductive rate	$\sum m_x$
R_0	Net reproductive rate	$\sum l_x m_x$
T	Mean generation time	$\sum(xl_x m_x/R_0)$
r_m	Intrinsic rate of increase (female/female/day)	$\ln R_0/T$
λ	Finite rate of increase (female/female/day)	e^{rm}
DT	Doubling time	$\ln 2/r_m$

3. Results

Bed bugs were fed on recently expired human blood (whole blood) by using an artificial feeding system[7,8,10] and left for about 1 h to ensure all the bed bugs have fed and are replete. The survivorship (l_x) of egg to adult stage life tables for the tropical bed bugs reared on human blood type A, B, O and AB were combined and illustrated in abridged form in Table 2. Bed bugs reared on blood type A, B and AB had the highest immature survivorship (~97%) with only ~3% of immature mortality. Bed bugs reared on blood type O had the lowest survivorship (~84%) as total mortality showed that more than 15% of immature stages had died before reaching adulthood. Stage-specific mortality was relatively high for bed bugs reared on blood type O during the 20 and 40 days of age interval.

Additionally, life expectancy (e_x) column displayed an interesting life history characteristic. A newly egg laid of tropical bed bugs reared on blood type A, B, O and AB had a life expectancy of 59.35, 59.65, 56.50 and 59.75 days, respectively. This simply implies that an egg from colony reared on blood type A, B and AB is expected to be alive ~3 days longer before becoming an adult compared with an egg from the colony reared on blood type O. The results also revealed that there was no mortality during egg stage for all colonies, but the number entering adult stage was varied with bed bugs reared on blood type O recorded the lowest number (76 individuals) followed by colonies reared on blood type A, B and AB (87 individuals). Furthermore, all colonies were fed more than five times before they emerged as adults.

Table 3 shows the mean duration of development for the immature life stages and the total development from egg to adulthood of tropical bed bugs reared on human blood type A, B, O and AB. Bed bugs reared on blood type AB had a longer development time (~49 days) compared to bed bugs that reared on blood type O (~45 days) as well as colonies reared on blood type A and B (~43 days). The third and fourth instar of bed bugs fed on blood type AB took the longest time to molt, with an average of ~10 days to complete the development. The fifth instar of bed bug reared on blood type B and AB took the shortest time of ~5 days to complete their stage development. Generally, egg to adult development duration for bed bugs reared on blood type A and B was shorter by ~6 days than for the bed bugs fed on blood type AB. Bed bugs reared on blood type O took ~45 days to reach adulthood, which was ~2 days longer than those reared on blood type A and B but ~4 days shorter than the ones reared on blood type AB. Overall, there was no significant difference (P > 0.05) between the duration of total development from egg to the emergence of adult for the tropical bed bug reared on blood type A, B, O and AB (Figure 1).

Table 2

Abridged survivorship life tables for the immature stages of *C. hemipterus* reared on human blood type A, B, O and AB.

Blood type	x	Life stage	n_x	l_x	e_x	Stage mortality (%)
A	0	Egg	90	1.00	59.35	
	10	N1	90	1.00	49.35	1.11
	20	N1, N2	89	0.99	39.80	
	30	N3, N4	89	0.99	29.80	
	40	N4, N5, Adult	89	0.99	19.80	1.11
	50	N5, Adult	88	0.98	9.95	1.11
	60	Adult	87	0.97		
B	0	Egg	90	1.00	59.65	
	10	N1	90	1.00	49.65	
	20	N1, N2	90	1.00	39.65	
	30	N3, N4	90	1.00	29.65	
	40	N4, N5, Adult	90	1.00	19.65	2.22
	50	N5, Adult	88	0.98	9.95	1.11
	60	Adult	87	0.97		
O	0	Egg	90	1.00	56.50	
	10	N1	90	1.00	46.50	1.11
	20	N1, N2	89	0.99	36.92	5.56
	30	N3, N4	84	0.93	28.98	
	40	N4, N5, Adult	84	0.93	18.98	5.56
	50	N5, Adult	79	0.88	9.83	3.33
	60	Adult	76	0.84		
AB	0	Egg	90	1.00	59.75	
	10	N1	90	1.00	49.75	
	20	N1, N2	90	1.00	39.75	
	30	N3, N4	90	1.00	29.75	
	40	N4, N5, Adult	90	1.00	19.75	1.11
	50	N5, Adult	89	0.99	9.90	2.22
	60	Adult	87	0.97		

N1: 1st instar; N2: 2nd instar; N3: 3rd instar; N4: 4th instar; N5: 5th instar.

Table 3

Mean duration of each life stage (days) of *C. hemipterus* feeding on blood type A, B, O and AB.

Life stage	Development time (Days ± SE)			
	A	B	O	AB
Egg	8.33 ± 0.67^a	7.33 ± 0.33^a	6.67 ± 0.33^a	7.00 ± 0.00^{ab}
1st instar	8.33 ± 0.33^a	7.67 ± 1.45^a	7.00 ± 1.00^a	7.00 ± 1.00^{ab}
2nd instar	6.33 ± 0.33^a	6.00 ± 0.58^a	7.67 ± 1.33^a	9.00 ± 0.00^{bc}
3rd instar	6.67 ± 0.33^a	8.33 ± 0.67^a	9.33 ± 0.33^a	10.33 ± 0.33^c
4th instar	7.33 ± 1.20^a	8.00 ± 1.16^a	8.33 ± 0.33^a	10.33 ± 0.88^c
5th instar	6.33 ± 0.33^a	5.67 ± 0.33^a	6.33 ± 0.33^a	5.67 ± 0.33^a
Egg – Adult	43.33 ± 1.20^a	43.00 ± 2.00^a	45.33 ± 2.33^a	49.33 ± 0.67^a

Means followed by the same letter in each row are not significantly different (P > 0.05).

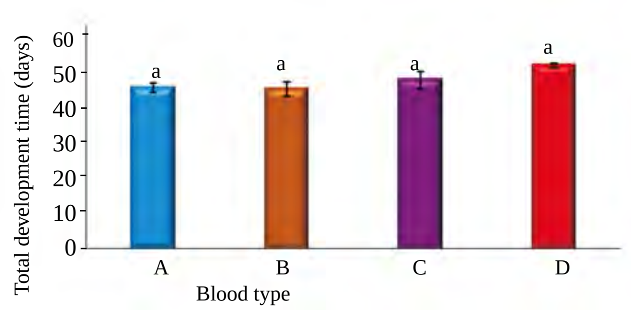

Figure 1. Mean duration of total development from egg to the emergence of adult (P > 0.05).

Stage specific life tables of the tropical bed bugs reared on human blood type A and AB were presented in Tables 4 and 5, respectively. The tropical bed bugs reared on human blood type A have a life expectancy (e_x) of 88 days in the egg stage and bed bugs reared on blood type AB have a life expectancy of 106 days.

Table 4
Stage specific life table of the tropical bed bug, *C. hemipterus* reared on human blood type A.

Stage (*n* = duration in days)	x	N_x	d_x	l_x	$_np_x$	$_nq_x$	$_nd_x$	$_nL_x$	T_x	e_x
Egg (*n* = 8)	0–8	90	0	1.00	1.00	0.00	0.00	8.00	88.26	88.26
1st Instar (*n* = 8)	8–16	90	1	1.00	0.99	0.01	0.01	7.96	80.26	80.26
2nd Instar (*n* = 6)	16–22	89	0	0.99	1.00	0.00	0.00	5.94	72.30	73.03
3rd Instar (*n* = 7)	22–29	89	0	0.99	1.00	0.00	0.00	6.93	66.36	67.03
4th Instar (*n* = 7)	29–36	89	1	0.99	0.99	0.01	0.01	6.90	59.43	60.03
5th Instar (*n* = 6)	36–42	88	11	0.98	0.88	0.13	0.12	5.49	52.53	53.60
Adult (*n* = 40)	42–82	77	40	0.86	0.48	0.52	0.44	25.60	47.04	54.70
Adult (*n* = 40)	82–122	37	19	0.41	0.49	0.51	0.21	12.20	21.44	52.29
Adult (*n* = 40)	122–162	18	10	0.20	0.44	0.56	0.11	5.80	9.24	46.20
Adult (*n* = 40)	162–202	8	5	0.09	0.38	0.63	0.06	2.40	3.44	38.22
Adult (*n* = 40)	202–242	3	1	0.03	0.67	0.33	0.01	1.00	1.04	34.67
Adult (*n* = 4)	242–246	2	2	0.02	0.00	1.00	0.02	0.04	0.04	2.00

Table 5
Stage specific life table of the tropical bed bug, *C. hemipterus* reared on human blood type AB.

Stage (*n* = duration in days)	x	N_x	d_x	l_x	$_np_x$	$_nq_x$	$_nd_x$	$_nL_x$	T_x	e_x
Egg (*n* = 7)	0–7	90	0	1.00	1.00	0.00	0.00	7.00	105.79	105.79
First instar (*n* = 7)	7–14	90	0	1.00	1.00	0.00	0.00	7.00	98.79	98.79
Second instar (*n* = 9)	14–23	90	0	1.00	1.00	0.00	0.00	9.00	91.79	91.79
Third instar (*n* = 10)	23–33	90	1	1.00	0.99	0.01	0.01	9.95	82.79	82.79
Fourth instar (*n* = 10)	33–43	89	2	0.99	0.98	0.02	0.02	9.80	72.84	73.58
Fifth instar (*n* = 6)	43–49	87	6	0.97	0.93	0.07	0.07	5.61	63.04	64.99
Adult (*n* = 40)	49–89	81	27	0.90	0.67	0.33	0.30	30.00	57.43	63.81
Adult (*n* = 40)	89–129	54	33	0.60	0.39	0.61	0.37	16.60	27.43	45.72
Adult (*n* = 40)	129–169	21	13	0.23	0.38	0.62	0.14	6.40	10.83	47.09
Adult (*n* = 40)	169–209	8	2	0.09	0.75	0.25	0.02	3.20	4.43	49.22
Adult (*n* = 30)	209–239	6	5	0.07	0.17	0.83	0.06	1.20	1.23	17.57
Adult (*n* = 5)	239–244	1	1	0.01	0.00	1.00	0.01	0.03	0.03	3.00

While bed bugs reared on human blood type A had a 86% chance of surviving to adulthood and were expected to live an additional 54 days once reaching the adult stage, bed bugs reared on human blood type AB had a higher chance of reaching the adult stage (90%) and were expected to live another 64 days once reaching the adult stage.

Figures 2 and 3 illustrate the survivorship curve (l_x) and the number of daughter eggs produced every ten days by each female (m_x) reared on blood type A and AB, respectively. The highest egg production per female was observed during the interval of Day 50 for bed bugs reared on blood type A and Day 60 for tropical bed bugs reared on blood type AB. The GRR for bed bugs reared on blood type A and AB was ~15 and ~14 daughter eggs per female, respectively (Table 6). When female survivorship was considered, bed bugs reared on blood type A and AB, both had a R_0 of ~12 daughter eggs per female. This suggests that bed bugs reared on blood type A or AB, are expected to have a 12-fold increase in the population per generation, with a T of 68 and 83 days, respectively. The approximate value of the r_m for bed bugs reared on blood type A and B, were 0.04 and 0.030 daughters/female/day, respectively. These indicate that colonies of bed bugs reared on blood type A and B multiply ~1 time per female per day (λ), but will double in size on different interval, 19 days for blood type A and 23 days for blood type AB.

Table 6
Population parameters of Cimex hemipterus reared on blood type A and AB.

Parameter	Blood type A	Blood type AB
Gross reproductive rate (GRR)	15.24	14.42
Net reproductive rate (R_0)	12.24	12.58
Mean generation time (T)	67.84	83.36
Intrinsic rate of increase (r_m)	0.04	0.03
Finite rate of increase (λ)	1.04	1.03
Doubling time (DT)	18.73	23.10

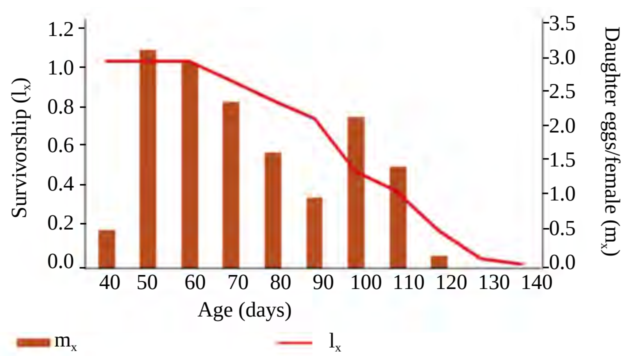

Figure 2. Survivorship curve and the number of daughter eggs produced every 10 days by each female of the tropical bed bug, *C. hemipterus* fed on human blood type A.

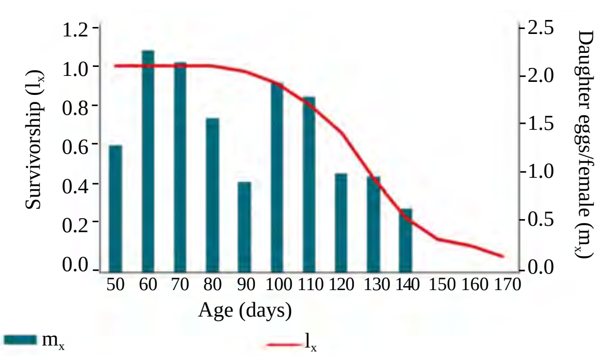

Figure 3. Survivorship curve and the number of daughter eggs produced every 10 days by each female of the tropical bed bug, *C. hemipterus* fed on human blood type AB.

4. Discussion

Construction of life tables is a convenient way of describing insect population dynamics. Entomologists constructed life tables in order to obtain insect life characteristics and population parameters such as survivorship and mortality, net reproductive rate, mean generation time, intrinsic and finite rate of increase, instantaneous birth and death rates, stable age distribution and reproductive values[17-21]. All these information on the biology, ecology, and population dynamics of an insect which can be derived from life tables, is very useful and essential in developing better control measures and improving management techniques towards certain insect pests. Researchers have employed application of life tables to illustrate the life history characteristics of populations over time in their studies on various insect species in many orders including Diptera[18], Lepidoptera[19], Hymenoptera[22], and Coleoptera[23].

Recent studies on life history of bed bugs were evaluated by Barbarin *et al.* and Polanco *et al.* but both were using common bed bug, *C. lectularius*, as their subject[11,14,24]. Polanco *et al.* constructed life tables for three strains of *C. lectularius*, a pyrethroid-susceptible laboratory strain, a highly resistant field strain, and a field strain with a declining level of resistance, fed on the arm of a human volunteer[14]. Barbarin *et al.* reared two strains of *C. lectularius* on two blood regimens, human and rabbit blood[11]. On the other occasion, Barbarin *et al.* compared the developmental rates and life tables of a laboratory strain and a field strain of *C. lectularius* reared on human blood[24]. Comparisons were made with these studies as *C. hemipterus* would display the same population parameters as *C. lectularius*[3,4].

Polanco *et al.* reported that the resistant field strain developed significantly faster from egg to adult (~35 days) than the other two strains (~40 days), while bed bugs in this study showed slower development from egg to adult (~43–49 days)[14]. The development time recorded in this study was more comparable with bed bug colonies reared on rabbit blood which took around ~52 days to reach adulthood[11]. Barbarin *et al.* reported that development time of subsequent offspring and generations were determined by the mother's strain[24]. How and Lee reported that total length of nymphal development period for tropical bed bugs was 17–20 days to achieve adulthood when reared on a human host[16]. These results are significantly shorter compared to the results achieved in this experiment. Suwannayod *et al.* also recorded shorter duration of various developmental stages of *C. hemipterus* and *C. lectularius* with an average of total development from egg to adult was ~40 days and ~37 days, respectively[25]. They reported that egg of both species recorded the shortest duration of development time, which is around 4 days. They also reported that the number of nymphal stages was five to six for both bed bug species[25] which was similar to this study where all tropical bed bug colonies were fed more than five times before the first emergence of an adult.

Barbarin *et al.* reported that bed bugs reared on human blood had a longer life expectancy from the egg stage than those reared on rabbit blood[11]. They also showed that different bed bug strain would have different life expectancy even when both colonies were reared on the same blood regimen. On the other study, Barbarin *et al.* reported that hybrid offspring from two strains have longer total survival time than their parents[24]. Additionally, a highly resistant field strain has the average of life expectancy of ~143 days from the egg stage[14] which is longer than the life expectancy of tropical bed bugs reared in this study where *C. hemipterus* reared on blood type A and AB had a life expectancy of 88 and 106 days, respectively. Studies on the longevity of mated and unmated adults of tropical bed bugs revealed that unmated adults lived significantly longer than mated adults, up to 216 days for unmated females[16]. Both colonies in this study had the last individual survived up to 240 days. Shorter longevity of mated adults, especially in females, compared to unmated adults, was probably because of their mating behavior, namely, traumatic insemination[3,14,26]. Suwannayod *et al.* reported that *C. hemipterus* and *C. lectularius* adult longevity may reach 122 and 127 days, respectively, when bed bugs were reared on rabbit blood[25].

Many factors may affect bed bug development and ultimate survival including sex, mating status, temperature, type of blood sources, nutritional status of the blood meal, duration of the blood meal and amount of blood imbibed[3,4,11,14,24]. This study demonstrates that survivorship and mortality of bed bugs were impacted by the blood they ingested. Studies by Barbarin *et al.* suggested that human blood may be more suitable to be used in rearing bed bug than blood from other animals[11], and this study further clarify that human blood type may also have influence on the immature development and adult longevity. However, biochemical differences may exist for blood sources used in this study since expired donor blood was used and fed through an artificial feeding system. Bed bugs reared via direct feeding method may present a better result as the bed bugs feed on fresh blood meals.

Romero and Schal, in their efforts to identify chemicals that stimulate acceptance and engorgement responses in various life stages of bed bugs reared via an artificial feeding system, revealed that adenosine triphosphate was more stimulatory than adenosine diphosphate, which was more effective than adenosine monophosphate[27]. Deng *et al.* demonstrated that blood feeding on an artificial feeder was not significantly different from blood feeding on live guinea pigs in a report outlining a novel feeding system for the dengue vector mosquito rearing[28]. Both mosquito colonies reared on a membrane feeding system and a live animal displayed no difference in the fecundity, survival, or the hatchability of eggs. Data derived from *Anopheles* standard research protocols suggest that glass feeding apparatus is just as effective as animal feeding[29,30].

Life tables of other insect pests also revealed that insects would have differences in their development when they fed or ate different food sources. For example, life table of *Cochlochila bullita* reared on *Orthosiphon aristatus* and *Ocimum basilicum* in laboratory condition found that the adult longevity of the bugs that feed on *Orthosiphon aristatus* was significantly higher than those bugs that feed on *Ocimum basilicum*. The pre-oviposition, oviposition and fecundity of *Cochlochila bullita* were also different between the host plants[20]. Tochen *et al.* reported that *Drosophila suzukii* reared on blueberries had lower fecundity than those reared on cherries at all temperatures where reproduction occurred[31]. Life table of the diamondback moth, *Plutella xylostella* on five host plants found that survival rate was the lowest when the moth reared on canola plant[32]. These imply that regardless of insect species, most of these pests would have differences in development time and other population parameters when they are reared on different food sources.

Recently expired whole blood was collected from blood bank and stored at 2–6 °C in a refrigerator. These sources of blood can be used successfully for up to 2 months when the blood was properly treated and stored at designated temperature[10]. Whole blood was selected as the blood source because the components of whole blood are the same as the component of blood in a human body which consists of a fluid intercellular material called plasma and formed elements which include erythrocytes (red blood cells), leukocytes (white blood cells) and platelets[33]. Human blood type is an inherited trait passed down by the parents. Parents' blood type will determine the types of their children's blood type. ABO blood groups are defined by the presence or absence of specific antigens that circulate in body fluids[34]. These differences in human blood components may be the key why there are differences in the nymphal development of bed bugs. However, further studies should be conducted to determine the specific components that have the nutritional differences on the bed bugs.

Various blood regimens may have different effects on the development time and life cycle of bed bugs. Studies on survivorship of bed bugs that fed on the other two blood type, B and O, should be conducted and full comparison can be made for all human blood type. Since this study was using expired blood, further studies could investigate the effects on bed bug development and survivorship when they were fed on host (direct feeding method) according to their blood type. Furthermore, studies should be conducted to explain the biochemical and nutritional differences between human blood types and other blood sources used to feed blood-sucking insect. The outputs from this study revealed that expired whole blood could be an alternative blood source for artificial feeding system. Bed bugs reared on this blood source can be used for other research objectives such as investigating insecticide resistance and improving their control techniques.

Conflict of interest statement

We declare that we have no conflict of interest.

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