1. Introduction

Bed bug is a hemaphagous ectoparasite that feeds primarily and exclusively on blood where all instar stages required blood meals for molting 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 hepaparized 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. A ge-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 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 (R0), mean generation time (T), intrinsic rate of increase (rm), 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 R0 = 12.58, T = 83.36, rm = 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.
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 D uran 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 harborage 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*) 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 (R0), intrinsic rate of natural increase (r), 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., Mankandan et al., Sowilem et al., Pati 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.
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 ($) 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 (~99%) 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 implied 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 A 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 4
Stage specific life table of the tropical bed bug, *C. hemipterus* reared on human blood type A.

| Stage (n = duration in days) | x  | N₀ | dₓ | lₓ | d₀ | d₁ | d₂ | lₘ | Tₓ | eₓ |
|-----------------------------|----|----|----|----|----|----|----|----|----|----|
| 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| 0.00| 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  | N₀ | dₓ | lₓ | d₀ | d₁ | d₂ | lₘ | Tₓ | eₓ |
|-----------------------------|----|----|----|----|----|----|----|----|----|----|
| 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 = 40)              | 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| 0.00| 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 (lₓ) and the number of daughter eggs produced every ten days by each female (mₓ) reared on blood type A and AB, respectively (Table 6). When female survivorship was considered, bed bugs reared on blood type A and AB, both had a R₀ 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 rₘ 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 (lₓ), 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 (R₀)         | 12.24        | 12.58         |
| Mean generation time (T)           | 67.84        | 83.36         |
| Intrinsic rate of increase (rₘ)    | 0.04         | 0.03          |
| Finite rate of increase (λ)        | 1.04         | 1.03          |
| Doubling time (DT)                 | 18.73        | 23.10         |

4. Discussion

Construction of life tables is a convenient way of describing insect
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. Suwannayot 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 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[13,14,26]. Suwannayot 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 bed bugs reared on human blood were fed through an artificial feeding system, revealed that adenosine triphosphate was more stimulatory than adenosine diphosphate, which was more effective than adenosine monophosphate[21]. 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]. Toczen 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 insects. 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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