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Laboratory rearing of *Cimex hemipterus* F. (Hemiptera: Cimicidae) feeding on different types of human blood compositions by using modified artificial feeding system

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ABSTRACT

Objective: To investigate the effects of three types of human blood compositions: whole blood, red blood cells and red blood cells mixed with plasma, and determine the suitable blood source that can be used to feed the bed bugs [*Cimex hemipterus* (*C. hemipterus*)], in comparison to the direct feeding method.
Methods: *C. hemipterus* were fed with the three types of blood compositions by using an artificial feeding system. Then the number of live and dead individuals as well as the number of adults produced was counted.
Results: Red blood cells caused 72.7% death of *C. hemipterus*, followed by red blood cells mixed with plasma (52.0%) and whole blood (48.7%). There were significant differences in the number of live individuals after seven weeks of feeding. However, there were no significant differences between the number of live individuals fed on whole blood, red blood cells and red blood cells mixed with plasma after seven weeks.
Conclusions: The components in the blood sources may be the key to their different effects on the growth dynamics of *C. hemipterus*.

1. Introduction

Global resurgence of bed bugs has sparked interest among scientists and the pest control companies for effective and efficient control and management strategies. Knowledge of biology and ecology of the bed bug, *Cimex lectularius* or *Cimex hemipterus* (*C. hemipterus*) under various environmental conditions is crucial to improve the management techniques. Bed bugs colonies are required to be tested and this is proved to be a problem since bed bug is an obligatory blood feeder[1-6]. These bugs feeding on blood of human or animal may cause discomfort to the host. Bed bugs bites can cause rashes and itchy skin, and repeated exposure to external allergens can lead to skin reactions[1,4,6-10]. Previous studies showed that bed bugs can be reared with bloods of vertebrates including human, mice, rabbit, guinea pig, bird and chicken[4,11-16]. The use of artificial feeding system to rear and maintain a large scale of bed bug colonies has major advantages in terms of convenience, productivity and cost over the direct feeding methods[14-16].

Montes *et al*. developed an artificial feeding system that could feed all the five instar stages as well as the adults of bed bug[16]. They found that heparinized blood was the most suitable blood meal for feeding bed bugs by using an artificial feeding system. They also investigated the effect of anticoagulated blood meal and found that bed bugs achieved better engorgement when fed on heparinized blood compared to defibrinated blood. Pharmaceutical-grade heparin is an anticoagulant derived from mucosal tissues of slaughtered meat animals such as porcine intestine (pig) or bovine lung (cow)[17]. 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[15].

When people donated blood at the blood bank, the blood was labelled as whole blood. Based on the volume of blood donated, the whole blood will be separated into various blood components. The blood components were obtained by physical separation or centrifuge and the most common ones are plasma and red blood cells[18]. The objectives of this study were to develop a new or modified bed bug feeding apparatus to rear bed bugs colonies in a laboratory and investigate the effects of different types of blood compositions (whole blood, red blood cells and red blood cells mixed with plasma) on the life cycle of bed bugs, with comparison to the direct feeding method (feed bed bug on host's arm).

2. Materials and methods

2.1. Bed bugs samples and rearing

The tropical bed bugs, *C. hemipterus*, were collected from

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Kuala Lumpur International Airport (KLIA), Malaysia. Collected samples were reared in plastic containers (8 cm in height and 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) and a rubber band to hold it in place. The bugs were fed on the arm or leg of a human volunteer for a few months to establish their colonies. Then, they were separated according to their developmental stages, first instar to adult stages. The colonies of *C. hemipterus* were cultured in an incubator with temperature at (26 ± 1) ˚C, (65 ± 5)% relative humidity and a photoperiod of 12 h: 12 h (light: dark) [12,14,19,20].

2.2. Blood collection

Recently expired human blood (donor blood that expired and no longer viable to patients) and fresh frozen plasma were collected from Blood Transfusion Unit, Penang Hospital. Hospital was not able to provide the fresh donor blood because it was reserved for patients. Three types of blood compositions were used in this study: whole blood, red blood cells, and red blood cells mixed with plasma. The bugs were fed with these blood compositions through an artificial feeding system in comparison to the direct feeding method. The blood meals were transferred to Schott Duran bottles for easy handling. All blood meals were stored in a refrigerator with the temperature maintained at 2–6 ˚C. Blood meals are usually good for at least a month. Although the blood had been screened for hepatitis B surface antigen, HIV, anti-hepatitis C virus and rapid plasma reagin (a screening test for syphilis) and was found to be non-reactive, gloves and other appropriate attires were worn when handling the blood meals.

2.3. Design for artificial feeding system

C. hemipterus were fed with the three types of blood compositions by using a modified artificial feeding system based on the studies of Chin-Heady *et al*. and Montes *et al*.[15,16]. As for direct feeding method, bed bugs were placed in a sample bottle and then placed on host's arm. An artificial feeding system consisted of a water bath, glass feeders (Figures 1 and 2) (10 cm in height × 6 cm in diameter and 5 cm in height × 9 cm in diameter) with a stretched parafilm M membrane (American National Can, Chicago, IL) placed at the bottom and a pump with hose to circulate the warm water through the glass feeder. Blood meal (5 mL) was placed through the hollow center of the glass feeder and pooled on the parafilm for design A. On the other hand, blood meal was placed first and then stretched parafilm was placed on top of the blood meal for design B. The parafilm M acted as an artificial membrane for the tropical bed bugs to feed through. All materials and apparatus were maintained regularly. The glass feeder was washed and cleaned after each feeding and new parafilm M was used for every feeding session.

The temperature of the water bath was set at 41 ˚C so that the blood meals will be maintained at 37 ˚C (the temperature of human body) during feeding time. The temperature of the blood meals was monitored regularly by using infrared thermometer. All materials used were assembled to set up the artificial feeding system. Parafilm was cut into the size of 10 cm × 12 cm and rubbed on a bare forearm for 10 s. The parafilm was stretched into a thin layer and placed at the bottom end of each glass feeder design A and on top of blood meals for glass feeder design B. Glass feeders were secured into places by using ring stands and hoses which were used to connect glass feeder to the water pump. The flow of water was directed into the glass feeder with the part closer to the blood meal first: bottom part for glass feeder design A and top part for glass feeder design B. After all hoses were connected firmly, water pump was switched on and water circulation was checked to ensure that the water does not leak. A total of 5 mL blood meals were poured through the hollow center of the glass feeder and pooled on the parafilm for design A. On the other hand, blood meals were placed first on top of glass feeder design B before stretched parafilm was placed on top of the blood meals. The blood meals were allowed to warm up to 37 ˚C first which took approximately 10 min. Lastly, bed bugs containers were placed under the glass feeder design A and on top of glass feeder design B. Glass feeders were shaken gently to remix the settled blood meals before placing the bed bugs containers.

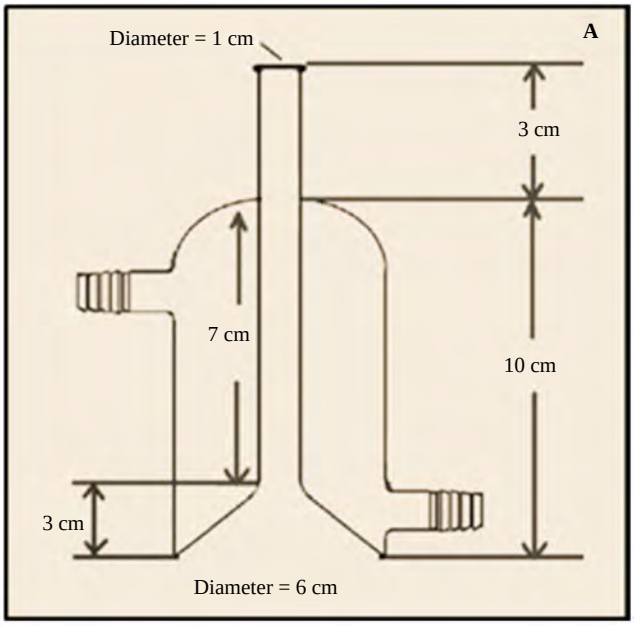

Figure 1. Glass feeder design A.

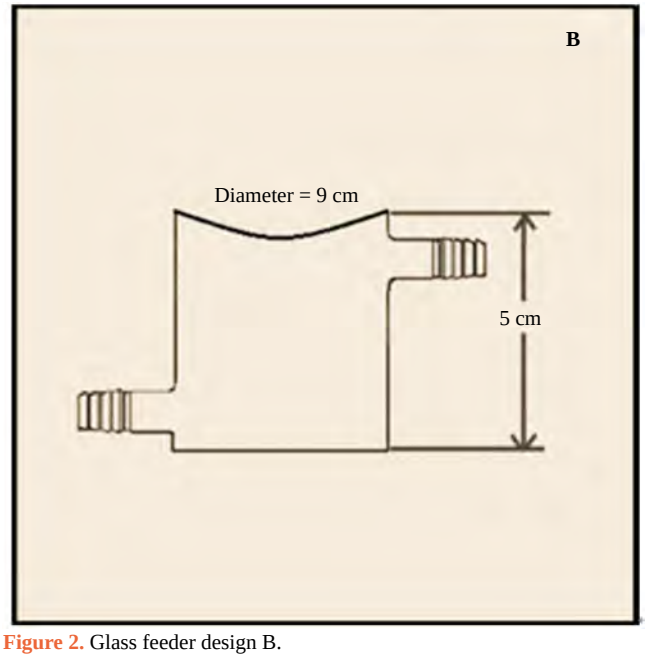

Figure 2. Glass feeder design B.

2.4. Feeding of C. hemipterus

The experiment was conducted through using *C. hemipterus* from KLIA that had been fed by using direct feeding method for a few months. After a few batches of offspring, newly hatched unfed first instar nymphs were randomly selected with 3 replicates for each type of blood compositions, and each replicate included 50 nymphs. The nymphs were placed in the plastic container containing one round A4 paper (5 cm in diameter) at the bottom and one folded A4 paper strip (6 cm × 15 cm). The folded paper strip was placed in such a way that would optimize direct contact with the net cloth, allowing the bugs to reach the blood meal through the net cloth and parafilm membrane. On the contrary, bed bug containers were placed on top of the glass feeder design B. The bugs were fed for 15–30 min every week. The tropical bed bugs were fed on the respective blood type until they were replete and had detached themselves from the sealing film. Observations included counts of the number of live and dead individuals, and number of adults produced.

2.5. Statistical analysis

Data obtained was checked for normality and the data was found normally distributed. The statistical analysis was performed by using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). The data were analyzed by using Two-way ANOVA to determine the significant difference between live individuals for each week. One-way ANOVA was performed to determine the significant difference between the live individuals fed on different blood sources after seven weeks.

3. Results

Tropical bed bugs were fed once every week by using both direct feeding method and artificial feeding method. Generally, tropical bed bugs required 10–20 min to complete feeding on a host's arm and reach engorged weight[2,3]. Additionally, all stages of the insects fed on blood in the artificial feeding system remained attached to the parafilm membrane for up to 1 h, until the completion of their blood meals. They climbed up the paper strips in the container to reach the stretched parafilm to feed on the blood meal. Most of the tropical bed bugs reached engorged weight when fed by using the artificial feeding system (Figure 3). The bed bugs with their bodies bloated were regarded as engorged[16]. Adult tropical bed bug emerged as early as Week 6 for direct feeding and red blood cells feeding. Direct feeding method produced the highest number of adults, a total of 115 individuals from three replicates. Tropical bed bugs that fed on red blood cells produced two adults after seven weeks and no adults emerged from the other two blood sources, whole blood and red blood cells mixed with plasma. Red blood cells caused 72.7% death, the highest record for dead tropical bed bugs, followed by red blood cells mixed with plasma (52%) and whole blood (48.7%). Tropical bed bugs fed on the host's arm (direct feeding method) showed the lowest mortality (4%).

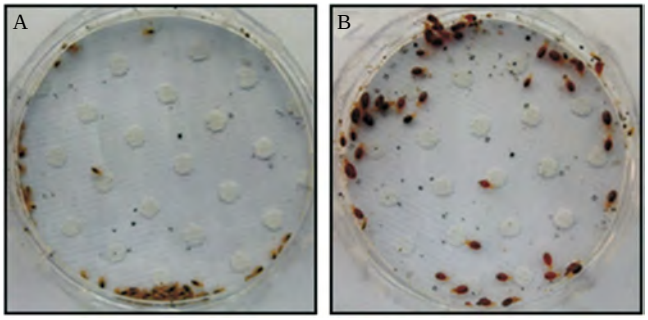

Figure 3. Bed bugs before and after feeding by using artificial feeding system.
A: Bed bugs before feeding by using artificial feeding system; B: Bed bugs after feeding by using artificial feeding system.

A Two-way ANOVA was performed to determine the effect of blood sources and time (in weeks) on the live individuals recorded in each week (Figure 4). There was a statistically significant difference between the effects of blood sources and time on the live individuals [F (18,56) = 2.745, P = 0.002]. There was also a significant difference in the mean of live individuals between weeks (P = 0.000) and between blood sources (P = 0.000). The mean

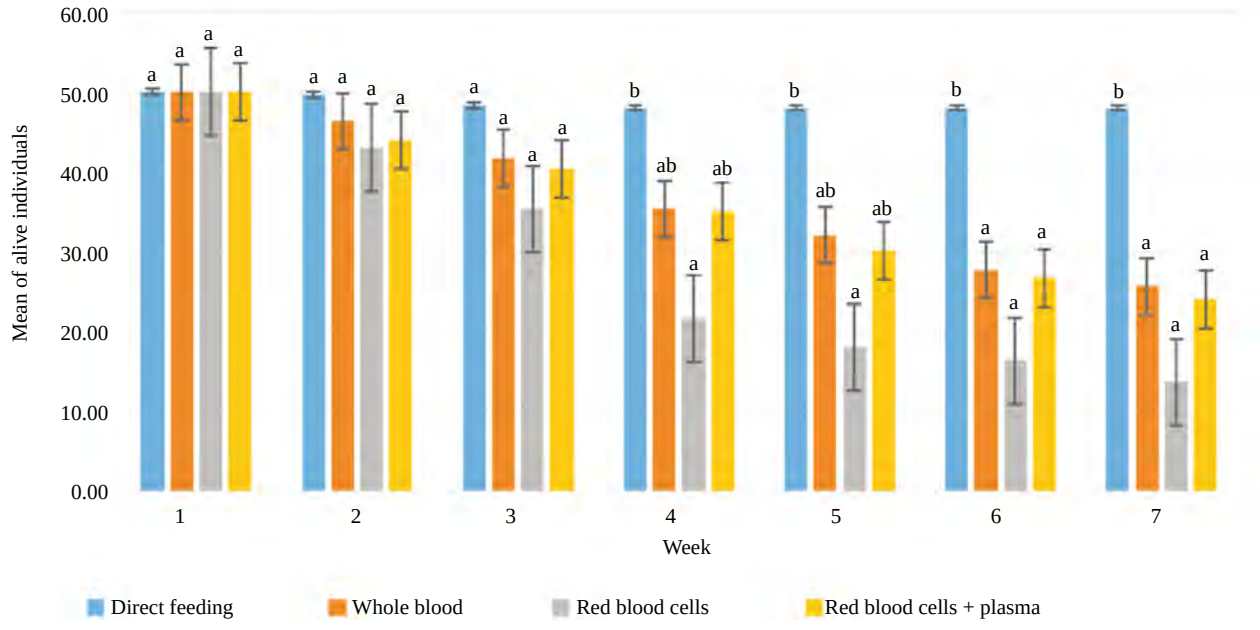

Figure 4. The number of alive individuals after being fed on different blood sources for seven weeks.
Data were presented as mean ± SEM. Bars with the same letter were not significantly different (ANOVA, P > 0.05).

of live individuals from the 1st week until the 3rd week showed no significant difference. From the 4th to the 5th week, there was a significant difference in mean of live individuals. However, in these two weeks, no significant difference between live individuals fed on whole blood and red blood cells mixed with plasma was observed. Furthermore, there was a significant difference in mean of live individuals between the 6th and 7th week, but no significance difference in live individuals fed on whole blood, red blood cells and red blood cells mixed with plasma was observed.

The initial number of individuals for each type of blood feeding was 50 newly hatched first instars. One-way ANOVA was performed to determine the effects of different blood sources on the live individuals after 7 weeks of feeding (Figure 5). There was a statistically significant difference in the number of live individuals after the tropical bed bugs were fed on different blood sources [F (3,8) = 13.877, P = 0.002]. Direct feeding method showed the highest number of live individuals followed by whole blood, red blood cells mixed with plasma and red blood cells. However, there was no significant difference between the number of live individuals fed on whole blood, red blood cells and red blood cells mixed with plasma.

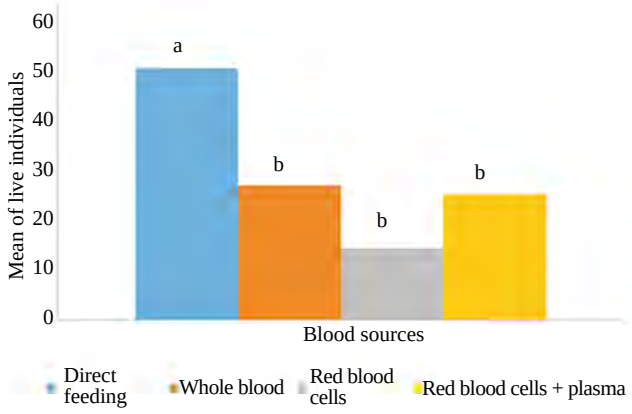

Figure 5. The mean of live individuals after being fed on different blood sources on Week 7.

Data were presented as mean ± SEM. Bars with the same letter were not significantly different (ANOVA, $P > 0.05$).

4. Discussion

The life span of bed bugs are significantly influenced by their diet and mating frequencies[11-13,19]. Bed bugs can be found worldwide because they are easily transported on or in luggage, furniture, boxes, and clothes. These thin and tiny insects can be found in residential houses, hotels, and public transports, and they have sparked major concerns to the hospitality and tourism industry. Bed bug is a hematophagous insect that feed primarily and exclusively on blood[4,5]. However, bed bug bite has always caused skin reactions such as rashes, redness and itchiness to their hosts, but they have not been linked to transmission of any disease[1,7-9]. This was the main reason why an artificial feeding system had been developed to feed and maintain blood-sucking insects such as mosquito and bed bug. Normally, the design of an artificial feeding system consisted of two basic features, a heating element and a membrane to hold the blood[14-16,21]. In this study, the heating element was the warm water at (37 ± 2) ℃ which circulated through the glass feeder, and parafilm M was stretched to make it thin enough so that the bed bug can pierce through and suck the blood.

Direct feeding is a method where bed bugs are placed on the skin of human volunteer to feed. This method resulted in the highest number of alive individuals after seven weeks. The result obtained might be due to the fact that host provide fresh blood meals and conditions as they fed naturally. Direct feeding method also produced the highest number of emerged adult compared to the other three blood sources that were fed through an artificial feeding system. The collected blood sources were recently expired, which might contribute to the death of the insects. Expired donor blood was chosen because the hospital was not able to provide the fresh donor blood. The fresh donor blood is reserved for their patients. Fresh donor blood usually expires after 42 days of blood donation[22]. Regular supply of blood sources is essential in order to rear a large scale of bed bug colonies by using artificial feeding system. It is also the cheapest way to rear bed bugs colonies since we do not need to pay to collect the expired blood. Human host and animal host would require payment for their services. In addition, the use of expired donor blood did not require ethics, unlike using human or animal host.

There are differences in the number of live bed bugs fed on the three blood sources by using artificial feeding system after seven weeks. The components in the blood sources may be the key to the different effects. Romero and Schal reported that adenine nucleotides are the most important feeding stimulants in bed bugs while the other blood components such as D-glucose, albumin, globulin, cholesterol and mixtures of vitamins and amino acids did not stimulate engorgement[23]. The components in the whole blood sachet is the same as the blood in the human body (red cells, plasma and platelets), which is why bed bugs that fed on whole blood may exhibit the same biological properties as the ones fed on human volunteer. Spinella *et al*. reported higher survival when warm fresh whole blood was transfused into patients with trauma compared to those therapy that transfused only stored component[24]. Meanwhile, the only component in the red blood cells sachet was red blood cells. When plasma is mixed with red blood cells, the appropriate or the exact ratio is hard to achieve. Studies on different ratios of fresh frozen plasma and packed red blood cells showed that higher ratios improved survival and the patients had a lower risk of death compared with those that received a low ratio[24-26]. Montes *et al*. reported that whole blood formulated with heparin was the most suitable blood meal for feeding bed bugs by using an artificial feeding system[16]. Pothikasikorn *et al*. suggested that expired human blood could be used as blood meals by up to 10 days where they found that feeding rate of *Aedes aegypti* and *Anopheles dirus* decreased to 18% and 7.3% on Day 25, respectively[27].

Temperature of the blood meals is maintained at 37 ℃ as it plays an important role in feeding these insect successfully through using the artificial feeding system. When the temperature is below 35 ℃ or above 38 ℃, the insects are not attracted to feed on the blood meals. These observations were comparable with those for *Cimex lectularius* where no bed bugs attached to the membrane when the blood was at temperature below 35 ℃[16]. Chin-Heady *et al*. reported that the optimum temperature for female bed bug was 37–38 ℃ unlike adult male and nymphs which will feed when the temperature ranged between 34 and 42 ℃, and they also reported a few drawbacks and challenges faced when using artificial feeding system, namely, blood or water could leak into the container, caused by the paper strip puncturing the parafilm or the parafilm was stretched too thin[15]. This problem could kill the bed bug colonies. The other problem is maintaining water circulation, which also means maintaining temperature of the blood meal. In our study, we used a Dophin submersible pump that could circulate the water

through two glass feeder.

The paper strips have to be placed in such a way to ensure that the insects can direct contact with the net cloth, thereby allowing them to reach the blood meal through the parafilm membrane. It is important to ensure that all parts of the parafilm are in contact with the blood meal so that no air pockets can occur[16]. Another aspect to be concerned is a reliable source of blood which can be collected from blood bank and a slaughter house. These sources of blood can be used successfully for up to 2 months if the blood is properly treated and stored at 2–6 ˚C. Since all blood should be handled with care and personal safety; the use of a membrane feeding apparatus is crucial and important. Proper attires such as latex gloves and lab coat should be worn when handling the blood and also during the setup of artificial feeding system.

Artificial feeding system is one of the alternative methods to feed bed bugs by using expired human blood or blood from ruminant animals. Finding suitable blood source is significant when feeding the insects in the laboratory so that their biological properties are the same as if they feed naturally. Whole blood showed promising results as an alternate source to feed bed bugs by using the artificial feeding system. However, the use of chemicals, natural additives, anticoagulant and phagostimulant can be investigated to get a better result.

Conflict of interest statement

We declare that we have no conflict of interest.

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