Isolation, Identification, and Time Course Human DNA Typing from *Cimex lectularius L.* (Bed bugs), Fed on Human Blood Meals

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Abstract

Bed bugs are parasitic insects that feed on the blood of mammals and are typically found in groups within residential settings. The goals of this study were to investigate whether sufficient amounts of human blood and DNA can be recovered from a Bed bug (Bb), *Cimex lectularius L.* and if the identity of a single and/or multiple individuals can be established by generating human genetic profile(s). Bed bugs were fed on human female, male and pooled (female + male) blood at North Carolina State University Entomology laboratories and were frozen at 12 hr time intervals starting at 0 hours up to 108 hours. Blood was extracted from each bed bug by homogenization, transferred onto Whatman® FTA cards and air dried. Rapid Stain Identification results confirmed the presence of human-specific Glycophorin A protein at various time points giving ratings from 0 to 5. DNA was isolated by an organic method, quantified by real-time PCR using Quantifiler® Duo Kit, and amplified using AmpFlSTR® IdentifilerFiler® Kit. Real-time PCR human DNA concentration ranged from 0.000395 ng/µL to 2.79 ng/µL. Full or partial Short Tandem Repeats (STR) DNA profiles for all 15 STR loci plus the Amelogenin locus were typed and compared with their respective reference DNA profile. Full profiles were generated for 0 to 48 hour female, male and pooled samples and partial profiles were generated for 60 to 108 hour samples. Our study demonstrates that bed bugs can be used as a reliable physical evidence in forensic investigations.

Introduction

Bed bugs (*Cimex lectularius L.*) are parasitic insects that feed on the blood of mammals and are typically found in groups within residential settings (1). They are easily translocated by passive dispersal and adapt to multiple host species (2, 3, 4). The recent resurgence of bed bugs, especially in North America, has gained interest in the field of forensics. Identifying an individual based on blood or tissue isolated from insects can be used to implicate a suspect in the time and place of a crime.

Research has revealed that human DNA can be recovered in bed bug blood meals and that can be amplified 7 days post-feeding (5). However, in other research, investigators were able to retrieve DNA up to 42 days post blood meal (6). Szalanski et al. (2006) demonstrated that DNA can be isolated from blood-fed bed bugs and it is qualitatively sufficient for the DNA typing. However to date, there are no documented reports about the successful human blood identification and/or full human STR typing from a bed bug fed on human; female or male or pooled (female:male) blood meals. Moreover, it is not known whether the identification of multiple humans can be made from a bed bug that had fed on multiple hosts. The objectives of this study were to establish a time-course for human blood identification, to quantify human DNA from bed bugs, and to generate DNA profile(s) of a host and/or multiple hosts from a bed bug that fed on human blood meals.

Methods

- Bed bugs were fed human female, male and pooled blood on membrane apparatus for 15-30 minutes. (Fig. 1)
- Bed bugs were homogenized and transferred to Whatman® FTA Cards. (Fig. 2)
- Rapid Stain Identification Testing was conducted to determine if sample was of human origin. (Fig. 3)
- DNA was extracted from Whatman FTA cards by the organic method.

Methods

- Extracted DNA was quantified by real time PCR using the Quantifiler® Duo Kit (Applied BioSystems).
- DNA was amplified using AmpFlSTR® IdentifilerFiler® Kit and analyzed using *GeneMapper ID v3.2.1* software on ABI Prism 310 Genetic Analyzer (Applied BioSystems)
- Sample profiles were compared to reference profiles.

Fig 1. Artificial feeder made to simulate bed bugs feeding on a human blood meal.

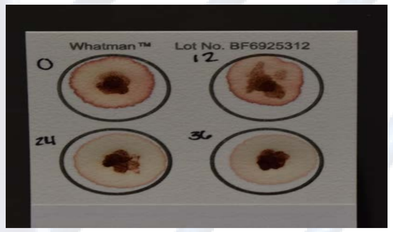

Fig 2. FTA card containing extracted blood meal fluid from a bed bug.

Fig 3. RSID example of sensitivity ratings. Left to Right : 5, 4, 3, 2, 1, and 0

Results

Time Intervals (Hrs)	Female	Male	Pool
0	3	3	2
12	3	4	4
24	3	3	5
36	3	4	2
48	1	2	1
60	4	1	1
72	1	1	1
84	1	1	1
96	1	2	1
108	1	1	-

Table 1. RSID ratings of female, male and pool. 0 indicates no detection, 1 is the weakest, and 5 being a strong detection.

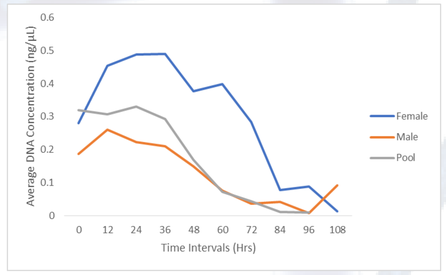

Fig. 4. Human DNA concentrations extracted from bed bugs fed female, male and pool human blood meals.

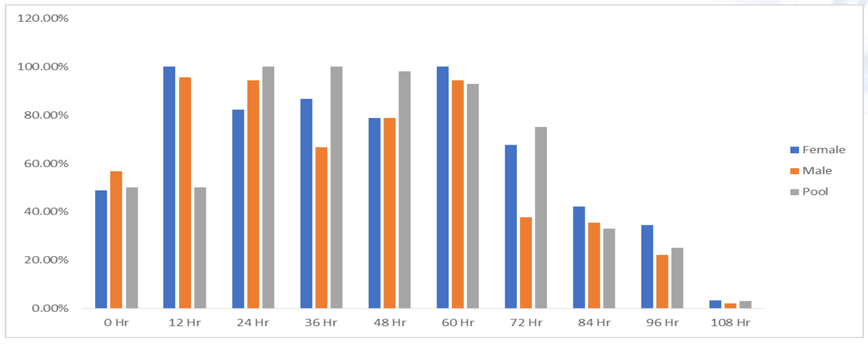

Fig. 5. Percentage of correct STR Alleles at 12 hrs time intervals for female, male and pool.

Results

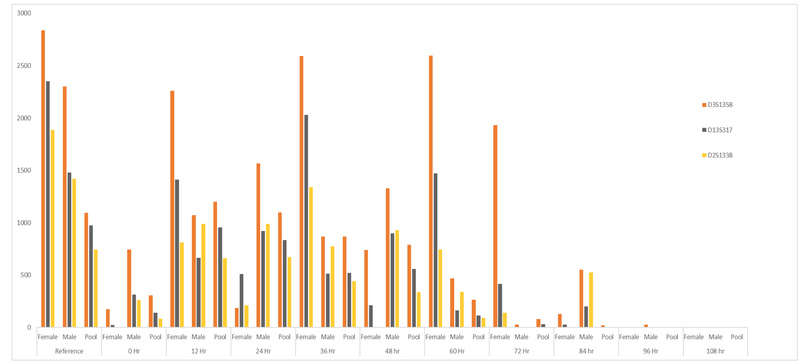

Fig. 6. Allele Peak Heights at designated markers. Demonstrates the degradation of DNA over time. D3S1358 is a low molecular weight marker, D13S317 is a medium molecular weight marker and D2S1338 is a high molecular weight marker.

Conclusion

This study demonstrates for the first time that the use of forensic biology and entomology methods empowers identification of the human blood as well as DNA profile source from a bed bug. It is also evident that multiple human hosts can be positively typed up to 48 hours. However, DNA degradation is a concern as time elapsed therefore, we recommend that crime scene technician collect multiple blood fed bed bugs from the crime scene to maximize the chances of generating complete DNA profile(s) from bed bugs.

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