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Body lice and bed bug co-infestation in an emergency department patient, Ohio, USA

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ABSTRACT

Body lice and bed bugs are hematophagous insects that parasitize humans. Body lice are established vectors of several bacterial pathogens (e.g. *Bartonella quintana, Borrelia recurrentis*). Bed bugs are biologically competent vectors of some of the same agents, but their vectorial capacity for these in nature is unclear. In particular, a lack of exposure to louse-borne pathogens in bed bugs in the field could be a factor that limits their contribution to transmission. Here, we describe a case of a patient seen in an urban emergency department who was suffering from infestation with both body lice and bed bugs. Insects were collected from the patient and tested for the presence of louse-borne bacterial pathogens using 16S rRNA gene amplicon sequencing. Although no *Bartonella, Borrelia,* or *Rickettsia* were detected, this case provides evidence of ecological overlap between body lice and bed bugs and highlights several potential risk factors for co-infestation. The ecological relationships between bed bugs, body lice, and louse-borne bacteria should be further investigated in the field to determine the frequency of co-infestations and identify possible instances of pathogen infection in bed bugs.

Introduction

The body louse, *Pediculus humanus humanus*, is a neglected but widespread blood-feeding human ectoparasite [1]. It is an established vector of three life-threatening bacterial pathogens: *Bartonella quintana*, *Rickettsia prowazekii*, and *Borrelia recurrentis* [2,3]. It is also a suspected vector of additional human pathogenic bacteria including *Yersinia pestis*, *Coxiella* spp., *Ehrlichia* spp., *Anaplasma* spp., *Serratia* spp., and *Acinetobacter* spp. [4–6].

In developed nations, body lice mostly affect populations living under crowded and unhygienic conditions (e.g. without access to clean clothes or frequent bathing opportunities). In particular, homelessness, sleeping outdoors, and heavy drinking are significant risk factors for body lice infestation [7,8]. Individuals with housing, but with inadequate hygiene, such as those with physical disability or mental illness, can also be affected. Recent reports of the prevalence of body lice infestation in the homeless population in the developed world have ranged from as low as 4 % in Boston, USA [8] to as high as 16 % in Tokyo, Japan [9] and 30 % in San Francisco, USA [7]. Additional surveys in France have estimated 12–22 % prevalence [10,11]. Among the homeless population in the developed world, *B. quintana* is the most common louse-borne pathogen, causing trench fever, chronic bacteremia, and endocarditis [12–14].

Body lice also affect the homeless in developing countries. For instance, 18 %–40 % prevalence has been reported in homeless individuals in Brazil, Colombia, and Russia [15–17]. However, body lice are common in other settings such as rural communities, refugee camps, and jails [18–21]. The pathogens present in lice in these settings differ from those commonly detected in developed urban environments, as in addition to *Bartonella* infection, louse-borne relapsing fever (*B. recurrentis*) and epidemic typhus (*R. prowazekii*) are concerns [22]. Lice that infest migrants and refugees can be a source of imported infections with these agents in developed nations [23–25].

Like the body louse, the common bed bug, *Cimex lectularius*, is an obligate blood-feeding insect with a strong host preference for humans [26]. It is a cosmopolitan species that lives in close association with its hosts, primarily within structures [27,28]. Although bed bugs are common in human dwellings across all

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socioeconomic levels, they can be particularly persistent in environments that lack the resources to prevent or treat infestations. For example, bed bugs are prevalent in settings of social and political disorder such as refugee camps [18,29]. They are also pervasive in low-income housing and homeless shelters [30–32]. In some cities, up to 30 % of homeless shelters have been found to be infested with bed bugs [31,31,32]. Moreover, bed bugs are being increasingly detected in urban emergency departments and a recent survey of patients confirmed that having a low-income and living in a group home are significant risk factors for bed bug infestation [33]. Bed bugs are biologically competent vectors for both *B. quintana* and *B. recurrentis* under laboratory conditions, but unlike body lice they have not been shown to transmit these agents in nature [34–36].

While the physiology and behavior of body lice and bed bugs differs in many ways, the risk factors that have been identified for infestation with each insect suggests a probable ecological overlap between the two species in living environments of high population density and/or low hygiene. This interaction could have important implications for infectious disease transmission by exposing bed bugs to louse-borne bacterial pathogens. However, direct evidence of spatiotemporal overlap between body lice and bed bug infestations is generally lacking and the hypothesis is based on independent surveys of the distribution of both insects. Similarly, little data on bed bug associated microbes in the field are available. Here, we describe a case of an urban emergency department patient suffering from co-infestation with body lice and bed bugs. We also report the results of long-read nanopore sequencing of bacterial 16S rRNA gene amplicons conducted to examine the bacterial communities associated with insects collected from the patient.

Case

A 56-year old disabled and low-income African American female presented to an emergency department (ED) in Cleveland, OH in 2018 acutely psychotic with insomnia and auditory hallucinations. She had a history of diabetes and hypertension and was noncompliant with her medications for schizophrenia and bipolar disorder. In the ED she was noted to have poor hygiene and both bed bugs and body lice were present (Fig. 1). Her triage vital signs were: temperature of 35.9 °C, blood pressure 116/69, heart rate of 125, respiratory rate of 16, and oxygen saturation of 94 % on room air. On a review of systems she complained of some vague abdominal discomfort without nausea or vomiting, but did report some loose stools. The physical exam was unremarkable except for superficial excoriations on her extremities, consistent with ectoparasite infestation. The ED blood laboratory test results are reported (Table 1). The urinalysis showed the presence of ketones, 63 epithelial cells, and 9 white blood cells per high powered field (HPF). The urine drug screen and blood alcohol tests were negative. Elevated blood urea nitrogen (BUN) and creatinine was consistent with dehydration, and she was given intravenous fluids. The hemoglobin and hematocrit values were low and eosinophils were elevated which has been previously reported with pediculosis [37]. She was given 12 mg of ivermectin in the ED for her ectoparasite infestations and was ultimately admitted to a psychiatric hospital for stabilization of her acute psychiatric condition. There, she was treated with permethrin 1 % topical lotion daily. On hospital day 14 she developed a fever and on hospital day 16, had three blood cultures drawn. Only one of these grew a gram-positive cocci, which was felt to be a skin contaminant, and no antimicrobials were administered. A nasopharyngeal swab polymerase chain reaction (PCR) for influenza A was positive and she was treated with oseltamivir phosphate (Tamiflu). The patient was discharged to her apartment after 19 days of inpatient treatment.

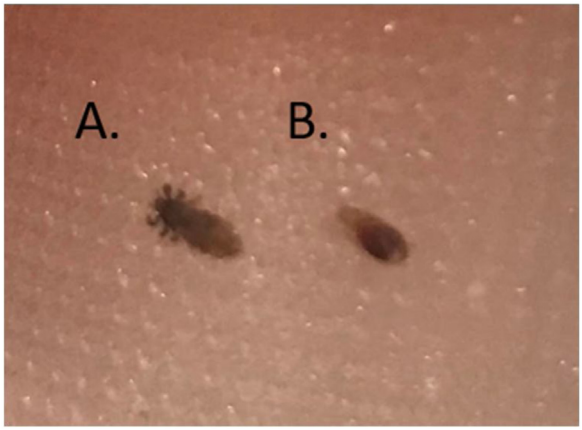

Fig. 1. Insect samples. Photo depicting (A) an adult body louse and (B) a bed bug nymph collected from a patient in the emergency department.

One adult *P. humanus* and one *C. lectularius* nymph were collected from the patient and preserved for further study. Immediately upon collection, the insects were placed in separate sterile microfuge tubes containing RNAlater solution for nucleic acid preservation (Thermo Fisher, Waltham, MA) and stored at −80 °C until further processing. Prior to DNA extraction, each insect was rinsed with a 10 % bleach solution and cell culture grade water to minimize surface contaminants. Extraction of bacterial DNA from each insect was performed with the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Venlo, Netherlands) using a modified protocol that included beating with glass and zirconium oxide beads and extended lysis incubation to enhance yields. Bead beating was performed for 1 min using lysis matrix H tubes (MP Biomedicals, Santa Ana, CA, USA) on a BeadBug homogenizer at maximum speed (Benchmark Scientific, Sayreville, NJ, USA). PCR was performed on extracted DNA samples using barcoded primers 27 F and 1492R from the Nanopore 16S Barcoding Kit (Oxford Nanopore, Oxford, UK) to amplify the full-length bacterial 16S rRNA gene. Cycle conditions were as follows: 1 min at 95 °C for initial denaturation, 25 cycles of 20 s at 95 °C, 30 s at 55 °C, and 2 min at 65 °C, and a final extension of 5 min at 65 °C. PCR products were run on an agarose gel to confirm amplification of the ~1500 base pair product. PCR products were subsequently purified using magnetic Ampure XP beads (Beckman Coulter, Brea, CA) and purified samples were quantified on a NanoDrop spectrophotometer (Thermo Fisher). The two barcoded libraries, one from the louse sample and one from the bed bug sample, were pooled at equimolar concentrations to a total of 100 ng and a rapid sequencing adapter was added. Sequencing, quality control, and base calling was performed on a MinION flow cell on the MinION sequencing device (Oxford Nanopore) using MinKNOW software according to the manufacturer's protocol [38–40]. The OmicsBox taxonomic classification workflow was used to taxonomically assign 16S reads [41]. The taxonomic classification algorithms used inside OmicsBox were Kraken 1.0 [42], with DB 2019.06 (archaea, bacteria, fungi, human, protozoa, viral), and Bowtie2 2.3.5.1 [43], run with default parameters. Raw sequences were deposited in the NCBI SRA (PRJNA594062).

Sequencing did not reveal the presence of *Bartonella, Borrelia,* or *Rickettsia* in either of the insects (Table 2). As expected, the primary louse endosymbiont *Candidatus* Riesia was the most abundant bacterium in the body louse, comprising 97.48 % of reads. Besides this bacterium, *Staphylococcus* and *Burkholderia* were detected in the body louse sample, but these were present at very low abundance. In the bed bug sample, *Wolbachia* was the most abundant bacterium, making up 45.42 % of reads. The bed bug

Table 1
Emergency department laboratory values.

Laboratory test	Normal range	Value
White blood cell count (WBC)	4.4–11.3	4.1 **L**
Nucleated erythrocyte count	0.00/100 WBC	0.0
Red blood cell count	4.00–5.20 × 10E12/L	3.59 **L**
Hemoglobin	12.0–16.0 g/dL	10.4 **L**
Hematocrit	36.0–46.0 %	29.7 % **L**
Mean corpuscular volume (MCV)	80–100 fL	83
Mean corpuscular hemoglobin concentration (MCHC)	32.0–36.0 g/dL	35.0
Platelet count	150−450 × 10E9/L	112 **L**
Red cell distribution width-coefficient of variation (RDW-CV)	11.5–14.5 %	15.8 % **H**
Neutrophil %	40.0-80.0 %	57.0 %
Immature granulocytes	0.0-0.9 %	0.7 %
Lymphocytes %	13.0–44.0 %	16.1
Monocyte %	2.0–10.0 %	19.7
Eosinophil %	0.0–6.0 %	6.3
Basophil %	0.0–2.0 %	0.2
Neutrophil count	1.20–7.70 × 10E9/L	2.34
Lymphocyte count	1.20–4.8 × 10E9/L	0.66 **L**
Monocyte count	0.10–1.00 × 10E9/L	0.81
Eosinophil count	0.00–0.70 × 10E9/L	0.26
Basophil count	0.00–0.10 × 10E9/L	0.01
Glucose, serum	74−99 mg/dL	129 **H**
Sodium, serum	136−145 mmol/L	137
Potassium, serum	3.5–5.3 mmol/L	3.9
Chloride, serum	98−107 mmol/L	102
Bicarbonate, serum	21−32 mmol/L	20 **L**
Anion gap, serum	10−20 mmol/L	19
Blood urea nitrogen, serum	6−23 mg/dL	23
Creatinine, serum	0.50–1.05 mg/dL	1.41 **H**
Glomerular filtration rate (GFR)	>60 mL/min/1.73m2	46 **L**
Calcium, serum	8.6–10.6 mg/dL	9.7
Albumin, serum	3.4–5.0 g/dL	4.1
Alkaline phosphatase, serum	33–110 U/L	54
Protein, total serum	6.4–8.2 g/dL	8.2
Bilirubin, serum total	0.0–1.2 mg/dL	0.3
Alanine aminotransferase, serum	7–45 U/L	31
Aspartate transferase, serum	9–39 U/L	42 **H**
Blood alcohol level	0.0	<10 mg/dL

*H = abnormal high result; L = abnormal low result.

contained a greater diversity of bacteria than the body louse. Additional genera detected in this sample included: *Klebsiella, Escherichia, Edwardsiella, Salmonella*, and *Enterobacter*. Reads from *Pectobacterium* and *Dickeya* likely represent the secondary endosymbiont of bed bugs (BEV-like endosymbiont), which is a close relative of these plant pathogenic bacteria [44]. Lastly, *Yersinia* was detected at low abundance in the bed bug sample.

Discussion

While it has been generally assumed that infestation with body lice and bed bugs may co-occur under certain conditions, no direct evidence of this has been reported in the literature. Here, we present the first clinical case report demonstrating co-infestation with the two ectoparasites. This report confirms this suspected phenomenon, which is likely underappreciated, and provides some insight on the factors that may promote co-infestation. Notably, the patient in this case suffered from mental illness and was socioeconomically disadvantaged, which may have limited hygiene practices and/or the means to treat infestations. Indeed, bed bugs do not nest on the host in the way that body lice do and finding the former directly on an individually is typically indicative of a heavy infestation in the home.

A screen of bacteria present in the insects did not reveal any known louse-borne pathogens or other known vector-borne pathogens. Therefore, it is unlikely that the patient suffered from a known vector-borne bacterial infection. The presence of *Burkholderia, Klebsiella, Escherichia, Edwardsiella, Salmonella*, and *Enterobacter* in tested samples may be the result of transient contamination from the environment or these may be stochastic colonizers of the insects. Regardless, data supporting their transmission to humans by arthropods are lacking. *Staphylococcus* is a possible exception, as several members of the genus having previously been detected in bed bugs and body lice [32,45] and are also able to colonize both insects [46, JEP unpublished data]. A retrospective study of emergency department patients found that those with bed bugs had higher rates of blood cultures positive for *Staphylococcus* than non-infested patients [JS unpublished data]. However, it is not clear if this increased rate of positive blood cultures is related to the direct physical transmission of *Staphylococcus* from *Cimex* to human, increased skin contamination of blood cultures, or other confounders associated with *Staphylococcus* positive blood cultures. The presence of *Yersinia* in the bed bug sample is also of potential interest. Only three species of *Yersinia* are pathogenic to humans, and only *Y. pestis*, which does not exist in Ohio, is vector-borne. The 16S sequence of the *Yersinia* we detected did not match any of the known pathogenic species and our analysis was not able to identify this bacterium to the species level. It is therefore unlikely that the *Yersinia* we detected is of public health significance.

Although our examination of a single co-infestation did not reveal the presence of any vector-borne pathogens, given their vector competence [35,36], we suggest that bed bugs could potentially acquire louse-borne pathogens and serve as secondary vectors under some circumstances. Environmental overlap between body lice and bed bugs provides this opportunity, but such a scenario is likely to be very rare and limited to foci in space and time where body lice, bed bugs, and pathogens such as *B. quintana* or *B. recurrentis* circulate in sufficient numbers. Indeed, one study detected *B. quintana* in bed bugs in a jail in Rwanda [34], an environment where outbreaks and infected lice are known to occur [2,21]. However, that study did not collect patient blood or lice samples from the same geographical site and thus was not able to examine whether bed bugs were transmitting the bacterium to humans.

Table 2
Relative abundance of bacteria in insect samples at the genus level.

Taxa	Body Louse Read Count	% Abundance	Taxa	Bed Bug Read Count	% Abundance
Candidatus Riesia	7511	97.48	*Wolbachia*	8339	45.42
Staphylococcus	49	0.64	*Klebsiella*	1950	10.62
Burkholderia	43	0.56	*Escherichia*	1667	9.08
Klebsiella	8	0.1	*Edwardsiella*	1429	7.78
Escherichia	5	0.06	*Salmonella*	1248	6.8
Buchnera	2	0.03	*Enterobacter*	674	3.67
Acinetobacter	2	0.03	*Pectobacterium*	518	2.82
Yersinia	2	0.03	*Serratia*	409	2.23
			Dickeya	188	1.02
			Yersinia	182	0.99

Ultimately, the restriction of body lice and louse-borne pathogens to small, often neglected populations presents a barrier to understanding their interactions with bed bugs. Future studies of at-risk populations such as the homeless should be conducted to determine the prevalence of co-infestation, its clinical impacts, and the possibility of pathogen acquisition by bed bugs.

Ethical approval

This study was approved by the University Hospital Cleveland Medical Center institutional review board. A copy of the IRB's determination is available for review by the Editor in Chief of the journal upon request.

Author contributions

JEP and JMS conceived the study. Data was collected by JAY and JEP. All authors were involved in data analysis and writing of the manuscript.

Declaration of Competing Interest

The authors report no conflicts of interest.

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