

Ignatzschineria larvae Bacteremia Following *Lucilia* sp. Myiasis in an Irregular Migrant: A Case Report

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Abstract: Bacteremia induced by wound myiasis is uncommon and therefore rarely suspected by clinicians when treating patients with neglected wounds. We present a case of *Ignatzschineria* larvae bacteremia as a complication of *Lucilia* sp. maggot wound myiasis in a young male migrant. This is the first reported human case of *Ignatzschineria* bacteremia in Slovenia and one of the 2 described in the literature where the fly larvae infesting the wounds of the patient with *Ignatzschineria* bacteremia were not only suspected to be *Lucilia* sp. but also entomologically identified.

Key words: *Lucilia* sp., *Ignatzschineria* larvae, myiasis, bacteremia, migrant

INTRODUCTION

Myiasis is an infestation of humans and other vertebrates by various dipteran larvae that feed on the host's dead or living tissues, body secretions, or ingested food [1,2]. Human myiasis is more common in poor socioeconomic regions of tropical and subtropical countries, whereas in industrialized countries of temperate climates it is usually associated with travel or precarious living conditions [1,3]. According to the classification of myiasis in relation to the location on the host, the infestation is classified into sanguinivorous or bloodsucking myiasis, cutaneous, furuncular, and migratory myiasis, wound myiasis, and cavitary myiasis [1]. The most important family of flies causing wound myiasis is Calliphoridae, with *Calliphora*, *Lucilia*, *Chrysomya*, and *Cochliomyia* as the most causative genera. *Lucilia* spp. are present in South Africa, Australia, Europe, and North America. Their larvae prefer to feed on dead tissue, usually causing facultative open wound myiasis [1,4]. Exposed lesions attract and stimulate females to oviposit [1]. Because flies and their larvae are hosts to bacteria, there are possibilities of secondary bacterial infections of the lesions and bacteremia if the bacteria invade the bloodstream. The

bacteria most often associated with myiasis-induced bacteremia are *Wohlfahrtiimonas* spp. and *Ignatzschineria* spp. [5]. Both genera are well known inhabitants of the larvae of many fly species [6]. In the salivary glands of *Lucilia* spp. bacteria of the genus *Ignatzschineria* have been found in relatively high abundance [7]. Here we describe a case of myiasis with subsequent *Ignatzschineria* larvae circulatory infection, presumably as a complication of a *Lucilia* sp. maggot wound infestation in a young male migrant.

CASE DESCRIPTION

The ethic review was exempted by the Institutional Review Board of the Institute of Microbiology and Immunology (IMI), Faculty of Medicine, University of Ljubljana, Slovenia (IRB No. 469-TJI/20). All procedures followed were in accordance with the ethical standards of the institutional and national committee on human experimentation and with the Helsinki Declaration as revised in 2013. The patient samples were collected for diagnostic purposes as a part of a routine diagnostic procedure of the IMI. The patient was informed about all diagnostic procedures, and steps were taken to preserve patient anonymity. All samples were anonymized and linked only to randomized numerical codes. Since no additional samples or data were collected, the study was deemed to be low risk and the need for additional ethical permission from the National Medical Ethics Committee was waived.

In September 2019, an 18-year-old male patient, an irregu-

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lar migrant from Asia, was admitted to the Department of Infectious Diseases, University Medical Centre Ljubljana, Slovenia, after being detained while crossing the border river between Slovenia and Croatia. He had left his home country approximately 2 years before this incident and was travelling on foot in rural areas and woodlands of the Balkans. After falling off a rock 3 days prior to admission, he reported subsequent abdominal pain and pain in the left leg. He had not eaten or had a bowel movement for several days; he had vomited once before admission but passed urine normally. He had no underlying comorbidities, but he reported an allergy due to which he was taking an antihistamine. Physical examination revealed that he was in pain, febrile (39.6°C), and normotensive (blood pressure 114/65 mm Hg) with tachycardia (130 beats/min), and tenderness in the right lower quadrant of the abdomen. There were multiple superficial wounds on both the calves and feet with surrounding cellulitis, which had spread proximally to the right thigh, and tenderness and swelling of the left knee. No accompanying lymphadenopathy was visible. The middle finger of his left hand was also swollen and tender. Numerous small moving white larvae were observed on the skin of the legs, in both armpits, in the perigenital region, and in the skin folds. The wounds were debrided, and all the visible larvae were removed. Urinary retention was noted, and a urinary catheter inserted. Laboratory results revealed an elevated C-reactive protein (CRP) level (499 mg/L), a procalcitonin (PCT) level of 8.94 ng/ml, and leukocytosis of $25.9 \times 10^9/L$ with a left shift (8% bands and 3% metamyelocytes). Acute renal insufficiency was present (plasma creatinine of 168 $\mu\text{mol/L}$, upper limit 97 $\mu\text{mol/L}$; and urea of 24.2 mmol/L, upper limit of 7.5 mmol/L). The patient had mild hyponatremia (130 mmol/L, lower limit 135 mmol/L), increased myoglobin of 553.4 $\mu\text{g/L}$ (upper limit 110 $\mu\text{g/L}$), mild elevations of transaminases (AST 2.63 $\mu\text{kat/L}$, upper limit 0.52 $\mu\text{kat/L}$; S-ALT 1.38 $\mu\text{kat/L}$ upper limit 0.57 $\mu\text{kat/L}$), and increased D-dimer (4,683 $\mu\text{g/L}$, upper limit 500 $\mu\text{g/L}$). A soft tissue ultrasound of the right thigh showed cellulitis. Blood cultures were taken, and the patient was started on empirical intravenous antibiotic therapy with flucloxacillin 2 g every 6 hr and ciprofloxacin 400 mg every 12 hr. Following treatment, the patient was afebrile the next day. The patient continued to receive supportive therapy with antipyretics, analgesics, and parenteral hydration, upon which the kidney function normalized within 4 days. A blood smear for malaria was negative. In accordance with our institutional guidelines, surveillance samples were taken on

admission. A rectal swab yielded ESBL-producing *Enterobacter cloacae*, and the pooled skin swab samples grew methicillin-resistant *Staphylococcus aureus*. Samples for other multidrug resistant organisms were negative.

The wounds were redressed 2 days later, with numerous larvae observed on the calf wounds, in the blisters surrounding the calf wounds, and under the toenails of several toes. All visible larvae were removed and additional debridement of dead skin surrounding the wounds was performed. Where the larvae were rooted in the nailbed, the overlying nails were ablated and the larvae extracted. The area was cleaned with a 3% solution of hydrogen peroxide and dressed. The patient became febrile again within hours after the second debridement, and therefore 2 blood culture sets, each containing one aerobic (BD BACTEC Plus Aerobic/F, Becton Dickinson, Franklin Lakes, New Jersey, USA) and one anaerobic (BD BACTEC Lytic Anaerobic/F, Becton Dickinson) bottle were drawn again and, due to an increase in leukocytosis to $33.1 \times 10^9/L$, antibiotic therapy was changed to tigecycline and vancomycin. The patient was hypotensive the next day (blood pressure 88/55 mm Hg) accompanied by vomiting. We changed tigecycline to imipenem/cilastatin 500 mg every 6 hr i.v. but continued with vancomycin 1 g every 12 hr i.v. Hypotension resolved after a fluid bolus. Acute bowel obstruction was excluded by abdominal radiograph and severe obstipation diagnosed, which was resolved by enema.

The larvae were sent to the IMI at the Faculty of Medicine in Ljubljana, Slovenia, for identification, where they were preserved in 10% formalin solution. The genus *Lucilia* was confirmed macroscopically and microscopically at the IMI through their morphological characteristics using the identification key to fly larvae [8]. The larvae were 9 to 10 mm long, round in cross section, tapered anteriorly, and white to cream colored. All of them were in larval stage III (3rd instar) of their life cycle. In the mouthparts an accessory oral sclerite was absent. The dorsal arm of the cephaloskeleton was longer than the ventral arm. The bodies of the larvae had short spines (Fig. 1A). The posterior spiracles were located on the face of the terminal segment, sessile with 3 distinct straight slits surrounded by a complete closed peritremal ring with one internal projection between the outer and middle slits and a distinct button (Fig. 1B, C). The spiracular area was surrounded by 12 tubercles.

The blood cultures drawn on admission were sterile. However, of the 2 blood culture sets drawn after the second wound

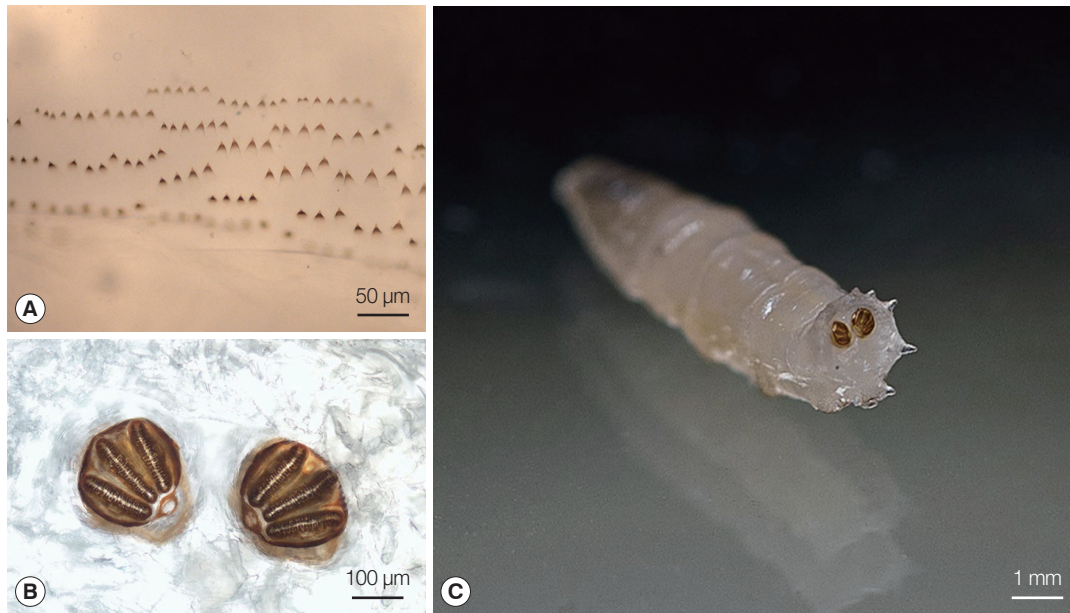


Fig. 1. *Lucilia* sp. 3rd instar larva from our patient. (A) Thoracic segment spines. (B) Posterior spiracles with 3 distinct straight slits surrounded by a complete closed peritremal ring with one internal projection between the outer and middle slits; the spines and the posterior spiracles were photographed using a Nikon Eclipse E600 (Nikon, Tokyo, Japan) microscope and DS-Fi1 (Nikon) camera. (C) Posterior spiracles located on the face of the terminal segment.

debridement, one anaerobic bottle was reported positive by the BD BACTEC FX blood culture system (Becton Dickinson), and Gram staining revealed Gram-negative rods (Fig. 2). Identification of bacteria directly from positive blood culture using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Server version 4.1.70 (PYTH) 48 2016-10-26_15-05-35, Billerica, Massachusetts, USA) [9] was not successful. Subculturing resulted in the growth of colonies with a yellowish pigment on Columbia and chocolate agar (Fig. 3) after 18 hr of incubation. No growth was observed on anaerobically incubated growth media. The identification of bacteria by MALDI-TOF MS was unsuccessful also from subculture. The strain was identified as *Ignatzschineria* sp. by partial (320 bp long region) 16S ribosomal ribonucleic acid (16S rRNA) gene sequencing [10]. By sequencing a longer 1,462 bp region of the 16S rRNA gene [11], the strain was identified as *Ignatzschineria larvae* with an identity match of 99.9%. The nucleotide sequence was deposited in the GenBank database under the accession number MW420873. The identity match with the most closely related species was 99.2% for *Ignatzschineria ureiclastica* and 98.5% for *Ignatzschineria indica* (Fig. 4). Testing of antibiotic susceptibility was done using Etests (Liofilchem, Roseto degli Abruzzi, Italy or bioMerieux, Marcy-l'Étoile, France). The isolated strain of *I. larvae* was re-

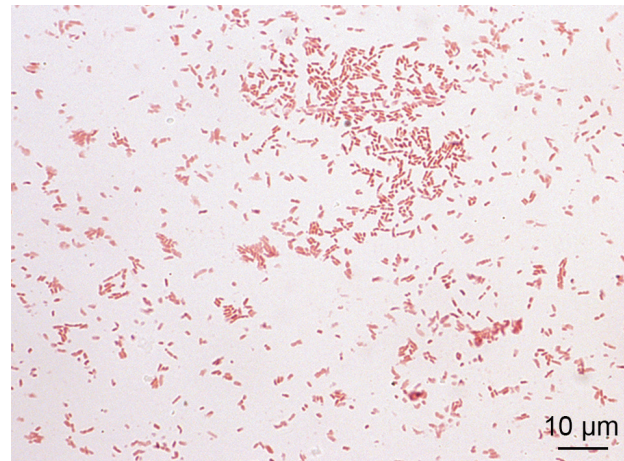


Fig. 2. *Ignatzschineria larvae* from pure bacterial culture: Gram stain.

ported susceptible to piperacillin-tazobactam, ceftazidime, ciprofloxacin, and imipenem considering pharmacodynamic/pharmacokinetic (non-species related) European Committee on Antimicrobial Susceptibility Testing (EUCAST) Clinical breakpoints [12].

After the second debridement, no more larvae were observed in the wounds, which were redressed regularly and were healing well. A swelling was observed on the proximal right thigh, and therefore an abscess was suspected and ultrasound per-

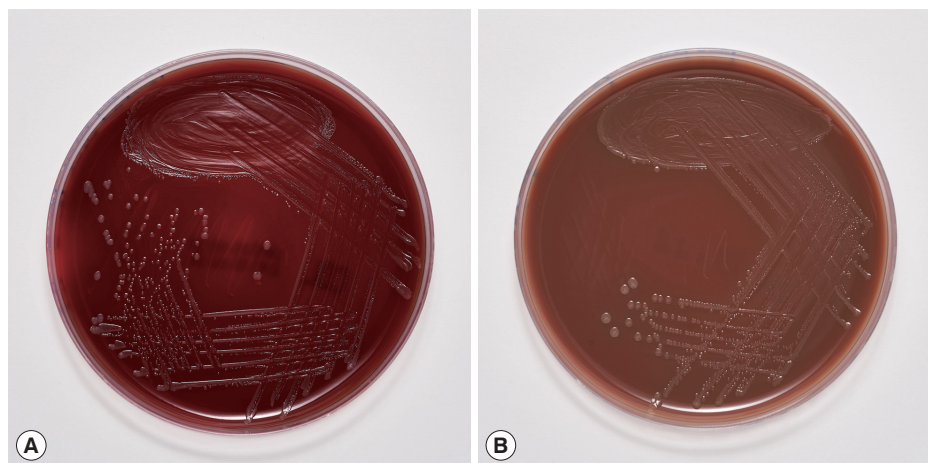


Fig. 3. Culture of *Ignatzschineria larvae* grown on (A) Columbia agar and (B) chocolate agar after 18-hour incubation.

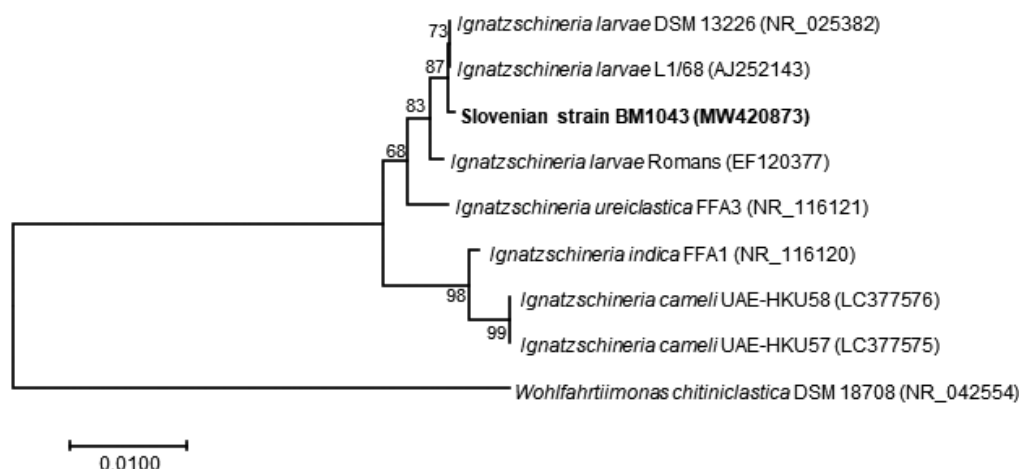


Fig. 4. Neighbour-Joining phylogenetic tree based on nearly full-length 16S rRNA gene sequence of *Ignatzschineria larvae* isolate BM1043 from our patient (in boldface) and representative *Ignatzschineria* sp. sequences retrieved from GenBank. The tree was created with MEGA 7.0 software using the Neighbour-Joining method and Jukes–Cantor model. Values on branches are percentage bootstrap values using 1,000 replicates. *Wohlfahrtiimonas chitinoclastica* was included as an outgroup organism.

formed, which showed only lymphadenopathy. Because tularemia is present in the rural Western Balkans [13], a punctate of the lymph nodes was sent to the IMI for PCR for *Francisella tularensis*, which was negative. Serology for *Bartonella* spp., *Francisella tularensis*, and *Toxoplasma gondii* were also negative. Thus, lymph node enlargement was likely only due to skin and soft tissue infections.

After receiving 10 days of appropriate antibiotic therapy, the patient’s clinical condition improved and biomarkers of inflammation resolved. He was afebrile from the 5th day onward and was discharged to a detention center. He was then lost to follow-up.

DISCUSSION

This is the first reported human case of *Ignatzschineria* spp. bacteremia in Slovenia. *Ignatzschineria* spp. are emerging aerobic, Gram-negative, non-sporulating, non-haemolytic, non-motile, rod-shaped bacteria that were first isolated from larvae of *Wohlfahrtia magnifica*, a parasitic flesh fly causing myiasis in several animal species and occasionally in humans [14-17]. Recent reports suggest that *Ignatzschineria* spp. may also be transmitted by flies from the genus *Lucilia*, which are more commonly associated with human wound myiasis [5,6]. Bacteria carried by fly larvae can spread into the bloodstream of

the infested host, causing serious systemic infections. However, bacteremia in humans caused by species of the genus *Ignatzschineria* has been rarely described and has only been documented in a few case reports [5,6,15,18-27]. The rarity of infections being detected may be due to the fact that these bacteria were first described only 2 decades ago and also the difficulty of their identification using standard medical microbiology identification methods, such as classical biochemical tests and commercial bacterial identification systems [18]. Recent inclusion of reference spectra for a representative strain of *Ignatzschineria indica* in the Bruker database may make possible more frequent identification of this species by MALDI-TOF MS in the future [19,28]. Indeed, as reported by Rodriguez-Zuniga et al. [24], Deslandes et al. [19], and Snyder et al. [26], *I. indica* from blood cultures of patients with sepsis or bacteremia has correctly been identified by MALDI-TOF MS. As shown in our case, the identification of the species *I. larvae* by MALDI-TOF MS was still not possible.

Currently, the most accurate method for identifying *Ignatzschineria* spp. is 16S rRNA gene sequencing. In fact, it was the only way for accurate identification of the species *Ignatzschineria larvae* in our case. Heddemma et al. [21] reported very high similarity among 16S rRNA sequences of *I. ureiclastica*, *I. larvae* and *I. indica*, 3 out of the 4 species described so far in the genus *Ignatzschineria*. By the initial sequencing of a 320 bp long region of the 16S rRNA gene [10] we were able to identify the bacterium, which caused bacteremia in our patient, at the genus level only. For this reason, sequencing of a 1,462 bp long region of the same gene [11] was required for accurate identification of *I. larvae*. To date, there have been 4 other case reports describing *I. larvae* bacteremia in patients with myiasis, all of them originating from France [20,22,25,27]. As in most of the described cases of *Ignatzschineria* spp. infections associated with myiasis, in these 4 case reports the maggots were discarded after wound cleaning and were not sent for entomological identification. To the best of our knowledge, there is only one case report in which the maggots were identified and *Lucilia sericata* connected to *I. indica* bacteremia was determined [6]. The maggots from the wounds of our patient were preserved in formalin immediately upon submission to the IML. The preservation killed the larvae, made them immotile, and thus made their identification possible. *Lucilia* sp. was identified based on the maggots' morphological appearance. Unfortunately, the preservation prevented their subsequent bacterial analysis. Hence, it was impossible to confirm that *I. larvae* causing bacteremia

in our patient formed part of the flora of these maggots. However, a relation between *Ignatzschineria* spp. infection and maggot infestation is well documented [5,6,25]. Thus, our hypothesis that the bacteremia originated from maggots infesting the patient's wounds is reasonable. Moreover, the bacterium grew only in the second set of blood cultures, which were drawn after wound debridement that involved nail ablation and necrectomy. We believe it is likely that the wound manipulation caused invasion of the bacteria into the bloodstream and a second spike of fever despite concomitant antibiotic therapy.

As discussed above, *Lucilia* spp. maggots are among the most common maggots infesting human wounds [6]. Community-acquired myiasis by these larvae poses a risk for serious systemic bacterial infections [5,18]. On the other hand, sterile medical-grade larvae of *Lucilia* species are very effective at wound cleaning and are therefore used in one of the oldest techniques in wound care, known as maggot debridement therapy or biosurgery [29]. Biosurgery has been acceleratingly reintroduced in healing of chronically infected wounds since the appearance of bacterial strains with antibiotic resistance [30].

Our patient was an irregular migrant from Asia using the western Balkan migration route to reach western Europe. This route is one of the main migration routes into Europe. In 2019, around 14,000 illegal crossings were identified on the European Union's borders on the western Balkan route alone, more than double the 2018 statistics [31]. In Slovenia, a total of 16,099 illegal crossings of the border were recorded in 2019. The number increased by 73.8% compared to 2018 [32]. Given the increasing number of illegal border crossings, myiasis and myiasis-induced bacteremia could become an important migrant health issue.

Clinicians should have a high level of suspicion when treating patients with neglected wounds. Just as myiasis may indicate an underlying bacterial infection, the presence of bacteremia with *Ignatzschineria* spp. or *Wohlfahrtiimonas* spp. – another maggot-associated bacteria that have caused episodes of bacteremia – may indicate myiasis [5]. We would like to emphasize that standard biochemical tests and automated identification systems do not always identify these emerging pathogens. We therefore believe that cases like ours need to be reported.

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CONFLICT OF INTEREST

The authors declare no conflict of interest related to this study.

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