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A Rare Dual Infection in a Homeless Patient: Diagnostic Challenges of *Naganishia diffluens* and *Bartonella quintana* Co-Infection

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Abstract

We report a case of dual infection with *Naganishia diffluens* (formerly *Cryptococcus diffluens*) and *Bartonella quintana* in a homeless patient in Mexico City. The patient exhibited sporotrichoid lesions along the lymphatic channels. This typical presentation initially suggested a fungal infection caused by *Sporothrix* species, which are common in such lesions in Mexico. Additionally, the presence of lice infestation raised suspicions of *Bartonella* infection, as human body lice often transmit it and is a concern in homeless populations. The infections were conclusively identified through a comprehensive diagnostic approach that included microbiological culture on PDA media, microscopic examination, molecular identification via PCR, and amplification and sequencing of the internal transcribed spacer (ITS) region for fungi, as well as the gltA gene for *Bartonella*. Immunological confirmation was also performed with a precipitin assay using patient serum tested against fungal extracts. *Naganishia diffluens* was isolated from skin lesion scrapings, and *Bartonella quintana* was identified from a blood sample. This case underscores the importance of employing thorough laboratory techniques for accurate diagnosis and effective management of similar cases.

Keywords

Naganishia, *Bartonella*, Lice, Homeless, Precipitin Assay

1. Introduction

People experiencing homelessness face a significantly higher risk of numerous diseases and health complications due to factors like exposure to the elements, poor hygiene, malnutrition, crowded or unsanitary living conditions, substance use, and limited access to healthcare [1] [2]. Homeless individuals are especially vulnerable to skin and soft tissue infections because they deal daily with injuries and immune system challenges. If not treated, these infections can become serious, making access to regular hygiene, medical care, and stable housing crucial for prevention.

The incidence of pathogens may vary depending on the geographic location and the population's health status [3]. The risk of opportunistic mycoses, lice infestations, and open wounds is very high and can lead to serious health complications. Environmental microorganisms from air, skin, and plants, especially primary pathogens, can enter the body through contact with the skin and subcutaneous tissues [1] [4], or by inhalation [5]. Fungal propagules such as yeasts, fragments of mycelium, pseudo-mycelium, or spores can be introduced by traumatic events, resulting in various clinical signs, including painful nodules on the axilla and trunk, as well as crusted papules on the arms and legs [6] [7]. Some of these manifestations resemble a lymphocutaneous sporotrichosis pattern, which a range of infectious agents can cause. Accurate diagnosis relies on clinical expertise, microbiological testing, and an understanding of the underlying pathology associated with each potential causative agent. Recently, molecular techniques have become valuable for the precise identification of pathogens [8]-[12].

Some studies have documented the isolation of *N. diffluens*, formerly known as *Cryptococcus diffluens*, from disease-related conditions, including subcutaneous cryptococcosis [10], onychomycosis [13], and epidermal colonization. Researchers have also isolated it from the skin of patients with atopic dermatitis, the oral mycobiome of healthy adults [14], and children with caries and healthy dentition [15]. A high prevalence of *C. diffluens* colonization in neonates hospitalized in the NICU of Bu Ali Sina Hospital has also been reported [16]. *N. diffluens*, a basidiomycetous yeast, is particularly intriguing due to its adaptability to extreme environmental conditions [17].

Pediculus humanus humanus is the vector for at least three intracellular bacteria, including *B. quintana*, the causative agent of trench fever [18] [19]. It is one of the most common problems among people experiencing homelessness. *B. quintana* infections are often asymptomatic and can persist if not detected. However, symptomatic cases may present fever, lethargy, and muscle pain lasting four to five days, known as "quintan" or "fifth-day fever" [20]-[22]. The potential for serious complications, including heart and neurological issues [18] [19], should be taken seri-

ously. Since 1955, *B. quintana* has been reported in Mexico City. A 2006 study found that 28.3% of body lice carried these bacteria [23] [24], and more recently, they were found in head lice from a Mayan community in southern Mexico [25].

Open wounds are common among homeless populations due to trauma, violence, injection drug use, and environmental hazards, which lead to the development and poor healing of both acute and chronic wounds [26]. The risk of developing myiasis increases significantly in these conditions. Exposed wounds attract flies to lay eggs, which hatch into larvae (maggots) that infest and feed on the tissue [27]. Additionally, mental health issues and substance use can impede adherence to wound care plans. This case describes a rare and unique instance of infection with *N. diffluens* and *B. quintana*, along with myiasis infestation in a homeless patient presenting with sporotrichoid skin lesions, open wounds on the feet infested with maggots, pediculosis, weakness, and disorientation. The process of isolating and identifying these two pathogens was complex and challenging, involving microbiology, molecular biology, and immunology. This case highlights the complex health challenges faced by people experiencing homelessness.

2. Case Report

In September 2023, a 53-year-old homeless man was taken to the emergency room at Dr. Gregorio Salas General Hospital in Mexico City after being immobile in a flower bed for five days. He had lived on the streets for 20 years, often in unsanitary conditions, and showed signs of mental changes such as confusion or disorientation.

Physical examination revealed several erythematous nodules arranged in a linear pattern, unevenly distributed on both sides and extending to the patient's lower limbs. Some nodules showed excoriation, a central blood crust, hyperpigmented brown areas, and hypopigmented, atrophic scars that had been present for a long time (**Figures 1(A)-(B)**). He also had a single tender and swollen nodule in the right axilla, which was not painful. The patient had a 5 cm lesion on the dorsal aspect of the right foot, characterized by greenish exudate, hyperthermia, edema, and myiasis (**Figure 2(C)**). Additionally, a significant infestation of body lice (*P. humanus humanus*) was observed.

(A) (B)

Figure 1. Lymphocutaneous sporotrichoid linear pattern after three weeks of itraconazole administration. (A) Cutaneous lesions of *Naganishia diffluens* infection (cryptococosis), ulcers spread with satellite lesions. (B) The pattern of the lesions presented surrounding hyperpigmented skin.

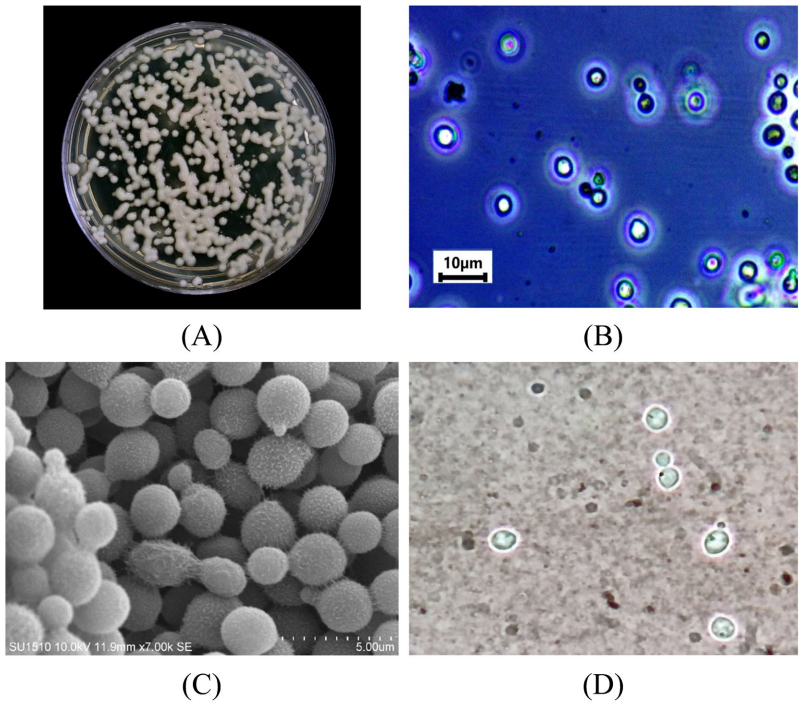

Figure 2. *Naganishia diffluens* phenotype. (A) *N. diffluens* from skin scraps grown on Sabouraud medium for 48 h at 30˚C shows white and creamy colonies with smooth surfaces. (B) Phase contrast microscopy shows a round cell (3 - 6 µm) exhibiting polar budding. (C) Scanning electron microscopy (Hitachi SU1510) of yeast cells shows cells coated with fibrils. Some yeast cells are budding. D. Yeast cells contrasted with India ink. Surrounding the cell body is a halo, indicating the presence of the polysaccharide capsule (Zeizz 100X).

The patient had normal vital signs. Hematological analysis showed an increase in leukocytes [18,000/mm^3 (reference 4500 - 11,000/mm^3)] and neutrophils [86.3% (reference 55% - 70%)], along with a decrease in lymphocytes [6.9% (reference 20% - 40%)] compared to reference values. He presented with anemia, characterized by a hemoglobin level of 9.58 g/dL (reference range 13.5 - 17.5 g/dL) and a hematocrit of 29.3% (reference range 41% - 53%). Platelet counts and electrolyte levels were within normal limits, and liver function tests were normal. A rapid HIV test was negative, and a chest X-ray appeared normal.

The presence of sporotrichoid lesions in the skin and evidence of louse infestation in clothing led us to suspect a fungal infection by *Sporothrix spp.* and louse-borne infectious diseases caused by *Bartonella.*

To identify the pathogens affecting the patient, samples were collected from skin wounds and blood. Initially, the skin scrapings were incubated in Sabouraud agar at 30˚C for 48 hours for colony morphology, microscopic analysis, and molecular biology. The colonies grown on solid Sabouraud medium from the ulcerated lesions were smooth, shiny, and creamy after 48 hours of incubation at 28˚C (**Figure 2(A)**). Microscopic morphological characteristics of the fungal cells showed individual round yeast cells (3 - 6 µm) that reproduce through budding (**Figure 2(B)**). Scanning electron microscopy revealed rounded cells, some bud-

ding, with visible extracellular fibrils (**Figure 2(C)**).

The colony morphology and the presence of yeast cells led us to suspect that *Sporothrix spp* was not the cause of the skin lesions. Because the colonies appeared smooth, we used Indian ink to check if the fungi had a polysaccharide capsule. The stain showed a clear halo around the yeast cells, where the ink particles moved away from the capsule; this confirmed the presence of a polysaccharide capsule (**Figure 2(D)**). These findings indicated the presence of a capsulated fungus from the *Cryptococcus* genus.

To identify the fungi in the patient's lesions, DNA was extracted from both the skin lesions and the colonies grown in culture media, then amplified by PCR using the fungal ITS1F-ITS4 R region of the ribosomal ITS region [28] and sequenced. BLAST and phylogenetic analysis of the 580 bp amplified fragment showed 99% identity with the sequence of *N. diffluens* from Europe, Australia, and South America. We deposited the sequences in GenBank under accession number OR976037.

Unfortunately, a biopsy was not performed on the patient to determine whether *Naganishia* caused the skin lesions; however, we conducted capillary precipitin assays using serial dilutions of extracts from various fungi, including *Naganishia*, with the patient's serum. This immunological reaction helps identify if the body has been exposed to and infected by the pathogen [29]. Antigens from *Aspergillus* (*flavus*, *niger* and *fumigatus*), *Coccidioides immitis, Histoplasma capsulatum*, and *N. diffluens* were included in this assay. The dilutions ranged from 1:2 to 1:64. Laboratory capillary tubes were used, with 20 μL of antigen and 20 μL of the patient's serum placed into each. For the negative control, 20 μL of 0.9% saline solution and 20 μL of the patient's serum were used. The results were read after 72 hours by assessing the agglutination of the sample [30]. The precipitin results showed that antibodies against *Naganishia* are detectable at the higher antigen dilution (1:64), while antibodies against *Coccidioides, Aspergillus*, and *Histoplasma* are only detectable at lower dilutions (1/2, 1/2, and 1/4, respectively).

The patient's skin lesions gradually improved after being diagnosed with *N. diffluent* and treated with itraconazole (100 mg/day). After 6 weeks of antifungal therapy, a linear pattern of lesions surrounding hyperpigmented skin with scarring was visible, and no new lesions had appeared since the treatment began (**Figures 1(A)-(B)**).

The presence of body lice (*P. humanus humanus*) in the patient indicated a potential *Bartonella* infection. This infection is now resurging, especially among vulnerable populations such as people experiencing homelessness. *Bartonella quintana* can cause various symptoms, including recurring fevers, bone pain, and in severe cases, endocarditis or bacillary angiomatosis, making early detection and treatment crucial. Diagnosing illnesses caused by *Bartonella* species with bacterial culture is challenging because these organisms grow slowly. To overcome this, we used PCR amplification and sequencing from blood DNA with primers targeting the citrate synthase [gltA] gene of the genus *Bartonella* [31]. The identity of *B.*

quintana was confirmed through BLAST and phylogenetic analysis which showed 99% identity with *B. quintana* from Europe and North America [32]. This sequence was deposited in GenBank under accession number OR976036.

Bartonellosis was treated with doxycycline capsules (100 mg every 12 hours) and injectable gentamicin (80 mg every 12 hours) for 30 days, successfully resolving his bacteremia. Among the antimicrobial agents considered, doxycycline and gentamicin are commonly used together for bartonellosis because of their synergistic effects and proven ability to target intracellular organisms [33] [34].

The necrotic tissue of the foot, infected with unidentified fly larvae (**Figure 3**), was debrided, and healthcare professionals surgically removed the larvae. After removing the larvae, the wound was cleaned daily, and amoxicillin-clavulanate (500 mg/125 mg tablet every 12 hours) was prescribed for 10 days to treat the secondary bacterial infection. The wound developed granulation tissue after being kept clean and dry, with no recurrence or complications.

Figure 3. Wound myiasis in the instep was treated by surgical debridement and antibiotics.

After eight weeks in the hospital, the patient regained mobility and the ability to walk, showing notable improvements in mood and language. A psychiatric evaluation diagnosed an unspecified mental disorder with cognitive deficits. He was discharged after 8 weeks and referred to a shelter.

3. Discussion

This case report details a rare and clinically significant mixed infection in a homeless patient, characterized by sporotrichoid skin lesions caused by *N. diffluens*, alongside *Bartonella,* likely resulting from body lice (*P. humanus humanus*) infestation. To our knowledge, this is the first documented case in Mexico City of a cutaneous *N. diffluens* infection with a sporotrichoid pattern in a human host. The patient was diagnosed through a combination of PCR and sequencing, which allowed us to identify specific pathogens. Additionally, the co-diagnosis of bar-

tonellosis raises essential questions about the interaction between environmental exposure, host immunity, and diagnostic vigilance.

Although we couldn't definitively confirm *Naganishia*'s presence in the skin lesions through biopsy, the immune test results (precipitin assays), the isolation from scrapes, and the patient's positive response to antifungal treatment strongly suggest that *Naganishia* caused the skin lesions.

N. diffluens, once classified among *Cryptococcus* species, [35] is an environmental basidiomycetous yeast with limited documentation as a human pathogen. Reports of *Naganishia* spp affecting the skin are rare. The sporotrichoid pattern seen in this case, characterized by linear nodular lesions following lymphatic drainage, is usually linked to *Sporothrix spp,* nontuberculous mycobacteria, and *Leishmania* spp. However, *Naganishia* should now also be considered in differential diagnoses, especially when standard diagnostic tests are inconclusive. The use of molecular techniques, such as PCR and sequencing, was essential in establishing a definitive identification, as phenotypic identification can be unreliable for rare yeasts. This underscores the importance of advanced diagnostic methods in detecting and understanding unusual and complex infections.

The presence of *Bartonella*, likely due to *B. quintana* transmitted through body lice, is not unexpected given the patient's homelessness and poor hygiene conditions. The clinical significance of this co-infection is emphasized by the possibility that *N. diffluens* and *B. quintana* may interact within the host, where fungal colonization and antigenic stimulation could influence the immune response, potentially supporting bacterial persistence. Additionally, the chronic inflammation and immune evasion strategies associated with *B. quintana* might alter host defenses, creating an environment conducive to opportunistic fungal pathogens. This highlights the importance of considering immunomodulatory interactions in polymicrobial infections to understand disease progression and patient outcomes better.

This emphasizes the importance of considering social factors that influence health in managing diseases. Body lice infestations and louse-borne illnesses are more prevalent among homeless populations, with *B. quintana* known to cause trench fever, bacillary angiomatosis, and endocarditis. The presence of skin lesions, along with systemic symptoms such as fever and malaise, should prompt clinicians to suspect these combined infections.

In conclusion, this case broadens the understanding of the clinical spectrum of *N. diffluens* and emphasizes the importance of increased awareness of potential mixed infections in socioeconomically disadvantaged populations. It also demonstrates the benefit of combining traditional serologic assays with molecular diagnostics. Clinicians should stay highly alert for multiple concurrent infections in vulnerable patients presenting with complex clinical syndromes, adopting a proactive approach to early diagnosis and effective management. From a public health perspective, this case highlights essential implications for vulnerable groups such as individuals experiencing homelessness, who face a greater risk of

multiple infections and injuries. Factors such as crowded living conditions, poor hygiene, malnutrition, and limited healthcare access increase the risk of co-infections and complicate diagnosis and treatment. Follow-up care is complicated in this population, as unstable housing, lack of social support, and barriers to healthcare often hinder treatment adherence and continuity. These challenges not only increase the chances of relapses and ongoing infections but also present broader risks of community spread. Tackling these issues requires integrated approaches that combine clinical care with public health efforts, such as improving access to diagnostic tools, implementing outreach programs, and establishing follow-up systems to reduce the recurrence of infections among homeless populations.

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Informed Consent Statement

The patient provided informed consent for publishing this case report.

Conflicts of Interest

The authors declare no conflicts of interest related to the publication of this paper.

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