Human *Brucella melitensis* infections in southern Vietnam

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Brucellosis is a collective term for infections caused by small Gram-negative coccobacilli belonging to genus *Brucella*. This genus incorporates the well-described animal pathogens *Brucella melitensis*, *Brucella abortus*, *Brucella ovis*, *Brucella suis*, and *Brucella canis*, which are associated with disease in goats, cattle, sheep, pigs, and dogs, respectively. *Brucella* are facultative intracellular pathogens, and are sequestered by monocytes and macrophages, spreading throughout the body to the liver, spleen, lymph nodes, and bone marrow [1]. These pathogens are synonymous with an aggressive disease syndrome in animals causing abortion, stillbirth, and the delivery of weak offspring. The organisms replicate to high concentrations in the affected tissues and are transmitted through contact with the placenta, fetus, fetal fluids, and vaginal discharge. Notably, goats can shed *B. melitensis* in vaginal discharge for up to three months after abortion and organisms can be shed in milk for the lifetime of an infected animal [2].

Many *Brucella* species have zoonotic potential and can be transmitted from animals to humans. Brucellosis in humans is typically contracted by contact with infected animals or through the ingestion of animal products prepared from infected animals. In symptomatic cases, disease presentation is highly variable and may arise rapidly or progressively. Classically, brucellosis in humans is a sub-acute, non-specific febrile disease characterized by high temperatures, headaches, malaise, night sweats, and body aches [3]. Some individuals recover quickly, while others develop more persistent, long-term complications including arthritis, spondylitis, endocarditis, dermatitis, and chronic fatigue, and neurological complications [3]. The disease is treated using antimicrobials; however, relapses are common, even after apparent bacteriological cure.

Many middle-high income countries employ successful control programs to reduce brucellosis in animals and humans. However, such control programs or surveillance infrastructures are less common in low-middle income countries (LMICs). Consequently, animal brucellosis is endemic in parts of Asia, the Middle East, East and North Africa, Latin America, and some southern and eastern European and poses a potential risk to human health in these locations [4]. Due to the complex variety of symptoms in humans,

the disease is difficult to diagnose and likely goes underreported. Vietnam is an LMIC in Southeast Asia where brucellosis is not a commonly reported cause of febrile disease [5], and no cases have been reported since three cases of *B. abortus* in 1962 [6].

The Hospital for Tropical Diseases (HTD) in Ho Chi Minh City (HCMC), serves as a primary and secondary facility for the surrounding local population and a tertiary referral center for infectious diseases for southern Vietnam. Non-specific febrile disease is a common reason for admission to HTD[7]. Blood culture is performed routinely on patients in whom an infection is suspected on the basis of fever (>38^{o}C) or clinical evidence of sepsis. For adult patients, 8-15 mL of venous blood is drawn and inoculated into BACTEC*plus* aerobic bottles (Becton Dickenson, USA), prior to incubation at 37^{o}C in a BACTEC9050 analyzer.

From 14th June 2016 to 18th January 2017, ten febrile patients attending HTD had a positive blood culture containing Gram-negative coccobacilli. These organisms were sub-cultured onto sheep chocolate blood agar and subjected to biochemical identification and antimicrobial susceptibility testing. The organisms stained red using a modified cold Ziehl-Neelsen stain, and were identified as *Brucella* spp. on a VITEK2 system (BioMerieux, France). All organisms were susceptible to amikacin, ciprofloxacin, gentamycin, doxycycline, imipenem, rifampicin, and trimethoprim sulphate. Nucleic acid was extracted from organisms and subjected to Bruce-ladder multiplex PCR to identify the infecting species [8]; all produced an identical collection of amplicons indicative of *B. melitensis.* We next performed MLVA-16 (Multiple Locus VNTR Analysis) on the ten *Brucella* isolates [9], which is comprised of three panels, panel 1 (8 minisatellite loci), panel 2A (3 microsatellite loci), and panel 2B (5 microsatellite loci). Panel 1 allows clustering the different Brucella species while panels 2A and 2B provide finer resolution characterization. The MLVA profiles of the Vietnamese isolates were compared with a global collection of various *Brucella* species (Fig.1a). The organisms were all confirmed as *B. melitensis* and produced independent

VNTR profiles falling into four subgroups, clustering with organisms originating from Southern Europe, the Middle East, and China.

The presumptive diagnoses of the brucellosis patients prior to bacterial culture and identification were sepsis(patients 1 and 2), non-specific viral infection(3), dengue(4), tuberculosis(5 and 7), and non-specific inflammatory disease(6); patients 8, 9, and 10 were correctly diagnosed with brucellosis after laboratory diagnosis of the first seven patients. These infections were additionally confirmed using Rose Bengal agglutination with titers in plasma ranging from 1/4 to 1/256. Differential blood counts were largely unremarkable but almost all patients had elevated AST, ALT, and GGT; several patients had elevated CRP and procalcitonin indicating systemic inflammatory response. On review of the medical histories, all patients had reported exposure to goats prior to the febrile episodes; eight kept goats, two had consumed goat meat, and one was a veterinarian who had been vaccinating goats. The ten cases originated from four provinces, with the primary cases occurring in Binh Phuoc and Tay Ninh near the Cambodian border, and later detection in the south (Ho Chi Minh City and Long An) (Fig.1b). All patients, apart from patient 5 (ceftriaxone 2g/day for seven days) received doxycycline (200mg/day for six weeks) with gentamycin (240 mg/day for seven days). All patients recovered without relapse with the exception of Patient 5, who was treated with imipenem (2g/day), gentamycin (240mg/day) for seven days and doxycycline (200mg/day) for six weeks. Patient 5 made a complete recovery without additional relapse.

B. melitensis is a known zoonotic pathogen that can cause an invasive febrile disease in humans exposed to infected animals[10]. Diagnosing brucellosis in humans is complicated by its non-specific presentation and may not be included in a differential diagnosis. The lack of a confirmatory diagnosis with appropriate antimicrobial therapy can lead to lasting physical effects through chronic infections. Persistent intracellular infection is associated with chronic arthritis, endocarditis and neurobrucellosis. Here we have reported the first identified cases of human brucellosis caused by *B. melitensis* in Vietnam. The organisms were susceptible to the advocated antimicrobial agents, again highlighting the importance of pathogen

isolation and identification. By performing MLVA genotyping, we found that the organisms fell into four different subgroups, suggesting that these organisms circulate widely in goats in southern Vietnam and were not part of an isolated outbreak. Therefore, we suggest that patients with non-specific febrile disease in Vietnam and comparable locations in Southeast Asia reporting contact with goats, sheep, and cattle receive a blood culture and a Rose-Bengal test. If the diagnosis is strongly suspected, empirical doxycycline can be given.

Vietnam is a hotspot for zoonotic infections and brucellosis is an important disease globally, stemming from the circulation of undiagnosed sick animals coming in contact with humans. Given the distribution of these cases and the collective exposure to goats, these findings suggest that these organisms are circulating widely in the goat population, which predicts that human cases may become increasingly common. We recommend that sick animals with a suspicion of brucellosis and their owners be screened in the identified provinces to assess the magnitude of the problem. This type of screening requires an interaction between the Departments of Preventative Medicine and Animal Health. Our work indicates the importance of zoonotic infections in Vietnam and highlights the need for sustained surveillance in human and animal populations.

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Figure 1. The locations and MLVA genotypes of ten human *Brucella melitensis* cases in southern Vietnam

A) Circular tree representing the global population structure of the genus *Brucella* using Multiple Locus VNTR Analysis-16 (MLVA-16) analysis. Nine different *Brucella* species (labeled) are shown and associated with main branches; *B. melitensis* forms the majority of the tree. The ten Vietnamese isolates are on black branches within the *B. melitensis* population. B) Map of southern Vietnam showing the locations of provinces where the ten cases of brucellosis originated between June 2016 and January 2017. The solid circle shows the location of the Hospital for Tropical Diseases in Ho Chi Minh City.