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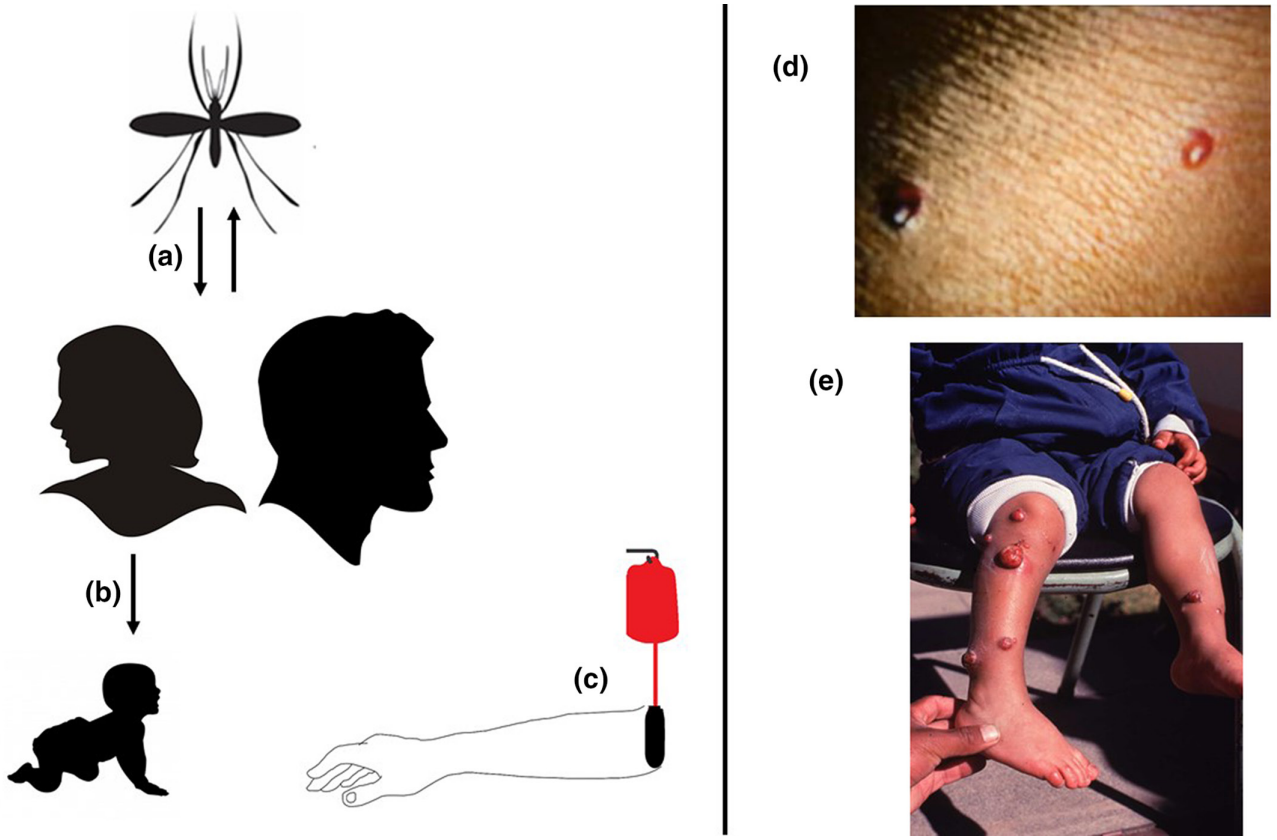
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MICROBIOLOGY SOCIETY

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JMM Profile: *Bartonella bacilliformis*: a forgotten killer

Joaquim Ruiz*



Graphical Abstract

Panel 1: Transmission routes of *Bartonella bacilliformis*. (a) *B. bacilliformis* is transmitted through the bite of several species of *Lutzomyia*, with *L. verrucarum* being the most representative. In turn, *Lutzomyia* species become infective vectors after feeding from a symptomatic or asymptomatic carrier. (b) Mother-to-child transmission has been reported with isolation of *B. bacilliformis* from newborns. Of note, *B. bacilliformis* infection during pregnancy may have severe effects, leading to premature births, fetal death and miscarriage, among others. (c) *B. bacilliformis* may be viable for years when blood is stored at 4 °C. This finding, together with the apparently healthy status of asymptomatic carriers, may result in post-transfusion infection. Panel 2: In the chronic phase or Peruvian wart, *B. bacilliformis* interacts with endothelial cells causing miliary, mular or subdermic warts. (d) Miliary presentation is characterized by the presence of numerous and usually painless reddish (and sometimes pruriginous) papules with a diameter <3 mm, in papillary and medial dermis. (e) Mular presentation is characterized by the presence of a low number of relatively large (often >5 mm) erythematous nodules which expand into the hypodermis, sometimes extending to subcutaneous tissue and muscle . All figures are drawn or extracted from Pixabay (Figures under 'Pixabay licence') except for the *Lutzomyia* silhouette which has been extracted from reference [13] and [18] [published under a Creative Commons Attribution (CC BY) licence], and wart images wich have been extracted from reference [19] (e) [published under a Creative Commons Attribution (CC BY) licence], and Public domain via Wikimedia commons (d).

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Abstract

Bartonella bacilliformis causes Carrión's disease, an infectious disease present in rural Andean areas of Peru and Ecuador. The disease has an acute and a chronic phase called Oroya fever and Peruvian wart, respectively. Oroya fever is potentially fatal if treated inadequately. Female *Lutzomyia verrucarum,* a phlebotomine sand fly endemic to South America, is the major vector. *B. bacilliformis* exhibits high susceptibility levels to a variety of antibacterial agents. *B. bacilliformis* is difficult to culture. Most endemic areas are remote with fragile health systems and poor communication. Thus, the true burden of the disease is difficult to ascertain.

HISTORICAL PERSPECTIVE

Bartonella bacilliformis was discovered in 1909 in patient blood by Alberto Barton, hence its name [1]. However, typical warty lesions caused by *B. bacilliformis* (see Clinical Presentation) are represented in ancient pre-Columbian pottery artwork pieces and compatible descriptions are present in the chronicles of the first third of the 16th century.

CLINICAL PRESENTATION

B. bacilliformis causes so-called Carrión's disease (honouring Daniel Alcides Carrión), a biphasic illness, accounting for acute (Oroya fever – named after a devastating 1870 outbreak affecting the construction of the Lima to La Oroya railroad) and chronic (Peruvian wart) phases [1]. The incubation period in humans varies from weeks to months. In the acute phase, *B. bacilliformis* invades erythrocytes, causing severe haemolytic anaemia, and inducing immunosuppression, favouring opportunistic infection as well as reactivation of latent infection [1, 2]. The acute phase is potentially fatal, with lethality rates of 40–85% in the absence or delay of adequate treatment [1]. In pregnant women, Carrión's disease can have serious maternal or fetal complications including premature birth, miscarriage, and fetal or maternal death. The chronic phase often takes place weeks to months after the acute infection, but may present in the absence of a prior acute phase. It is characterized by an angiogenic process leading to a proliferation of endothelial cells resulting in bleeding, miliary (usually painless), mular (frequently painful) or subdermic warts [1], which often present on the extremities, and is seldom fatal [2]. In the absence of treatment, the warts usually resolve after several weeks or months [1]. Asymptomatic carriers can serve as pathogen reservoirs in endemic areas [3], with some confirmed cases having ≥3 years of asymptomatic status [4].

MICROBIAL CHARACTERISTICS: PHENOTYPIC AND GENOTYPIC FEATURES

B. bacilliformis is a motile (having two to 16 flagella), slow growing Gram-negative alphaproteobacterium that varies in shape and size (0.2–0.5 µm by 1–2 µm). Primary culture usually needs up to 2 weeks to grow, with subsequent cultures growing faster (3–5 days). This slow growth may lead to plate contamination, requiring careful management, a temperature of 25–30 °C (usually 28 °C) and, ideally, microaerophilic conditions [1, 5]. *B. bacilliformis* culture requires the presence of haem, being possible to culture in solid media such as Columbia blood agar, or blood-supplemented *Leptospira* medium, Brain Heart Infusion (BHI), *Brucella* medium or others [1, 5]. The use of biphasic media [solid phase: Columbia agar plus 10% (v/v) sheep blood; liquid phase: RPMI 1640 plus L-glutamine and $NaHCO_3$] has been reported for sensitive cultures such as reactivation from frozen stocks [6]. While colonies vary from opaque to translucent, being usually small (0.2–1 mm), round and lenticular, they may have other presentations, which, in turn, may vary between consecutive cultures (Fig. 1a and b) [1, 5, 7].

B. bacilliformis has a small genome of ca. 1.4 Mb, which has been related to its obligate host-associated bacterial way of life [1]. To date, 16 *B. bacilliformis* isolates have been sequenced by whole genome sequencing, showing the presence of two main clades, which might constitute two subspecies or two closely related species. Strains KC583 (from Lima) and VER097 (from Ancash) are representative of each group. A seven-locus multilocus sequence typing (MLST) scheme for *B. bacilliformis* was described in 2011 and a database is available on the PubMLST.org website (https://pubmlst.org/organisms/bartonella-bacilliformis). To date, 14 distinct sequence types (STs) have been determined from 64 isolates. Of these, ST 8 and ST 12 are genotypically related to the VER097 group, and the remaining to KC583-like isolates [8].

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Keywords: bartonellosis; neglected disease; tropical disease.
Abbreviations: IFA, indirect fluorescent antibody; MLST, multilocus sequence typing; qPCR, quantitative PCR; ST, sequence type; T4SS, Type 4 Secretion System.

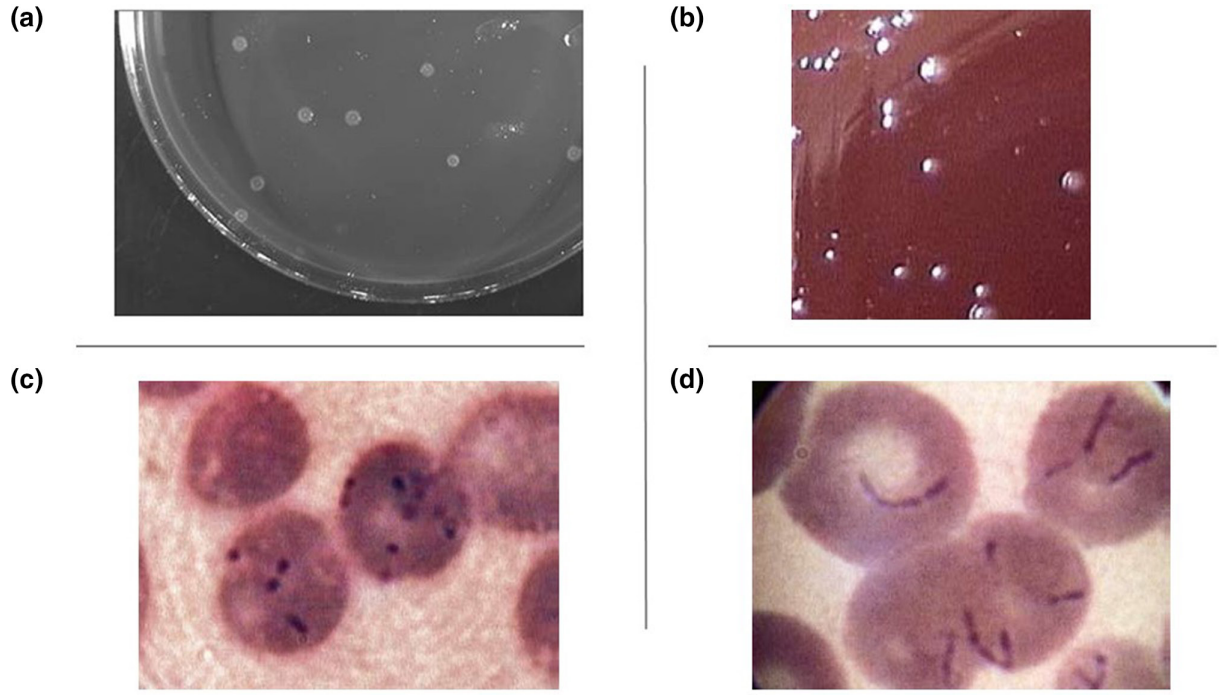


Fig. 1. Morphologies of *Bartonella bacilliformis* in culture and in Giemsa staining. (a) *B. bacilliformis* culture presenting the T1 morphology. Similar to classical presentation (see Fig. 1b), but with a characteristic centred 'bubble' (0.7x aprox) [reprinted from reference [7] (published under a Creative Commons licence)]. (b) Typical presentation of *B. bacilliformis* in blood culture: small colonies, usually translucent and round, with a regular edge (5x aprox) [reprinted from reference [19] (published under a Creative Commons licence)]. (c) Erythrocytes invaded by *B. bacilliformis* presenting coccoid forms (4000x aprox) [reprinted from reference [19] (published under a Creative Commons licence)]. (d) Erythrocytes invaded by *B. bacilliformis* presenting bacillar forms (5000x aprox) [reprinted from reference [19] (published under a Creative Commons licence)].

CLINICAL DIAGNOSIS, LABORATORY CONFIRMATION AND SAFETY

Clinical diagnosis

The clinical diagnosis of Carrión's disease has two main challenges: the high turnover of physicians in endemic rural areas, who are often recent university graduates, and the lack of symptom specificity. Oroya fever also shares symptoms with other infections such as malaria, typhoid fever or hepatitis [1]. Malaise, low-grade fever, headache, mild chills and pallor rank among the most frequent symptoms, while others including hepatomegaly or abdominal pain have also been described [1, 2]. These findings threaten patient survival when *B. bacilliformis* is introduced in naive areas, due to erroneous diagnosis and inappropriate treatment [1]. Nevertheless, the clinical diagnosis of Oroya fever is based on symptomatology and the use of Giemsa-stained peripheral blood smears [1]. Meanwhile, the diagnosis of Peruvian wart is made by visual inspection, with red-to-purple vascular wart-like lesions, frequently bleeding, occurring in clusters often at extremities. The presence of asymptomatic carriers remains hidden and undetected unless molecular tools are used [3].

Laboratory confirmation

The most common laboratory confirmation is the use of Giemsa-stained peripheral blood smears (Fig. 1c and d) [1]. While this technique has a high specificity (ca.96%), it has a low sensitivity (ca.30%), mainly being expertise-dependent [1]. When the samples are sent to reference centres, immunological tools, such as ELISA or immunofluorescence assay (IFA), and/or molecular tools, such as PCR, are used [1, 2]. Different PCR approaches have been proposed, including amplification of the 16S rRNA – 23S rRNA intergenic region or amplification and sequencing (or specific enzyme digestion) of a fragment of the 16S rRNA gene [1]. Other techniques such as real-time quantitative PCR (qPCR) have been used in research studies, but not for clinical diagnosis [3, 9]. In all these cases, the techniques are developed in-house as no commercial system is available. Bacterial culture has a limited diagnostic utility because of the above-mentioned slow growth and specific requirements. Antigenic candidates, able to be used with easy-to-use rapid diagnostic strips, have been explored, but, to date, none has been introduced for diagnostic purposes [3].

Laboratory safety

B. bacilliformis is classified as a Biosafety Level 2 (BSL-2), Risk group 2 or Hazard Group 2 biological agent, by the various national health organizations, requiring standard personal protective equipment such as gloves and laboratory coats. Clinical samples (usually blood) need to be processed within a microbiological safety cabinet and the potential presence of other blood-transmitted diseases must be taken into account during manipulation. At least one accidental *B. bacilliformis* infection during patient management has been described [1] and laboratory-acquired infections, whilst not reported in the literature to date, remain a possibility.

TREATMENT AND RESISTANCE

Treatment

Oroya fever is mainly treated with ciprofloxacin or chloramphenicol, and with amoxicillin plus clavulanic acid in pregnant women or small children. Meanwhile Peruvian warts are treated with macrolides (azithromycin, erythromycin). Severe cases are often treated with combinations of ciprofloxacin and cephalosporins, varying to chloramphenicol with cephalosporins in pregnant women. Other antibiotics such as rifampicin have been largely used in the past, and are currently among the second-line antibiotics [1, 10]. Severely affected patients with acute anaemia may need blood transfusion [1].

Resistance

While data about antibiotic resistance are limited to a scarce number of clinical isolates, *B. bacilliformis* displays susceptibility to a wide range of antimicrobial agents [1]. Nevertheless, despite fluoroquinolones being used as the treatment of choice, *B. bacilliformis* is intrinsically resistant to nalidixic acid, presenting diminished susceptibility and a relative ease to develop full resistance to fluoroquinolones [7, 11]. These findings are related to the presence of a wildtype alanine in positions 91 of GyrA and 85 of ParC and, recently, *B. bacilliformis* clinical isolates showing high levels of ciprofloxacin resistance related to mutations in GyrA and/or GyrB have been described [6]. To date, only one clinical isolate showing low-level resistance to chloramphenicol has been reported [6]. Interestingly, an *in vitro* study showed the difficulty in obtaining chloramphenicol- or azithromycin-resistant mutants, suggesting a high impact on the fitness of selected target mutations [7]. Of note, despite this high level of susceptibility, the presence of clinical failures with chloramphenicol treatment has also been reported [1].

PATHOGENIC STRATEGIES: HOST RANGE, TRANSMISSION, INFECTION AND VIRULENCE FACTORS

Host range

Although several potential mammalian animal and even plant reservoirs have been proposed, to date the only convincing evidence remains for that of humans together with a few *Lutzomyia* species as disease vectors. *Macaca mulatta* (rhesus macaque) and *Aotus nancymaae* (owl monkeys) may present with bacteraemia and/or warts if experimentally infected with *B. bacilliformis* [1].

Transmission

Carrión's disease is transmitted through the bite of *Lutzomyia* spcies, mainly *L. verrucarum*, with the carrier roles of *Lutzomyia peruensis*, *Lutzomyia maranonensis* and *Lutzomyia robusta* confirmed [1, 12]. The incubation time from the sand-fly bite to symptom onset ranges from 10 to 210 days (mean of 61 days) [1]. In addition, mother-to-child transmission and post-transfusion infection has been reported [1, 13]. To date, no case related to organ transplantation has been reported.

Infection

The presence of a primary niche, which allows *B. bacilliformis* to remain hidden from the immune system after infective bites, has been proposed [1]. The process of erythrocyte and endothelial cell interactions as well as mechanisms leading to transition from the acute to the chronic phase remain to be fully elucidated.

Virulence factors

While in other *Bartonellaceae* Type IV secretion systems (T4SSs) play a key role in virulence, infection and host adaptability, these are absent in *B. bacilliformis* [1]. Data regarding virulence factors are scarce and fragmented. Flagella, deformin, and IalA and IalB are involved in adherence to and invasion of erythrocytes during the acute phase, with GroEL being a key factor favouring vascular cell proliferation [1]. *B. bacilliformis* possesses a three haemin-binding protein, with Pap31 being by far the best characterized to date [1].

EPIDEMIOLOGY, PREVENTION AND RISK GROUPS

Epidemiology

B. bacilliformis is a pathogen restricted to the Andean areas of Peru and Ecuador. The presence of Peruvian warts in coastal Ecuador is probably related to an unidentified sister *Bartonella* species rather than to *B. bacilliformis* [1, 14]. Similarly, *Bartonella ancashensis* has been isolated from two patients with Peruvian warts [15]. In addition, a devastating outbreak in Southern Colombia occurred in the 1930s and 1940s, but no case has been confirmed in the country since the last century [1].

Though rarely reported, *B. bacilliformis* infections have been described outside endemic areas either in returning travellers or migrants from endemic areas [1].

Prevention

While early vaccine studies are from the 1930s, and several antigenic candidates have been proposed in recent studies, no effective vaccine has been developed [16, 17]. Vector-control measures are the most efficient prevention approaches, including insecticide spraying and maintenance of irrigation systems or avoidance of stagnant waters. Use of insect repellents and avoiding evening exposure may limit *Lutzomyia* bites. Blood banks from endemic areas include *B. bacilliformis* detection in their screening prior to use [18].

Risk groups

As *B. bacilliformis* infection results in semi-immunity, the main risk groups are immunocompromised patients, small children and pregnant women. Foreigners in endemic areas may also be considered as a population at risk, but are often outside of or have limited activities in the most exposed areas.

OPEN QUESTIONS

(1) What takes place between the infectious bite of *Lutzomyia* and onset of symptoms?
(2) What is the cause of frequent microbiological failures (presence of viable *B. bacilliformis* in blood) after clinical success of antimicrobial treatments?
(3) Are some *B. bacilliformis* clones more prone to causing the acute or chronic phase of the disease?
(4) Does erythrocyte invasion precede endothelial cell interaction?
(5) What mechanisms facilitate *B. bacilliformis* to induce immunosuppression, evade the immune system, and remain silent, hidden and chronic?

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Conflicts of interest
The author declares that there are no conflicts of interest.

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