Bartonella bacilliformis

e1

A 49-year-old patient was admitted to hospital with diarrhea, fever, and rigors. A blood culture was taken. On examination, it was noted he had many red vascular cutaneous nodules on his trunk and legs. His laboratory results were Hb 8.3 g/dl WBC 13.2 X 10^9/L, pl 160 x 10^9 /L, ALT 55 U/L, ALKP 150 U/L, CRP 210, demonstrating an anemia with an inflammatory response and disturbed liver function. He gave a history of visiting Peru 8 weeks previously, remaining on the coastal plane. On returning home, he began to feel unwell with fever, myalgia, and shivering which lasted about one week. He was given amoxicillin by his GP for a presumed chest infection. Shortly after recovering, he began to have fever and rigors again and nodules began to develop on his legs. The blood culture had gram-negative bacilli which proved to be *Salmonella* enteritidis. Given the travel history and presentation, polymerase chain reaction (PCR) demonstrated the patient was infected with *Bartonella bacilliformis* and, given the clinical presentation, had the chronic phase of Oroya fever: verruga peruana. The patient was negative for HIV, *Babesia*, Malaria, Zika, and Dengue.

1. WHAT IS THE CAUSATIVE AGENT, HOW DOES IT ENTER THE BODY AND HOW DOES IT SPREAD A) WITHIN THE BODY AND B) FROM PERSON TO PERSON?

CAUSATIVE AGENT

Bartonella belongs to the alphaproteobacter in the Order *Rhizobiales*, Family *Bartonellaceae*. The genus consists of more than 40 species, three of which are important human pathogens: *Bartonella bacilliformis* (Oroya fever), *Bartonella quintana* (Trench fever), and *Bartonella henselae* (cat scratch disease). It is a rapidly evolving genus with new members being identified.

Bartonella are fastidious, slowly growing gram-negative pleomorphic bacilli and are intracellular pathogens affecting erythrocytes and vascular endothelium. The organisms possess a Type IV secretion system (except *B. bacilliformis*) and surface autotransporter adhesins. It is proposed there are four phylogenetic lineages with *B. bacilliformis* in L1 and *B. quintana* and *B. henselae* in L4. The genome is 1.45–2.65 Mb and, as well as plasmids, some *Bartonella* carry linear DNA derived from the genome packaged into bacteriophage-like particles.

ENTRY INTO THE BODY

In 2015 at the G7 summit, the politicians set a "One Health" approach to human and veterinary medicine recognizing that many organisms infect both humans and animals. One such example is *Bartonella* as they are found in a wide variety of animals and also cause important disease in both humans and animals. Many of these infections can be transmitted from animal reservoirs to humans by insect vectors. *Bartonella bacilliformis* is transmitted to humans by the sandfly *Lutzomyia* (**Figure e1.1**) the vector of *Bartonella bacilliformis* (Oroya fever) and *Leishmania* spp. (leishmaniasis). In the blood, the organism circulates round the body penetrating erythrocytes and vascular endothelium.

PERSON-TO-PERSON SPREAD

B. bacilliformis is transmitted to humans by the sandfly either from another human or an animal reservoir causing Oroya fever. Other *Bartonella* species have rarely been linked to Oroya fever and other vectors may also transmit the illness.

B. quintana (Trench fever) is a disease of the homeless and is transmitted by the body louse. The louse acquires the organism after feeding on an infected person and the organism is excreted in the feces which can remain infectious for months. An individual acquires the infection from the

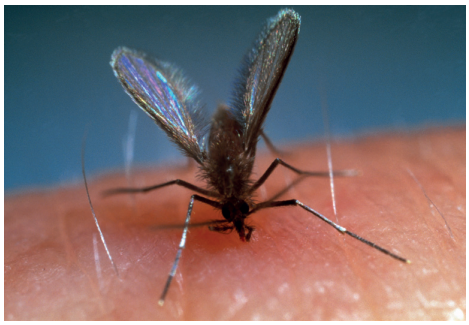

Figure e1.1 American sandfly, the insect vector for Oroya fever.
From Sinclair Stammers / Science Photo Library.

infected lice feces following minor trauma caused, for example, by scratching.

B. henselae (Cat scratch disease [CSD]) is a disease transmitted by cats (or dogs) biting, scratching or licking the broken skin of a human. The cat becomes infected after the bite of an infected cat flea. The organism has no effect on the cat. The organism may also be transmitted to humans by fleas or ticks.

EPIDEMIOLOGY

Oroya fever is restricted to South America with Peru having the highest number of cases, in part, because there is mandatory reporting of cases. Oroya fever is common in regions of poverty and poor education within areas where the vector is found often in relation to coffee plantations. Males are more frequently affected compared to females although pregnant women and children are particularly vulnerable to the acute disease.

In Peru, more than 60% of the population are seropositive. The department of Ancash has the greatest endemicity, mainly as verruga peruana [Peruvian warts] whereas Oroya fever is more common in less endemic areas. There was a peak in 2004 with 11130 cases (40.4/100 000). Since then, preventive measures of spraying insecticide has reduced the number of cases. Between 2002 and 2016, there were 247 deaths from Oroya fever. The annual incidence of disease was lower than malaria or Dengue although the death rate was higher. Figures indicate that 80% of the cases occur in 20% of the households and the relatives of an index case have a higher risk of acquiring the disease. Outside of Peru, Colombia has the highest prevalence of bartonellosis (2009–2013 = 3.0/100 000): -2.9% Oroya fever, 13.1% verruga, and 85.3% other Bartonella diseases. Bolivia had a prevalence of 2.5/100 000 for Oroya fever.

B. quintana was originally identified as Trench fever in 1915 in soldiers during WW1. It has a global distribution and has been the cause of outbreaks in the US, Russia, and Europe (Urban Trench Fever) and is regarded as an "emerging" disease. It is a disease associated with overcrowding and poor hygiene.

B. henselae occurs mainly in young persons and has a global distribution. The global prevalence is wide, from 1% to 36% depending on the cat population. Most infections in temperate climates occur in autumn/winter, whereas in the tropics there is no seasonal change.

The disease characteristics of Bartonella infections are changing because:

1. New *Bartonella* species are being identified (*B. rondoniensis*) some causing human disease such as Oroya fever. Disease has been identified in new areas in Peru (Cajamarca) and in the Highlands (Huanuco).
2. The territory of the sandfly *Lutzomyia* is extending from the typical habitat of between 500 m and 3000 m above sea level. Infections have recently occurred at 3500 m and below 500 m.
3. There are other potential vectors for transmission. Several arthropod vectors, such as fleas, ticks, bedbugs and lice, have been found carrying *Bartonella* spp. *B. quintana* (normally carried by lice) has been found in bedbugs.
4. A wide range of animal reservoirs occur, e.g. raccoons (*B. rochalimae*), kangaroos (*B. australis*), and even bees (*B. apis*).
5. There is a correlation between outbreaks of Oroya fever and the El Nino Southern Oscillation relating to meterologic changes (particularly sea surface temperatures) affecting the vector distribution.
6. Bartonellosis has previously been referred to as Oroya fever caused by *B. bacilliformis* but currently the term is applied to diseases caused by all *Bartonella* species.

2. WHAT IS THE HOST RESPONSE TO THE INFECTION AND WHAT IS THE DISEASE PATHOGENESIS?

INNATE IMMUNITY

Very little is known about the immunology of Oroya fever, being a "Neglected Disease" and having no animal model. The organism targets the innate immune system stimulating the production of IL-10 and thus limiting the innate immune response (see pathogenesis). Up to 30% of patients with acute Oroya fever develop opportunist infections with typically Salmonella or other gram-negative bacteria, pneumocystis, or reactivation of tuberculosis or toxoplasma. Additionally, neither the flagellae of Bartonella activate TLR5 nor does the **LPS** activate TLR4, thus limiting further the inflammatory response.

ADAPTIVE IMMUNITY

T-Cell Responses

With the acute infection, there is a decrease in white cells (lymphopenia) and CD4 T cells owing to the increase in IL-10 which persists throughout the acute infection into the chronic phase. This immune response is very similar to that of HIV.

Antibody Responses

In acute Oroya fever, there is an initial increase in IgM followed by an increase in IgG by week 2–4 similar to many other infections. In the chronic phase of Oroya fever, high IgG levels correlate with increased **VEGF** and **eotaxin**. Long-term humoral immunity develops although the targets are unknown.

PATHOGENESIS

B. bacilliformis can bind and penetrate a number of different cell types but principally the anucleate erythrocyte and the nucleated vascular endothelial cell. In acute Oroya fever, the organism binds to and penetrates the erythrocyte. Adhesion

appears to be through the polar flagella although in other species of *Bartonella* (e.g. *B. quintana* and *B. henselae*), the main erythrocyte adhesin is the T4SS. Adhesion to nucleated cells by *Bartonella* spp. is mediated by trimeric autotransporter (TAA) proteins and *B. bacilliformis* has three Brp's (Bartonella repeat proteins) which belong to the TAA family. In non-*B. bacilliformis* these three proteins a) affect actin changes and the formation of the invasosome b) activate NFkB and downstream inflammation, and c) inhibit apoptosis. Attachment to nucleated cells also involves actin filaments as inhibition of actin filament formation inhibits cell uptake.

Three factors are associated with invasion of erythrocytes and vascular endothelial cells. Motility is an important factor because if motility is inhibited so is cell penetration. Deformin is an extracellular protein that induces invagination of the erythrocyte membrane which is believed to be the entry site for the organism. Other factors are proteins coded for by the invasion-associated locus (ialA and B) which is a nucleotide phosphate hydrolase that aids intracellular survival by hydrolyzing "stress" markers in the cell.

The invasion of the erythrocytes leads to a hemolytic anemia and an array of symptoms including pallor, fatigue, cardiac murmurs, jaundice, tachycardia, and hepatomegaly.

In the chronic phase of Oroya fever, the organism invades the vascular endothelium causing proliferation of vascular endothelial cells. The pathologic sequence is mitogenesis of endothelial cells, with angiogenesis and inhibition of apoptosis. The organ mainly affected is the skin and the raspberry-like vascular lesions present as verruga peruana. With other *Bartonella* spp., a similar sequence can occur which also affects the skin but may occur in other organs, for example, the liver (Bacillary angiomatosis).

3. WHAT IS THE TYPICAL CLINICAL PRESENTATION AND WHAT COMPLICATIONS CAN OCCUR?

Oroya fever is a biphasic illness with a nonspecific febrile illness of fever, myalgia, headache, and hemolytic anemia followed by a chronic illness, called verruga peruana (Peruvian warts). Red/purple proliferative vascular lesions develop on the skin. Long-term colonization can occur following infection. Infected patients are prone to opportunistic infections with *Salmonella* spp.

The disease is named after the city La Oroya following an epidemic in migrant workers in the vicinity of the city in the 1850s where 7–10 000 individuals died. It is also called Carrion's Disease in honor of a Medical Student Daniel Alcides Carrion who contracted the disease and died of the acute illness after injecting himself with material from a patient with veruga peruana thus confirming the disease had an acute and a chronic phase.

Trench Fever presents with a fever, headache, rash, and bone pain typically on the shins.

Cat-scratch disease presents with a low-grade fever, lymphadenopathy in relation to the inoculum about three weeks after the incident and an erythematous nodule at the site of inoculum.

Other clinical presentations of bartonellosis are:

- bacillary angiomatosis (vascular proliferation in any area but mainly the skin);
- peliosis hepatis (lesions in the liver) in HIV positive individuals;
- infective endocarditis in any individual.

4. HOW IS THE DISEASE DIAGNOSED, AND WHAT IS THE DIFFERENTIAL DIAGNOSIS?

LABORATORY DIAGNOSIS

Laboratory diagnosis is mainly by microscopy of Giemsa-stained blood films revealing the organism in association with erythrocytes or vascular endothelium, but it has poor sensitivity. It is possible to culture *Bartonella* spp. from blood or tissue on blood agar medium at 28°C in 5% CO_2 incubated for up to 10 weeks but this is not usually part of the routine because of the length of time to diagnosis. Specific PCR is available and is probably the best available test. Antibody assays are available but suffer from cross-reaction with other *Bartonella* spp. and other bacteria. For tissue specimens, for example, lymph nodes in cat scratch disease, immunostaining is available.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of acute Oroya fever is: malaria, Dengue fever, Babesia and for the chronic stage other Bartonella infections (bacillary angiomatosis) and Kaposi's sarcoma.

For cat scratch disease (*B. henselae*), other conditions to consider are lymphoma tularemia, plague toxoplasmosis, and EBV infections.

5. HOW IS THE DISEASE MANAGED AND PREVENTED?

MANAGEMENT

Analysis of a small number of strains of *B. bacilliformis* indicates the organism is generally sensitive to a wide range of antibiotics including beta-lactams, macrolides, fluoroquinolones, aminoglycosides, tetracyclines, clotrimoxazole, rifampicin, and chloramphenicol. However, there are indications

of intrinsic resistance in some strains to ciprofloaxacin and acquired resistance developing to some other antibiotics.

The treatment of Oroya fever depends on whether it is acute or chronic. The drug most used for acute Oroya fever has been chloramphenicol although many of the antibiotics (above) have also been used such as ciprofloxacin, doxycycline, ampicillin, erythromycin, and cotrimoxazole. For the chronic phase of Oroya fever, rifampicin or azithromycin are used.

Trench fever is treated with gentamicin plus doxycycline.

Cat scratch disease is usually self-limiting and doesn't usually require antibiotics.

Bacillary angiomatosis and peliosis hepatis is treated with erythromycin or doxycycline.

Infective endocarditis is treated with doxycycline and gentamicin.

PREVENTION

There is no vaccine for *B. bacilliformis* and thus control of the disease depends on control of the vector *Lutzomiya*. The approach is similar to any flying insect vector: physical barriers such as nets and insecticides such as pyrethroids. In the case of this sandfly vector, the life cycle is not clear as the breeding grounds are not clearly identified and the flies, when resting, do so in inaccessible areas making environmental control difficult. An alternative approach is to use bait for the sandflies that contain lethal agents where the baits can be sprayed on surfaces. An alternative approach is to feed bait to rodents containing fipronil which does not affect the rodent but is present in the rodent feces, making the feces toxic to sandfly larvae.

SUMMARY

1. WHAT IS THE CAUSATIVE AGENT, HOW DOES IT ENTER THE BODY, AND HOW DOES IT SPREAD A) WITHIN THE BODY AND B) FROM PERSON TO PERSON?

- A gram-negative bacillus *Bartonella bacilliformis*.
- Transmitted by the South American sandfly *Lutzomyia*.
- Replication at the site of entry.
- Penetration of erythrocytes and distribution round the body.
- Transmitted from animals to humans by the sandfly:
  - Human >vector> human is also possible;
  - New *Bartonella* spp. are being discovered;
  - Alternative vectors may transmit the disease.

2. WHAT IS THE HOST RESPONSE TO THE INFECTION AND WHAT IS THE DISEASE PATHOGENESIS?

- A poor innate immune response.
- Lack of activation of both TLR4 and TLR5 by flagella.
- Bacterial stimulation of IL-10 and subsequent inhibition of immune response.
- Replication within erythrocytes (hemolysis) and vascular endothelium (skin nodules).
- ialA/B hydrolyses stress markers in cells.
- Flagella are important for adhesion.

3. WHAT IS THE TYPICAL CLINICAL PRESENTATION AND WHAT COMPLICATIONS CAN OCCUR?

- Biphasic illness.
- Fever.
- Hemolytic anemia.
- Lethargy.
- Superinfection with *Salmonella* spp. or other gram-negative bacteria
- Chronic phase characterized by skin lesions – verruga peruana.

4. HOW IS THE DISEASE DIAGNOSED, AND WHAT IS THE DIFFERENTIAL DIAGNOSIS?

- PCR.
- Microscopy.
- Of acute stage: malaria, Babesia, Dengue.
- Of chronic stage: bacilliary angiomatosis (*B. henselae*), Kaposi's sarcoma.
- Of cat scratch disease: lymphoma, EBV, toxoplasma.

5. HOW IS THE DISEASE MANAGED AND PREVENTED?

- Chloramphenicol, doxycycline, fluoroquinolone can be used.
- Veruga peruana: azithromycin, rifampicin.
- Trench fever: gentamicin, doxycycline.
- Cat Scratch Disease usually self-limiting.
- There is no vaccine.
- Vector control.