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REVIEW

Carrion's Disease: the Sound of Silence

Cláudia Gomes,[a] Joaquim Ruiz[a]

[a]Institute for Global Health, Barcelona Centre for International Health Research, Hospital Clínic, Universitat de Barcelona, Barcelona, Spain

SUMMARY Carrion's disease (CD) is a neglected biphasic vector-borne illness related to *Bartonella bacilliformis*. It is found in the Andean valleys and is transmitted mainly by members of the *Lutzomyia* genus but also by blood transfusions and from mother to child. The acute phase, Oroya fever, presents severe anemia and fever. The lethality is high in the absence of adequate treatment, despite the organism be-

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Address correspondence to Cláudia Gomes, claudiasofiapgomes@gmail.com, or Joaquim Ruiz, joruiz.trabajo@gmail.com.

ing susceptible to most antibiotics. Partial immunity is developed after infection by *B. bacilliformis*, resulting in high numbers of asymptomatic carriers. Following infection there is the chronic phase, Peruvian warts, involving abnormal proliferation of the endothelial cells. Despite potentially being eradicable, CD has been expanded due to human migration and geographical expansion of the vector. Moreover, *in vitro* studies have demonstrated the risk of the development of antimicrobial resistance. These findings, together with the description of new *Bartonella* species producing CD-like infections, the presence of undescribed potential vectors in new areas, the lack of adequate diagnostic tools and knowledge of the immunology and bacterial pathogenesis of CD, and poor international visibility, have led to the risk of increasing the potential expansion of resistant strains which will challenge current treatment schemes as well as the possible appearance of CD in areas where it is not endemic.

KEYWORDS *Bartonella bacilliformis*, Carrion's disease, Oroya fever, Peruvian warts

INTRODUCTION

The Pre-Inca and Inca Periods

Although no written documents from the pre-Inca and Inca cultures are available, there is evidence that Carrion's disease was known to the pre-Columbian cultures (1). Among the most ancient suggestive findings is the description of a pre-Incan mummy from the Nazca area (southern Peru) which had granulomatous lesions containing Giemsa-staining microorganisms which were also present in blood vessels and in internal organ granulomas. These lesions were compatible with the typical chronic-phase warty lesions (see "Peruvian Warts" within Clinical Presentation of Carrion's Disease below), and the microorganisms were compatible with *Bartonella bacilliformis*, the etiological agent of Carrion's disease (2). Some pottery artwork pieces made by the Mochica and Chimú cultures in the pre-Columbian period (huacos) show images of people with warty lesions, as do some stone figures from the Huaylas culture. Moreover, Quechua, the language of the ancient Peruvians, has words to describe fever ("rupha"), anemia ("sirki"), and eruption ("kcepo" and "ticti") (1).

In 1525, during the Inca period, the imperial city of Cusco suffered an epidemic during which more than 200,000 lives were lost. At that time the Incan emperor was in Tumibamba (present-day Cuenca, Ecuador), and he and his heirs and other family members were also victims of this epidemic. Some authors have suggested that he was infected by the messenger who delivered the reports of the epidemic (1, 3), but others believe that it was from the transfer of his mummy to Cusco after his death that the epidemic later arose (4). Although some authors attributed this epidemic to Carrion's disease, it is not completely clear because the warty phase was never described (1, 3). In fact, the presence of the first American outbreak of smallpox or measles (also supported by the occurrence of rash) is highly probable, being imported either through the Spanish settlements in Panama (4) or from the Plate river settlements by the expedition of the Portuguese explorer Aleixo Garcia to the Inca emporium in 1524 (5).

The Spanish Conquest

In Coaque (current Manabi Province, Ecuador) in 1531, Pizarro lost a quarter of his men to an outbreak of a warty epidemic involving muscle and skeletal pain similar to what is described in Carrion's disease (1, 6). Later, Juan Calvete described that during the invasion in 1547 to end the Gonzalo Pizarro rebellion, many soldiers became sick with skin eruptions (7). For some authors, these epidemics were associated with bartonellosis (6, 8) (see "Carrion's disease in other countries where it is endemic" below), but for others, they were not. Some of the reasons to the contrary are that Coaque is a coastal area almost at sea level and that the Carrion's disease in Ecuador was not firmly described until the 1920s (9).

The 17th and 18th Centuries

In 1630, Gago de Vadillo described the first report of the presence of a warty disease in an area in Huaylas, in the Ancash department, and raised the hypothesis that the warts were the consequence of the drinking water (10).

The next landmark in the history of bartonellosis was in 1764, when Cosme Bueno mentioned two important new facts: (i) the disease is very bothersome and dangerous in the absence of warts, which are related to the development of the chronic phase, which causes low mortality and therefore is a good prognostic indicating benignity, and (ii) the origin of the illness seems to be a small insect named uta (6, 11).

The 19th Century: the Tragic Era

The event that first sparked medical attention to this disease was the appearance of a febrile disease that killed thousands of Chinese and Chilean men in 1870 while they were working on the railroad in Cocachacra, a village between Lima and Oroya city. The unexplained epidemic appeared suddenly. Fever and anemia were rampant, as was the number of deaths, which, according to some reports, was more than 7,000. Indeed, it was said that "every railroad tie has cost a life." The new disease became known as Oroya fever despite Oroya city being quite far away and the absence of cases there (6).

The most remarkable contribution was made by Daniel Alcides Carrion, a young Peruvian medical student who inoculated himself with wart blood from a hospitalized child on 27 August 1885. The young researcher wanted to describe and understand the preeruption symptoms of the Peruvian warts because of the difficulty in diagnosing the disease before the appearance of the wart (11–13). The results of this experiment were a huge surprise for everybody, including himself. Some expected the eruption of a normal wart with no associated dangers, while others considered that the inoculation would not lead to anything. However, 21 days after inoculation, the first symptoms appeared, followed by rapid disease progression, which was completely compatible with the clinical features of Oroya fever (1, 6, 11). Despite his rapidly failing health, Carrion had the wisdom to clearly understand what was happening and said to his medical student friends:

"Up to today, I thought I was only in the invasive stage of the verruga as a consequence of my inoculation, that is, in the period of anemia that precedes the eruption. But now I am deeply convinced that I am suffering from the fever that killed our friend, Orihuela. Therefore, this is the evident proof that Oroya fever and the verruga have the same origin, as Dr. Alarco once said" (12).

Unfortunately, this experiment ended tragically. Daniel Carrion died on 5 October, 39 days after inoculation. In his honor, 5 October was declared National Medicine Day in Peru, and the disease was named after him. His experiment of self-infection proved that Oroya fever and the Peruvian warts are 2 different phases of the same illness (1, 6, 8).

The 20th Century and Modern Times

In 1909, Alberto Barton announced the discovery of the causal agent of Carrion's disease. He also described that the bacillus multiplies in patients with Oroya fever and decreases until its practical disappearance in the Peruvian wart phase of the illness (14).

Nonetheless, some researchers were not convinced. Richard Strong led the first Harvard Expedition to South America in 1913 and was able to find the bacillus in erythrocytes and other body tissues only in patients in the acute phase of the disease, and not in Peruvian warts, thereby contradicting previous findings determining that Oroya fever and Peruvian warts were 2 different illnesses (3).

In the same period, Charles Townsend attempted to discover the vector of the disease, spending several months unsuccessfully studying the possible role of ticks, acarus, and diurnal bugs. Thereafter, he decided to investigate the traditional native belief which considered a nocturnal sand fly called "titira" the vector. After several studies, he confirmed the traditional knowledge proposing that the vector was the

"titira," a member of the *Phlebotomus* genus named *Phlebotomus verrucarum* (currently known as *Lutzomyia verrucarum*) (15).

Further doubts finally vanished in 1927, when Hideyo Noguchi isolated the etiological agent from blood from patients with both Oroya fever and Peruvian warts (16, 17). Furthermore, the microorganisms isolated from the acute phase of the disease were inoculated into monkeys, producing both anemia and the characteristic eruptions on the skin (18). Noguchi had decisively proven the work of Daniel Carrion, and his results were later confirmed in 1937 by the second Harvard Expedition to Peru. The etiological agent of Carrion's disease was then named *Bartonella bacilliformis* in recognition of the discovery by Alberto Barton years previously (3).

Meanwhile, several other human inoculations were carried out with *B. bacilliformis*. In 1913, Strong inoculated a volunteer. Fortunately, the outcome was only the appearance of a Peruvian wart at the site of the inoculation. In 1928, Garcia Rosell suffered accidental inoculation when making a blood transfusion in a patient with Oroya fever, developing moderate fever and some time later a Peruvian wart. This was the inverse of Carrion's experiment; the formation of a Peruvian wart was also possible from inoculation with blood from a patient with Oroya fever. Nevertheless, Kuczynski-Godard and Garzón went further and inoculated themselves with *B. bacilliformis* in 1937 and 1942, respectively. While Kuczynski-Godard presented the Oroya fever phase 19 days after inoculation, which progressed to the Peruvian wart phase (6, 19), Garzón died on 12 September (20).

Until now, only the chapter on human infectious diseases related to smallpox has been definitively closed. Poliomyelitis is being actively eliminated through intensive vaccination, and yaws, measles, and several other infectious diseases have been included in eradication agendas. However, a series of chapters on the history of Carrion's disease have been lost, and several issues have apparently been forgotten, with a large amount of information being accessible only in local publications or unpublished theses. This illness continues to be present in the poorest regions and affects the most disadvantaged populations, far from the developed world, and is consequently completely outside international focus. Indeed, the study of Carrion's disease shows that this is a truly neglected disease, which only a few have attempted to describe and understand (Fig. 1).

EPIDEMIOLOGY

Peru

Carrion's disease is considered to be of special relevance in Peru, with the reporting of new cases being mandatory. Information about the situation outside Peru is scarce and mostly outdated. Thus, the main data regarding the ecology and sociology of the disease are limited to Peru, although they can likely be extrapolated to the neighboring countries affected.

Peru has an area of 1,285,216 km^2. It is the 19th largest country in the world and the third largest in South America (21). Moreover, its orography favors its great diversity of climates and habitats. The south and center Pacific coastal areas are desert, which transform to humid areas of mangrove woodlands on the northern coasts. Parallel to the Pacific Ocean, the center of the country is fully occupied by the Andes mountain range, with the Huascaran peak towering 6,768 m above sea level (masl). Meanwhile, at the center and north of the eastern side of the mountains, there are humid woodlands that give way to a deep jungle in the border areas of the country (Fig. 2).

According to the World Bank classification, Peru is an upper-middle-income country (http://www.worldbank.org/en/country/peru), with a population of about 31 million, ranking 84th in the 188 positions of the human development index (22). The high percentage of poverty in this country is of note, accounting for 21.7% of the population in 2015 (23) and being extremely high in the rural mountain areas (49.0% of inhabitants).

The internal migratory phenomenon during the 20th and 21st centuries resulted in an enormous population concentration in both the capital, the metropolitan area of

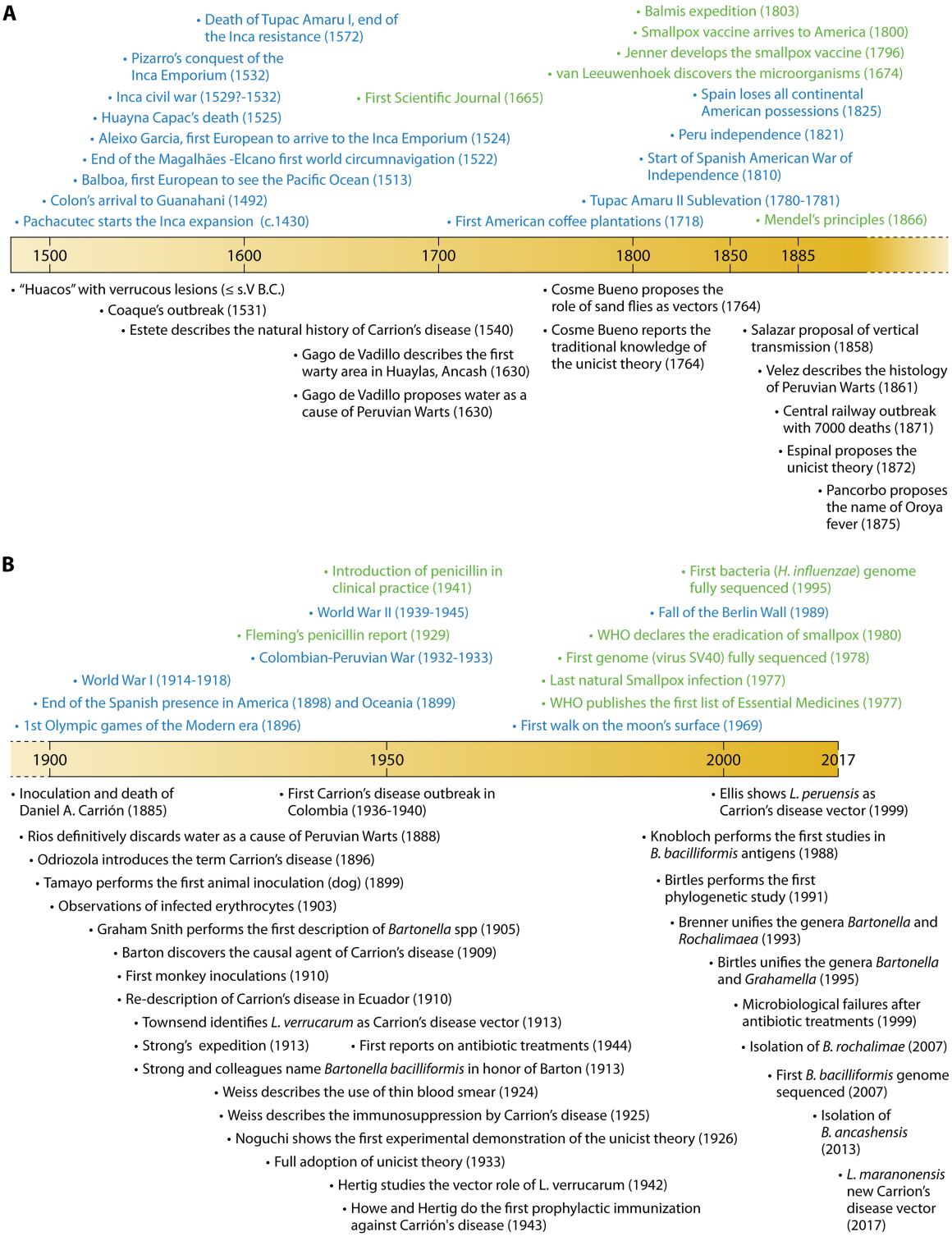

FIG 1 Chronology of Carrion's disease. (A) From the pre-Columbian period to Carrion's death (1885). (B) From 1885 onwards. Historic events (blue) and scientific milestones (green) are shown above the timeline bar, while relevant achievements in the knowledge and understanding of Carrion's diseases are shown below the bar.

Lima (9,886,647 inhabitants in 2015; 31.4% of total country inhabitants), and the coastal areas, which are home to approximately 55% of the total country inhabitants (21, 23).

Illiteracy has markedly decreased and now affects only 5.7% of the population, although it continues to affect up to 15.5% of the population in some rural areas (23).

FIG 2 Maps of areas of endemicity. (A) Geography of Peru. (Reprinted from https://es.wikipedia.org/wiki/Archivo:Mapa_fisico_Peru_fondo_transparente.png [published under a Creative Commons license].) (B) Administrative divisions of Peru. (Adapted from http://d-maps.com/m/america/perou/perou22.pdf.) (C) Administrative divisions of Ecuador. (Adapted from http://d-maps.com/m/america/equateur/equateur22.pdf.) The coast, mountain, and jungle cover 11.7,

(Continued on next page)

Moreover, the rate of infant mortality has decreased from 56.6/1,000 live births in 1990 to 12.9/1,000 live births in 2013 (24). Despite the high inequity between rural and urban areas, malnutrition has also decreased in the last 10 years. Overall, the level of chronic malnutrition in children under five is 18.1%, being 10.1% in urban areas and reaching 31.9% in rural areas (http://www.minsa.gob.pe/portada/Especiales/2015/Nutriwawa/directivas/005_Plan_Reduccion.pdf). Access to health care facilities in rural areas remains one of the unsolved challenges for this country. The availability of hospitals and an effective national health system to the poorest populations is very low in Peru. Access or proximity to the closest health care center is often lacking, leading to a lack of appropriate medical assistance in some areas. Another important issue is the uncontrolled and illegal sale of medications (25).

Characteristics of the Affected Population

The persistence of Carrion's disease in regions where it is endemic is associated mainly with poverty, warm weather, living conditions, low levels of education, and the characteristics of the region that define the presence of the vector. Andean areas have been largely neglected during the 19th and 20th centuries, resulting in small communities which are difficult to reach, and access to higher education requires migration to urban areas.

Carrion's disease usually affects men, with a slightly higher prevalence than for women (26–29). Furthermore, Carrion's disease has been considered an occupational disease in light of vector exposure of seasonal workers, mainly men, in most of the affected areas (e.g., coffee plantation workers living outside and traveling to areas of endemicity) (30). Moreover, the relationship between coffee plantations and the presence of *Lutzomyia* spp. has also been described (31).

Both children and pregnant women are especially affected. Fetal deaths, miscarriages, and premature births rank among the most serious complications affecting pregnant women (32). Children are the most affected by the acute phase of the disease (28, 33). Additionally, high levels of malnutrition enhance the severity of this disease (34).

Age and the presence of family members with bartonellosis are the best predictors of the possible development of *B. bacilliformis* infection (35). Patients are clustered in households. Eighteen percent of cohort households account for 70% of the cases. This pattern of bartonellosis implies that 80% of cases take place in 20% of households ("20/80 rule"). Thus, people living in a patient's household have a risk to become infected that is 2.6 times greater that that for the remaining area inhabitants (35). This finding has been related to the lack of intermediate hosts and person-to-sand fly to person transmission (36). It has also been shown that cases were more likely to report bites than controls in houses near or far away (37).

Carrion's Disease in Peru

Carrion's disease is an endemic illness found mostly in the inter-Andean valleys located between 500 and 3,200 masl (38). Peru is the country most affected by this disease, with the department of Ancash having the greatest endemicity. It is estimated that >1.6 million inhabitants live in areas of endemicity, which account for around 145,000 km^2 (39), with the seroprevalence among the general population of these areas being >60% (6). Although there are exceptions, the warty phase of the disease seems to prevail in regions of endemicity, whereas severe Oroya fever appears to be more common in areas of nonendemicity (40).

FIG 2 Legend (Continued)
27.9, and 60.3% of the Peruvian territory, respectively. Peru is the country most affected by Carrion's disease. In the period from January 2004 to May 2017, only the departments of Arequipa, Moquegua, and Tacna did not report Carrion's disease cases; in addition, in Pasco there was only 1 possible case reported. It is necessary to mention that in some departments, such as Tumbes, the number of reported cases was minimal, while in others, such as Callao, the cases were probably imported or reported as a result of patient movement. Regarding cases in Ecuador, the most recent cases have been described in the Peru-bordering province of Zamora-Chinchipe (bordering San Ignacio, one of the most relevant Peruvian areas of endemicity), as well in Guayas and Manabi (both of which are non-Andean areas). In Colombia during the last decades, only sporadic cases have been reported (9).

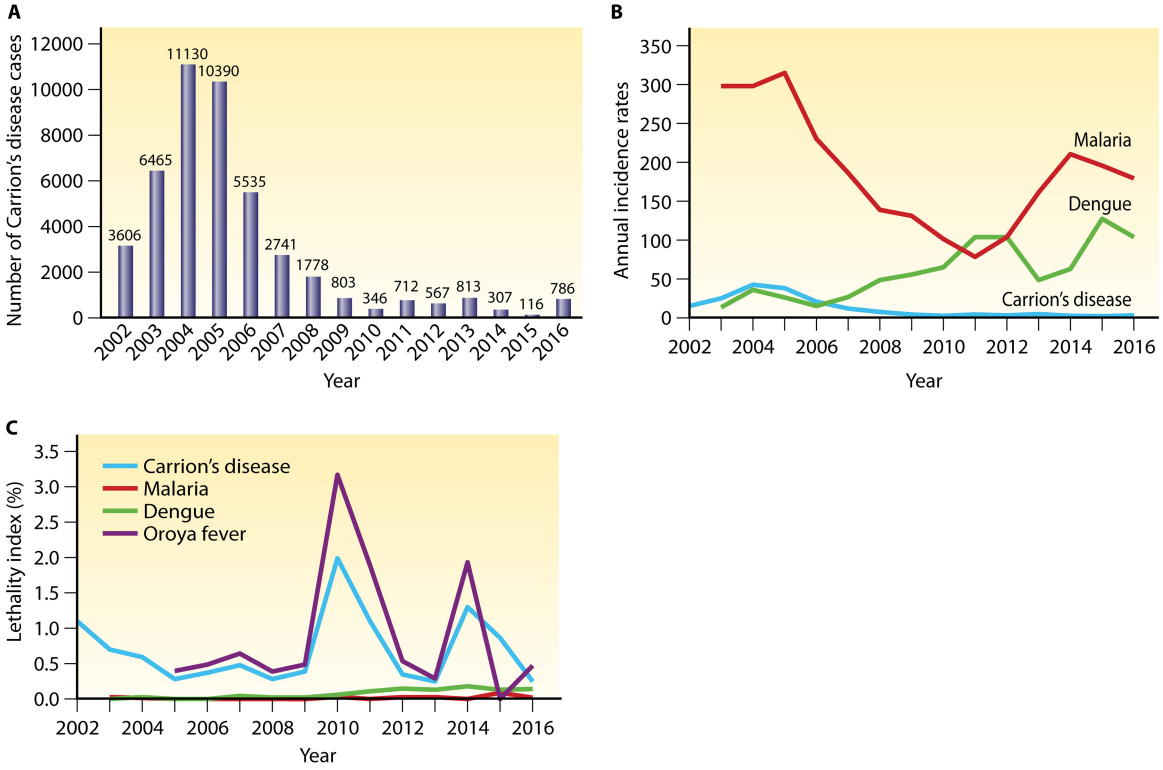

FIG 3 Essential data on Carrion's disease (2002 to 2016). (A) Number of Carrion's disease cases in Peru between 2002 and 2016. In this period, the maximum peak was of 11,130 cases reached in 2004. (B) Comparison of annual incidence rates (×100,000 inhabitants) of Carrion's disease, malaria, and dengue in Peru. (C) Comparison of Carrion's disease, malaria, and dengue lethality in Peru. The annual lethality index (LI) was established as LI = number of deaths/total cases. No data for cases of or deaths from malaria and dengue were available in 2002. From the early years of the 21st century years onwards, the number of cases and incidence of Carrion's disease showed a trend to a decrease in Peru. In 2016, the annual incidence rate of Carrion's disease cases was around 50 times lower than that of dengue and >70 times lower than that of malaria. Nonetheless, in the period from 2003 to 2016, the lethality index of Carrion's disease (LI = 0.487) was even greater than those of malaria (LI = 0.008; $P < 0.0001$) and dengue (LI = 0.095; $P < 0.0001$). During all of the period analyzed, cases of Peruvian warts represented around 30% of the overall Carrion's disease cases, while only 1 death related to this illness phase was reported. Thus, the specific lethality levels of Oroya fever are even greater, reaching 3.18% in 2010. The graphs were constructed using data from the Boletín Epidemiológico de Peru (http://www.dge.gob.pe/portal/index.php?option=com_content&view=article&id=347&Itemid=249).

Despite a trend toward a decrease in the number of cases over the last decade, there was a peak in the diagnosis of Carrion's disease in 2004, with 11,130 cases being reported and a cumulative incidence of 40.4 per 100,000 inhabitants (Fig. 3). From 2004 to 2010, about 134,000 sprayings with insecticides were done in order to control the disease (41). Nonetheless, the number of cases increased in the provinces of Cutervo (Cajamarca, 2010 and 2011), Pataz (La Libertad, 2011) and Huancabamba (Piura, 2011 and 2013) (41) after discontinuation of this activity from 2010 onwards. Overall, 247 Carrion's disease-related deaths were officially reported in Peru from 2002 to 2016, with most of these deaths belonging to 4 departments (Cajamarca, Ancash, Amazonas, and Piura) (Fig. 2 and 3) (http://www.dge.gob.pe/portal/index.php?option=com_content&view=article&id=347&Itemid=249). These data provide a general picture of the situation of Carrion's disease in Peru. Nonetheless, we believe that not all cases are reported or even diagnosed and thus that these numbers are likely underestimated; therefore, the real extent of the problem remains largely unknown.

Human bartonellosis is considered an emerging disease which is expanding to new areas (42), influenced by climate changes such as El Niño (15, 43, 44). El Niño causes a warming in sea temperature every 5 to 7 years, which favorably affects the vector ecology, increasing the number of cases in regions of endemicity as well as outbreaks in areas of nonendemicity (38, 45). Elevation of the sea surface temperature seems to happen just before the appearance of an increase in the number of cases of bartonellosis (44). Indeed, Chinga-Alayo et al. reported an almost 4-fold increase in monthly

cases during an El Niño cycle (45). Additionally, geographic expansion and the development of outbreaks should be taken into account in the epidemiology of the disease (40). In the last years, several outbreaks have been described in the literature (26, 28, 37, 46–49). Thus, Gray et al. (26) described a febrile outbreak in 1987 with a mortality rate of up to 88% in untreated cases. Maguiña reported the first outbreak in indigenous communities in the jungle in 1992, with 10 to 11% lethality (48). Kosek et al. described an outbreak in the Amazonas department in 1996 with both acute and chronic cases, with a low case-mortality rate of 0.7% (49). Ellis et al. reported a case-control study during an outbreak of acute bartonellosis in the Urubamba Valley (Cusco department) in 1998 (37), and more recently, Sánchez Clemente et al. (28) described a 2003 outbreak in Caraz (an area of endemicity in the Ancash department). In 2013 to 2014, 428 cases were reported in the Piura department, with an outbreak in the Lalaquiz district (44, 50, 51). Finally, in 2016, 33% of the total number of cases were reported in Huanuco (52).

Masuoka et al. (36), showed that Carrion's disease in the Caraz area was not more frequent near rivers but was related to agrarian activities; these agrarian areas, not necessarily next to rivers, are where sand flies encountered the most adequate living conditions, including the presence of vegetal sugars and humans.

Carrion's Disease outside Peru

Carrion's disease in other countries where it is endemic. Some neighboring countries, such as Ecuador and Colombia, are also affected by Carrion's disease. There has been a cutaneous form of the disease in Ecuador possibly since pre-Columbian times (9). Despite being reported in Zaruma in the 1880s, it has only been since the 1930s that more reports of bartonellosis started to reappear in Ecuador (9, 53, 54). The distribution of the disease is widespread throughout the country (9), and a less aggressive form seems to be present, with an unrecognized acute phase and a milder warty phase (54). Although no further data are available, in 1997 Amano and coworkers (55) showed the presence of patients with verrucous lesions as well as a high seropositivity proportion of 21% in contacts of index cases in the coastal northern Ecuadorian province of Manabi (Fig. 2); as mentioned above, in 1531 Pizarro reported an outbreak of a warty epidemic in this area (1, 6). Nonetheless, molecular studies showed that the amplified fragment of the 16S rRNA gene had only 96.5% of identity with that of *B. bacilliformis* (55). In this regard, the recent description of other *Bartonella* spp. able to produce Carrion's disease-like symptoms should be taken into account (56, 57). Nonetheless, the illness seems to be underreported. In fact, one of the most relevant Peruvian areas of endemicity is on the Ecuadorian border (54), where only sporadic cases have been reported (D. Larreategui, "Caso de bartonelosis," presented at the Primer Encuentro Andino de Infectología, Quito, Ecuador, 24 to 28 November 2010) since an outbreak in 1997 (58). In Colombia, the disease was absent until 1936, when a devastating outbreak leading to more than 6,000 deaths started (9, 59). It was proposed that the disease had been imported from Peru by soldiers after the war between Colombia and Peru in 1932 to 1934 (9). Thereafter, only sporadic cases, and none during the present century, have been reported (9).

Carrion's disease in areas where it is not endemic. In addition, sporadic cases have been reported in Chile and Bolivia (26). In distant geographical areas, such as Southeast Asia or Guatemala, compatible clinical symptoms have also been described (38). Once again, the possible role of other *Bartonella* species cannot be ruled out, and the cosmopolitan distribution of *Bartonella rochalimae* should particularly be considered (60) (see *Bartonellaceae* below).

Peru receives more than 700,000 tourists yearly (37). Although the illness seems to be outside the most relevant tourist destinations, several imported *Bartonella*-like cases have been described in travelers to and immigrants from areas of endemicity (57, 61–63). Thus, in 1992, a chronic travel-related case of Carrion's disease was diagnosed in Italy 4 months after return from a lengthy journey (7 months) to Ancash, Peru. A retrospective analysis of the clinical history showed the presence of an empirically treated febrile illness immediately after the patient's return from Peru (63). In this

regard, it should be taken into account that >35% of traveler's febrile illnesses remain without a definitive diagnostic, mostly receiving empirical treatment with antimicrobial agents (64). Other cases described involved immigrants from areas of endemicity, such as a chronic case reported in an Ecuadorian immigrant to Spain (61). Moreover, relevant periods of migration to other countries have taken place since the end of the 20th century. Thus, it was estimated that the negative net migration rate of Peruvian inhabitants to other countries in the period 1990 to 2012 was >2.5 million, with the number of Ecuadorian people living in other countries in 2008 also being around 2.5 million (http://www.migracionoea.org/index.php/es/parte-iii-informes-por-pais.html) (65). This and the high prevalence of carriers in areas of endemicity (50), together with the periodic visits of emigrants to related areas of endemicity and the possible long duration of asymptomatic infections (50, 62), underline the undoubtable presence of asymptomatic carriers in countries where the disease is not endemic.

Control and Prevention

In the last third of the 19th century and the beginning of the 20th century, the first actions to prevent this infection in foreign workers (e.g., railway or road construction workers) involved attempts to remain outside areas of endemicity during the evening (66). This was followed by the use of different insect repellents. Among these, the use of petroleum-based insecticides was tested after the observation of the apparent lack of cases among workers on night trains traveling though areas of endemicity. A reduction in the presence of *Phlebotomus* was achieved by spraying over approximately 1 week (67).

Thereafter, the main actions were aimed at vector control, considering that the vector is the primary target for the control and prevention of Carrion's disease. The use of dichlorodiphenyltrichloroethane (DDT) sprayings was efficiently used to control sand flies after World War II, and it has continued to be applied in areas of endemicity (1). This use of DDT in Peru was successful in reducing the sand fly population to negligible numbers as well as virtually eliminating the appearance of new cases of bartonellosis, and this remnant effect persisted for at least 1.5 years (68). In a study done in Peru between 1945 and 1947, DDT spraying of stone walls and houses reduced the sand fly population to an extremely low level (68, 69), as did DDT spraying of school buildings in a zone with Peruvian warts (70) and the spraying of village dwellings during an Oroya fever epidemic (26). However, it is not clear if DDT sprayings continue at present and, if so, which areas are covered.

Other preventive measures have been taken by health authorities. Although they are not always directed against Carrion's disease, these measures also play a role in the control of the sand fly population and are useful in the fight to stop this illness. These measures include regular maintenance of irrigation canals or gullies together with campaigns aimed at making the population aware of the risks of stagnant water in the transmission of other vector-borne diseases such as dengue. Moreover, albeit not frequent, the use of mosquito nets in windows or beds in areas of endemicity has also been described (71). However, a great deal of work is still necessary in order to make the population truly aware of a problem which can be prevented and treated (71).

While vaccination approaches will be the best option, only few attempts along this line have been made line (see Immunology below). Recent studies on antigenic candidates open the door to better diagnostic tools (see "Serological Techniques" below) but also to the future development of vaccines.

Indeed, the described association between climate phenomena and illness outbreaks (45) open the door to better program of preventive actions which may be developed for hot areas prior to predictable outbreaks.

BARTONELLACEAE

Although *Bartonella* spp. were first described in 1905 by Graham-Smith (thus the former name *Grahamella* for the genus) as a parasite present in mole blood (72), the genus *Bartonella* was proposed after the description of *B. bacilliformis*. Afterwards,

phylogenetic studies demonstrated a close phylogenetic and phenotypic relationship between *B. bacilliformis* and *Rochalimaea quintana*, (73, 74). Following these observations, the genera *Bartonella* and *Rochalimaea* were combined to become only *Bartonella* (75), and later on the genus *Grahamella* was also added to the genus *Bartonella* (76). Therefore, the genus *Bartonella* belongs to the *Bartonellaceae* family, a member of the alpha-2 subgroup of the alphaproteobacteria (77), along with *Rickettsia* and *Brucella* (38). *Bartonella* organisms are widely dispersed throughout nature (78). Most of these bacteria are pleomorphic Gram-negative coccobacillary or bacillary rods (0.6 μm by 1.0 μm) and are considered facultative intracellular pathogens. Among other characteristics, they are erythrocyte adherent, fastidious, and aerobic. When cultured, usually from 5 to 15 days, and up to 45 days, in primary culture is needed to form colonies on blood-containing media (77, 78).

Until 1993, the genus *Bartonella* contained only 1 species, *B. bacilliformis* (79). Currently, 35 species of *Bartonella* have been identified (http://www.bacterio.net/index.html) and have international standing in nomenclature, and other species have been proposed, several of which are classified as "*Candidatus*" species (80). New species of *Bartonella* continue to be discovered, but frequently only partial genetic data obtained by molecular analysis are available (81–83). *B. rochalimae* was reported in 2007 to have caused an Oroya fever-like disease in a traveler returning from Peru (57), and a retrospective study also identified this species in the blood of a Peruvian patient with Carrion's disease (84). *B. ancashensis* was first reported in 2013 isolated from the blood of 2 patients with Peruvian warts (56, 85). Moreover, preliminary data have shown several new *Bartonella* species to have a close phylogenetic relationship with *B. bacilliformis*, such as in the case of the recently described "*Candidatus* Bartonella rondoniensis," which shares between 84 and 91% identity with *B. bacilliformis* (82). Although there are few studies on these species, *B. rochalimae* has been described to be present in fleas (86) and to be widely disseminated (87–90). On the other hand, *B. ancashensis* has been described only in the area of Ancash, and no information about its vectors or reservoirs is currently known. "*Candidatus* Bartonella rondoniensis" has been described in Chagas illness vector kissing bugs (*Eratyrus mucronatus*) in French Guiana (82).

The members of this genus have a small genome, which is especially of note in *B. bacilliformis* (ca. 1.4 Mb). This finding is common in different microorganisms living as obligate host-associated bacteria, in which genome shrinkage has been proposed (91). The different environmental pressures with respect to free-living microorganisms might underlie this finding. With regard to *Bartonellaceae*, it has been estimated that the current species have lost around 1,500 genes compared to their original ancestor (92).

Different approaches for the study of the *Bartonella* genus phylogeny have been carried out in the last years. Thus, the use of the 16S rRNA gene sequence was first investigated, albeit with unsatisfactory results, because of the high degree of conservation of this gene. Thereafter, phylogenic approaches based first on alignments of single genes or noncoding DNA regions, such as the *gltA*, *nuoG*, or g*roEL* genes or the 16S-23S intergenic spacer (ITS) (92–96) and then on the use of concatenated gene sequences (97) have been proposed.

Different authors have proposed to separate the genus *Bartonella* into different phylogenetic lineages based on type IV secretion systems (T4SS) acquired by the different species (97–99), which favor distinct host adaptability. Thus, lineage 1 includes *B. bacilliformis*, whereas lineage 2 contains ruminant-specific species. Lineage 3 is composed of species infecting diverse mammals; lineage 4 includes, for example, *Bartonella henselae* or *Bartonella quintana*. Regarding *B. ancashensis*, while some authors classify it as the first member of a new lineage (100), others include this species within lineage 1 together with *B. bacilliformis* (101) (Table 1).

The T4SS are involved in adherence to erythrocytes, facilitating host-specific adhesion (102). The exception is lineage 1, in which the T4SS is considered to be absent, thereby implicating other virulence proteins in erythrocyte adherence and invasion (see Pathogenesis and Virulence Factors below). The presence of an independently acquired

TABLE 1 Essential information on *Bartonella* spp.[a]

Microorganism[c]	Human disease[d]	Reservoir(s)[e]	Vector(s)[f]	Yr[g]	Fla	T4SS[b]			Lin
						VirB	Vbh	Trw	
CD-related *Bartonella* spp. and closely related "*Candidatus*" species									
B. ancashensis[h]	CD-like	ND	ND	2015	+	+	−	−	5[i]
B. bacilliformis[j]	CD	Humans[k]	Sand flies	1913	+	−	−	−	1
B. rochalimae	CD-like, Bact	Canids	Fleas	2012	+	+	−	−	3
"*Candidatus* Bartonella rondoniensis"[l]		ND	Kissing bugs	—[m]	ND	ND	ND	ND	ND
Bartonella spp. causing other human illness									
B. alsatica	End	Rabbits	Rabbit fleas	1999	−	+	−	+	4
B. clarridgeiae	CSD, End	Cats	Cat fleas	1996	+	+	−	−	3
B. doshiae	Bact	Rodents	Fleas	1995	−	+	+	+	4
B. elizabethae[n]	End, Nrt	Rats	Rat fleas	1993	−	+	+	+	4
B. grahamii	Nrt	Wild mice	Rodent fleas	1995	−	+	+	+	4
B. henselae[n]	BA, Bact, CSD, End, Nrt	Cats	Cat fleas	1992	−	+	+	+	4
B. koehlerae	Bact, End	Cats	Cat fleas	2000	−	+	+	+	4
B. mayotimonensis	End	Bats	Fleas, flies	—	ND	ND	ND	ND	ND
B. melophagi	CF, Per	Sheep	Sheep ked	—	+	−	+	−	ND
B. quintana[n,o]	TF, BA, End	Humans	Body lice	1917	−	+	+	+	4
B. schoenbuchensis	Bact	Cervids	Deer flies	2001	+	−	+	−	2
B. tamiae[p]	Fever	Rodents?	Ticks, flies	—	−	−	−	−	ND
B. tribocorum	Bact	Rats	Rat fleas	1998	−	+	+	+	4
B. vinsonii arupensis	End, Bact	Mice	Rodent fleas	2000	−	+	−	+	4
B. vinsonii berkhoffi	End	Dogs	Fleas	1996	−	+	−/+[q]	+	4
B. washoensis	End, Myo, Men	Rodents	Rodent fleas	—	−	+	+	+	ND
Other *Bartonella* spp.									
B. apis		Honey bees[r]	Honey bees[r]	2016	+	−	−	−	ND
B. acomydis		Rodents	ND	2013	−	ND	ND	ND	ND
B. birtlesii		Rodents, shrews	Ticks	2000	−	+	+	+	ND
B. bovis[s]		Cattle	Ticks	2002	+	−	−/+[t]	−	2
B. callosciuri		Rodents	ND	2013	−	ND	ND	ND	ND
B. capreoli		Cervids	Deer flies	2002	+	−	+	−	2
B. chomelii		Cattle	Ticks	2004	+	−	+	−	2
B. coopersplainsensis		Rodents	Fleas	2009	−	ND	ND	ND	ND
B. florencae		Shrews	ND	2014	−	+	+	+	ND
B. fuyuanensis		Rodents	ND	2016	−	ND	ND	ND	ND
B. heixiaziensis		Rodents	ND	2016	−	ND	ND	ND	ND
B. jaculi		Rodents	ND	2013	−	ND	ND	ND	ND
B. japonica		Rodents	ND	2010	−	ND	ND	ND	ND
B. pachyuromydis		Rodents	ND	2013	−	ND	ND	ND	ND
B. peromysci[w]		Rodents	ND	1942	−	ND	ND	ND	ND
B. queenslandensis		Rats	ND	2009	−	+	+	+	ND
B. rattaustraliani		Rodents	Ticks	2009	−	+	+	+	ND
B. senegalensis		Rodents?	Rodent ticks	2014	−	+	−	+	ND
B. silvatica		Rodents	ND	2010	−	ND	ND	ND	ND
B. talpae[w,x]		Rodents	ND	1911	−	ND	ND	ND	ND
B. taylorii		Rodents	ND	1995	−	+	−/+[y]	+	4
B. vinsonii vinsonii[n]		Mice	ND	1982	−	+	−	+	4

[a]Abbreviations: T4SS, type IV secretion system-coupling protein (in some cases data have not been reported but were determined from analysis of GenBank sequences); Fla, flagellum; VirB, VirB/VirD4 system; Vbh, VirB homolog (sometimes referred to in the literature as Vbh/TraD); Lin, Lineage or clade (only those microorganisms previously classified in the literature as belonging to any of the described clades [92, 97–101] are indicated); BA, bacillary angiomatosis; Bact, bacteremia; CD, Carrion's disease; CF, chronic fatigue; CSD, cat scratch disease; End, endocarditis; Men, meningitis; Myo, myocarditis; Nrt, neuroretinitis; Per, pericarditis; TF, trench fever; ND, no data.

[b]The presence of T4SS is presented according to different reports (92, 97–101). Note that in some cases the presence (+) or absence (−) of T4SS has been reported in species and strains which are not present in GenBank. In addition, these systems have also been reported as being encoded on plasmids and chromosomes (373), which may lead to differences in their presence/absence within members of the same species. This should be taken into account, especially in light of the low number of available *Bartonella* genomes present in GenBank (see footnote *c*) or *Bartonella* strains in which the presence of T4SS has been explored. In addition to examining previously published data, a search for the presence of these systems in GenBank was made using the *B. tribocorum* (GenBank accession number AM260525.1) VirB/VirD4, Vbh, and Trw operon sequences was made. Some "*Candidatus*" species not included in this table (see criteria in footnote *c*), such as "*Candidatus* Bartonella australis" or "*Candidatus* Bartonella rattamassiliensis," possess one or more of these T4SS.

(Continued on next page)

VirB4/D system in *B. ancashensis* (101) may lead to a modification of this concept even if its classification in lineage 1 becomes definitive.

All these proposals usually classify *B. bacilliformis* as an outlying species, but a study analyzing 428 concatenated gene sequences described this species as being closely related to *Bartonella bovis* and *Bartonella schoenbuchensis*, both of which are found in ruminants, with *Bartonella tamiae* being an outlier species (92). A similar outlying classification is also given to *Bartonella apis* when included in the classifications (101). It is of note that an analysis of the *B. tamiae* and *B. apis* genomes showed the lack of T4SS (identical to the case for lineage 1) as well as the absence of flagella in *B. tamiae* (Table 1).

Several arthropod vectors, such as fleas, ticks, flies, and lice, have been described for *Bartonella* spp. (86–90, 103–105). The recent identification of *B. quintana* in bedbugs such as *Cimex lectularius* and *Cimex hemypterus* also makes these insects potential new vectors of *Bartonella* (106, 107). *Bartonella* coinfections are frequent, with a rate of coinfection in fleas from rats of 90% (104).

In animals and humans, the diversity of clinical and pathological manifestations associated with *Bartonella* infections may be related to the wide variety of cells which may be infected, including erythrocytes, pericytes, endothelial cells, dendritic cells, $CD34^+$ progenitor cells, and various macrophage-type cells (108). This finding might have facilitated the presence of coevolution phenomena. In fact, almost all *Bartonella* spp. have a mammalian reservoir host (109). Thus, *Bartonella vinsonii* subsp. *berkhoffii*, "*Candidatus* Bartonella melophagi," and "*Candidatus* Bartonella australis" have co-evolved with canids, sheep, and kangaroos, respectively (108). *B. rochalimae* has been described in raccoons, coyotes, and red foxes (60), and in addition, different reports have described the presence of closely related *B. rochalimae* microorganisms in rats and rat fleas (110, 111). Nonetheless, the newly described *B. apis* is considered a honey bee symbiont (112). In fact, the evolution from insect-associated gut symbiont to a mammalian pathogen through the loss of genetic material and the acquisition of horizontal

TABLE 1 (Continued)

[c]Species listed in alphabetical order within groups. Only those names with standing in nomenclature (80), as well as those causing human infection or closely related to *B. bacilliformis*, are presented (see more in-depth information at http://www.bacterio.net/-foreword.html). Species with at least one genome sequence present in GenBank (https://www.ncbi.nlm.nih.gov/genome/?term=Bartonella) are highlighted in bold. Note that at the time of writing this review, only 27 *Bartonella* species (either with or without standing nomenclature) and an additional 12 unnamed *Bartonella*-related microorganisms fulfilled this criterion. Of these, *B. henselae* has 23 genomes, *B. bacilliformis* has 15 genomes, *B. quintana* has 14 genomes, *B. apis* and *B. birtlesii* have 6 genomes each, *B. vinsonii* has 5 genomes (2 belonging to *B. vinsonii* subsp. *arupensis* and 3 belonging to *B. vinsonii* subsp. *berkhoffii*), and *B. elizabethae* has 4 genomes; no more than 3 genomes are present for the remaining *Bartonella* spp. sequenced.

[d]Some of these species have been only sporadically reported as a cause of human illness.

[e]In most cases, the *Bartonella* spp. have also been reported in other reservoirs; e.g., macaques act as a *B. quintana* reservoir (374).

[f]In most cases, other vectors have been described.

[g]Year of official recognition as a new species (previously published reports may be found in the literature) (http://www.bacterio.net/-foreword.html). Note that despite having been reported in the literature some time ago, some species described earlier, such as *B. talpae*, have only recently been correctly defined from bacterial cultures.

[h]In some articles reported as *Bartonella ancashi*.

[i]New lineage proposed by Mullins et al. (100). Other authors considered *B. ancashensis* to belong to lineage 1 (101).

[j]Also present in early literature as "*Bartonia bacilliformis*."

[k]Humans are the only known reservoir of *B. bacilliformis*.

[l]To date, only a single description of "*Candidatus* Bartonella rondoniensis" in kissing bugs has been reported, with the use of molecular tools. No isolate is available to perform further studies. This *Bartonella* species is closely related to *B. bacilliformis* and *B. ancashensis* (82).

[m]—, not yet officially recognized as a new species (80).

[n]Prior to 1993 classified within the *Rochalimaea* genus.

[o]This species may also be found as *Rickettsia quintana*, *Rickettsia pediculi*, *Rickettsia wolhynica*, *Rickettsia weigli*, *Burnetia* (*Rocha-limae*) *wolhynica*, or *Wolhynia qintanae*.

[p]The *in silico* analysis does not show the presence of any characterized T4SS or a flagellin-encoding gene.

[q]Although some *B. vinsonii* subsp. *berkhoffi* strains have been reported to possess only the VirB4/VirD system (97), the Winnie strain (GenBank accession number CP003124.1) has both the Virb4/VirD and Vbh systems (92).

[r]Considered a honey bee symbiont.

[s]Should not be confused with the microorganism "*Bartonella bovis*" described in 1934 by Donatien and Lestoquard (375). In addition, it may also be found in previous reports as "*Bartonella weissi*," "*Bartonella weissii*," or "*Bartonella* sp. FC7049UT" (77, 96).

[t]Although reported to be Vbh operon positive (97), *B. bovis* shows no identity when genomes present in GenBank (https://www.ncbi.nlm.nih.gov/GenBank/) are analyzed using the Blast tool. Note that the positive *B. bovis* strain reported (strain A96) is neither totally nor partially present in GenBank.

[w]Prior to 1995 was classified within the genus *Grahamella*.

[x]This species may also be found in previous reports as *Grahamia talpae*.

[y]*B. taylorii* strain 8TBB (GenBank accession number AIMD01000055.1) possesses 2 T4SS.

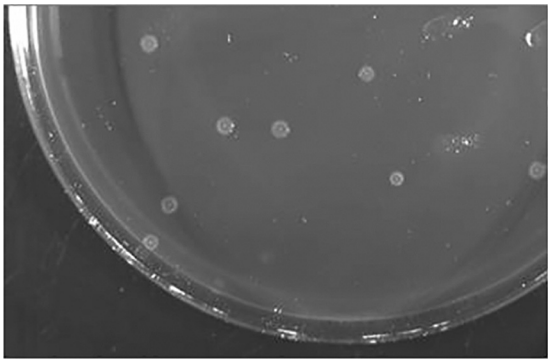

FIG 4 *Bartonella bacilliformis* culture presenting the T1 morphology. (Reprinted from reference 123 [published under a Creative Commons license].)

virulence factors has been proposed, with *B. tamiae* being representative of a transition stage (113, 114). *Bartonella* spp. have a close phylogenetic relationship with nitrogen-fixing soil microorganisms such as *Rhizobium* spp. This finding, together the symbiont role of *B. apis*, which seems to maintain the nitrogen fixation capacity, as well as the recent detection of *Mesorhizobium* spp. and *Bradyrhizobium* spp. in sand flies and other insects (suggested to be beneficial in female insects in the absence of blood) (115), suggests the possible evolution of these soil microorganisms to different *Bartonella* spp. in insects.

Several species have been associated with different diseases and syndromes in humans, with *B. bacilliformis*, *B. henselae*, *B. quintana*, *Bartonella elizabethae*, and *Bartonella clarridgeiae* being the most relevant. In addition *B. rochalimae* and *B. ancashensis* have recently been related to Carrion's disease-like syndromes (56, 57, 78, 85, 116). Moreover, the role of several *Bartonella* spp. in the development of endocarditis in immunocompetent persons has been shown (108). Furthermore, other *Bartonella* spp. have sporadically been described as a cause of different diseases (117–121) (Table 1). The term "bartonellosis," historically attributed to infections with *B. bacilliformis*, at present has a wide definition and includes infections caused by any *Bartonella* spp. However, if not expressly indicated, in the present article "bartonellosis" always means infection caused by *B. bacilliformis*.

Bartonella bacilliformis

Classically *B. bacilliformis* has been considered a common "ancestor" of the *Bartonella* genus, but this evolutionary scenario has recently been questioned, and *B. bacilliformis* has been related to *B. schoenbuchensis* and *B. bovis*, as mentioned above. Indeed, it has been proposed that *B. bacilliformis* might have originated from a small bacterial population after successful accidental transfer from local camelids (*Lama* spp. and *Vicugna* spp.) to humans (92). Nonetheless, despite the presence of *Bartonellaceae* in Old World camelids (122), at present no *Bartonella* sp. has been recovered from any of the current 4 local camelid species.

B. bacilliformis is a Gram-negative, nonfermentative, aerobic, pleomorphic coccobacillus, with dimensions of 0.2 to 0.5 μm by 1 to 2 μm and 2 to 16 flagella that confer high mobility. Biochemical tests are not useful for presumptive identification of *Bartonella* species, but peptidase activity in L-proline and L-lysine is useful for that of *B. bacilliformis* (39). The growth of this microorganism is slow in blood-containing media such as *Leptospira* medium with rabbit, sheep, horse, or human blood (16) or Columbia blood agar with 5% sheep blood. The optimum temperature is 28 to 30°C, and although the organism is aerobic, microaerophilic conditions provide better results (15, 16). The colonies are typically small, round, and lenticular, ranging from translucent to opaque (39). Occasionally, the colonies may adopt other forms, such as the so-called T1 morphology (colonies having regular edge, a small halo, and a "bubble" centered on the colony) (123, 124) (Fig. 4). *B. bacilliformis* can remain viable at 4°C for long periods

of time (125, 126), similar to what has been described for other *Bartonella* spp. (127). As mentioned above, the *B. bacilliformis* genome consists of a single circular DNA molecule ranging from 1.39 Mbp (strain Peru-18; GenBank accession no. KK097689 to KK097708) to 1.44 Mbp (strain ATCC 35685D-5; GenBank accession no. NZ_CP014012.1), which has a GC content of 38.2 to 38.3% (https://www.ncbi.nlm.nih.gov/genome/genomes/524). The high percentage of AT content is also observed in other *Bartonellaceae*, being a distinctive trait of host-dependent microorganisms (39).

Genetic Diversity

The phylogenetic relationships and genetic diversity among *B. bacilliformis* isolates have not been extensively studied, although several studies have addressed this aspect. Interestingly, in a study assessing the differentiation of *B. bacilliformis* isolates by the evaluation of *gltA* and 16S-23S ribosomal DNA intergenic spacer regions as well as an amplified fragment length polymorphism (AFLP), *B. bacilliformis* strains causing acute disease were also found to cause asymptomatic infection (40). This finding may be related to different compatible scenarios, including alterations in the expression of different genes related to host-pathogen interactions but also possible horizontal acquisition/loss of genetic material or previous exposure to the pathogen leading to acquisition of partial immunity. Moreover, the same study revealed that 3 different outbreaks in new illness expansion areas were caused by 3 different minority genotypes of *B. bacilliformis* and not by those genotypes most often encountered in regions of endemicity (40). The use of infrequent restriction site PCR has shown a relative high degree of genotypic variation within populations of *B. bacilliformis* (128).

More recently, a multilocus sequence typing (MLST) approach consisting of the amplification of 7 different genetic loci (*ftsZ*, *flaA*, *ribC*, *rnpB*, *rpoB*, *bvrR*, and *groEL*) was developed (129), and in 2017 a webpage devoted to bringing order to and information about *B. bacilliformis* MLST was created (https://pubmlst.org/bbacilliformis/). To date, phylogenetic analyses have identified up to 14 distinct sequence types among *B. bacilliformis* strains (129–131). A comparative genomic approach revealed that the evolution of *B. bacilliformis* is shaped predominantly by mutations. Mutational divergence leads to subspeciation, but mutational convergence between clones of a subspecies shows evidence of common adaptive evolution (131).

Currently up to 15 *B. bacilliformis* genomes are present in GenBank. Of these, although present in GenBank as a "representative genome" together with strain KC583 (NC_008783), the genome of strain Ver097 (NZ_KL503802 to NZ_KL503807) differs greatly from the remaining *B. bacilliformis* genomes, suggesting that it may, in fact, be a nonrecognized new *Bartonella* species or a *B. bacilliformis* subspecies, similar to what has been also proposed for other strains (Ramirez, Vega, and Peru53) which are currently not included in GenBank (131, 132). Confirmation of the Ver097 strain as a new species will make it a new *Bartonella* species recovered from Carrion's disease-like syndromes.

CLINICAL PRESENTATION OF CARRION'S DISEASE

The clinical presentation of Carrion's disease involves 2 syndromes that occur independently or sequentially (39): an acute phase called Oroya fever and a chronic phase named Peruvian warts. However, the presence of Peruvian warts has also been described in the clinical care of acute or recovery phases of Oroya fever (133, 134).

Once a person is bitten by an infected sand fly, an asymptomatic infection or a mild to severe disease is presented. While the average incubation time of Carrion's disease is 61 days (between 10 and 210 days) (78), the severity of the disease is probably determined by individual predisposition and the specific virulence of the strain causing infection. According to Noguchi, strains isolated from people with very mild anemia or Peruvian warts are presumably less virulent (135). Similarly, Kosek et al. proposed that a strain of *B. bacilliformis* with diminished virulence had caused the infection when disease manifestations were milder than the typical manifestations of Oroya fever, (49). In light of the description of *B. rochalimae* and, especially, *B. ancashensis* and the

TABLE 2 Different names for Carrion's disease

Names for Carrion's disease[a] in:	
Acute phase	**Chronic phase[b]**
Oroya fever (fiebre de Oroya)	Peruvian wart (verruga Peruana)
Anemia de Carrión	Cutaneous bartonellosis[c]
Anemia perniciosa de las quebradas	Mucocutaneous bartonellosis[c]
Bartonellosis[d]	Botón de los Andes
Fiebre aguda verrucosa	Verruga Andícola
Fiebre de Castilla	Verruga blanda
Fiebre del Guáitara[e]	Verruga de Ancash
Fiebre grave de Carrión	Verruga de Castilla
Fiebre maligna de las quebradas	Verruga de los conquistadores
Fiebre maligna verrucosa	Verruga de crapaud[f]
Fiebre verrucosa de Guáitara[e]	Verruga de los libertadores
Tifus palúdico de la Oroya	Verruga de quinua
	Verruga de sangre
	Verruga de zapo/sapo[f]
	Verruga Ecuatoriana[g]
	Verruga hemorrágica Peruana
	Verruga mular
	Verruga nodular
	Verruca Peruviana

[a]Most of these names are present in Spanish scientific literature.
[b]It should be highlighted that in several articles, especially early articles, the use of the term Peruvian wart and other names including the word "verruga" (wart) as synonyms for Carrion's disease is usual, irrespective of the exact illness phase.
[c]From 1993 onwards, this name was extended to infections by other *Bartonella* spp.
[d]Until 1993 this name was exclusively used to designate infections by *B. bacilliformis*. Thereafter, it may refer to human infections related to any *Bartonella* sp. Additionally, note that bartonellosis may also not be used exclusively to designate systemic infections.
[e]Term used mainly in Colombia.
[f]Note that "crapaud" (used in some early French literature) and "sapo" mean "toad" in English. Similarly, "zapo" is phonetically closely similar to "sapo."
[g]Proposed in Ecuador to explain the apparent lesser severity of local clinical cases.

possible misidentification of presumptive new Carrion's disease-causing *Bartonella* species as *B. bacilliformis*, studies are needed to evaluate whether these species have different virulence potentials to cause severe Oroya fever, mild infections, or Peruvian warts.

Nomenclature and International Classifications

Carrion's disease is referred to by a great variety of names (Table 2). This is related to the history of the disease, including the lack of consensus as to a single or dual nature of differently related syndromes (present until the first third of the 20th century) (see History above), as well as the particularities of the areas and inhabitants mainly affected (see Epidemiology above).

Carrion's disease is a notifiable disease in Peru (http://www.minsa.gob.pe/renhice/documentos/normativa/RM506-2012-MINSA%20-%20DS%20046%20DGE%20Notificacion%20Enfermedades%20Eventos%20Vigilancia%20Epidemiologica%20Salud%20Publica.pdf), and according to the guidelines of the World Health Organization, it is internationally reported under the codes A44.0 (Systemic bartonellosis—Oroya Fever) and A44.1 (Cutaneous and mucocutaneous bartonellosis—Verruga Peruana) or the code A44.9 (Bartonellosis, unspecified) (http://apps.who.int/classifications/icd10/browse/2016/en).

In the Medical Subject Headings (MeSH) of the National Center for Biotechnology Information, *B. bacilliformis* infections are classified together with those caused by other *Bartonellaceae* under the code D001474, while in the Universal Medical Language System (UMLS), Carrion's disease is codified under the codes C0029307 (including other *Bartonella* infections) and C0348974 (systemic bartonellosis). The illness is also codified in other disease-normalizing international coding systems, such as Disease Ontology developed by Northwestern University, Institute for Genome Sciences and Center for

Genetic Medicine, and the University of Maryland School of Medicine under the code DOID:0050398. In addition, it is of interest that this illness is considered to be an orphan disease and is included in the ORPHANET list under the code ORPHA64692.

Oroya Fever

The major clinical consequences of infection with *B. bacilliformis* result from invasion of the erythrocytes by the bacteria in the acute phase of Carrion's disease. *B. bacilliformis* infects the erythrocytes, which are subsequently destroyed in the spleen and liver, leading to severe hemolytic anemia and transient immunosuppression. Although not all the infected erythrocytes are removed from the circulation, hemolytic anemia involves the destruction of the infected erythrocytes by the reticuloendothelial system, with the erythrocytes remaining within the circulation for a shorter time than normal, thereby leading to the development of anemia (136). Although increased production of erythrocytes in response to the great destruction of these cells may be up to five times greater than normal, in Oroya fever there is a substantial reduction in the number of erythrocytes with a compensatory increase in plasma, so that the total blood volume is not severely affected (136). On the other hand, a deregulation of the immune system causes a deterioration of cellular immunity leading to immunosuppression, predisposing the patients to opportunistic infections (137, 138). The amount of microorganisms in the blood is higher in patients with severe anemia than in patients with warts (135). In fact, in a study by Maguiña et al., the mean percentage of infected erythrocytes in this phase of the disease was 61% (range, 2 to 100%), and in 25% of patients, >90% of the erythrocytes were infected at the time of admission (139).

The symptoms of Oroya fever are indistinguishable from the initial symptoms of other infectious diseases such as malaria, typhoid fever, dengue, tuberculosis, or even viral hepatitis. The onset is usually gradual, with malaise, fever, headache, and mild chills, and can include pallor, hepatomegaly, abdominal pain, and other unspecific symptoms (29, 33, 78, 140). The acute phase seems to have a greater effect on children and teenagers up to the age of 15 years (6, 29, 35, 43, 49). Overall, patients in the acute phase are younger than those in the eruptive phase; thus, in a study by Maguiña et al. (139), it was observed that patients in the acute phase have a mean age of 14.6 years while those in the eruptive phase has a mean age of 18.4 years, suggesting the development of acquired immunity following exposure (35). The mortality rate in the acute phase is 40 to 85% in untreated patients; however, in reference centers (where severe cases are treated) with appropriate and timely treatment, this rate can be reduced to values of around 10% (6, 15, 78, 139, 141, 142). Complications or secondary opportunistic infections can dramatically worsen the clinical outcome, and unfortunately, they are quite common (29, 33, 78, 142–144). There are several reports on noninfectious hematological, cardiovascular, and neurological complications (29, 33, 140). In regard to opportunistic infections, the most common pathogens involved are *Salmonella* spp., which are responsible for 90% of the deaths in Oroya fever (1). Moreover, mortality is increased during outbreaks, mainly in new transmission areas because health personnel are unaware of the disease and laboratory staff members are not trained to diagnose the disease (6). For example, in the late 1990s in La Convencion, a district of the Cusco department, acute cases of Carrion's disease were systematically reported as viral hepatitis, with mortality rates of 39% in some hospitals (27). As indicated above, the risk of mortality is high in pregnant women during acute bartonellosis, with the possible presentation of severe complications, including fetal death (32, 78, 141).

Peruvian Warts

The chronic phase of Carrion's disease, called Peruvian warts, is characterized by the development of dermal eruptions, occurring weeks or months after Oroya fever, and persists from 1 month to 1 year (1, 78). Nonetheless, the chronic phase may occur in the absence of previous reported acute illness (145). Lesions vary in size and number, mainly affecting the arms and legs, although other body areas can also be affected

(145). The microorganisms are observed within the warts, both intracellularly and within the extracellular matrix (145). The lesions are classified as follows (141, 145): (i) miliary, i.e., small (diameter, $<$3 mm), often numerous, reddish papules situated at the papillary and medial dermis, may be pruriginous; (ii) mular, i.e., erythematous nodules (diameter, $>$5 mm) which tend to extend deeper into the hypodermis with the possible involvement of subcutaneous tissue and muscle; and (iii) subdermic, i.e., diffuse subdermic nodules showing no changes in the overlying skin, larger and more prominent than the miliary and mular lesions.

The eruptions are most often miliary (145), although different types of warts may commonly be found in the same patient (6). Miliary lesions are normally painless, while the mular lesions may be painful, and two-thirds of patients complain of bleeding warts.

The eruptive phase is a more common manifestation in inhabitants of regions of endemicity, and the risk of developing Peruvian warts increases with age (49). This phase tends to heal spontaneously, and the mortality rate is insignificant. These eruptions are frequently accompanied by mild systemic manifestations, including fever, malaise, osteoarticular pain, lymphadenopathy, and headache (78, 145). Notwithstanding, in more severe cases, complications, including bleeding, may be observed in 66% and secondary infection in 12% of the patients, occasionally leading to fatal outcomes in the absence of timely blood transfusions (6, 139). In fact, in the period from 2005 to 2016, only 1 death related to the chronic phase of Carrion's disease was reported (146).

Around 50% of patients with Peruvian warts are bacteremic (35, 147), with bacteremia being significantly correlated with the onset of the lesions as well as low hemoglobin levels. On the other hand, the number and distribution of the lesions and the clinical symptoms are not associated with bacteremia (147).

Although scarcely described, some reports have also indicated the presence of internal granulomas, which may be present in different organs, including the lungs, brain, or spleen (62, 133). By far the most ancient description of the presence of these granulomas was in a Tiahuanaco mummy, being suggestive of Carrion's disease (2). Although the relationship of these lesions with *B. bacilliformis* has not been definitively established, confirmation of the extension and the clinical parameters of the disease should be described and their true clinical relevance evaluated.

In a recent study on a patient with Peruvian warts, the presence of a concomitant infection by *B. ancashensis* (detected by molecular tools) and *B. bacilliformis* (detected by molecular and culture methods) was observed. After treatment with rifampin, a blood culture of *B. ancashensis* was obtained in one patient (100). This report highlighted the possible coinfection by different strains or closely related microorganisms, as well as the need for in-depth analysis of the real role of *B. ancashensis* in the development of chronic Carrion's disease. Moreover, this study lead to the question as to whether the two microorganisms were transferred together from the same vector or whether the patient had two different infectious processes.

Asymptomatic Carriers

It has long been known that these microorganisms can persist in blood for many years after the infection (70). Indeed, one study described the isolation of *B. bacilliformis* from an individual who had visited Ecuador 3 years previously (62). Moreover, asymptomatic carriers have also been described in areas of endemicity (35, 50, 148, 149). It is believed that these carriers perpetuate the disease and can introduce it into new areas, either within a stable setting with a vector in the area (as described in Colombia in the mid-1930s) or within an unstable setting in which no vector is present. Indeed, Chamberlin and coworkers analyzed the prevalence of asymptomatic *B. bacilliformis* carriers in an area of endemicity by PCR and found that 0.5% were positive (35). More recently, a study including an area of endemicity (Huancabamba) and an postoutbreak area (Lalaquiz), both in the northern Peruvian department of Piura, showed that approximately half of the volunteers in the area of endemicity and about 40% of the individuals in the postoutbreak area had a positive result by quantitative real time-PCR

(qPCR), demonstrating the high number of asymptomatic carriers in these zones (50). It is of concern that the positivity observed was near the qPCR detection limit, showing that the presence of bacteria in the blood may have been very low. This suggests that the real number of *B. bacilliformis* carriers may be underestimated when less-sensitive techniques are used. Although reinfections cannot be ruled out, the presence of microbiological failures seems evident, as all volunteers from the postoutbreak area were treated with ciprofloxacin during the outbreak (50).

PATHOGENESIS AND VIRULENCE FACTORS

Successful infection of a mammalian host by a bacterial pathogen typically involves a series of intimate host-pathogen interactions. *B. bacilliformis* can invade a variety of different human cell types *in vitro* (150), but it is known that *B. bacilliformis* infects the erythrocytes and endothelial cells in Oroya fever and Peruvian warts, respectively. Erythrocyte infection has several advantages, such as being hidden from the humoral immune system, the absence of lysosomes, and a reasonably long life (141). Mature erythrocytes are nonendocytic, and thus, erythrocyte involvement in the invasion process is necessarily passive (150), with several determinants and virulence factors being involved in the pathogenesis of *B. bacilliformis*, including motility, erythrocyte deformation, and invasive factors (151–155).

In contrast, the endothelial cells actively participate in the uptake of *B. bacilliformis* by a process similar to phagocytosis (150). The objectives of this complex adaptation to the human host are perhaps to achieve the persistence and maintenance of a reservoir state for vector-based transmission and immune evasion.

Erythrocytes and Oroya Fever

The first study reporting the ability of *B. bacilliformis* to penetrate erythrocytes was performed in 1969 (156). Erythrocyte invasion is probably the most important step in the pathogenesis of Carrion's disease, since it is related to the worst outcome of the illness. It is possible that competence for erythrocyte invasion must be acquired by an adaptation process in the primary niche (157). Although it is controversial, some authors have described colonization of a primary niche after intravenous inoculation and before erythrocyte infection (38, 151, 157, 158). Moreover, the incubation period (60 days on average) of Oroya fever after infection is long (158). Erythrocytes lack the normal actin-based structures employed by most cells during endocytosis, and therefore, as mentioned above, *B. bacilliformis* plays an active role during erythrocyte invasion (159, 160). Infection of erythrocytes by *B. bacilliformis* involves at least 2 important steps: (i) the binding of the bacteria to the surface of the erythrocytes and (ii) deformation of the erythrocyte membrane, including deep invaginations and membrane fusion leading to the formation of intracellular vacuoles containing bacteria (161).

Polar flagella, composed of multiple 42-kDa flagellin subunits, provide the bacterium with a high degree of mobility during its search for erythrocytes (160, 162), which is essential for erythrocyte binding and deformation by potentially providing mechanical force (158, 161). A mutant lacking flagella has a nonmotile phenotype (163), and a significant decrease in the efficiency of invasion has been described after preincubating *B. bacilliformis* with antiflagellum antibodies (162). Nonetheless, spontaneous mutants (e.g., with T1 morphology) which despite being flagellated have reduced motility or nonmotility it has been described (124). These flagellated nonmotile variants can be bound to erythrocytes, promoting surface contact interaction. The optimal binding of bacteria to the surface of erythrocytes requires *B. bacilliformis* energy, most likely proton motive force-dependent motility (123, 140), with the whole *in vitro* process taking approximately 6 h (161).

Beyond flagella, the extracellular protein deformin plays a fundamental role in erythrocyte invasion. This protein is able to deform human erythrocytes by producing deep invaginations in their membranes (155, 164). Studies with purified deformin have shown the capacity of this protein to achieve these invaginations even in the absence

FIG 5 Deformation of erythrocytes as seen by scanning electron microscopy. (Adapted from reference 161.)

of bacteria (155). It is likely that erythrocyte invasion is related to the concomitant action of flagellum-based motility and deformin (Fig. 5).

In addition, the *ialA* and *ialB* genes seem to facilitate erythrocyte invasion. The proteins encoded by these genes have shown the ability to invade human erythrocytes in the absence of the other *Bartonella* virulence factors (165).

Studies cloning the *ialAB* locus in *Escherichia coli* have shown an invasion increase of 6- to 39-fold when both genes were present, highlighting that both genes are needed during the invasion processes. The *ialA* gene reduces stress-induced dinucleotide levels during invasion, thereby enhancing pathogen survival, and *ialB* has been shown to probably encode a secreted protein with a direct role in human erythrocyte parasitism (153, 155, 165, 166). It has also been shown that anti-IalB antibodies can block the interaction between IalB and erythrocytes (166). A study by Coleman and Minnick (167) showed that higher *ialB* mRNA levels occur under acidic conditions (pH 5.0) or in bacteria grown at low temperatures (20°C). Therefore, the erythrocyte invasion would be diminished at 37°C and near neutral pH by downregulation of *ialB* expression, emphasizing the chronic nature of Carrion's disease. Since the insect midgut becomes acidified at around 3 days postingestion and that female sand flies need to ingest a blood meal every 4 to 5 days, these data suggest that *ialB* expression is upregulated, leading to maximization of the *B. bacilliformis* erythrocytic invasion capacity when the sand fly feeds again. Those authors also suggested that if sand fly feeding is interrupted, the *ialB* expression of ingested *B. bacilliformis* would be upregulated, then being under optimal conditions to adhere to and invade erythrocytes when transferred to another person during the insect's next feed (167).

Erythrocyte adherence and invasion undoubtedly involve other proteins and factors, some of which have already been identified. For example, actin, α- and β-spectrin, band 3 protein, glycophorin A, and glycophorin B are erythrocyte proteins implicated in the specific binding of *B. bacilliformis* to the human erythrocyte membrane (152, 164). Moreover, the *B. bacilliformis* interaction with actin and spectrin suggests that erythrocyte internalization may be related to alterations in cytoskeletal structure (152, 164). *B. bacilliformis* repeat proteins also seem to play a role in the adherence of the bacteria to host cells by sharing common domains and structural characteristics with the trimeric autotransporter adhesion proteins of *B. henselae* and *B. quintana*; these proteins are involved in adhesion to host cells and extracellular matrix proteins (168).

The severity of the hemolytic anemia characteristic of Oroya fever seems to be unique among *Bartonella* spp. and could be explained by massive infection of the erythrocytes (79). Hemolytic activity is contact dependent and is due to a *Bartonella* protein, not requiring direct involvement of the erythrocyte proteins.

B. bacilliformis has 3 hemin-binding proteins (Hbps) (168). These proteins are located on the bacterial surfaces and act as hemin receptors. Moreover, data obtained from the analysis of other *Bartonella* spp. also suggest their possible role as adhesins (169). Other studies have suggested that these proteins may favor the development of the most adequate microaerophilic conditions (170). Two interesting aspects have also been observed in *B. quintana* and may probably be extrapolated to *B. bacilliformis*. HbpA (also known as Pap31) is the most frequently expressed of the 5 *B. quintana* Hbps irrespective of the culture or hemin temperature used. This is of special concern since the equivalent HbpA is an immunologically dominant protein of *B. bacilliformis* and is

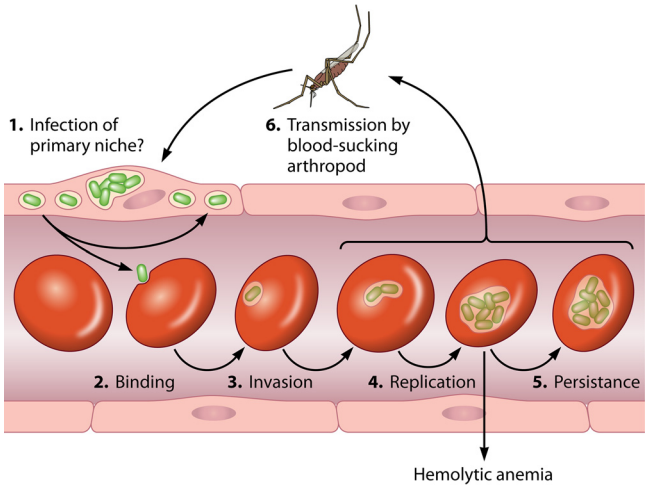

FIG 6 Model of the course of acute *Bartonella* infection. (1) In infections by *B. bacilliformis* primary niche remains controversial; in other *Bartonella* species, it is considered to include the vascular endothelium as a major constituent. From the hypothetical primary niche, *Bartonella* is released into the bloodstream, in which it may reinfect the primary niche (if it exists) again. (2) Binding to erythrocytes. (3) Invasion of erythrocytes. (4) Replication. (5) Finally, the infection leads to hemolytic anemia or the organisms persist in a nonreplicative intraerythrocytic state. (Adapted from reference 372 with permission of Macmillan Publishers Ltd.)

considered a good candidate for use in enzyme-linked immunosorbent assay (ELISA) (171) (see "Serological Techniques" within Diagnosis of Carrion's Disease below). Moreover, it has been observed that while the levels of HbpA, HbpD, and HbpE expression increased under simulated human conditions, those of HbpB and HpbC rose when insect conditions were simulated.

In addition, *Bartonella* spp. also possess another heme acquisition system, the so-called hemin utilization (Hut) system (172); however, to our knowledge, no study has been done for *B. bacilliformis*.

Lysis of erythrocytes results in intact ghost cells that may contain numerous highly motile *B. bacilliformis* organisms. *B. bacilliformis* might use these ghosts to allow host immune system evasion, facilitating the invasion of the microvasculature endothelial cells (173).

In vivo experiments in rats have shown a prolonged period of intraerythrocytic colonization, demonstrating for the first time the persistence of *B. tribocorum* in erythrocytes (157). The persistence of intracellular erythrocyte parasitism is central in the pathogenesis of all *Bartonella* spp., allowing them to persist within an immunological environment and increasing the possibility of transmission by blood-sucking arthropods (38, 157, 158) (Fig. 6).

The role of other proteins, such as the 3 Brp proteins belonging to the trimeric autotransporter adhesion (TAA) family, remains to be elucidated, but TAA-related proteins in other *Bartonella* spp. have been involved in adhesion processes in addition to autoagglutination, inhibition of phagocytosis, and induction of a proangiogenic response (168).

Endothelial Cells and Peruvian Warts

The Peruvian wart appears in the chronic phase of Carrion's disease, with infection of the endothelial cells and their pronounced proliferation resulting in the characteristic skin eruptions. Epithelial or endothelial cells participate more actively in bacterial uptake than erythrocytes, which is facilitated, in part, by the microfilament-dependent activity of these host cells (140, 150). Definite morphological evidence of the presence of *B. bacilliformis* in Peruvian wart lesions is achieved by the finding of Rocha-Lima's inclusions by light microscopy. Moreover, the bacteria are also found in abundance in the extracellular spaces when there are florid lesions, while they are absent in the resolving nodules (174).

The existence of numerous capillaries within Peruvian warts suggests that infection of endothelial cells induces a local angiogenic response, that is, the formation of new blood vessels. This fact was experimentally demonstrated with a report that *B. bacilliformis* stimulates endothelial cell proliferation by up to 3 times, as well as the production of the tissue type plasminogen activator (t-PA), in human vascular endothelial cells (HUVECs). *B. bacilliformis* extracts also stimulated the formation of new blood vessels in an *in vivo* model for angiogenesis (175). t-PA is involved in different physiological and pathogenic processes, including transformation of plasminogen in plasmin and subsequent fibrinolysis, which, although controversially (because of lack of full *in vitro*/*in vivo* correlation), has been reported to be involved in angiogenic processes (176). Further studies confirmed and expanded these results, identifying a heat shock and highly immunogenic protein of *B. bacilliformis*, GroEL, which plays a key role in the induction of vascular cell proliferation (177). GroEL is actively secreted by the supernatants of *B. bacilliformis* cultures and acts in a dose-dependent manner by increasing HUVEC numbers by 6- to 20-fold. Cell proliferation is inhibited in the presence of anti-GroEL antibodies (177). The levels of expression of this protein are regulated by different factors, such as temperature or DNA supercoiling relaxation (178), which may be produced at different levels during the different infection phases. Furthermore, the increase in cell numbers could be caused by either increased cell division or reduced cell death. An antiapoptotic mechanism has been reported in *Bartonella*, accounting in part for its ability to induce vascular proliferation *in vivo* and enhancing the survival of the host cells and, therefore, itself (179) (Fig. 7).

In situ hybridization has shown that high levels of expression of angiogenesis factors such as vascular endothelial growth factor (VEGF) receptors and angiopoietin-2 are observed in the endothelium of Peruvian warts. Cerimele and colleagues showed that VEGF was produced in the overlying epidermis, demonstrating cooperation between infected endothelium and the overlying epidermis to induce angiogenesis (180). *In vitro* infection of human endothelial cells by *B. bacilliformis* results in activation of Rho family GTPases (Rho, Rac, and Cdc42, which are key signaling proteins in pathways involving actin organization), and subsequently the endothelial cell cytoskeleton is remodeled to form filopodia and lamellipodia, which lead to the bacterial invasion of cell. This demonstrates that *B. bacilliformis* stimulates its own entry into endothelial cells (181–183). *B. bacilliformis* is a paradigmatic example of strong and specific host adaptation, having the ability to manipulate the host cell with a refined elegance for the purpose of bacterial entry. Indeed, despite its lethality in the acute phase, it has developed the capacity to modify its virulence characteristics during infection, allowing it to survive for a long time in intimate interplay with the host immune system.

IMMUNOLOGY

Little is known about the immunology of Carrion's disease, and more studies in this field are definitely warranted in the future. In fact, most of the present knowledge is inferred from what has been obtained by analyzing other members of the genus. Interleukin-10 (IL-10) secretion is involved in immune modulation by the *Bartonella* genus (158). IL-10 is an anti-inflammatory cytokine involved in the downregulation of the inflammatory response induced by bacterial products (158, 184). It interferes with the innate immunity by suppressing inflammatory mediators such as T helper cells, monocytes/macrophages, and dendritic cells, among others, interfering with the adaptive immune response (158, 185). Accordingly, the few studies on Carrion's disease have shown a significant elevation of IL-10 and gamma interferon (IFN-γ) in patients in the acute phase, especially in the systemic inflammatory response, which would explain the severe disease course developed by some patients (186). Elevation of IL-10 levels was also described in 2 patients in the acute phase of Carrion's disease during pregnancy, being more prominent in the patient with a more serious clinical outcome (187). Finally, a case of neurobartonellosis with 98% parasitemia showed the highest IL-10 levels among 13 patients diagnosed with acute disease. The patients showed an initial elevation of IFN-γ and significantly low $CD4^+$ and $CD8^+$ T lymphocyte counts, which

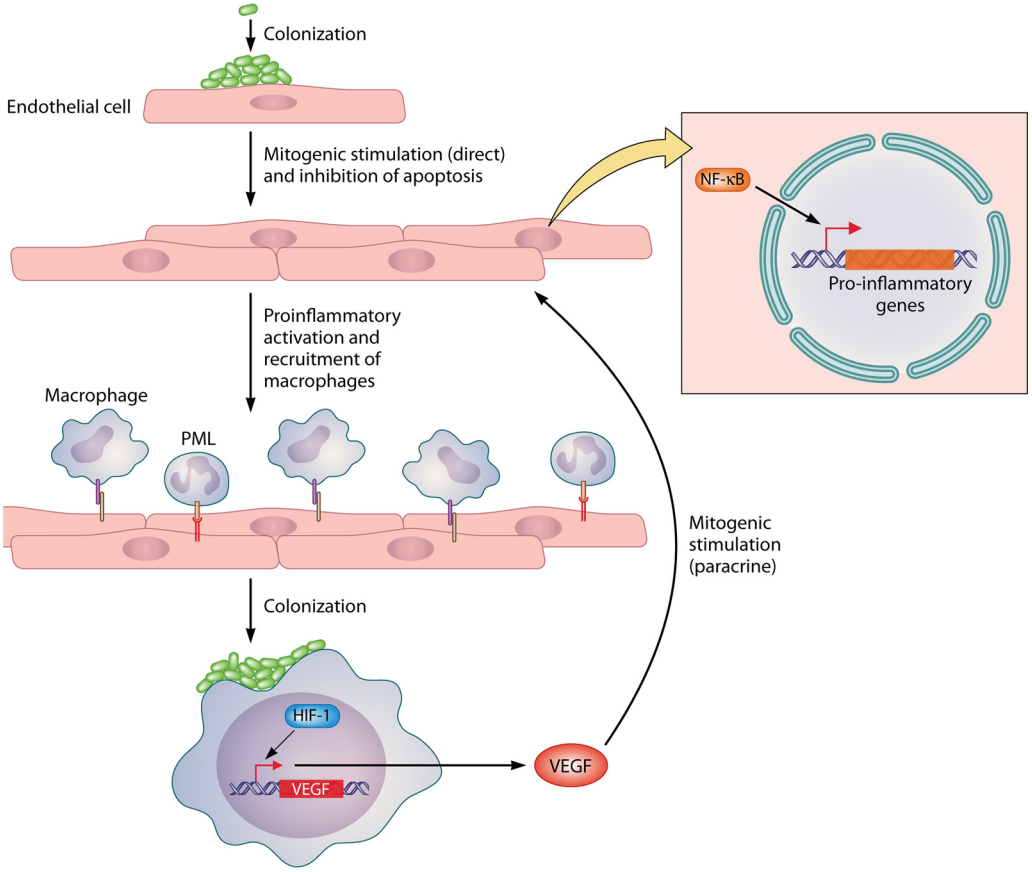

FIG 7 Model of *Bartonella*-triggered vascular tumor formation. PML, polymorphonuclear leukocyte; NF-κB, nuclear factor κB; HIF-1, hypoxia-inducible factor 1; VEGF, vascular endothelial growth factor. The adhesion to and invasion of endothelial cells by *Bartonella* lead to apoptosis inhibition and direct endothelial cell proliferation. In parallel, *Bartonella* triggers an NF-κB-dependent proinflammatory phenotype resulting in the recruitment of macrophages and other lymphocytes, which may also be colonized. This colonization results in the activation of HIF-1, therefore upregulating VGEF, which also stimulates endothelial cell proliferation. All these phenomena are thought to mediate vascular tumor formation. (Adapted from reference 372 with permission of Macmillan Publishers Ltd.)

resolved after appropriate treatment (188). The elevated IL-10 secretion leads to an immune peripheral tolerance which greatly favors asymptomatic infection and persistent infection (158, 188, 189). An analysis of up to 30 different cytokines and chemokines from inhabitants of both postoutbreak areas and areas of endemicity showed the presence of significant differences in some cytokine levels related to the presence or absence of asymptomatic bacteremia and immunoglobulin M (IgM) and IgG levels (190). Thus, in asymptomatic carriers, lower levels of hepatocyte growth factor (HGF) ($P = 0.005$), IL-15 ($P = 0.002$), IL-6 ($P = 0.05$), IFN-γ-inducible protein 10 (IP-10) ($P = 0.008$), MIG ($P = 0.03$), and MIP-1α ($P = 0.03$) were observed, showing a trend to higher IL-1RA levels ($P = 0.059$). When age was considered, all these cytokines except IL-6 remained significantly underexpressed. In addition, IL-12 was also negatively correlated with the presence of asymptomatic *B. bacilliformis* bacteremia Moreover, EGF and eotaxin levels were positively, albeit moderately, correlated with bacteremia. HGF, IL-15, IP-10, MIG, and MIP-1α have been shown to be involved in inflammatory processes and T cell and NK cell activation (191–193). Thus, this cytokine profile leads to attenuation of inflammatory processes as well as a reduction in the immune response (190). In the same study, a multimarker analysis developed to show an association with the presence of markers found that HGF, IL-6, IL-15, and IP-10, as well as granulocyte colony-stimulating factor (G-CSF), IFN-γ, MIG, MIP-1α, and RANTES, were negatively associated with the presence of asymptomatic bacteremia, while IL-2, IL-8, IL-10, MCP-1, MIP-1β, and tumor necrosis factor (TNF) had a positive association (190).

Similarities have been observed between Carrion's disease and infections with other *Bartonella* spp. Circulating IL-10 levels have been described to be significantly higher in patients with cat scratch disease (caused by *B. henselae*) and in asymptomatic homeless people with *B. quintana* bacteremia. Moreover, this specific immune profile and an attenuation of the inflammatory response may also account for the chronic persistence of *B. quintana* (194, 195). *In vivo* studies showed IL-10 production in culture supernatants of spleen cells from mice inoculated with *B. henselae* (196). The lack of bacteremia in IL-10 knockout mice infected with *B. birtlesii* supports the key role of IL-10 in the establishment of *Bartonella* infection (197).

High rates of seroprevalence are observed in regions of endemicity, and native populations seem to be less susceptible to infection and advanced hemolytic disease than foreigners. It is believed that antibodies may provide long-term protective immunity (198). In addition, an opposite correlation between patient age and disease incidence or severity in regions of endemicity suggests that humoral immunity confers partial immunological protection related to the patient's immune status (35, 50, 198). In the first stages of the acute phase of bartonellosis, total IgM antibody levels rise, while IgG and IgA levels remain normal. Total IgG levels increase after the second to fourth week of treatment. In the eruptive phase, there is a significant, albeit not very marked, increase of total IgA, IgG, and IgM levels (43, 199).

The transient depression of the cellular immunity that occurs during the acute hematic phase is of note (200), with patients presenting lymphopenia with a decrease in $CD4^+$ and a slight increase of $CD8^+$ T lymphocytes. Recent data support the possibility that this immunosuppression remains long after the end of the acute phase (190). These findings may reflect an immune tolerance (lack of a specific immune response to an antigen) which might be triggered by *B. bacilliformis*, leading to an immune evasion and installation of persistent bacteremia. In fact, a resemblance between this immunosuppression and AIDS has been established (138). Immunosuppression develops in both diseases, causing a deterioration of cellular immunity, which could thereby explain the predisposition to opportunistic infections (6, 138, 140). Treatment may induce immune reconstitution and favor the manifestation of atypical symptoms. The intensity of this inflammatory response probably depends on previous exposure to *Bartonella* and on individual genetic predisposition (138).

Data on the immune response in the eruptive phase are very scarce. Leukocyte levels remain normal, with a slight tendency to present lymphocytosis (6, 140). Significantly elevated IFN-γ and IL-4 levels were reported in 21 patients in the chronic phase of the disease (187). Interestingly, for healthy preexposed and asymptomatic carriers it was observed that those having elevated IgG levels were more prone to have high eotaxin levels ($P = 0.006$), while IgG seropositivity was correlated with VEGF levels ($P = 0.047$), both of which are involved in angiogenic processes (190).

The development of a vaccine inducing both humoral and cellular immune responses is perhaps the most valuable approach to advance toward disease eradication and, overall, to improve the lives of people living along with Carrion's disease as well as to improve public health strategies to manage this disease (39, 166, 168). Several proteins involved in the interactions of *B. bacilliformis* with the human host, such as flagellin, FtsZ, or IalB, have been proposed as good candidates for the development of a vaccine (166, 168).

During the Colombian outbreak of the 1930s to 1940s, glycerinated and phenolizated *B. bacilliformis*-rich blood was studied as a possible vaccine, but unsuccessfully (201). The only in-field study regarding vaccines for Carrion's disease was carried out in 1943 by Howe and Hertig (202) using a suspension of 4 inactivated strains of *B. bacilliformis* and consisted of the active immunization of 22 military guards posted to zones with warty disease. During 1 month of exposure, 75% of nonimmune individuals developed Peruvian warts, and in the other group with 4 months of exposure, approximately 90% of the guards became infected, with two-thirds becoming incapacitated by severe illness (202). Eighty-six percent of the guards developed high titers of agglutinins for *B. bacilliformis* as a result of one or more inoculations. Although active

immunization did not prevent the development of infection and positive cultures in 55% of the guards, only one guard required hospitalization. These results showed improvement in the course of potentially severe Carrion's disease on comparison with the controls. On the other hand, the sensitization of rabbits with *B. bacilliformis* increased the lethality when they were subsequently administered *Bartonella* metabolites, indicating that hyperreactive states are dependent on the degree of sensitization (203).

TRANSMISSION

Different routes of transmission of *Bartonella* have been described or proposed. Nonetheless, the most common and relevant route of Carrion's disease transmission is vector-borne transmission.

Vectors

Townsend identified *P. verrucarum* as the vector of Carrion's disease by observing its habits and characteristics, the conditions of zones of endemicity, and the distributions of the disease and the insect (15, 204). Nevertheless, the role of *L. verrucarum* as the vector of Carrion's disease was not firmly supported until the studies of Noguchi et al. in the late 1920s (205), being corroborated by Hertig in 1942 (206) and definitively established in 2004 (207) (see below). Currently, *Lutzomyia* and *Phlebotomus* are two different genera within the *Phlebotominae* subfamily, belonging to the *Psychodidae* family. While *Phlebotomus* members live in temperate regions of the Old World, the *Lutzomyia* genus is present in tropical and subtropical areas of the Americas, from Argentina and Chile to the United States (208).

According to the Theodor classification, the *Lutzomyia* genus has at least 26 subgenera, accounting overall for approximately 400 species (209). According to both morphological and molecular criteria, a subclassification has been proposed in which *L. verrucarum* and at least 40 related species, including *Lutzomyia maranonensis* and *Lutzomyia columbiana*, have been placed in the so-called verrucarum group (210, 211). The *Psychodidae* family has a complex taxonomy which is under constant revision; the most recently proposed taxonomy by Galati has resulted in new genera and in the reclassification of different species, including *L. verrucarum* (classified within the *Pintomyia* genus, *Pifanomyia* subgenus, and verrucarum series) (212) (Fig. 8). The Theodor classification (genus *Lutzomyia* and species *L. verrucarum*) is used in the present article.

Although *L. verrucarum* lives in relatively highly arid zones, the presence of humidity and organic material facilitates the establishment of *Lutzomyia* (31). These findings, together with the presence of sugar sources, have been related to the association between traditional coffee plantations and the presence of *Lutzomyia* spp. (31). Both male and female *Lutzomyia* organisms feed on honey dew from aphids or vegetable sugars, but females (except for some autogenous species) also have blood requirements for egg development (208). Thus, *L. verrucarum* females are responsible for the transmission of *B. bacilliformis* to humans. The mechanism of this transmission is still unclear (39).

L. verrucarum has classically been distributed at altitudes of 500 to 3,200 masl in inter-Andean valleys and slopes of the center and northern Peruvian Andes (39, 78). *L. verrucarum* and other *Lutzomyia* species have a unimodal annual distribution pattern, reaching the population maximum prior to the onset of the summer rainy season (39). Sand fly population densities are directly correlated with the average minimum environmental temperatures and relative humidity. Sand flies are usually found inside houses (35, 37, 213). They have nocturnal habits, feeding from dusk, taking advantage of the decrease of temperature and relative increase of humidity (15, 39, 140). In fact, unusually high numbers of sand flies were collected after El Niño affected Peru in 1997 to 1998 (35, 44).

In the brilliant study by Noguchi et al. (205), several insect species were collected, including three species of *Lutzomyia* (*L. verrucarum*, *Lutzomyia noguchii*, and *Lutzomyia peruensis*), from districts of Peru in which the disease prevails. The

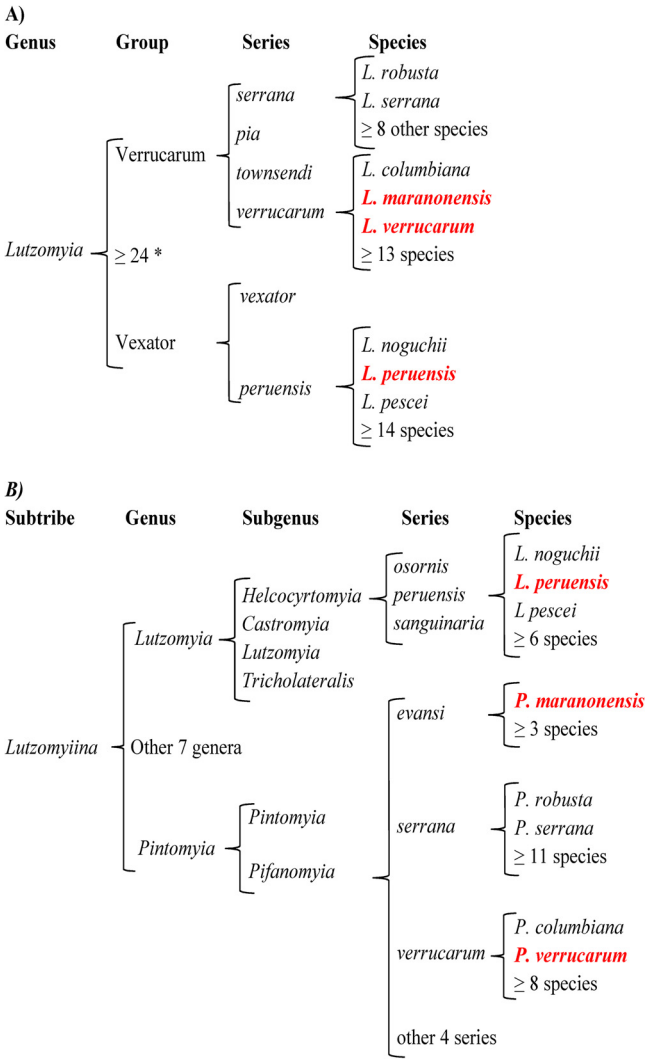

FIG 8 Suspected and confirmed Carrion's disease vectors. (A) Carrion's disease vector classification following the 1965 scheme of Theodor (209). (B) Carrion's disease vector classification following the 2014 scheme of Galati (212). Incertae sedis are not included. Confirmed Carrion's disease vectors are in red. *, among groups and subgenera.

presence of *B. bacilliformis* in the insects was established by infecting *Macaca mulatta* (rhesus macaque) with the extract of the crushed insects. To corroborate the results, the blood from the animals was cultured *in vitro*, yielding cultures of *B. bacilliformis* which when inoculated in other monkeys, produced verrucous lesions. After recovering, the monkeys showed resistance to a human strain of *B. bacilliformis* that was later inoculated. The results showed that *L. noguchii* very likely carried *B. bacilliformis* and that *L. verrucarum* was a probable vector. However, there continue to be reservations with respect to *L. peruensis* (205). Studies in which monkeys were directly bitten by wild *L. verrucarum* sand flies were later performed by Hertig, resulting in infection of 5 out of 8 monkeys (206). The presence of *B. bacilliformis* in *L. verrucarum* was later confirmed by PCR and qPCR (207). In a later study done in Cusco using PCR, 1% of the *L. peruensis* organisms collected were infected with *B. bacilliformis*, and this vector was implicated in the transmission of bartonellosis to humans (37).

These data, together with the geographical distribution of *L. peruensis*, highlight the risk of introducing the illness in Bolivia, from which sporadic cases have been reported (38).

TABLE 3 *Lutzomyia* species confirmed or proposed as Carrion's disease vectors according to the 1965 Theodor classification

Species	Group	Series	Status	Distribution[a]
L. verrucarum	Verrucarum	Verrucarum	Confirmed[b]	PE
L. peruensis	Vexator	Peruensis	Confirmed	BO, PE
L. maranonensis	Verrucarum	Verrucarum	Confirmed	EC, PE
L. noguchii	Vexator	Peruensis	Highly probable[c]	PE
L. columbiana	Verrucarum	Verrucarum	Potential[d]	CO
L. pescei	Vexator	Peruensis	Potential	PE
L. robusta	Verrucarum	Serrana	Potential	EC, PE
L. serrana	Verrucarum	Serrana	Potential	BO, BR, BZ, CO, CR, EC, GF, GT, HN, MX, NI, PA, PE, VE

[a]BO, Bolivia; BR, Brazil; BZ, Belize; CO, Colombia; CR, Costa Rica; EC, Ecuador; GF, French Guyana; GT, Guatemala; HN, Honduras; MX, Mexico; NI, Nicaragua; PA, Panama; PE, Peru; VE, Venezuela.
[b]The presence of *Bartonella bacilliformis* has been confirmed by use of molecular tools.
[c]There were early studies on infecting *Macaca mulatta* with the extract of the crushed insects, but in the absence of molecular confirmation, there is a risk of insect misidentification.
[d]Present in areas of endemicity without established vectors; distribution and vector ecology support the proposal.

The distribution of Carrion's disease and the presence of *L. verrucarum* and *L. peruensis* do not always seem to match, raising the possibility of the involvement of other *Lutzomyia* species in disease transmission (213, 214). In Peru, in zones of endemicity in the Cajamarca and Amazonas departments, *L. maranonensis* and *Lutzomyia robusta* were considered the probable vectors (213, 215). On the other hand, in the Peruvian jungle of the Huanuco department, *Lutzomyia serrana* was found to be the most probable vector (216). Along this line, the presence of *L. verrucarum* in Ecuador and Colombia has never been reported (9). As indicated, Colombia was free from Carrion's disease prior to 1936. Indeed, in the first outbreak more than 6,000 deaths were reported (59), highlighting the possible adaptation of another arthropod to the vector role in Carrion's disease; it was postulated that the closely related *L. verrucarum* species *L. columbiana* was involved in disease transmission. The results were based on the collection of insects in areas of endemicity using traps followed by the identification of the species collected. However, *B. bacilliformis* was not identified in any of these studies.

More recently the vector role of *L. maranonensis* has been confirmed in northern Peru (Cajamarca). Thus, Ulloa-Urizar et al. (217), analyzing 97 pools containing 5 females of *L. maranonensis* each by PCR, found 2 positive pools, which were confirmed by sequencing as *B. bacilliformis*. These results expand the area of known established vectors, describing the vector presence in different areas of northern Peru and Ecuador (Table 3).

Vectors may play a role in the maintenance of vertical transmission by vector-borne microorganisms. This has been described for some viruses, such as dengue virus or chikungunya virus, among others (218, 219), as well as in bacteria, including *Bartonella* spp., such as *B. schoenbuchensis* (220) or *B. quintana* (107). Two compatible pathways have been proposed to explain this phenomenon: transovarial transmission and the contact of eggs and larvae with contaminated insect feces or diuretic fluids (221).

The role of *L. verrucarum* in the maintenance of the microorganism by vertical transmission was assessed by Ponce and Solorzano (222). *L. verrucarum* from an area with a high prevalence of insects and illness were fed blood from two patients diagnosed with Oroya fever, with a positive blood smear with 3 and 80% of infected erythrocytes, respectively. After oviposition and the death of the insects, PCR was performed to detect the microorganism. The results showed that insects fed blood with a higher bacteremia had a shorter life, and most of the infected females (35 out of 36) were unable to perform oviposition, while no *Bartonella*-positive descendant of the remaining infected female was obtained, supporting the hypothesis of a higher mortality of *L. verrucarum* in the presence of higher bacteremia and the absence of vertical

transmission of *B. bacilliformis* in *L. verrucarum* (222). A recent report compared *B. bacilliformis* colonization in a competent (*L. verrucarum*) and noncompetent (*L. longipalpis*) vectors. Initially, no differences in the colonization of the two fly species were observed. However, at day 3 the bacteria remained in the abdominal midgut of *L. longipalpis*, being progressively digested and disappearing at day 7. In *L. verrucarum*, *B. bacilliformis* colonizes the digestive tract lumen, persisting for more than 14 days. Thus, *L. longipalpis* eliminates *B. bacilliformis*, while bacteria in *L. verrucarum* survive on blood meal digestion, colonizing the entire digestive tract of the sand fly (223). Although sporadic transmission of Carrion's disease related to *L. longipalpis* cannot be ruled out because the microorganism remains viable within *L. longipalpis* for up to 11 days, no mechanical transmission or isolation of viable *B. bacilliformis* has been obtained from feces or diuretic body fluids (223). *B. bacilliformis* was not observed in feces or diuretic body fluids of *L. longipalpis* or in eggs of *L. verrucarum* and *L. longipalpis* (223). Unfortunately, analysis of feces or diuretic body fluids was not carried out in the *L. verrucarum* group.

Other types of vectors cannot be excluded. One report described the transmission of *B. bacilliformis* from 2 experimentally infected to 2 healthy rhesus monkeys by the bite of the tick *Dermacentor andersoni*. The infection was mild, and the bacteria were recovered from the lymph nodes and blood of the animals (224). Members of the *Eratyrus* genus, *E. mucronatus* and *E. cuspidatus*, are present in some coastal Ecuadorian areas, and *E. mucronatus* can also be found in jungle areas of Peru (225). Taking into account the currently expanding distribution of Carrion's disease, this finding, together with the previously mentioned description of "*Candidatus* Bartonella rondoniensis," which is closely related to *B. bacilliformis*, in *E. mucronatus* make it necessary to evaluate the role of these or other kissing bugs as potential vectors of Carrion's disease.

While nothing is known about the vectors of *B. ancashensis*, *B. rochalimae* is widely distributed worldwide through fleas and also ticks. In areas where Carrion's disease is endemic, *B. rochalimae* has been recovered in ticks and fleas from cats and dogs in both Cajamarca and Lima (86, 90). Moreover, although uncultured, *B. rochalimae* was detected before to its first full description (57) in a flea from a human from the Cuzco department (226).

Vertical Transmission

To the best of our knowledge, the first proposal of the vertical transmission route was made by Tomas de Salazar as far back as 1858 (142, 227), with the report of a case leading to the death of both the mother following labor and the newborn several days after birth (227). Afterwards, Campodonico and Odriozola described similar findings in 1895 and 1898, respectively (228, 229). Thereafter, in the first third of the 20th century, Monge and Strong in 1912 and 1913, respectively, and both Malpartida and Colaretta in 1937 also reported different cases of vertical transmission (3, 48, 227, 230). More recently, several authors have also reported different cases (15, 48, 231–233), with the most evident description being of a *B. bacilliformis*-positive culture obtained from the blood of a preterm child collected 90 min after birth (231) (Table 4).

Although the phase of infection is not stated in some of the reports and involves the acute phase in others (48, 227), it seems that in several cases the mother was asymptomatic or presented Peruvian warts (142). This finding may also be related, in part, to the serious adverse events associated with mother or fetal deaths, miscarriages, or preterm births related to acute phase of the disease during pregnancy (15, 32, 142, 234). In fact, in 1898 Odriozola explicitly described the extreme severity of the illness in newborns (229).

Blood Transfusion

Although direct blood contact with contaminated blood is a direct pathway of infection, the number of reports on *B. bacilliformis* transfusion transmission is scarce and limited to countries where it is endemic. Thus, in 1972 a Carrion's disease-related death of a newborn after a blood transfusion was reported (48), while in 2015 the

TABLE 4 Proposed vertical transmission cases[a]

	Mother		Children		
Yr[b]	Diagnosis	Outcome	Diagnosis	Outcome	Reference(s)[c]
1858	OF	Death	OF	Death	227
1894	NR[d]	NR	PW[e]	Death	228, 229
1898	PW	NR	PW	NR	228
1937	OF	Recovery	OF	Death	230
1937	OF	NR	OF	Death	51
1993	OF	Recovery	OF	Recovery	232[f]
2003[g]	PW	NR	AB?	NR	231
2015	PW	NR	OF	Recovery	233

[a]Abbreviations: OF, Oroya fever; PW, Peruvian warts; AB, asymptomatic bacteremia; NR, not reported.
[b]If the year of the case was not explicitly reported, the publication year is indicated.
[c]Other reports, such as that by Monge (376), highlight the presence of cases in newborns but do not present any specific case. In addition, note that in most of the cases a possible natural sand fly bite transmission cannot be ruled out. In addition, the existence of unreported cases or reports in local congresses or journals is highly probable.
[d]In the report it is stated that the parents were healthy, but it is not indicated whether this was stated after physical examination; thus, the presence of Peruvian warts cannot be ruled out.
[e]The patient presented a high number of verrucous lesions, but the remaining symptoms may be related to the acute phase of Carrion's disease.
[f]A later publication of this case in *Laborat-Acta* (380) has been reported in different revisions, but despite intensive efforts, we have been unable to find this article.
[g]The clearest and an undoubtable case. A *B. bacilliformis* culture-positive blood sample was collected from a newborn 90 min after birth.

development of Oroya fever in a polytransfused immunosuppressed patient was described (235). Perhaps the frequent associated use of antimicrobial agents in transfused patients diminishes the risk of disease development.

Blood donors are usually apparently healthy people, and as such those who are infected are actually asymptomatic carriers who probably have a low bacterial load in blood, thereby hindering the detection of this microorganism. Moreover, there are no studies on how long a person can be an asymptomatic carrier, with as long as 3 years having been described in a specific case of an Ecuadorian expatriate (62). This, together with the ability of the microorganism to survive for long periods of up to 30 months in blood stored at 4°C (124), leads to a true risk when transfusions are made in areas of endemicity. In fact, in a PCR analysis of 42 samples from blood donors in an area of nonendemicity in the north of Peru, 2.4% of the samples were found to be positive for *B. bacilliformis* (236). To avoid this risk, blood banks from Peruvian areas of endemicity include Carrion's disease among infectious diseases to be considered, while no data about other countries are available (237, 238). Although not considered, *B. bacilliformis* testing in blood banks outside countries where it is endemic would be extremely valuable due to the natural movement of people who may provide unexpected infected donations in far-away areas. In fact, as indicated above, it has been estimated that more than 2.5 million Peruvians live outside the country, with 22.1% of these individuals being from mountain areas (65).

Organ transplantation. To the best of our knowledge no *B. bacilliformis* infection related to organ transplantation has ever been described. Nonetheless, it should be noted that strong evidence of other posttransplant *Bartonella* sp. infections has been reported (239).

RESERVOIRS OF *B. BACILLIFORMIS*

Various candidates have been suggested as the reservoir of Carrion's disease. Euphorb plants were initially proposed as reservoirs because of their geographical correlation with warty zones as well as the seasonal incidence of the disease and the period of greatest plant growth. Herrer tried to recover *B. bacilliformis* from euphorb plants in zones of endemicity and to experimentally infect these plants with cultures of this microorganism (240). Thereafter, he determined whether latex from plants allowed the development of *B. bacilliformis* in culture. The results of all of the above-described experiments were negative: *B. bacilliformis* was not recovered from the *Euphorbiaceous*

plants, no infection was produced in the plants, and the conditions for the *in vitro* development of the bacteria in latex were adverse. Altogether, these results demonstrated that euphorb plants do not act as a reservoir of Carrion's disease (240).

Several studies have been performed in animals in the search for other potential reservoirs. Noguchi successfully demonstrated that *M. mulatta* is susceptible to infection by *B. bacilliformis*. The microorganisms were found in the red blood cells of the animals and recovered in *in vitro* cultures. The clinical manifestations in monkeys are less severe than those of human disease, and the nodules which developed resembled those of the Peruvian wart (18). Similarly, in 1942, Hertig (206) was able to isolate *B. bacilliformis* from blood of rhesus macaques experimentally exposed to the bite of wild *L. verrucarum*. More recently, it has been shown that owl monkeys (*Aotus nancymaae*), which are present in the South American jungles, including those of northern Peru, may be experimentally infected. Six monkeys were inoculated, 3 intradermally (in order to simulate the bites of the sand fly) and 3 intravenously. In 4 out of the 6 monkeys (2 from each group) the presence of the microorganism was observed within the erythrocytes at 21 days after inoculation. Moreover, a more-than-4-fold increase in IgM levels was observed in the 3 intradermally and 1 intravenously inoculated animals. Similar to the results of the previous study, the symptoms were less evident, and no positive PCR or successful culture was obtained (241).

These data warn about a latent risk of the expansion of Carrion's disease, with the possible arrival of infected sand flies to areas inhabited by owl monkeys (present in different areas of northern Peru) which might be infected and act as natural dispersing agents toward neighboring areas in Brazil.

Cooper et al. proposed that human bartonellosis is a zoonosis in which wild animals, most probably rodents, are the natural reservoir (58, 242). However, this proposal was based on case-control studies using questionnaires. No animal was tested for *B. bacilliformis*, and the results were never confirmed in later studies. The isolation of *B. bacilliformis* from the blood of a single rodent belonging to the genus *Phyllotis* from an area of endemicity in central Peru (Rimac valley) was reported, but no attempts to infect other rodents were successful (68). To our knowledge no other isolation of *B. bacilliformis* from blood samples of an animal has been reported, and possible misidentification cannot be ruled out (6). It is known that inhabitants of zones of endemicity usually have pets which are susceptible to infection with *Bartonella* spp., and these animals can present warts similar to those of patients with Peruvian warts. However, the presence of *B. bacilliformis* in warts from these animals has never been microbiologically demonstrated (6). A survey in which the blood of 50 animals from 11 homes of families with children who had recently had bartonellosis was collected was unable to identify an animal reservoir for *B. bacilliformis* (243). Moreover, in a study done by Solano and colleagues (244), blood smears and blood cultures were obtained from dogs, guinea pigs, and wild mice as well as healthy individuals from areas of endemicity in Peru. No positive results were obtained for blood smears, and no positive cultures were obtained from the wild animals. On the other hand, 12% of human samples were found to be positive for *B. bacilliformis* (244). Different studies showed that between 47% and 54% of patients with warty disease were bacteremic (35, 147), while Gomes et al. (50) observed 40 to 50% of bacteremic asymptomatic carriers, suggesting that patients with chronic disease and asymptomatic carriers are the most likely reservoirs.

Despite the proposal involving camelids as the original source of primitive *B. bacilliformis* (92), humans are currently the only established reservoir for Carrion's disease, although the absence of other vegetal or animal reservoirs for *B. bacilliformis* has never been definitively demonstrated. Most studies have been carried out with techniques with a low sensitivity, and thus, studies using techniques able to detect low bacteremia, such as serological techniques and/or qPCR, are needed.

No reservoir has yet been described for *B. ancashensis*, while the main reservoirs of *B. rochalimae* include a variety of wild and domestic canids, as also described in other carnivores (60, 245–249).

DIAGNOSIS OF CARRION'S DISEASE

Carrion's disease affects the poorest populations from remote isolated rural areas, which have poor communications and poorly equipped laboratories (38). Currently, the diagnosis of Carrion's disease presents important limitations. The initial febrile stage is often misdiagnosed because its symptomatology is similar to that of other illnesses (27, 37–39, 139, 250). Although the warty phase is easier to diagnose, incorrect diagnosis can be made, especially in regions where the disease is not present and in cases imported from areas where it is endemic. One example was reported by Maguiña et al., in which erroneous initial diagnoses were made in 19% of patients, including skin tumor in 7 patients, hemangioma in 5 patients, polyarthralgias in 2 patients, and systemic lupus erythematosis in 1 patient (139). Moreover, without the diagnosis of asymptomatic carriers, perpetuation of the disease will never end.

Routine Diagnostic Techniques

In rural areas of endemicity, the diagnosis of Oroya fever is based mainly on clinical symptoms and Giemsa-stained peripheral blood smears, a low-cost and easy-to-use technique. Nonetheless, this method is requires expertise, and despite the high specificity of 96%, a very low sensitivity (24 to 36%) has been reported, especially in mild cases of disease and in the subclinical and chronic phases of illness (37, 38, 251). Moreover, the unspecific initial symptoms of Carrion's disease should be taken into account, since they are common to several pathologies present in these areas, such as different arboviral diseases, malaria, or tuberculosis, thereby making diagnosis based only on clinical symptoms difficult (15). In fact, outbreaks of Carrion's disease have been reported using microscopy tools and clinical diagnostic techniques (http://www.promedmail.org/direct.php?id=20130304.1569888) although analysis by molecular tools revealed another etiological cause (252). In the chronic stage of the disease, clinical diagnosis is made by the presence of cutaneous angiomatous skin lesions. While microscopic techniques have 36% sensitivity in the acute phase, a decrease to less than 10% is observed in Peruvian warts (140), related to lower blood bacterial carriage. Histopathological diagnosis is possible, albeit difficult, with staining of sections with Warthin-Starry silver or Giemsa stain demonstrating the presence of bacteria in the skin eruptions (39).

B. bacilliformis culture does not have clinical utility in diagnosis due to the culture requirements and especially because of the low bacterial growth rate (1 to 6 weeks). Moreover, it is cumbersome and time-consuming, and contaminations have been described in 7 to 20% of the cultures (15, 139). Additionally, the sensitivity of this method is extremely low. In one study a considerable portion of Peruvian wart cases yielded negative blood cultures (70), and another study showed that only 13% of patients with Peruvian warts had a positive culture or blood film (139). Moreover, a study in which cultures from persons known to be infected with *B. bacilliformis* were performed over a 6-month period supported the suggestion that the proportion of positive cultures is much higher in the first months of infection than later (70). Finally, different researchers have evaluated the use of different insect-based liquid culture media (253–255) to isolate and grow *Bartonella* spp., but to our knowledge, no study on the utility of these media in the isolation and growth of *B. bacilliformis* has been developed.

Molecular Techniques

Several PCR approaches have been described. However, these studies usually involve a small number of samples, and additionally, as occurs with the remaining diagnostic tools, they are hampered by the lack of a standard case definition. Moreover, it is necessary to identify genetic targets able to differentiate human-pathogenic strains from those that are less virulent (256). PCR approaches are more effective than optical microscopic and culture techniques, being able to diagnose Carrion's disease patients in the acute phase previously classified as negative by thin blood smear (250). Nonetheless, a critical issue is the detection limit of these techniques, raising doubts about

their usefulness in the detection of low bacteremia or asymptomatic carriers. Moreover, it should be kept in mind that routine implementation of molecular techniques in remote endemic rural areas is difficult because of the lack of technical resources and trained personnel (38). In fact, molecular techniques are used only in reference centers, located mainly in Lima (Peru). This fact also leads to another problem, which is the need for rapid, consistent transfer of samples and the return of diagnostic results from reference centers to local health facilities.

PCR can be done directly in affected tissues and/or blood or in enrichment cultures (39). With enrichment, the conventional PCR positivity increases by 55% compared with that for original blood samples (257). This enrichment technique needs 14 days for development, which leads to an unaffordable delay in the processing of samples, making it impracticable in clinical diagnosis. The use of dried blood spots (DBS) may make transportation of samples from areas of endemicity to reference laboratories easier. Moreover, the small blood volumes make the use of DBS especially interesting in the pediatric setting.

Several PCR assays for the detection and identification of *Bartonella* species have been reported in the literature. A nested PCR using the cell division gene *ftsZ* to differentiate among *B. bacilliformis*, *B. quintana*, and *B. henselae* was described by Kelly et al. (258). Additional approaches include several PCR restriction fragment length polymorphism (RFLP) methods, including use of RNA polymerase beta subunit (*rpoB*) gene analysis (259), the citrate synthase (*gltA*) gene (260, 261), the *ftsZ* gene (262), and the 16S rRNA gene (263). Molecular analyses based on only one-step PCRs have also been described. The riboflavin synthesis genes, such as *ribC*, *ribD*, and *ribE*, are absent in humans and present in bacteria and therefore are useful either for bacterial DNA detection in human samples or for the differentiation of *Bartonella* species (264). The *ialB* gene has been proposed for the diagnosis of *B. bacilliformis* on demonstrating high sensitivity and specificity in 10 blood samples from acute-phase patients with confirmed thin blood smears (265). Nonetheless, further studies showed its presence in other members of the genus, such as *B. henselae* and *B. birtlesii*, thus making it a useful tool for *Bartonella* sp. infection (166) but not discriminatory for *B. bacilliformis*. The amplification of the 16S rRNA gene has also been proposed for the diagnosis of Carrion's disease in Peru (250). However, several studies have warned about the utility of the 16S rRNA gene as a means of differentiating *Bartonella* species due to the high conservation of this gene (266, 267). Hypervariable intergenic transcribed spacer (ITS) 16S-23S rRNA amplification allows differentiation between *B. bacilliformis* and *B. ancashensis* from the main pathogenic *Bartonella* spp. (268). In fact, the ITS region has been used in different studies of *Bartonella* spp. (89, 269). Another PCR approach is multiplex PCR based on amplification of the ITS gene combined with reverse line blotting, which allows distinguishing among 20 different *Bartonella* species (270).

The detection limits of 3 different PCR approaches have recently been determined in blood and DBS artificially infected with *Bartonella*. Two of these approaches, targeting the 16S rRNA and *fla* genes, had a detection limit of 5 CFU/μl when infected blood was tested. This sensitivity was maintained in the case of 16S rRNA gene amplification when applied to DBS but decreased to 500 CFU/ml in the case of *fla* amplification. The third approach targeted the ITS region and showed a detection limit of 500 CFU/μl in both artificially infected blood and DBS. The sensitivity of the 16S rRNA PCR approaches seems to be high enough to diagnose acute cases. Nonetheless, there continue to be doubts regarding the usefulness of PCR in the detection of asymptomatic carriers (271).

Finally, recent studies have demonstrated the utility of more sensitive molecular techniques such as qPCR. Using this technique, Smit et al. showed that 24.6% of 65 children were positive whereas only 3% were blood culture positive (272), and Gomes et al. detected 38% of asymptomatic carriers in a postoutbreak zone and 50% in an area of endemicity (50). In another study with animal blood samples, the sequences of a region of the *ssrA* gene amplified by qPCR were able to discriminate *Bartonella* species (256). Along the same line, a study on the detection of the ITS region using qPCR, which is able to detect a minimum of 13 *Bartonella* spp., including *B. bacilliformis*, *in silico*,

showed a sensitivity of 50 genomes per reaction (273). A two-step protocol consisting of a genus-specific qPCR with the *gltA* gene as the target followed by pyrosequencing of qPCR-positive specimens directed against a segment of the *rpoB* gene was also described for differentiation of at least 11 medically relevant *Bartonella* spp. Despite being a two-step protocol, it can be done in 5 h, making it an alternative to the time-consuming sequencing of a PCR product (274). On the other hand, a qPCR targeting the NADH dehydrogenase gamma subunit gene (*nuoG*) was found to be sensitive and specific enough to detect diverse *Bartonella* species, maintaining minimal cross-reactivity to mammalian host and arthropod vector organisms (94). Angkasekwinai et al. (275) described a loop-mediated isothermal amplification with *pap31* as the target gene. This is a simple method to detect the presence of *B. bacilliformis* DNA within 1 h and requires less specialized equipment. This method has a detection limit of between 1 and 10 copies/μl, depending on whether bacterial genomic DNA is used alone or in the presence of human DNA, respectively. Nonetheless, its usefulness remains to be validated in human clinical samples.

Serological Techniques

Serological tests are useful diagnostic tools for Carrion's disease, especially when combined with other techniques such as PCR and blood culture. However, immune diagnostic methods for this disease are relatively underdeveloped (39), and as mentioned above, the most-studied approaches, such as indirect fluorescence antibody assay (IFA) or enzyme-linked immunosorbent assay (ELISA), have the disadvantage of limited technical resources in areas of endemicity. In this context, the characterization of the antigens expressed during *B. bacilliformis* infection, which is essential in the elucidation of Carrion's disease pathogenesis, may also lead toward the development of rapid diagnostic tools (38, 276). A rapid diagnostic test which does not need experienced personnel or high-technology machinery could be easily introduced in low-income areas after basic training. Moreover, results can be obtained quickly, thereby facilitating early initiation of antibiotic treatment.

In 1988 the first study identifying *B. bacilliformis* antigens was published (277), which described 24 proteins, including two main antigens of 65 and 75 kDa. BB65 is a heat shock protein later identified as GroEL, and the 75-kDa antigen corresponds to the cell division protein FtsZ (277–280). Later studies have described the identification of GroEL, leading to the identification of antigenic candidates using anti-human IgG as a secondary antibody (50). Although Gomes et al. (50) detected anti-GroEL IgM antibodies, other authors have found that GroEL does not bind to *Bartonella* IgM, binding to IgG antibodies 2 weeks after infection and remaining detectable at least until the third year after *B. bacilliformis* infection. However, no anti-GroEL antibodies are detected in the 40% of Peruvian wart patients (278). GroEL has mitogenic activity against HUVECs, resulting in the development of warts. The partial inhibition of this mitogenic activity when anti-GroEL antibodies are present suggests their protective role in asymptomatic carriers (177). Recently, it has been reported that this protein has been tested in a rapid immunochromatographic assay, using rabbit IgG polyclonal anti-GroEL antibodies with a sensitivity equal to or lower than 10^2 CFU/ml (281).

The first study which attempted to identify the outer membrane proteins (OMPs) of *B. bacilliformis* led to the identification of 14 OMPs ranging from 11.2 to 75.3 kDa. The most prominent immunoprecipitants with rabbit anti-*Bartonella* hyperimmune serum were proteins of 31.5, 42, and 45 kDa, which may represent the immunodominant OMPs of the pathogen (159).

An antigenic lipoprotein of 43 kDa was found by Padmalayam et al. by performing a screening of a genomic DNA lambda library with serum from a patient in the chronic phase of the disease (276). An ELISA using the 43-kDa lipoprotein in its recombinant form was described. The sensitivities of IgG and IgM ELISAs were 70.4 and 85.2%, respectively, and the specificity for both IgG and IgM was 90%. Despite the good sensitivity and specificity, 3 sera from chronic-phase patients had high titers of IgM,

indicating that the IgM ELISA does not discriminate between the two phases of the disease (282).

Another ELISA using the recombinant flagellin gene (*fla*) of *B. bacilliformis* was assessed against sera from patients with bartonellosis and healthy controls. The sensitivity of this method was 58%, and when sera from people with other pathologies were tested, a specificity of 69% was obtained. Moreover, 15% of sera from healthy people also gave a positive result (283). One possible explanation for the worse results on comparison with the 43-kDa lipoprotein ELISA may be that the flagellin of *B. bacilliformis* is very similar to other flagellins and may share epitopes with other bacteria (282). More recently the immunological dominance and high expression of Pap31 (HbpA) in *B. bacilliformis* cultures was described, and this was considered a good candidate for use in ELISA (171). Furthermore, Hbps have been proposed as serodiagnostic tools for *B. quintana* infections (284), and this usefulness probably could be extrapolated to other *Bartonella* infections. More recently, anti-Pap31 IgM has been detected in serum samples from healthy inhabitants of both postoutbreak areas and areas of endemicity irrespective of their asymptomatic carrier status (50). That study also described 2 new antigenic candidates: the two subunits of succinyl coenzyme A (succinyl-CoA) synthetase (SCS-α and SCS-β) (50). These new antigenic candidates are implicated in the tricarboxylic acid cycle. They are described as immunogenic protein of *Brucella melitensis* (SCS-α) (285) and as being involved in the pathogenesis of *B. henselae* infection (SCS-β) (286). Finally, through *in silico* analysis, the antigenic potential of 16 outer membrane or secreted proteins has been described (287).

Although further studies are needed, the use of more than one antigenic candidate may be the best option for a rapid diagnostic test. Indeed, other authors have suggested the need to evaluate other proteins of *B. bacilliformis* in order to improve the sensitivity or specificity of the serological test, and in order to achieve this goal, a cocktail of recombinant proteins or synthetic peptides would be necessary (283).

B. bacilliformis immunoblot sonication of whole organisms is remarkably sensitive to *B. bacilliformis* antibodies from sera of patients with chronic disease (94%) and also yields reasonable results in acute disease (70%). However, some cross-reactions with sera with antigenically similar bacteria such as *Brucella* spp. and *Chlamydia psittaci* were reported in 34% and 5% of the cases, respectively (251). Indeed, an intense cross-reactivity between *B. bacilliformis* and *C. psittaci* due to common surface epitopes has also been reported previously (288).

Chamberlin et al. (148) described an IFA with irradiated *B. bacilliformis* whole-cell antigen preparation cocultivated with Vero cells in which the 81% of acute confirmed cases were positive. Interestingly, 74% of the volunteers having a positive IFA reported bartonellosis within the last year, which decreased to 39% when a more distant or nonbartonellosis episode was reported. Overall, 45% of the volunteers from an area where Carrion's disease is endemic were seropositive for *B. bacilliformis* by this technique (148).

TREATMENT OF CARRION'S DISEASE

The treatment of Carrion's disease differs depending on which phase of the disease is presented and includes blood transfusions and antimicrobial agents. With regard to antimicrobial therapy, different antibacterial agents have been tested since the beginning of the antibiotic era (289). Nonetheless, the current clinical practice relies mostly on personal experience and expert opinion (290). In any case, no clinical assays have been developed to define the real usefulness of the different antibiotic schedules proposed to treat Oroya fever, and only one clinical assay (www.isrctn.com/ISRCTN16597283?q=&filters=recruitmentCountry:Peru&sort=&offset=2&totalResults=24&page=1&pageSize=10&searchType=basic-search) has been developed for the chronic phase (291).

Treatment of Oroya Fever

It is often considered that *B. bacilliformis* was the most lethal bacterium in the preantibiotic era. Left untreated, Oroya fever is frequently referred to as having one of the highest death rates of all infectious diseases, being from 40 to 85% (141) and achieving 88% in a study by Gray and colleagues (26). Fortunately, effective treatment is now available, and the mortality rates have decreased.

The first treatments were based on traditional folklore, including herbal extracts such as "uña de gato" (*Uncaria tormentosa*) or "agua de mote con vino o chancaca" (water in which maize has been cooked and then mixed with wine or chancaca) (292), as well as dubious procedures based on the use of oral or endovenous glycerin, tripaflavin, chaulmogra oil, or *para*-aminobenzoic acid, among other substances (293, 294). Repeated injections of citrate blood from the same patient were also made (295). It is of note that in several areas of endemicity, some of these traditional treatments were reported to remain in use as late as 1978 (6). In order to "provoke the sprout" of warts, herbal extracts such as those mentioned above or others such as "quisuar" (*Buddleja incana*) infusion were used. Moreover, for the treatment of symptomatic pain and discomfort, thermal baths were made or methods to "absorb negative energies" such as the so-called "sobada de cuy" (rubbing a black guinea pig all over the patient's body) were applied.

Salvarsan and quinine were also tested for use during acute infections, with unsatisfactory results (289, 294). Immunotherapies were also tested, with immune rabbit serum with high antibodies titers being used in the early 1940s without conclusive results (134).

Blood transfusions are perhaps the most classical treatment and are recommended to treat the severe anemia typically found in the acute phase of the illness (33, 62, 145, 296). Thus, although the life of transfused erythrocytes is shortened, the survival is normal in at least 50% of cases (297).

Although several studies were developed in the 19th century, it is largely considered that the first antimicrobial discovered was penicillin by Alexander Fleming in 1929, which was introduced in clinical practice coinciding with World War II (298–300). Regarding Carrion's disease, the first treatments with penicillin were reported in 1944 (289, 379). Subsequently, several antibiotics were tested. A series of case reports in the literature described the use of antimicrobial agents such as streptomycin, chloromycetin (chloramphenicol), aureomycin (chlortetracycline), ilocitin (erythromycin), furadantin (nitrofurantoin), or acromycin (tetracycline) with satisfactory results, while terramicin (oxytetracycline), neomycin, or sulfamides showed poor or unsatisfactory results (294, 301–303).

Since 2003 and according to the Ministry of Health of Peru recommendations and guidelines, ciprofloxacin is the drug of choice for adults in the acute phase of Carrion's disease, while in severe cases, ceftriaxone should be added to the treatment (304) (Table 5). However, as mentioned above, *B. bacilliformis* is intrinsically resistant to nalidixic acid (305), and it has been suggested that ciprofloxacin is not adequate for treatment of the acute phase and should be removed from the current guidelines (38, 306). Nonetheless, the issue of the most adequate antimicrobial remains controversial. On one hand, successful treatment has been reported, as in the case of a patient with a massive erythrocyte infection (more than 95%) who received a 10-day course of ciprofloxacin and ceftriaxone with full recovery (307), while on the other hand, therapeutic failure and persistent bacteremia of up to 22.6% have been described in patients who have completed treatment schedules (308).

Prior to the inclusion of ciprofloxacin in the guidelines, some studies reported the use of chloramphenicol with good results (6, 139, 294, 309–311). In fact, although chloramphenicol had been extensively used since 1956 (48), a medical consensus considered this drug to be the treatment of choice for bartonelosis in acute cases from 1998 to 2003. It was then replaced by ciprofloxacin and relegated to rescue therapy. The reason for the change was the lack of clinical response in some patients (312–314),

TABLE 5 Current Carrion's disease treatment schedules in Peru[a]

			First-line treatment					Second-line treatment				
Demographic group	Phase	Illness	Antibiotic(s)	Dose[c]>	Interval	Duration	Route	Antibiotic(s)	Dose[c]	Interval (h)	Duration (days)	Route
Children[b]	OF	Uncomplicated	AMC	20	12 h	14 days	p.o.	CIP	5	12	14	p.o.
								CHL[d]	16.6/10	8	3/11	p.o.
								SXT	5	12	14	p.o.
		Severe	CIP + CRO	CIP, 5–7.5	12 h	14 days	i.v./p.o.	CIP + CAZ	CIP, 5–7.5	12	14	i.v./p.o.
				CRO, 70	24 h[e]	7–10 days	i.v.		CAZ, 16.6–33.3	8	7–10	i.v.
								CIP + AK	CIP, 5–7.5	12	14	i.v./p.o.
									AK, 7.5	12	7–10	i.v./i.m.
	PW		AZM	10	24 h	7 days	p.o.	RFP	10	24	21–28	p.o.
								ERY	7.5–12.5	6	14	p.o.
								CIP	5	12	14	p.o.
Teenagers and adults	OF	Uncomplicated	CIP	500 mg	12 h	14 days	p.o.	AMC	1 g	12	14	p.o.
								SXT	800 mg	12	14	p.o.
								CHL[d]	16.6/10	8	3/11	p.o.
		Severe	CIP + CRO	CIP[f], 400/200 mg	12 h	3 days/11 days	i.v./p.o.	CIP + CAZ	CIP[f], 400/200 mg	12	3/11	i.v./p.o.
				CRO, 2 g	24 h	7–10 days	i.v.		CAZ, 1 g	8	7	i.v.
								CIP+AK	CIP[f], 400/200 mg	12	3/11	i.v./p.o.
									AK[g], 7.5	12	7–10	i.v./i.m.
	PW		AZM	500 mg	24 h	7 days	p.o.	RIF	600 mg	24	21–28	p.o.
								ERY	500 mg	6	14	p.o.
								CIP	500 mg	12	14	p.o.
Pregnant or breast-feeding women	OF	Uncomplicated	AMC	1 g	12 h	14 days	p.o.	CHL[d]	16.6/10	8	3/11	p.o.
								SXT	800 mg	12	14	p.o.
								AMX	1 g	8	14	p.o.
		Severe	CRO + CHL	CRO 1 g	12 h	10 days	i.v.	CRO + AK	CRO, 1 g	12	10	i.v.
									AK, 500 mg	12	7–10	i.v./i.m.
				CHL[h] 1 g/500 mg	8 h/6 h	5 days/11 days	i.v./p.o.	CAZ + AK	CAZ, 1 g	12	10	i.v.
									AK, 500 mg	12	7–10	i.v./i.m.
	PW		AZM	1 g	1 day/1 wk[i]	3 w		RFP	600 mg	24	21–28	p.o.
								ERY	500 mg	6	14	p.o.

[a]Based on current official guidelines (34, 377). Abbreviations: OF, Oroya fever; PW, Peruvian warts; p.o., oral; i.v., intravenous; i.m., intramuscular; AK, amikacin; AMC, amoxicillin plus clavulanic acid; AMX, amoxicillin; AZM, azithromycin; CAZ, ceftazidime; CIP, ciprofloxacin; CRO, ceftriaxone; CHL, chloramphenicol; ERY, erythromycin; RFP, rifampin; SXT, co-trimoxazole.

[b]Individuals up to 14 years old or weighing <45 kg (if the weight is higher the teenager/adult guidelines will be followed). Doses for children are reported in milligrams per kilogram of body weight. Note that specific situations or complications may require schedule modifications (34, 377). In addition, for adults/teenagers and for pregnant women when no units are indicated, the doses are reported in milligrams per kilogram of body weight.

[c]For amoxicillin plus clavulanic acid or co-trimoxazole, the dose is in reference to the first antibiotic compound of each combination.

[d]Fifty milligrams per kilogram per day for 3 days; thereafter, 30 mg/kg/day for 11 days.

[e]In the guidelines 70 mg/kg/day is indicated, but no dosage information is provided.

[f]Four hundred milligrams every 12 h for 3 days; thereafter, 200 mg every 12 h.

[g]The maximum dose is 1.5 g/day.

[h]One gram every 8 h for 5 days (i.v.) followed by 500 mg every 6 h for 11 days (p.o., if possible).

[i]One gram per day once a week for 3 weeks.

which had been sporadically reported early in the 1950s (310). Moreover, similar to the case for chloramphenicol (15, 145) ciprofloxacin provided good coverage against *Salmonella* infections (315). In the study by Gray and colleagues mentioned above, none of 10 patients treated with chloramphenicol died, compared with 88% mortality among nontreated patients (26). In another study, 19 patients received chloramphenicol for 5 days and similar results were obtained; the temperature returned to normal within 24 h, and the erythrocyte count and size were reestablished. Relapse was infrequent, and when it occurred, further administration of chloramphenicol resulted in recovery (316). Moreover, in a retrospective report on 215 patients, clinical cure was obtained with chloramphenicol in 89% of the cases (313). A good response to therapy was also achieved in 23 patients with the use of chloramphenicol and another antibiotic (139).

The acute illness is often complicated by other infections, usually with *Salmonella* spp., which greatly intensifies the clinical condition of the patient (33, 143). In fact, the lack of improvement after 72 h of treatment may allow suspicion of a coinfection. A treatment scheme based on chloramphenicol has the advantage that it is a low-cost, broad-spectrum antibiotic which also covers potential coinfections (39). However, despite the effectiveness of chloramphenicol in some patients, therapeutic failures and persistent bacteremia, leading to asymptomatic carrier status, have been reported in other patients receiving this drug (37, 139, 308). In a study by Maguiña et al., 3 out of 42 patients did not respond well to chloramphenicol therapy, and it is notable that these 3 patients had an initial microorganism burden of $>$80% (139). Another study reported 50% persistent bacteremia after chloramphenicol treatment in 66 patients; 28.8% presented posterior Peruvian warts, and the remaining presented positive blood cultures or PCR results (308). Moreover, in Europe as well as other locations, the use of chloramphenicol in humans is restricted, and its use in livestock production is strictly forbidden because of its potential to produce side effects in the bone marrow (317).

Regarding *Salmonella* coinfections and ciprofloxacin, in the last years the percentage of clinical isolates of *Salmonella* spp. resistant to nalidixic acid has been on the rise worldwide (318–321), and it is extremely high in Peru (322, 323). This may lead to therapeutic failure during ciprofloxacin treatment of bloodstream *Salmonella* infections (324), being an established risk for the development of ciprofloxacin resistance (325). Moreover, although has been postulated that the development of ciprofloxacin-resistant *Salmonella* is difficult because of the severe effects on bacterial fitness, with impaired growth and decreased virulence capacity (326, 327), reports on ciprofloxacin-resistant isolates and the emergence of successful ciprofloxacin-resistant *Salmonella* clones have been described worldwide in the last years (319, 320).

These findings, together with the relevant presence of extended-spectrum β-lactamase (ESBL)-carrying *Salmonella* spp. in the area (323), indicate a scenario in which correct and early diagnosis and analysis of resistance levels of both *B. bacilliformis* and concomitant opportunistic pathogens are needed.

Treatment of Peruvian Wart

From 1969 to 1975 streptomycin was the drug of choice for the treatment of the chronic phase of Carrion's disease, and in the mid-1970s rifampin was introduced and became the first-line drug, showing better results than streptomycin (139, 328). In a study of 260 chronic-phase patients receiving rifampin, clinical cure was observed in 93.1% (313). Nonetheless, treatment failure with rifampin for Peruvian wart has also been reported (309, 329, 330), and alternatives have been used in the treatment of chronic-phase patients. A good response was obtained in an eruptive case treated with chloramphenicol (63), and in another case, a 12-year-old child was treated with sultamicillin and deflazacort for 10 days and showed rapid improvement of the overall symptoms and complete remission of skin lesions at 21 days (331). More recently, after the successful use of azithromycin (309), the Ministry of Health of Peru proposed the use of this antimicrobial (304) for the eruptive phase of the disease (Table 5).

MECHANISMS OF ANTIMICROBIAL RESISTANCE

In intracellular bacteria, such as *B. bacilliformis*, antibiotic resistance is related mainly to wild-type-specific target-encoded gene sequences or those acquired by inactivation or structure modification of antibiotic targets related to encoding DNA events such as insertions, deletions, or punctual mutations.

Although no plasmid has yet been reported in *B. bacilliformis*, their presence has been determined in other members of the genus *Bartonella*, with the first being described in 2003 in *Bartonella grahamii* (332). Thereafter, the presence of plasmids has been reported in other *Bartonella* spp., such as *B. tribocorum*, *B. schoenbuchensis*, and *Bartonella rattaustraliani*; interestingly, most of these plasmids encode proteins belong to T4SS (85, 92, 333). Moreover, it has been shown that other species of *Bartonella* may exchange plasmids with other microorganisms within amoebae (333). This finding suggests that the gut eukaryotome of *Lutzomyia* spp. might potentially act to favor the horizontal exchange of genetic material between *B. bacilliformis* and other microorganisms.

Fortunately, *Bartonella* spp. continues to be highly susceptible to antibiotics (334). Antimicrobial resistance levels have been established in a few clinical or collection isolates of *B. bacilliformis* (123, 305, 314, 335–337), and it should be taken into account that *in vitro* susceptibility does not consistently correlate with the *in vivo* outcomes for patients (330). In addition to the constitutive nalidixic acid resistance and related diminished fluoroquinolone susceptibility described previously (305, 338), in a recent report 26% of isolates presented resistance to ciprofloxacin (314). The current scenario is completed with the relatively high MICs of clindamycin and colistin (335), the sporadic low resistance to chloramphenicol, and the trend toward diminished susceptibility to some aminoglycosides (314, 336).

Despite the description of antibiotic treatment failure as early as 1948 (339), information about the mechanisms of antimicrobial resistance in *B. bacilliformis* is scarce and is mostly focused on constitutive quinolone resistance. Indeed, studies analyzing the quinolone resistance-determining regions (QRDRs) of *B. bacilliformis* clinical isolates, including several recovered prior to the introduction of quinolones, showed an alanine at both position 91 of GyrA and position 85 of ParC, which are responsible for the intrinsic resistance of *B. bacilliformis* to quinolones (305, 337). This characteristic has also been reported in other members of the *Bartonella* genus from which the DNA sequence encoding GyrA has been determined (338), as well as in other microorganisms having constitutive resistance to quinolones, such as *Brevundimonas diminuta* (340).

To our knowledge only four *in vitro* studies have been developed with *B. bacilliformis*, selecting *in vitro* resistance to different antimicrobial agents, including chloramphenicol, ciprofloxacin, coumermycin A1, erythromycin, and rifampin (123, 330, 341, 342). In these studies, ciprofloxacin resistance ranked among the most easily selected, while chloramphenicol resistance ranked among the most difficult to obtain (123). Additionally, 3 reports have also analyzed the presence of antibiotic resistance-related mutations in clinical isolates of *B. bacilliformis* (337, 343, 344), but 1 of these studies did not include the MICs of the antibiotic (344) (Table 6).

In both *in vivo* (343, 344) and *in vitro* (123, 329, 342) studies, the most frequently described amino acid substitutions involved in the development of ciprofloxacin resistance are in the classical QRDR of GyrA, affecting positions 91 and 95. This is in accordance with what has been widely described in the equivalent amino acid positions (e.g., positions 83 and 87 in *E. coli* numeration) in other microorganisms and related to the mechanisms of quinolone target-DNA interactions (345). In addition, unusual mutations, such as those affecting codon 89 (Gly89→Cys) and 90 (Asp90→Gly), have been described *in vivo* and *in vitro*, respectively (40, 344). The same and other amino acid substitutions have been reported, albeit rarely, at equivalent positions in other microorganisms, such as *E. coli* (345, 346). Interestingly, the equivalent Asp90→Gly substitution has previously been described in *E. coli* (Asp82→Gly) having

TABLE 6 Antibiotic resistance mutations described in *B. bacilliformis*

	Mutation(s) causing resistance to[a]:									
	Cip		Azm			Chl	Rif	Cou A1	Strep	Trim
Study type	GyrA	GyrB	L4	L22	23S rRNA	23S rRNA	RpoB	GyrB	S7	DHFR
In vitro	**D90→G**, **A91→V**, **D95→G**, **D95→N**	**E475→K**	**Δ62-65**, **Q66→K**, **G70→R**, **H74→Y**[b]	83::VSEAHVGKS	**A1983→G**	**G2372→A**	**Q527→R**, **H540→Y**, **S545→F**, **S588→Y**	**G124→S**, **R184→Q**, **T214→A**, **T214→I**		
In vivo[c]	**G89→C**, **A91→V**, **S92→P**, **D95→N**, **D95→Y**	**S472→F ?**[d]							A39→T[e], D48→E[e]	H115→R[e]

[a]Abbreviations: Cip, ciprofloxacin; Azm, azithromycin; Chl, chloramphenicol; Rif, rifampin; Cou A1, coumermycin A1; Strep, streptomycin; Trim, trimethoprim; DHFR, dihydrofolate reductase. Data are reported in the following format: wild-type amino acid (or DNA base for the 23S rRNA gene), protein (or gene for the 23S rRNA gene) position→mutant amino acid (or DNA base for the 23S rRNA gene) (e.g., for D90→G, D is the wild-type amino acid, 90 is the position in the GyrA protein, and G is the amino acid found in the resistant strain). Bold indicates that alterations (the same or other) involved in antibiotic resistance development have been found in an equivalent position in other microorganisms (either *in vitro* or *in vivo*). In addition, the presence of polymorphisms has been observed in some of these genes (e.g., T13→A in L4 or R9→C in L22) (123).

[b]Although not alone, a mutation has been described in the equivalent position of *in vitro*-obtained erythromycin-resistant mutants of *E. coli* and *B. henselae* (363, 378).

[c]The MIC has been reported for only one ciprofloxacin-resistant clinical isolate with an Ala91→Val substitution (343). In the remaining cases, the presence of mutations has been reported without data on antibiotic resistance.

[d]The authors indicated the change Ser472→Phe. *B. bacilliformis* possesses a Leu at amino acid codon position 472. A probable scenario is a reading mistake and the presence of the change in position 473 or 474 (both presenting Ser as the wild-type amino acid).

[e]The role in the development of antibiotic resistance remains to be determined.

the atypical nalidixic acid-susceptible, ciprofloxacin-resistant pattern (346). Unfortunately, in the study describing this mutation in *B. bacilliformis*, only an increase in the MIC of ciprofloxacin from 0.3 to 0.9 μg/ml was reported, with no determinations of the MIC of nalidixic acid (342). Regarding other quinolone targets, the Glu475→Lys substitution in GyrB causes a slight increase in the MICs of ciprofloxacin (123). The region surrounding the equivalent amino acid position is largely involved in the development of decreased susceptibility or resistance to fluoroquinolones. Thus, amino acid substitutions have been observed in the equivalent Glu475 position of other microorganisms, such as *Salmonella* spp. (Glu465→Leu), *Streptococcus pneumoniae* (Glu474→Lys), *Bacteroides fragilis* (Glu478→Lys), and *Helicobacter pylori* (Glu463→Lys) (347–350). Although there are no data on quinolone susceptibility, the Ser472→Phe substitution in GyrB has also been described (344). Nonetheless, careful review of all the *gyrB* genes of *B. bacilliformis* recorded in GenBank showed the presence of Leu as a wild-type amino acid 472, while Ser is present at both positions 473 and 474, suggesting that mutations are located at one of the two latter points. Accordingly, an analysis of what has been described in other microorganisms showed the presence of the same and other amino acid substitutions in the equivalent Ser474 position of *Mycobacterium* spp., *Proteus mirabilis*, *Pseudomonas* spp., and *Salmonella* spp., among others (350–354). Furthermore, the GyrB amino acid changes Gly124→Ser, Arg184→Gln, and Thr214→Ala/Ile have been involved in the development of coumermycin A1 resistance (341).

Regarding rifampin resistance, three "hot" regions have been established in the *rpoB* gene in other microorganisms. These regions are the so-called cluster I, cluster II, and cluster III (following *E. coli* numeration, amino acid 505 to 537, 563 to 575, and 684 to 690, respectively), in which mutations leading to rifampin resistance have been described (355, 356). Regarding *B. bacilliformis*, 4 different mutations in the *rpoB* gene were observed in the 2 *in vitro* studies carried out to date (123, 329). Accordingly, three of these mutations are found in the equivalent cluster I region, i.e., Glu527→Arg, His540→Tyr, and Ser545→Phe, with the remaining Ser588→Tyr being located in the equivalent cluster II region. Amino acid substitutions leading to rifampin resistance have been described in equivalent positions in other *Bartonellaceae* as well as microorganisms such as *E. coli* or *Mycobacterium tuberculosis* (357–360). In accordance with what has been widely described in other microorganisms (361, 362), including other members of the *Bartonella* genus (363, 364), macrolide resistance has been related to the 23S rRNA gene mutation A1983G (equivalent to A2058G following *E. coli* numeration) (329). This finding is frequently found in microorganisms with a small number of *rrn* loci (361, 362, 365). Nonetheless, in a larger study selecting independent azithromycin-resistant mutants, only alterations affecting L4 and/or L22, including insertions and deletions, were found (123). These alterations were located within the regions involved in macrolide resistance development in other microorganism and were at the same positions in several cases (123). In one study, the fitness cost of antibiotic resistance was clearly highlighted on analyzing 2 chloramphenicol-resistant *B. bacilliformis* mutants with the G2372→A 23S rRNA gene mutation (123). Thus, in both mutants the 23S rRNA substitution reverted, and the resistance levels substantially fell after 5 passages without antibiotic pressure (123). It has been suggested that structural changes at the peptidyl transferase center affecting chloramphenicol binding impair ribosome function (366). This involvement at the fitness level also agrees with the enhanced difficulty in selecting chloramphenicol resistance and with the high persistence of bacteremia of up to 50% following chloramphenicol treatment (308) even if transitory chloramphenicol-resistant low-fitness *B. bacilliformis* is selected *in vivo* during treatment. Thereafter, immune system pressure may lead to the control of the disease but not necessarily to its clearance. In fact, on assessing the stability of the resistance acquired to chloramphenicol, ciprofloxacin, azithromycin, and rifampin, only resistance to rifampin, which was related to different mutations in the *rpoB* gene, remained constant. This emphasizes the serious risk of selection of stable rifampin resistance during treatment (123).

Additionally, mutations in aminoglycosides and folate inhibitor targets have also

been observed in *B. bacilliformis* clinical isolates. However, their association with resistance remains to be established (344).

Efflux Pumps

Efflux pumps are involved in the expulsion of toxic substances from the bacteria, including antimicrobials, contributing to the intrinsic resistance to antibiotics. Efflux pumps can be specific, extruding a single substrate, or generalist, affecting a variety of compounds, including antibiotics from different families. This is important for the development of multiresistance, since only one type of pump can confer simultaneous resistance to a wide range of antimicrobial agents (367). Although not specifically studied, different efflux systems have been identified by sequence homology during the genomic sequencing of different *B. bacilliformis* strains (131).

To our knowledge, only one study has analyzed the role of 2 efflux pump inhibitors, phenylalanine-arginine-β-naphthylamide (PAβN) and artesunate, in resistant mutants obtained *in vitro* (123). This study showed the first evidence of the role of efflux pumps in the levels of resistance to ciprofloxacin, azithromycin, and rifampin, being especially of note for chloramphenicol; thus, one chloramphenicol-resistant mutant presenting a decrease in the MIC from 4 μg/ml to 1 μg/ml and 1.5 μg/ml (with PAβN and artesunate, respectively) was described.

ERADICATION OF CARRION'S DISEASE

In 1997, the Dahlem criteria on the feasibility of eradicating an illness were described (368). These criteria consider that the effectiveness of an intervention, the sensitivity of the diagnostic tools, and the fact that humans are the only vertebrate reservoir are the 3 main factors which should be taken into account when determining the actions to be implemented.

Based on these criteria, Carrion's disease is a potentially eradicable illness, mainly due to its restricted geographic location, the lack of nonhuman reservoirs, and other factors such as the advances toward obtaining better, inexpensive, and sensitive diagnostic tools. Some of the tools proposed are based on antigenic candidates, which may also lead to the design of preventive strategies and enhance the current good activity of antibacterial agents (50, 271, 272, 369).

Illness eradication may be approached from different flanks, including the use of vaccines, as in the successful history of worldwide smallpox eradication in 1980 (370), or by massive antibiotic treatments, as has been proposed to eradicate yaws (371).

Regarding Carrion's disease, the characterization of several antigenic candidates, which have been explored in the field of development of new diagnostic tools (50, 159, 276, 277), might also be useful for the development of vaccines. Meanwhile, the current high levels of antibiotic susceptibility presented by *B. bacilliformis* can support the mass treatment approach, but the possibility of resistance development, which might underlie the high number of microbiological failures in clinical treatments and lead to the emergence of resistant strains, precludes the exclusive use of mass treatments for Carrion's disease eradication purposes. Probably the most efficient approach will be a mixed formula: the use of a protective vaccine combined with massive antibiotic treatment.

Nonetheless, the absolute lack of visibility of this illness, related to the nature of the affected populations, is the Achilles heel which prevents strong social and political commitments to efforts to eradicate Carrion's disease. Therefore, it is necessary to construct a narrative involving groups from policymakers to field-based physicians and to obtain the cooperation of community leaders and local educators, the support of international health authorities and developed world-based public and private fundraisers, and the collaboration of local and foreigner researchers and of journalists. It is necessary to act but also to listen: to listen to local inhabitants, because they are the target population of any action which will be designed, and to eliminate their doubts, their suspicions, and their fears.

EPILOGUE

While the hands of the research clock point to mark the future, and exciting new scientific and technical developments allow the development of direct methods to prevent, diagnose, and treat infectious diseases, Carrion's disease remains lost in time, broken and stranded, submerged in darkness and oblivion. Carrion's disease is an illness which is restricted to small isolated rural areas of middle-income countries far from the tourist routes and the developed world and consequently completely outside international focus and forgotten by the great majority of funding sources. It affects mainly the poorest populations and thus may not be perceived as relevant or generate sufficient interest to receive funding or encourage scientific interest. This lack of visibility is clearly the biggest obstacle to achieve the eradication of Carrion's disease. Notwithstanding this, although the number of people affected is low compared with those affected by other infectious diseases and the clinical management of Carrion's disease is relatively easy with the administration of antibiotics, this disease is currently expanding to new areas, and albeit few for now, people are dying from this disease, especially during outbreaks. Indeed, any effort to improve the diagnosis and treatment of Carrion's disease would have a great impact on the communities living side by side with this disease, and in the end, we should ask the question, "Will only those who are affected benefit, or will we all?"

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Cláudia Gomes obtained an Ms.C. in microbiology at the Universidade Nova de Lisboa, Portugal, and a Ph.D. at the Barcelona Institute for Global Health (ISGlobal), University of Barcelona, Spain. She has been a research fellow at ISGlobal with a grant from the Instituto de Salud Carlos III and at the National Institute of Infectious Diseases in Tokyo, Japan, with a grant from the Canon Foundation in Europe. Her research interests have been focused on infectious diseases from low- and middle-income countries, and during the last 4 years she had been working on Carrion's disease, with an emphasis on diagnosis as well as the mechanisms of antimicrobial resistance.

Joaquim Ruiz, Ms.C., Ph.D., is a microbiologist who graduated from the Science Faculty of the Universitat Autònoma de Barcelona. He has developed his scientific career at the Hospital Clinic of Barcelona, being Associate Research Professor at the Barcelona Institute for Global Health and Visiting Lecturer at the Universidad Peruana Cayetano Heredia in Lima. During recent years he has focused his research on the knowledge of the dynamics of the development of antibiotic resistance and dissemination through different environments and on the study of infectious diseases in low- and middle-income countries, mainly diarrheagenic pathogens affecting children. Since 2008 he has been leading a research line devoted to the understanding and knowledge of Carrion's disease.