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Microbiology: a dangerous profession?

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The history of science contains many cases of researchers who have died because of their professional activity. In the field of microbiology, some have died or have come close to death from infection by agents that were the subject of their research (Table 1). Infections that had a lethal outcome were usually accidental. Sometimes, however, researchers inoculated themselves with the pathogen or did not take preventive measures against the potential pathogen because they wanted to prove their hypotheses—or disprove someone else's—regarding the origin of the infection. Here is an overview of several episodes in the history of microbiology since the mid nineteenth century involving researchers or workers in fields related to microbiology who have become infected. They are considered here in their historical context to provide insights into some of the pillars of modern microbiology—the giants on whose shoulders several generations of microbiologists have stood to see further.

From the Lady with the Lamp to the Lady with the Microscope. Mediterranean, Malta, Gibraltar, and Crimean fever are several of the names by which the disease called undulant fever after 1913—now brucellosis—was traditionally known. It was predominant in the Mediterranean region and its history has been associated to military medicine. Hoover and Friedlander refer to British army surgeon George Cleghorn (1716-1789), who was sent to Minorca during the British domination of the Balearic island, and described cases of chronic, relapsing febrile illness [16]. Cleghorn, who was in Minorca from 1736 to 1749, practiced medicine not only among British soldiers but also among the locals, and gathered a wealth of data about the most frequent diseases in the island. The result of his observations was a 311-page book titled *Observations on the epidemical diseases in Minorca from the year 1744 to 1749 to which is prefixed, a short account of the climate, productions, inhabitants, and endemical distempers of that island* (T. Cadell, D. Wilson and G. Nicol, London, 1751), which he dedicated to the Society of Surgeons of His Majesty's Royal Navy. Minorcan historian of science Josep M. Vidal Hernández has described and carefully analyzed Cleghorn's work in Minorca and his report [43]. According to Vidal, what Cleghorn describes is "tertian" fever, which was the name given at the time to fever caused by malaria parasites with a periodicity of 48 hours. In fact, Cleghorn used quinine to treat tertian fever (i.e, malaria), which was not eradicated from Minorca until the 1940s [43,44]. It was Spanish army surgeon Manuel Rodríguez y Caramazana (1765-1836), appointed *Cirujano Mayor* (Chief Surgeon) of the Militar Hospital of Minorca in 1802, who clearly referred to Malta fever in Minorca in 1813. He published an eight-page pamphlet titled *Modo de precaver a Menorca de la peste de Malta* (How to protect Minorca from the plague of Malta). The pamphlet was not received warmly because of the strict preventive measures he proposed [45].

The British army surgeon Jeffrey Allen Marston (1831-1911) contracted Malta fever in 1861 and described his own case in great detail in the statistical report of the British Army Medical Department that Marston wrote that same year, but published in 1863. Marston first distinguished Malta fever from other Mediterranean fevers [16,19], even though he did not know what the causal agent of the disease was. Another British army physician and microbiologist, Surgeon Captain David Bruce (1855-1931), with his wife and close collaborator Mary, succeeded in culturing the causal agent from the spleen of a patient who had died from Malta fever. Bruce named it *Micrococcus melitensis*, now known as *Brucella melitensis* [42]. The discoveries that apparently healthy goats could be the reservoir for the bacterium, and that milk of nat-

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Table 1. Some microbiologists or people who worked in fields related to microbiology who contracted the disease in which they worked (see text for details of each case)

Disease	Causal agent	Researcher or worker infected	Year
Brucellosis	*Brucella melitensis*	Florence Nightingale[s]	1855
	Brucella melitensis	Jeffrey A. Marston[s]	1861
	Brucella melitensis	Alice C. Evans[s]	1922
Carrion's disease	*Bartonella bacilliformis*	Daniel Alcides Carrión[d]	1885
	Bartonella bacilliformis	Ovidio García Rosell[s]	1928
	Bartonella bacilliformis	Maxime Kuczynski-Godard[s]	1937
Cholera	*Vibrio cholera*	Louis Thuillier[d]	1883
	Vibrio cholera	Max von Pettenkofer[s]	1892
Epidemic typhus	*Rickettsia prowazekii*	Howard Taylor Ricketts[d]	1910
	Rickettsia prowazekii	Stanislaus von Prowazek[d]	1915
Rocky Mountain spotted fever	*Rickettsia ricketsii*	Thomas Bailey McClintic[d]	1912
	Rickettsia ricketsii	Henry Cowan[d,f]	1924
Yellow fever	Yellow fever virus	Elihu H. Smith[d]	1798
	Yellow fever virus	Jesse W. Lazear[d]	1900
	Yellow fever virus	James Carroll[s]	1900
	Yellow fever virus	Adrian Stokes[d]	1927
	Yellow fever virus	Hideyo Noguchi[d]	1928
	Yellow fever virus	William A. Young[d]	1928
Eastern equine encephalitis	EEE virus	Richard E. Shope[s]	1959
Lassa fever	Lassa fever virus	Jordi Casals-Ariet[s]	1969
	Lassa fever virus	Juan Roman[d,t]	1969
Gastric ulcer	*Helicobacter pylori*	Barry Marshall[s]	1982
Sever acute respiratory syndrome	SARS virus	Carlo Urbani[d]	2003

[s]Survived the disease. [d]Died from the disease. [f]Field worker. [t]Technician.

urally infected goats was the source of human infection were made in 1905 by Themistocles Zammit (1864-1935), a Maltese physician whose role in the history of brucellosis has not always been credited. Zammit carried out experiments and epidemiological studies to prove his hypothesis, and found bacteria in the milk, blood and urine of infected goats. He even devised tests that were later used by others [47].

A few years before Marston's illness, Florence Nightingale (1820-1910), the British founder of modern nursing who made major contributions to public health and epidemiology during the Crimean War, had fallen ill with what was then called Crimean fever. After reading an account of the conditions of the British Army at Scutari (the district of Istanbul now known as Üsküdar), Nightingale had volunteered to go to Crimea leading a team of 38 nurses. British soldiers who had been wounded in the battlefront in the Crimean Peninsula, and diagnosed with medical diseases (in the mid-nineteenth century the cause of infectious diseases was still to be discovered) in the hospitals of Balaclava on the north shore of the Black Sea, were transported to Scutari to isolate them from healthy troops. Many soldiers succumbed during the journey across the Black Sea, which took around thirteen days [13]. Those who reached Scutari alive were more likely to die from infections in the hospital than as a

result of the war (for every soldier who died from his wounds, seven died from diseases contracted during their hospital stay).

Although Nightingale did not know about the role of bacteria and viruses in infection, she clearly understood the concept of contagion, the transmission of disease from person to person. Her accurate records and statistics proved the relationship between filthy conditions and mortality. The work she and her team of nurses carried out in Scutari resulted in a dramatic reduction in mortality among hospitalized soldiers [26]. The death rate among patients, which in February 1855 was 42.7%, decreased to 2.2% in June 1855 after the reforms in hygiene Nightingale introduced. Many current health-care practices, including cleansing of patient areas, aseptic preparation of foods, ventilation of wards, disposal of human and medical wastes, isolation of patients with antibiotic-resistant pathogens, and measures to prevent cross-contamination, are based on the work Nightingale undertook in Scutari [29]. At night, when orderlies substitued for the nurses, she was the only nurse in the wards. To check on the wounded soldiers she carried a lamp to see them in the dark, so patients called her "the lady with lamp."

On May 2 1855, while Nightingale was in Balaclava to inspect the hospitals there, she fell ill. Her severe symptoms and high fever forced her to stay in bed. The following weeks her condition was typical of undulant fever: she felt better in the morning and very ill again in the evening. By May 24, doctors considered that although she was still very weak, she was out of danger. Even though Nightingale died at the age of 90, after the Crimean war she became an invalid and was bedridden for many years before her death. Physicians of her time tended to consider her a malingerer or maintained that she was suffering from neurasthenia. Among twentieth-century authors who have studied her life, several have assumed that no organic disease could account for her illness. Others, however, have considered the symptoms she suffered after her return to Britain consistent with chronic brucellosis, especially brucellosis caused by *Brucella melitensis*.

Evidence for the existence of chronic brucellosis was obtained during the first half of the twentieth century, when Alice Catherine Evans (1881-1975), a microbiologist who studied the bacteria in fresh milk, became infected herself with *Brucella*. In 1918, Evans established the close relation between the causal agent of undulant fever in humans—*Brucella melitensis*—and that of abortion fever in cows—*Brucella abortus*. She found them indistinguishable in morphology and in culture requirements, and found that when inoculated into pregnant guinea pigs, the proportion of abortions was the same in both species. (Currently *Brucella* is considered to be a monospecific genus that should be named *B. melitensis*, and all other species are subtypes, with an interspecies homology greater than 87% [34]). Evans then suggested that raw cow's milk might be involved in the transmission of undulant fever to humans. In 1922, even though she had used precautions in her research, Evans became infected, and the microorganism was isolated from her blood. (The respiratory tract might have been the route of infection.). She subsequently suffered years of poor health or disability alternating with periods in which she felt that she recovered. At the meeting of The Society of American Bacteriologists (currently American Society for Microbiology, ASM) held in December 1927, she was elected President but could not attend the meeting because she was bedridden due to a relapse of her brucellosis. When Evans suffered the last disabling episode of the disease in 1943 she had already developed hypersensitivity to brucellar antigens and was no longer able to work with live cultures. Nonetheless, her own experience as a patient lead to valuable insights in her study of chronic brucellosis [9,32,35,48].

***Bartonella*, the missing link.** La Oroya is a Peruvian mining town located 180 km east of Lima in a deep, narrow valley 3371 meters above the sea level, where the rivers Mantaro and Yauli met. With a population of around 35,000, the town is notorious for being ranked among the ten most polluted locations in the world [8] due to emissions from a smelter facility. During the second half of the nineteenth century La Oroya was also notorious because of another health problem. In 1870, an epidemic of fever and anemia with a mortality of almost 100% killed thousands of the workers who were building the railway from Lima to the mining centers in the central Andes [6]. Because the disease was restricted to La Oroya and its valley, medical authorities called it "Oroya fever" until the eponym "Carrión's disease" became in use to honor Daniel Alcides Carrión (1857-1885), the medical student who proved that Oroya fever was another clinical manifestation of the disease known as "Peruvian wart," endemic in the region, but benign. The price he paid to prove the connection was, however, very high: his own life. Carrión was a student at the School of Medicine of San Marcos University, the only medical school at that time in Peru, and like his classmates and instructors, felt attracted to the study of diseases unique to a region of his country. In August 1885, he asked a classmate to inoculate him with blood drawn directly from the warts of a young female patient. Three weeks later, instead of warts on his skin, Carrión developed the first symptoms of Oroya fever. He was aware of his condition and understood that the two endemic diseases were actually the same. He made notes on the progress of his disease until he felt so ill that he was no

longer able to write. Then he asked his classmates to continue his medical diary on his behalf. He died on October 5, 1885; the date now marks the annual celebration of "Peruvian Medicine Day" [6].

In 1909, the Peruvian physician Alberto E. Barton (1870-1950) published an article in *La Crónica Médica* describing what he considered to be infectious particles in red blood cells of Carrion's disease patients, and called them "X particles" or "endoglobular elements." They were shaped like bacilli and differed from any other known microorganism. He noted that in Oroya fever the bacilli proliferated, whereas they almost disappeared when the patients developed warts [6]. Other researchers, including physicians Richard P. Strong and Hideyo Noguchi, and entomologist Charles Towsend, who worked in the USA and participated in expeditions to Peru, contributed to a better understanding of the causal agent of Carrión's disease [6], which nowadays is "viewed as a medical curiosity" [2]. Strong confirmed that the endoglobular bodies Barton described were bacilli and proposed a new genus and species, which he named *Bartonella bacilliformis* in honor of Barton. Noguchi traveled to Peru in 1919 as a member of the Rockefeller Foundation commission that was studying yellow fever (see the section on yellow fever below) and became familiar with Oroya fever and Peruvian wart, which he studied along with Telémaco Battistini, a Peruvian student at the Rockefeller Institute in New York. During the 1920s they published a series of articles describing their experiments, which confirmed that the diseases were indeed two distinct clinical manifestations of the same infection. (The final report was signed by Noguchi alone in 1927.) Towsend identified a nocturnal sandfly, known in Peru as the *titira* (*Phlebotomus verrucarum*, now *Lutzomya verrucarum*), as the vector of Oroya fever.

The history of bartonellosis records two other cases of researchers who became self-infected: Ovidio García Rosell and Maxime Kuczynski-Godard (1890-1967). García Rosell, whose medical specialty was phthisiology, fell ill with symptoms that were first attributed to tuberculosis, which he was assumed to have contracted from a patient. However, his fever soon disappeared and some time later he broke out in Peruvian warts. Before falling ill, he had pricked himself accidentally with a needle contaminated with the blood of a patient with the severe anemia of Carrión's disease, and this must have been the cause of the infection. In contrast, Kuczynski-Godard, a German-Peruvian physician, intentionally inoculated himself with infected blood 11 times over 37 days. Fortunately, the outcome was only the development of warts, as he later described in an article published in 1937 [5].

The power of faith. In 2003, *Hospital Doctor*, a British weekly magazine aimed at the hospital sector, organized a poll to identify the "greatest doctor ever." John Snow (1813-1858), who carried out seminal epidemiologic research to trace the source of a cholera outbreak in London in 1854, won the poll [Dr John Snow named the greatest doctor. John Snow site. UCLA http://www.ph.ucla.edu/epi/snow/ snow-greatestdoc.html]. John Snow's study, along with two other contemporary studies of the same cholera epidemic—a study of a localized outbreak in Broad Street, and an official study of the whole epidemic—took place a quarter-century before Robert Koch's description of *Vibrio cholera* as the causal agent of this fearsome disease.

Two of the scientists who participated in these studies described the presence of *vibriones* (in conventional nineteenth-century medical terminology, 'vibriones' were motile, elongated microscopic organisms) in several samples they analyzed. Richard Dundas Thomson (1810-1864), professor of chemistry at St. Thomas Hospital, observed them in distilled water in which air from a ward full of cholera patients had been trapped. Arthur Hill Hassall (1817-1894), author of the first English textbook on microscopic anatomy of the human body, also saw vibriones in rice-water stools of cholera patients. These findings well fit with the hypothesis of Snow, who believed that the origin of cholera was most probably a live organism which could be ingested accidentally from sources contaminated with feces, and then multiply in the body to be expelled in the rice-water stools, which were the cause of transmission. He even calculated an incubation period of 24–48 hours before the onset of the disease. However, the prevailing paradigm was that the origin of epidemic diseases lay in the influence of decaying organic matter, and that the "miasmas" that emanated from such matter caused the disease when inhaled. Hence, the presence of vibriones was considered an epiphenomenon of the disease, not an explanation of its origin [33].

Thomson and Hassall were not the only ones to have noticed that vibriones were related to cholera before Robert Koch (1843-1910) did. Italian physician Filippo Pacini (1812-1883) became interested in the disease when the Asiatic cholera pandemic of 1846–1863 reached Florence, his hometown. He described the vibriones, found in myriads when he observed tissues of dead cholera patients, in his article *Osservazioni microscopiche e deduzioni patologiche sul cholera asiatico*, published in 1854. Only in 1965 was Pacini's discovery recognized, when the Judicial Commission of the International Committee on Bacteriological Nomenclature added his name to that of the species, so that now the correct name of the species is *Vibrio cholerae* Pacini 1854 [17].

In 1883, cholera threatened to spread throughout Europe. By then Robert Koch had already found the causal agents of anthrax and tuberculosis, and the Koch-Henle postulates for the definition of microbial pathogens had been established. Germany and France sent their best bacteriologists to Egypt to study the disease [21]. Koch traveled there with George Gaffky (1850-1917) and Bernard Fischer (1852-1915), whereas the French team included Isidore Strauss, Emile Roux (1853-1933), Edmond Nocard (1850-1903), and Louis Thuillier (1856-1883). In Alexandria, where the European research teams established their headquarters, misfortune accompanied the French expedition. On September 17, 1883 the young bacteriologist Thuillier became infected with cholera, and neither of his physicians colleagues could save his life. Thuillier died on September 19, a victim of the disease he had studied [17,21]. When the Egyptian epidemic faded, the German team traveled to India to continue work in Calcutta, which seemed to be a promising location for their research. It was in Calcutta that Koch finally succeeded in isolating the cholera bacterium in pure culture.

When Koch's team returned to Germany, they stopped in Munich, on their way to Berlin, to pay a visit to the Bavarian medical chemist Max von Pettenkofer (1810-1901), a noted experimental hygienist and strong believer in the miasma theory. He was convinced that the microbe played only a secondary role, and claimed that there must be a specific factor in soils for the germ to develop and produce the infectious material that eventually would cause cholera. The disease, according to von Pettenkofer, could not be transmitted person-to-person; instead, transmission must depend mainly on the condition of the soils. Koch presented to von Pettenkofer the results of his research in Egypt and India, but did not succeed in convincing him. Even though von Pettenkofer was wrong, the efforts he made to improve hygiene and sanitation in Munich reduced the incidence of cholera in that city, which reinforced his belief in the miasma theory. In 1892 he wrote to Koch to ask him for a sample of cholera germs. When von Pettenkofer received the sample, he diluted it in a flask of a meat gravy and swallowed the whole contents of the flask. The dose might have killed him, but he was fortunate enough to suffer only diarrhea over the following several days. Once he had recovered, he sent a note to Koch to thank him for the sample. In the note he also wrote that "*Herr* Doctor von Pettenkofer has now drunk the entire contents and is happy to be able to inform *Herr* Doctor Professor Koch that he remains in his usual good health" [17,46]. Von Pettenkofer's conclusion from not having developed symptoms of cholera was that *Vibrio cholera* did not cause the disease. What he actually proved was that exposure to the bacterium was not enough for a person to develop the disease.

Rickettsial diseases. At the turn of the twentieth century a mysterious deadly disease of unknown origin afflicted the settlers of the Bitterroot Valley in western Montana, USA, in spring and summer. The main symptoms were high fever, chills, muscle aches and headache. A rash that looked like small red spots or blotches and usually appeared on the third to fifth days after the first symptoms gave the disease its name: Rocky Mountain spotted fever. The Montana State Board of Health, set up in 1901, placed high priority on investigating the cause of the disease. During the first decades of the twentieth century, researchers from other regions of the country came to Montana hoping to find the cause of the disease and to develop an effective treatment. Among these researchers was Howard Taylor Ricketts (1871-1910), a pathologist and excellent microscopist from the University of Chicago. In 1906, Ricketts showed that the agent of spotted fever was present in the blood of infected humans and that it could be removed by filtration. Other researchers, including Louis B. Wilson and William M. Chowning from the University of Minnesota, suggested that the wood ticks were the vector of spotted fever, based on the facts that the disease was seasonal and that in all cases there was a history of exposure to a tick of the genus *Dermacentor* [41]. Ticks, like mosquitoes, were then thought to carry only protozoan parasites. In 1904, Wilson and Chowning assumed that the causal agent was a protozoan, and they even reported the presence of *Pyroplasma* (currently *Babesia*) in the blood of patients suffering from spotted fever.

In 1909, Ricketts reported that freshly laid eggs of infected *Dermacentor* ticks were "laden with astonishing numbers of an organism which appears typically as a bipolar staining bacillus of minute size," which seemed to confirm Wilson and Chowning's claim [38]. In contrast to the attitude of other researchers, however, Ricketts was prudent in his claims regarding the role of the microorganism he had found , and wrote that "[i]n spite of the constancy with which these bodies were found, it did not seem justifiable to claim that they represent the microparasite of the disease." He did not even name the microorganism but suggested only that it might "be referred to tentatively as the bacillus of Rocky Mountain spotted fever" [38].

A state budget shortage in Montana in 1910 made the continuity of Ricketts' funded research in Bitterroot Valley uncertain, so he accepted an offer to go to Mexico to study epidemic typhus [History of Rocky Mountain Labs, http:www3.niaid.nih.gov/about/organization/dir/rml/history/htm]. He had been invited to move to the Rockefeller Institute in New York, but he declined because he did not want to give up the subject of diseases transmitted by mosquitoes, fleas, and other arthropods. In addition, he was eager

to work in Mexico, especially when he learned that two researchers employed by the Mexican Government had been able to transfer epidemic typhus fever to monkeys by inoculating them with blood from a patient diagnosed with the disease. In Mexico, Ricketts fell ill on April 18, 1910, when he was about to return home. He made notes describing the development of his disease, and although he thought he was recovering, he eventually died from heart failure. He had already described the pathogen that killed him: a tiny bacillus he had found in the lice and the blood from patients suffering the disease, which very much resembled the one causing Rocky Mountain spotted fever. In 1916, Brazilian bacteriologist Henrique de Rocha Lima (1879-1956) named the typhus causal agent *Rickettsia prowazekii* in honor of Ricketts and parasitologist Stanislaus von Prowazek (1875-1915), another researcher who died from epidemic typhus. Von Prowazeck, who had studied the disease in the Balkans in 1913, was asked to study it in a Russian prisoner-of-war camp near Cottbus, southeast from Berlin. There he became infected and died [18,25,36].

Thomas Bailey McClintic, who also worked for the Montana State Board of Health supervising the measures for the eradication of Rocky Mountain spotted fever, was another victim of the disease he studied. An infected tick bit McClintic as he was working in the laboratory in 1912. He died on his way back to Washington to be with his wife and children [American Journal of Public Health, 2(9), September 1912, Editorial, p. 713]. Also in Montana, Henry Cowan, a field worker who gathered ticks for researchers from 1918 to 1924, and William Gittinger, a young researcher with whom Cowan worked, were victims of spotted fever. Cowen died in 1924 just as a vaccine for the disease was being tested. For him, the vaccine arrived too late [Devlin S. Spotted fever researchers. In: The 100 most influential Montanans of the Century. Missoulian Online http://www.missoulian.com /specials/100montanans/list/051.html]

The pioneers of yellow fever research. Among researchers who worked on the discovery of the cause of yellow fever, several were victims of the infectious disease they had tried to fight. Among the best known is the case of Japanese-American bacteriologist Hideyo Noguchi (1876-1928), who worked at the Rockefeller Institute. Noguchi, disregarding previous work by other researchers who had reported that yellow fever was caused by a filterable virus, claimed that the causal agent was a spirochete. He even described the isolation and characteristics of the bacterium, which he called "*Leptospira icteroides*" [30,31]. Repeated attempts by other researchers to prove that *L. icteroides* was also the cause of the disease in Western Africa had failed, and Noguchi was determined to prove that the bacterium was the only cause of the disease [28]. Noguchi died from yellow fever on May 28, 1928 in Accra, Ghana, where he had traveled to study the disease. His death preceded by two days that of William A. Young, director of the British Institute for Medical Research in Accra. Other researchers from the Institute suspected that Young might have inoculated himself with blood from Noguchi during the initial days of Noguchi's disease.

On September 19, 1927, several months before the deaths of Noguchi and Young, the Irish physician Adrian Stokes (1887-1927) died from laboratory-acquired yellow fever in Lagos, Nigeria. Stokes was on a sabbatical with the West African Yellow Fever Commission, which was funded by the Rockefeller Foundation. Along with two American colleagues (Johannes H.Bauer and N. Paul Hudson), he worked with an animal model, the rhesus macaque (currently *Macaca mulatta*), to which they applied Koch's postulates to prove that yellow fever was caused by a virus and not by Noguchi's leptospira spirochete. The experiment succeeded and was to become a milestone in virology. However, when the work was close to completion, Stokes contracted the disease in the laboratory and died [40].

Other researchers had died earlier from yellow fever. In 1798, Elihu H. Smith (1771-1798), a physician from Connecticut, succumbed to the disease, which he had described in several articles published in *Medical Repository*, a journal he had co-founded with Samuel L. Mitchill and Edward Miller. (*Medical Repository* was published from 1797 to 1824, and is considered the first medical journal published in the USA) [20]. In 1900, Jesse William Lazear (1866-1900), member of the US Army Yellow Fever Board—the other members were Walter Reed (1851-1902), James Carroll (1854-1907) and Arístides Agramonte (1868-1931)—died in Cuba after having been bitten by a mosquito infected with the disease that the Board was studying.

Officially, Lazear was infected "incidentally." Some of his notes, however, suggested that he intended to prove that the mosquito was actually the vector of the disease. [Bauer & Hudson; Philip S. Hench Walter Reed Yellow Fever Collection: yellowfever.lib.virginia.edu/reed/lazear.html]. In a letter that Agramonte wrote to the Cuban microbiologist Carlos J. Finlay (1833-1915) on April 12 1905, he recalled the cases of Lazear and James Carroll, the two members of the Board who had become infected during the experiments in which he himself also participated. According to Agramonte, Carroll did not believe that mosquitoes were the vectors of yellow fever and he did not do anything to prevent them from biting him. He contracted the disease but was fortunate enough to survive.The case of Lazear was different. He had been in Europe in 1894 and 1895; in addition to stay-

ing for some time at the laboratories of Robert Koch (1843-1910), Emil Roux (1855-1933) and Elie Metchnikoff (1845-1916), he also worked at the laboratory of Giovanni Battista Grassi (1854-1925) in Rome, where he became acquainted with Grassi's discoveries on the role of a mosquito species in the transmission of malaria [3,23]. Lazear became convinced that a mosquito was also the vector of yellow fever. On September 13, 1900 he allowed one of the infected mosquitoes caught by the staff of the Board at Las Animas Hospital to bite him. Five days later he fell ill and realized he had yellow fever. He soon developed albuminuria and jaundice and eventually died on September 25, 1900 "a victim of scientific zeal and indeed as a hero" [Letter by Agramonte to Finlay, reproduced in Cuadernos de Historia de la Salud Pública (Cuba) 2002, 92:87-88].

Eastern equine encephalitis. Equine encephalitis is a zoonosis that was first reported in Massachusetts in 1831, when 75 horses died from an unknown type of encephalomyelitis. Until the 1930s it was a veterinary disease never recorded in humans. In 1931, Swiss-born veterinarian Karl Friedrich Meyer (1884-1974) and his colleagues at the George Williams Hooper Foundation for Medical Research-University of California described the virus causing equine encephalomyelitis and reported that they had noted three people with symptoms that might be due to the virus. Meyer suggested the possibility that humans could also contract the equine disease. In a memorable lecture, he called attention to the major role that the animal kingdom could play as a reservoir of human pathogens. [39].

In 1933, Carl Ten Broeck (1885-1966) and Malcom H. Merrill (1903-1987) isolated the virus during an epizootic on the North American Atlantic coast [15]. In 1938, coinciding with an outbreak of encephalomyelitis in horses in Massachusetts, eight cases of human encephalitis occurred in the same area as the equine disease. The analysis of brain tissue from patients who died revealed the presence of the eastern strain of the equine encephalomyelitis virus. These were the first cases of human equine virus for which there was microbiological evidence [10]. Since then, cases have occurred sporadically as well as in small epidemics, mostly along the eastern and Gulf coasts of the USA (hence the adjective "eastern" in the name of the disease). From 1955 to 1993 there were 223 cases reported to the US Center for Disease Control and Prevention (CDC) with a peak of 36 cases in 1959, when there was an outbreak in New Jersey [7,14].

Richard E. Shope (1901-1966) was a brilliant virologist at the Rockefeller Institute for Medical Research in New York who studied the eastern equine encephalitis virus and became infected with it in 1959. Shope had worked in Ocean County, New Jersey, where there had been 18 cases with 10 deaths [New Jersey Mosquito Control Agency, at http://www.rci.rutgers.edu/~insects/oceprof.htm], and had collected mosquitoes suspected of carrying the virus. He was bitten and, when he started to have chills with muscle and joint pain, he asked a colleague, Delphine Clarke, to take a sample of his blood and examine it to search for the encephalitis virus, which she found. Up to then the virus had usually been isolated from brain and other tissues after the patient's death; so that was the first time the virus had been found in the blood of a patient. The virus did not attack Shope's brain; in fact, he survived and escaped paralysis and other frequent consequences of the disease such as mental impairment.

Out of Africa. Among the emerging diseases of the twentieth century, Lassa fever virus has become a growing public health concern, not only in the African countries where major outbreaks take place, but also in developed countries that have recorded patients with imported Lassa fever. Along with Hantavirus fevers, Lassa fever affects more people that any other hemorrhagic fever. Lassa fever owes its name to the Nigerian village of Lassa, where the first cases were detected in the 1950s [12]. The virus was not isolated, however, until 1969. Sonia Buckley (1918-2005), Jordi Casals-Ariet (1911-2004) and Wilbur G. Downs (1913-1991), from The Yale Arbovirus Research Unit at Yale University, isolated it from three missionary nurses from the USA who worked in northern Nigeria and became ill. Two of the nurses died, and the third one was sent to the USA, where she recovered after a nine-week stay in Columbia-Presbyterian Hospital in Manhattan, NY. In June 1969, Casals-Ariet fell ill, but did not initially relate his severe thigh-muscle pain to the disease because the nurses had not manifested this symptom. The physicians who were treating him were soon convinced that he had Lassa fever, but confirming the diagnosis would have taken 96 hours, and Casals-Ariet, who was already severely ill, would surely not have survived. His doctors took a risky decision: they called the nurse who had survived the first outbreak of the disease, took some of her blood, separated the plasma fraction that contained antibodies, and injected them into Casals-Ariet, who then recovered. Had he not been infected with the Lassa virus, however, the injection of the antibodies could have caused a dangerous cross-reaction [22; Altman LK (2004) Jordi Casals-Ariet, who found the Lassa virus, dies at 92. The New York Times, February 21, 2004]. Around Thanksgiving that same year, Juan Roman, a technician working on Casals-Ariet's team, fell ill and died a few weeks later. When it was confirmed that he had died from Lassa fever the team stopped working with live virus, and the remaining samples they had were sent to the CDC, where

they were kept in a biosafety laboratory. How Casals-Ariet and Roman became infected has never been discovered, which is disturbing because Roman, as a technician, had never worked directly with the virus. Some of their colleagues suggested that they might have inhaled viruses from dust in the cages of infected mice at Casals-Ariet's laboratory [Powell K (2004) A life among viruses. originally published in Yale Medicine, Fall/Winter 2004. Available online at http://yalemedicine.yale.edu/ym_fw04/capsule.html].

Event though Lassa fever is a rodent-borne disease, person-to-person transmission is also possible, and major outbreaks have occurred in homes and hospitals in Western Africa, associated with inadequate sanitary conditions and direct contact with infected blood and contaminated needles and syringes [12]. Of the 24 cases reported worldwide of patients with Lassa fever imported from Western Africa from 1969 to 2004, four occurred in physicians, four in nurses, and three in aid workers [24], who are often in contact with the poorest people or work in hospitals. In a study published in 1995 on cases of nosocomial Lassa fever in two hospitals in Nigeria [11], the authors concluded that the nosocomial outbreaks they had studied were the result of introducing modern medicine, mainly parenteral drugs and surgery, in inadequately equipped hospitals (where scarce disposable equipment was often shared or reused), whose staff had received little formal education or professional training acquired on the job. In fact, no outbreaks have been reported in the community or in better-managed hospitals in Nigeria.

Checking Koch's postulates. The incidents at The Yale Arbovirus Research Unit during research on Lassa fever led to changes in biosafety practices. In the USA, and also in other countries, strong measures to prevent infections when handling highly hazardous microbials have been adopted. Nowadays no one expects any microbiologist to infect himself or herself to prove or disprove a hypothesis. However, in 1982, Barry Marshall, codiscoverer, with J. Robin Warren, of the role of *Helicobacter pylori* as causal agent of gastric ulcers—for which they were awarded the 2005 Nobel Prize of Physiology or Medicine—swallowed a culture of the bacterium to prove his and Warren's hypothesis, which had been greeted with skepticism by their colleagues.

In the twentieth century, with the development of psychosomatic medicine, which linked the psyche and the body, many physical disorders of unknown etiology were considered to be biological manifestations of psychological disturbances. In the 1940s, peptic ulcer was considered a chronic psychosomatic disease attributed to stress and lifestyle [27]. Around 1970 the application of fiber optics to endoscopy made it possible to easily examine the stomach. The diagnosis of gastric and duodenal ulcers became easier, and gastroenterologists could take small biopsies from the lining of the stomach and study the pathophysiology of ulcer disease. Soon it became obvious that most people suffering from ulcer had also gastritis. The discovery of H2 receptors in the 1970s was a great advance in the treatment of peptic ulcers. Tagamet®, the first H2-receptor antagonist, was released for sale around 1975. It was a sort of magic drug capable of healing ulcers. No longer was it necessary for patients to take massive doses of antacids, nor did they need to be hospitalized except in extreme cases. However, Tagamet and other H2-receptor antagonists that were produced later were effective only if administered regularly. This characteristic, and the fact that peptic ulcer was a widespread disease in human populations made Tagamet the most profitable drug in the history of pharmacology between 1975 and 1980.

In 1979, Robin Warren, from Royal Perth Hospital, in Perth, Western Australia, reported finding spiral bacteria in samples from gastric biopsies of patients who had endoscopies. Warren found no previous reports in medical texts of these bacteria, which were apparently associated to gastritis, and his discovery was overlooked by his colleagues, probably because gastritis was not recognized as a major disease. In July 1981 Marshall joined Robin Warren's team, as postdoc. Although other researchers had found spiral bacteria in the stomach, Warren and Marshall were the first to suggest that they might be the causal agent of peptic ulcers. Marshall was asked to study the clinical records of patients suffering from gastritis and try to find any potential correlation between the presence of the bacteria and food, oral medication, or lifestyle, but he found no such associations. Apparently, anybody could become infected, but only people with the bacteria in their stomach developed ulcers. He and Warren succeeded at growing the bacterium, which turned out to be a new species similar to members of the genus *Campylobacter*, and that was not exclusive to Australia. In fact, Marshall studied histological preparations of gastric mucosasamples from the USA and the Netherlands, and the *Campylobacter*-like (now *Helicobacter pylori*) bacteria were also there.

To prove that these *Campylobacter*-like bacteria were the causal agents of gastritis and ulcers, it was necessary to inoculate an animal with the bacterium and show that it became infected. Marshall carried out these experiments but did not succeed in proving his and Warren's hypothesis. So he decided to infect himself with the bacterium, and swallowed the contents of a culture of the *Campylobacter*-like bacteria. After a week he felt the first symptoms of acute gastritis, confirmed by a biopsy that revealed the presence of the bacterium. Koch's postulates had been proved. Fortunately, gastric

ulcer is not a lethal disease. In addition, the treatment that they envisaged for the disease—a combination of bismuth with antibiotics—was successful, and Marshall soon recovered. Warren could not experiment on himself because he was already suffering from gastric ulcer! [Rose J (2000) Marshall J. Robin Warren - Interview, available at http://nobelprize.org; Marshall BJ (1998) Peptic ulcers, stomach cancer and the bacteria which are responsible (lecture given by Marshall as the recipient of the 1998 Heineken Prize for Medicine, available at http://www.knaw.nl/heinekenprizes/pdf/37.pdf].

The Vietnam hero. Severe acute respiratory syndrome (SARS) is an emerging disease of the twenty-first century caused by an unusual influenza-like virus. The first outbreak occurred in the Vietnam-French Hospital in Hanoi, Vietnam in 2003, and more than half of the 60 patients affected by the disease belonged to the hospital staff. Aware of the potential danger of the infection, many of them decided to quarantine themselves inside the hospital, to protect their families and the community. Carlo Urbani (1956-2003), from the World Health Organization (WHO) office in Hanoi, had been called in as an epidemiological and clinical expert to examine the patients at the Vietnam-French Hospital. He recognized the severity of the disease and the need to establish infection control safeguards, and warned the WHO, which made it possible to promptly develop coordinated global actions to keep the new disease under control [4].

Urbani was a member of the SARS working group that identified a novel coronavirus associated with the outbreak, and participated in the preparation of the manuscript describing the virus. Unfortunately, he could not see the printed article that reported the group's discovery, which was published in the March 31, 2003 issue of the *New England Journal of Medicine*: he died in Bangkok two days before the release of the issue. Urbani's wife was very concerned about his husband's close contact with patients suffering from such a severe and highly contagious disease. Urbani told her "If I cannot carry out my work as a physician under these circumstances, why I am here? To answer e-mails, attend cocktail parties representing the WHO, and write mission reports?" [1]. On March 11, during a flight to Bangkok, where he was to attend a meeting, Urbani felt the first symptoms of the disease, and on his arrival he told a colleague from the CDC no to approach him too closely and to call an ambulance. [37,4]. He was hospitalized and died on March 29. The abundant correspondence and reports he sent to colleagues, mainly at the WHO and the CDC from March 4 to March 10—his last week at work before falling ill—illustrates his uncommon abilities as a clinician, his competence as an epidemiologist and expert in infectious diseases, and his devotion to patients. The report he wrote on March 10 was the first report to be released internationally [1].

Concluding remarks. Nowadays, laboratories where infectious microorganisms are studied have adopted strict biosafety standards regarding practices, equipment and facilities to prevent any possible hazard to the staff, the environment and the community. In the last few years, some countries including the USA seem to be more concerned over biosecurity than over biosafety, and have adopted measures to prevent access to microorganisms that could be used as bioweapons. After several cases in which scientists have been charged with infringing the rules for the handling of hazardous biological materials, others have given up their research and considered that dangers coming from political interference can be even worse than those intrinsic to their own work in the laboratory.

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