Juan Ernesto Ludert · Flor H. Pujol · Juan Arbiza
Editors

Human Virology in Latin America

From Biology to Control

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Foreword

The publication of this book, which includes the main aspects of human, animal and environmental virology in the Latin American and Caribbean region (LAC) in 2016, coincides with important achievements and challenges in public health in the region.

According to the Pan American Health Organization/World Health Organization, in September 27, 2016, "The Region of the Americas is the first to have eliminated measles in the world, a viral disease that can cause severe health problems, including pneumonia, brain swelling and even death. This achievement culminates a 22-year effort involving mass vaccination against measles, mumps and rubella throughout the Americas. Measles is the fifth vaccine-preventable disease to be eliminated from the Americas, after the regional eradication of smallpox in 1971, poliomyelitis in 1994, and rubella and congenital rubella syndrome in 2015". Another important achievement and innovative action in LAC occurred from March 2006, when rotavirus A vaccines were introduced in national immunization programmes, considerably reducing hospitalizations and mortality related to rotavirus A diarrhoea.

Despite these important achievements, new challenges have arisen in the region. Considering only arboviruses, the four dengue virus serotypes circulate here, and recently the introduction and the pandemic dispersion of the Chikungunya and Zika viruses were observed. In this context, both the original and scientifically robust contributions of virologists and the regional scientific community who generated knowledge of these viruses should be highlighted. Thus, considering the achievements and challenges, it is possible to state that virologists and virology in LAC followed an innovative course and contributed in expressive ways in the generation of scientific knowledge and to the understanding of different phenomena related to the existing "virosphere" in LAC.

This book, *Human Virology in Latin America: From Biology to Control*, edited by J. E. Ludert, F. H. Pujol, J. Arbiza, contains 22 chapters that have been contributed by expert virologists on topics of great relevance to Public Health in the LAC. Starting with the history of virology in the region that occurred between the sixteenth and twenty-first centuries, Chap. 1 describes the scientific knowledge generated over time about the main viruses, and their impact on public health and

prevention actions relevant to the region. This volume covers five fields of virology: viral gastrointestinal diseases, including the unprecedented environmental virology (Chaps. 2, 3, 4, 5, 6 and 7); mosquito-borne viral diseases (Chaps. 8 and 9); hemorrhagic, skin and respiratory viral diseases (Chaps. 10, 11, 12, 13, 14 and 15); sexually and blood-borne transmitted diseases (Chaps. 16, 17, 18, 19 and 20); prevention and treatment (Chaps. 21 and 22). This book undoubtedly reflects the resizing of virology in LAC. We hope that this initiative will be the catalyst for the creation of a Latin American and Caribbean Society for Virology (LACSVI), similarly to the European Society for Virology.

The LAC countries bear a rich socio-biodiversity that concentrates the greatest extension of forests dedicated to biodiversity conservation (26%). In addition, out of the ten countries with the largest freshwater reserve on the planet, three are in LAC (Brazil, Colombia and Peru). The concept of One Health aims to reduce the risks of emergence and spread of infectious diseases resulting from the interface between animals, humans and ecosystems. In this context, viruses are important agents of emerging and re-emerging diseases, since they do not occupy any specific and permanent ecological niche in a conservative way. On the contrary, due to their intrinsic capacity for evolutionary mechanisms, they present potential to parasitize alternative host species.

Considering the infectious diseases of viral aetiology presented in this text, integration between human health, animal health and environment propels new challenges for LAC, mainly in qualified human resources that can generate new knowledge of new multidisciplinary approaches and translational researches with respect to socio-biodiversity and maintenance of healthy ecosystems for a most just, responsible and sustainable economic and social development.

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Preface

Viruses were discovered at the very end of the ninetieth century, and although many known viruses to date cause no harm to humans, some of them, like Ebola virus, influenza or the human immunodeficiency virus (HIV) for example, are among the most fearful pathogens affecting humankind. Moreover, viruses have the ability to affect large areas or populations in short periods of time causing epidemic outbreaks that in occasions may become pandemic. Out of the 11 most important epidemics affecting the world in the last 14 years, 9 have been caused by viruses.

Latin America is an extensive region populated by more than 600 million inhabitants, containing an exuberant biological richness constituting one of the most diverse ecological regions on Earth. Since the 1980s the Latin America region has been affected, like the rest of the world, by the HIV, but also by the hemorrhagic manifestation of dengue. More recently the region was affected by a large outbreak of chikungunya and currently the region is battling to control the Zika epidemic. All these emerging viral diseases add to the more "classical" endemic viruses such as papilloma, viral hepatitis and those causing respiratory and gastrointestinal infections. Moreover, the region is under the constant threat of the emergence or reemergence of highly pathogenic human viruses such as yellow fever or Mayaro, some of which are currently silent under well-established Amazonia sylvatic cycles. Another threat is the introduction of viruses from elsewhere such as the Middle East Respiratory Syndrome (MERS).

Through the 22 chapters of this book, some of the most respected virologists working in Latin America provide their views of the state-of-the-art of virology in the region. They address issues that range from history to biology, pathogenesis, epidemiology, prevention and treatment of the most important human viral diseases in the region. Almost in every case, the answer to an emerging disease in the region has been reactive, even though lessons from past epidemic experiences, in combination with the current epidemiological, medical and scientific knowledge, should allow for a more proactive, early and, therefore, more efficient reaction. It is the hope of the Latin American community of virologists to generate original and valuable scientific knowledge that will not only impact the universal knowledge, but that

will also provide effective tools to alleviate the current burden caused by viral diseases in the region and to be better prepared for future contingencies.

We like to express our gratitude to all the authors who so generously and enthusiastically contributed their chapters. We hope that their work reaches and informs graduate students, scientists and public health authorities with updated, authoritative and useful information about the virology endeavor in Latin America, and no less important, that they inspire a new generation of scientists to become virologists!

Mexico City, Mexico — Juan Ernesto Ludert
Caracas, Venezuela — Flor H. Pujol
Montevideo, Uruguay — Juan Arbiza

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Part I
The Early Days

Chapter 1
Viral Epidemics in Latin America from the Sixteenth to the Nineteenth Centuries and the Early Days of Virology in the Region

José Esparza

1 Introduction

The known epidemiological history of Latin America started with the encounter between American and European populations after the voyages of Christopher Columbus [24, 30]. In the sixteenth and seventeenth centuries, newly introduced viral epidemic diseases rapidly spread among the American aborigines, causing "virgin soil" epidemics and affecting populations without previous exposure to the new pathogens, causing high mortality rates and resulting in the decimation of the American aboriginal populations [9, 10, 42]. To some extent, those early American epidemics biologically resembled the more recent "virgin soil" epidemics of chikungunya and Zika in the Americas [16].

By the second half of the nineteenth century, the concepts of epidemics and contagion began to be understood more scientifically, especially after the germ theory of disease was formalized thanks to the work of Louis Pasteur (1822–1895) and Robert Koch (1843–1910). This new understanding of disease led to the discovery of many microorganisms that were found to be specifically associated with different diseases. Although a vaccine against smallpox was developed in 1796 by Edward Jenner (1749–1823) [26] and one against rabies was developed by Louis Pasteur in 1885 [3], it was not until 1892 that the concept of filterable viruses was proposed by the Russian botanist Dmitri Ivanovsky (1864–1920) for the agent of the tobacco mosaic disease [25]. This discovery led to the identification of other filterable agents of disease (now known as viruses), which also resisted cultivation in standard bacteriological media. The first observation that an animal disease is caused by a filterable

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virus was that in Germany by Friedrich Loeffler (1852–1915) and Paul Frosch (1860–1928) in 1898 of food-and-mouth disease [28]. In 1901, Walter Reed (1851–1902) and collaborators were the first to identify, in Cuba, a human filterable virus as the cause of yellow fever [34]. It was during this exciting time of the birth of virology that the first steps in this science were taken in Latin America.

2 Viral Epidemics in Latin America (Sixteenth to Nineteenth Centuries)

2.1 Influenza

The Spanish medical historian Francisco Guerra [23] proposed that the first viral epidemic introduced by the Spanish explorers in the Americas may have been swine influenza, which broke out in Santo Domingo (Hispaniola) during the second voyage of Columbus in 1493. The epidemic might have been originated from infected pigs brought from the Canary Islands. Guerra suggested that the influenza epidemic spread to a number of islands in the Caribbean, causing large mortality among the aboriginal population.

Since 1510 and until the end of the nineteenth century, there appeared to have been ten or more pandemics of influenza [32], with evidence that most of them reached Latin American countries where they received different popular names: zamparina (Brazil, 1771), pasa diez (Colombia, 1808), susto de la pinacata (Mexico, 1826), la corcunda (Brazil, 1826), la jardinera (Peru, 1875), emisión (Peru, 1878), etc.

During the last influenza pandemic of the nineteenth century in 1889–1890, researchers used the new methods of medical microbiology in an effort to identify the microbial cause of influenza. An apparent breakthrough came in 1892 when Richard Pfeiffer in Germany, a protégé of Robert Koch, announced that he had found a bacillus as the cause of influenza, known as Pfeiffer's bacillus or *Bacillus influenzae*, which was probably *Haemophilus influenzae.* Moreover, bacterial vaccines were prepared and used at that time in an attempt to prevent influenza. Another 30 years later, the British investigators Andrewes, Smith, and Laidlaw [1] isolated, in 1933, the virus that causes influenza in humans.

2.2 Smallpox

However, the major epidemic killer in the Americas was smallpox, first introduced from Africa to Santo Domingo in 1518. From there, the smallpox epidemic spread in 1519 to Puerto Rico and Cuba, reaching Mexico in 1520 [14]. It has been repeatedly proposed that this first smallpox epidemic in the Americas contributed to the fall of the Aztecs and, a few years later, to that of the Inca Empire, where the epidemic arrived in 1525–1527.

That first American smallpox epidemic was followed by other epidemics in different countries in Central and South America, caused by multiple reintroductions of the disease from slaves of African origin. One of the worst smallpox epidemics of the sixteenth century took place between 1585 and 1591, affecting the Andean region, from Colombia to Argentina, causing great mortality among aborigines and Spaniards. The Amazonian forest may have protected Brazil from these initial epidemics that ravaged the Spanish territories; however, an epidemic introduced directly from Portugal affected all the Brazilian coast in 1562–1563. It is believed that epidemic diseases, especially smallpox, were the major cause of the demographic catastrophe of the American Indian population in the sixteenth and seventeenth centuries. By 1650, when censuses became more accurate, the American indigenous population was estimated as 6 million, down from an estimated of 50 to 100 million in 1492.

Multiple epidemics of smallpox occurred in the Americas during the seventeenth to the twentieth century. The standard societal response to those early epidemics was based on the isolation of cases to prevent further spread of the disease. However, from the 1760s, variolation (the inoculation of smallpox) began to be used in Latin America, some 40 years after the procedure was first introduced in England by Lady Mary Wortley Montagu [44]. The beginning of the end of the smallpox epidemics in Latin America came in 1804 with the introduction of vaccination (inoculation of cowpox) by the Royal Philanthropic Expedition of the Vaccine [15, 33]; the last case of smallpox in the Americas occurred in Brazil in 1971.

2.3 Yellow Fever

Yellow fever was one of the most feared epidemic diseases in the Americas from the seventeenth to the end of the nineteenth century [7]. The origins of the disease most likely lie in Africa, and the virus and its mosquito vector were probably brought to the Caribbean and South America by ship after 1492 [4]. Although epidemics that resemble yellow fever had been described in the Caribbean and Mexico since 1527, one of the first well-described outbreaks, mentioned in the *Chilam Balam* de Chumayel, was in 1648 in Yucatan, Mexico. During the sixteenth and seventeenth centuries, yellow fever became endemo-epidemic in the Americas, causing numerous outbreaks mostly in the Caribbean, Mexico, Venezuela, and Brazil. By the late seventeenth century, yellow fever had also reached ports in the United States, and in the nineteenth century, the epidemics also made inroads in European ports with commercial contacts with America. In 1871, a severe epidemic of yellow fever occurred in Buenos Aires, killing about 8% of the inhabitants of the city [37].

Today we know that yellow fever is caused by a virus and transmitted by mosquitoes, but that knowledge was only acquired at the very beginning of the twentieth century. In the urban cycle of yellow fever, the virus is transmitted from human to human by the *Aedes aegypti* mosquito. In the sylvatic or forest cycle, different species of *Aedes* serve as a vector between nonhuman primates and humans. The sylvatic cycle of yellow fever was described in Brazil in 1932 by the Rockefeller Foundation epidemiologist Fred Soper (1893–1977) [40].

2.4 Other Viral Epidemics

In addition to influenza, smallpox, and yellow fever, many other infectious diseases, bacterial and viral, affected colonial Latin America [24]. Their precise identification remains difficult because of the limited clinical information available and also because very often two or more epidemic diseases occurred at the same time.

What seems to be clear is that the third emerging viral infection in colonial Latin America was measles, which, similar to smallpox, was first introduced in the Caribbean around 1529 [9]. Measles arrived in Mexico in 1531, soon extending to all Mesoamerica and the Andes. Epidemics of a disease compatible with mumps were described in Mexico in 1550–1560, also extending to Mesoamerica. Both measles and mumps remained in Latin America as endemo-epidemic diseases. Dengue may have been introduced in the Caribbean as early as in 1635 [21]. However, cases compatible with poliomyelitis were only reported for the first time at the end of the nineteenth century, in Argentina (1860) and in Cuba (1879).

3 The Early Days of Virology in the Region

The Latin American society responded to those epidemic diseases with a number of activities that were pioneers in the field.

3.1 The Royal Philanthropic Expedition of the Vaccine

On May 14, 1796, Edward Jenner performed his best known experiment when he showed that the inoculation of an 8-year-old boy, James Phipps, with cowpox "material" protected him from developing disease after a smallpox challenge. This observation and others were privately published by Jenner in 1798 [26]. Information about Jenner's experiments was initially known in Spain in 1799, and the vaccine itself (cowpox material) reached Spain in December 1800; by the end of 1801, several thousand vaccinations had been performed in that country. At that time, dozens of booklets about vaccination were published in Spain and widely circulated in Spanish America. Moreover, several attempts were made to bring the vaccine to the New World using cowpox material in impregnated silk threads or sealed between small glass plates, but these methods proved unreliable on lengthy journeys and in warm climates.

In 1802, the Viceroy of New Granada (now Colombia) asked His Majesty the King of Spain for help after informing him of an epidemic of smallpox that had caused thousands of deaths. Consequently, on March 1803, King Charles IV instructed the Council of Indies to evaluate the means to introduce the vaccine to his American and Asian possessions. The process resulted in the selection of Francisco

Fig. 1.1 Francisco Xavier de Balmis, the director of the expedition that in 1804 brought the smallpox vaccine from Spain to Latin America

Xavier de Balmis (1753–1819) (Fig. 1.1) as director of an expedition that would take the vaccine to all corners of the Spanish colonies and around the world [12, 29, 33, 41]. The expedition was carefully planned and executed with three objectives: (1) to provide cost-free vaccinations to the general population, (2) to train local physicians in correct vaccine administration, and (3) to establish central and regional vaccination boards to ensure the preservation and distribution of the vaccine. The expedition left the Spanish port of La Coruna on November 30, 1803, aboard the corvette *María Pita*. It was directed by Balmis with the assistance of Joseph Salvany as Vice Director, two physicians, two surgeons, and four male nurses. To transport the vaccine on the 1-month voyage to the Americas, 22 nonimmune orphan boys, aged 3–9, would be sequentially vaccinated during the crossing by serial arm-to-arm inoculation. The expedition also included the rectoress of the La Coruna foundling house, Isabel Zendal Gómez, who took care of the children. The expedition lasted for 3 years, and new children were recruited along the way to maintain the arm-to-arm transfer of the vaccine.

The corvette *María Pita* made a first stop in the Canary Islands, and from there the expedition continued westbound to reach the island of Puerto Rico in February 1804. In March, the expedition sailed to Caracas, Venezuela, where it was received with open arms and enthusiastic collaboration. In less than 1 month, more than 12,000 people were vaccinated in Venezuela, where the first Central Vaccination Board was established on which other Spanish American boards were modeled. In Caracas, the expedition was divided into two groups. One group, led by Salvany, proceeded south to the Viceroyalty of Nueva Granada (Colombia) and Peru, with the final goal of reaching Rio de la Plata (Argentina). The other sub-expedition, directed by Balmis himself, sailed from Caracas to La Havana, Cuba, and from there to the Viceroyalty of New Spain (Mexico), where it arrived in June 1804. From there, the vaccine was taken to different places in Mexico and neighboring regions. In February 1805, Balmis sailed from Acapulco to the Philippines accompanied by

26 Mexican children to serve as vaccine carriers. For health reasons, Balmis sailed to the Asian mainland in September 1805, reestablishing vaccination in the Portuguese colony of Macau. Balmis returned to Europe by sailing around the African continent, finally landing in Cadiz on September 7, 1806.

An estimated 250,000 people were directly vaccinated by the expedition, with many more reached by the programs they established in different countries. When Jenner learned of the expedition, he said that he does not *imagine the annals of history furnish an example of philanthropy so noble, so extensive as this*.

3.2 Discovering the Etiology and Mechanism of Transmission of Yellow Fever

The full emergence of the germ theory of disease during the 1880s gave impetus to the search for the "germ of yellow fever," and many putative microorganisms were proposed at that time [11]. In Brazil, Domingos José Freire (1843–1899) reported that the pathogenic agent of yellow fever was *Cryptococcus xantogenicus* [6], whereas in the same country, João Baptista de Lacerda (1846–1915) proposed that the cause was the fungus *Cogumelo*. In Mexico, Manuel Carmona y Valle (1827–1902) believed that the cause was the mold *Peronospora lutea*. And even Carlos Finlay (1833–1915) (Fig. 1.2) in Havana had a candidate that he called *Micrococcus tetragenus febris flavae*.

In view of those multiple claims, in 1887, the U.S. government commissioned George Miller Sternberg (1838–1915) to clarify the situation. Sternberg was an army physician who is considered to be the first U.S. bacteriologist. After traveling to two endemic countries, Brazil and Cuba, in 1890, he emitted the verdict stating that none of the proposed microorganisms was the cause of yellow fever. However, Sternberg himself suggested that perhaps a new germ that he identified, known as *Bacillus x*, could be the cause. After these very definite conclusions by Sternberg, it came rather as a surprise when in 1897 Dr. Giuseppe Sanarelli (1864–1940)

Fig. 1.2 Carlos Finlay, the Cuban physician who in 1881 formally proposed the mosquito transmission of yellow fever, information that was used in 1900–1901 by Walter Reed and collaborators to advance our knowledge regarding the etiology and transmission of the disease

Fig. 1.3 Giuseppe Sanarelli, a distinguished Italian bacteriologist working in Uruguay, conducted pioneering work on the etiology of yellow fever and in 1898 discovered the viral etiology of rabbit myxomatosis

(Fig. 1.3), a distinguished Italian bacteriologist and professor of experimental hygiene in Montevideo, Uruguay, formerly from the Institut Pasteur in Paris where he was a disciple of Élie Metchnikoff (1845–1916), announced that he has discovered the true cause of yellow fever, *Bacillus icteroides* [38]. This time some American experts believed Sanarelli to be right, and even Sternberg considered the possibility that *Bacillus icteroides* and *Bacillus x* were one and the same.

Consequently, in 1900, Sternberg, who by then was the U.S. Army Surgeon General, sent to Cuba a new Yellow Fever Commission (the fourth one) with the main goal of confirming or refuting the claim that yellow fever was caused by *Bacillus icteroides*. It is important to remember that at that time Cuba was occupied by the United States as a consequence of the conflict known as the Spanish-American War that was initiated in 1898. The commission was led by Major Walter Reed; acting Assistant Surgeon General, James Carroll (1854–1907); Aristides Agramonte (1868–1931); and Jesse Lazear (1866–1900). After conducting the necessary research, the conclusion of the Commission was that *Bacillus icteroides* was simply a contaminant. Walter Reed concluded that *At this stage of our investigation… the time had arrived when the plan of our work should be radically changed*. So, the Commission went through a major paradigm change and decided that rather than studying the causes of the disease, they would focus on what transmits it. At this point, in August 1900, the Commission decided to consult with Carlos Finlay and to test his mosquito theory in human volunteers [17].

Carlos Finlay, a Cuban physician trained in Europe and the United States, had sent in 1865 a paper to the Academy of Sciences of Havana outlining his theory on weather conditions and its relationship to yellow fever. However, after a more careful study of the epidemics, he finally made the correct observation that the appearance of epidemics in the hot and wet summer months was not caused by a worsening of some of the miasmatic conditions but rather by an increase in the population of a

mosquito that Finlay referred to as the *Culex* mosquito, known at that time as *Stegomyia fasciata* and today as *Aedes aegypti*.

On August 14, 1881, Finlay read a paper before the Academy of Sciences of Havana, proposing that yellow fever was propagated by mosquitoes. He began by admitting that the cause of the disease was a *material transportable cause which may be either an amorphous virus, an animal or vegetable germ, bacterium, etc., but which consists in all cases of a tangible something which has to be communicated from the sick to the healthy in order that the disease may be propagated*. By the 1880s, the germ theory of disease was gaining followers within the medical community, and it was clear that Finlay accepted that concept rather than the popular miasmatic theory ("something in the air"). Finlay continued his presentation to the Academy, proposing that *it seems natural* that the agent that transmits the pathogenic material of yellow fever *could be found in that class of insects which, by penetrating in the interior of the blood vessel, could suck up the blood together with any infecting particles contained therein, and carry the same from the diseased to the healthy*. Through acute observation, Finlay correctly identified *Aedes aegypti* as the vector of the yellow fever virus [18, 19].

His presentation was received with total indifference, and for many years, Finlay was ridiculed because of his ideas. Nevertheless, that same year of 1881, Finlay commenced experimental inoculations on a series of 20 nonimmune Spanish soldiers, completing in 1900 a total of 102 cases. It has been argued that Finlay could have not convincingly transmitted the infection because his volunteers were not adequately isolated and the observed infections could have occurred by natural exposure to the infection rather than by the experimental mosquito transmission. Moreover, it has been argued that the experiments were not optimized for the "extrinsic" and "intrinsic" incubation periods of the virus. Transmission of yellow fever from human to human requires a competent mosquito to feed on an infected human and survive an extrinsic incubation period in which the virus replicates in the mosquito and disseminates to its salivary glands, whereupon it finally feeds on a susceptible human. Similarly, disease in humans occurs after an intrinsic incubation period in which the virus replicates and disseminates within the person. The extrinsic incubation period in *Aedes aegypti* has a median of 10 days at 25°C, and the intrinsic incubation period had a median of 4.3 days. Those concepts were unknown to Finlay, who did not consider the mosquitoes as biological vectors of the yellow fever virus, but rather as only "flying pins" that mechanically carried the yellow fever agent from human to human.

In 1900, Carlos Finlay had an opportunity to have his mosquito theory tested by the American Commission, and he personally provided the eggs of the mosquitoes to be used in the experiments [8, 19]. This time, the Commission was careful in isolating the volunteers to ensure that any infection observed in the volunteers was indeed experimentally transmitted by the mosquitoes. After some initial failures, the Commission achieved positive results when the experiments were designed to allow for the appropriate extrinsic and intrinsic incubation periods. Significantly, the mosquitoes had fed on cases within the initial 3 days of the disease and had been allowed to ripen for at least 12 days before the transmission experiments were conducted. One of the volunteers was a member of the Commission, Jesse Lazear, who developed a severe infection and died of the disease.

With these initial results, Walter Reed rapidly prepared a preliminary note that he presented on October 23, 1900, at the annual meeting of the American Public Health Association in Indianapolis, Indiana (USA) [35]. Reed returned to Cuba where he conducted additional experiments in "Camp Lazear," west of Havana near the adjacent suburban towns of Quemados and Marianao. Most of the volunteers were recent Spanish immigrants who were not immune to yellow fever (were not "acclimatized") and who signed what are probably the first examples of informed consent. More than 30 volunteers participated in the experiments, and 22 developed yellow fever.

On August 1, 1901, less than 1 year after the transmission experiments in Camp Lazear, James Carroll, from the Walter Reed Commission, who returned to Cuba and was aware of the foot-and-mouth experiments of Loeffler and Frosch, proceeded to demonstrate that filtered serum from one yellow fever patient can induce yellow fever when injected in a healthy volunteer. The subsequent filtration and passage of serum from the second subject to a third, with the same results, provided the evidence of a replicating infection agent rather than a toxin, and this was the demonstration that the yellow fever agent was a filterable virus [34].

Soon after the yellow fever work of Walter Reed and collaborators was publicized, there was a search in the literature for visionary individuals who have previously suggested a possible role of insects in the transmission of disease. Several such "predecessors" were identified, but among those one remains apart, Louis Daniel Beauperthuy (1808–1871) [5, 13, 20, 27] (Fig. 1.4). Beauperthuy was born in the island of Guadalupe in the French Antilles and studied in Paris, graduating as physician and surgeon in 1837 with a thesis entitled "De la climatologie" in which he analyzed the environment and its relationship to diseases. In 1839, he moved to Venezuela where he worked for a brief period as a naturalist for the Musée d'Histoire Naturelle de Paris. During a yellow fever epidemic that occurred in 1853 in Cumaná, Venezuela, he made the connection between mosquitoes and the spread of the disease, pointing out that among the many species of mosquitoes he studied, *the agents of this infection come in many varieties that are not all harmful to the same extent. The silly mosquito*

Fig. 1.4 Louis Daniel Beauperthuy, based on observations made in Venezuela, suggested in 1853 that yellow fever is transmitted by mosquitoes

variety, with white streaks on its legs, a somehow domestic variety, is the most common. Entomologists accept that Beauperthuy correctly identified the *Aedes aegypti* vector. In 1853, he published his observations locally in Cumaná, Venezuela, but also in the *Comptes Rendus des Séances* and in the *L'Abeille Médicalle* of Paris.

A major difference between the 1853 theory of Beauperthuy and the 1881 suggestion of Finlay was that Beauperthuy believed that the mosquitoes mechanically carried the yellow fever agent from unhealthy swamps to humans, whereas Finlay correctly proposed the human-to-human transmission of the yellow fever virus by mosquitoes, although it took several years for virologists to fully understand the biological role of mosquitoes in the epidemiology of arboviruses.

3.3 Early Identification of Rabbit Myxomatosis as a Viral Disease by Sanarelli in Uruguay

Myxomatosis was first recognized when it killed European rabbits *(Oryctolagus cuniculus)* in Giuseppe Sanarelli's laboratory in Montevideo, Uruguay, in 1896. After failing to detect bacteria in the lymph from the vesicles of diseased rabbits, Sanarelli described the myxomatosis agent as invisible. In 1898, Sanarelli reported his findings at the Ninth International Congress for Hygiene and Demography in Madrid, classifying the myxomatosis agent as a virus on the basis of its submicroscopic size, even though its filterability was not reported for some years [39]. However, Sanarelli noted in 1898 that centrifugation produced an infectious serum that did not contain microorganisms. Sanarelli's difficulties in demonstrating a filterable virus were probably the result of using a fine filter and the relatively large size of the myxomatosis virus (a poxvirus). It is noteworthy that the initial identification of the myxomatosis was made the same year that the German scientists Loeffler and Frosch reported the agent of foot-and-mouth disease as the first filterable animal virus [25, 43].

In 1911, workers in the Oswaldo Cruz Institute in Rio de Janeiro, Brazil, correctly further classified the agent of myxomatosis as a large virus, and Henrique de Beaurepaire Rohan Aragão (1879–1956) showed that it could be transmitted mechanically by insect bite.

3.4 Rabies Vaccination Arrives in Latin America as a Sign of Modernity

In 1880, Louis Pasteur began to work on rabies, a disease that plagued Europe in the nineteenth century. Starting in 1884, he presented the successful results of preventive rabies vaccines in dogs, establishing the principle of vaccination before exposure to rabies in animals. Pasteur then sought to improve his method and developed a means of attenuating the virulence of the rabies microorganism, which consisted in exposing the spinal cords of rabies-infected rabbits to the air in specially designed

flasks. Pasteur then had the idea of using this vaccine to create immunity after a dog bite and to give it to humans [3].

Pasteur took the next step in 1885 with the vaccination of a 9-year-old child, Joseph Meister, who was presented to Pasteur in his laboratory at the Ecole Normale in rue d'Ulm in Paris. The young boy arrived from Alsace presenting multiple deep dog bites, received 13 injections of rabbit medulla homogenate, 1 per day, and survived. Three months later, Pasteur repeated the experiment on a young shepherd, Jean-Baptiste Jupille, severely bitten by a dog. On October 26, 1885, Pasteur showed the promising results of his treatment against rabies in humans to the French Academy of Sciences. From then on, patients bitten by rabid animals flocked to Pasteur's laboratory. On March 1, 1886, Pasteur presented a paper to the French Academy of Sciences with the results from the inoculation of 350 people, concluding that *rabies prophylaxis after a bite is justified. There is a cause to create a rabies vaccine establishment*. He immediately launched an international fund, and as a result, in November 1888, the Institut Pasteur was created, dedicated not only to rabies treatment but to Pasteur's study of science.

News about Pasteur's vaccination spread rapidly, and people from all over the world began to arrive in Paris to receive the rabies vaccine. Pasteur also opened several vaccination centers in Russia. In 1887, Dr. Valentine Mott (1852–1918) opened a center to administer the rabies vaccine in New York. That year, Dr. Mott went to Paris as the representative of the "American Pasteur Institute," and when he returned to the United States, Pasteur permitted him to bring back a rabies-inoculated rabbit. In 1891, Albert Calmette (1863–1933) was sent to Saigon to administer the rabies vaccine, leading to the creation of the first overseas Pasteur Institute [22].

It appears, however, that the first vaccination against rabies in Latin America was done in Argentina on September of 1886, only 1 year after the vaccination of Joseph Meister in Paris. The vaccination was done by Dr. Desiderio Davel (1857–1943) using a strain provided by Pasteur *which was maintained by repeated passages in rabbits during the steamship travel from Paris to Buenos Aires*. Some claim that the Pasteur Laboratory of Buenos Aires, now the Institute of Zoonosis, was the first institution outside Europe that conducted rabies vaccination in humans [2].

Rabies vaccination was introduced in Mexico in 1888 by the eminent physician Eduardo Liceaga (1839–1922), the most distinguished hygienist of late nineteenth-century Mexico, who brought the vaccine in the brain of an inoculated rabbit maintained in glycerin during the travel from Paris [36].

The accounts presented here are interesting examples of the early transfer and adoption of vaccine technologies in Latin America.

4 Conclusions

The Latin American colonial society was exposed to numerous emerging viral infections that were imported from Europe or Africa during the process of conquest and colonization, resulting in significant cultural and demographic impact. Society

today is confronting some of the same diseases with vaccines that were mostly developed during the twentieth century. Smallpox was declared eradicated in 1980 thanks to a vaccine first developed in 1796 by Edward Jenner. Poliomyelitis may be eradicated by 2018 using vaccines developed by Jonas Salk (1914–1995) in 1955 and Albert Sabin (1906–1993) in 1961. Although a highly effective yellow fever vaccine was developed in 1937 by Max Theiler (1899–1972) [31], we are still experiencing severe epidemics of yellow fever, especially in Africa. Similarly, rabies causes tens of thousands of deaths every year, mostly in Asia and Africa, and 15 million people every year receive post-bite vaccination with new versions of the vaccine first developed by Louis Pasteur in 1885, thus preventing hundreds of thousands of deaths.

The growth of the global population from 2 to 7.4 billion during the past century and the increased mobility of the population will surely allow new pathogens to emerge and spread locally, regionally, and globally. Vaccines will continue to have a role in the control of current and future epidemics and pandemics.

We reviewed how, at the beginning of the nineteenth century, it took only 7 years from the discovery of smallpox vaccination to the launching of the Balmis expedition, the first-ever global health campaign. We also reviewed how, soon after the germ theory of disease was formulated in Europe, scientists working in Latin America reported their experiences in identifying new viruses, specifically those causing yellow fever and rabbit myxomatosis. The rapid transfer of rabies vaccination from Paris to Argentina and Mexico was interpreted as a sign of progress and modernity and of the commitment of the new nations in Latin America to become an integral part of the civilized world.

The twentieth century continued producing examples of excellent virological work in Latin America. The younger generations of Latin American virologists, those who are working in the twenty-first century, should find encouragement in the successes of the past to address the challenges of the future.

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Part II
Viral Gastrointestinal Diseases

Chapter 2
Rotavirus Biology

Susana López and Carlos F. Arias

1 Introduction

Acute, infectious diarrhea is one of the most common causes of morbidity and mortality among children living in developing countries. In 2010, 1.7 billion cases of diarrheal events were estimated to have occurred worldwide in children under 5 years of age [123]. Diarrheal diseases are the third cause of death in this age range, after perinatal problems and respiratory infections [1]; however, it is complicated to calculate the number of deaths associated with a particular enteric pathogen [39, 115]. Updated global estimates of rotavirus mortality in children less than 5 years of age indicate a decline from 296,000 deaths in 2008 to 215,000 in 2013, with a slight decrease in the proportion of diarrheal deaths caused by rotavirus, from 39% to 37% in this same period [115]. Developing countries bear the major burden of mortality from rotavirus, with about 85% of these cases occurring in six countries in Africa and Asia and very few in industrialized nations [39, 115].

Rotaviruses continue to be the leading etiological agent of severe diarrheal disease, even though two live attenuated vaccines have been licensed in more than 100 countries since 2006,[115]. These live oral vaccines have shown a lower efficacy in countries with a high burden of diarrheal disease [17, 39, 114], and the majority of those currently using rotavirus vaccines are low-mortality countries, so the impact of vaccine use on global estimates of rotavirus mortality has been limited [115]. Furthermore, the recent Global Enteric Multicenter Study showed that rotavirus was the leading cause of infant diarrhea among more than 20,000 children studied in seven sites across Asia and Africa [60]; this study also reported that each episode of severe diarrhea in children increased the risk of delayed physical

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and intellectual development as well as increased mortality by 8.5 fold [60, 114]. Thus, the development of improved vaccines and therapeutic strategies is needed to efficiently control rotavirus infection, and in fact new rotavirus vaccines are under investigation or have recently been licensed in various parts of the world [121]. Fundamental to these developments is a basic understanding of the molecular mechanisms by which rotaviruses interact with their host cell.

Although rotavirus can infect older children and adults, severe diarrheal disease is primarily observed in children less than 2 years of age [37]. Rotavirus infection is primarily restricted to mature enterocytes located at the tip of intestinal villi. However, additional extraintestinal spread of rotavirus during infection of animals indicates a wider host tissue range than previously appreciated [88, 99]. In vitro, rotaviruses bind to a wide variety of cell lines, although only a subset of these, including cells of renal or intestinal origin and transformed cell lines derived from breast, stomach, bone, and lung, are productively infected [23]. The initial stages of rotavirus interactions with the host cell are complex and are the focus of intense current research. Most of these studies have been performed using model cell culture lines, the monkey kidney epithelial cell line MA104 and the human colon carcinoma cell line Caco-2, both of which are highly permissive to rotavirus infection and are the most commonly employed.

The mature rotavirus infectious particles are formed by a triple-layered protein capsid that encloses the genome, composed of 11 segments of double-stranded RNA (dsRNA). The innermost layer, formed by 120 dimers of VP2, contains the viral genome and 12 copies each of VP1, the virus RNA-dependent RNA polymerase (RdRP), and VP3, a protein with guanylyltransferase, methylase, and phosphodiesterase enzymatic activities; these viral elements constitute the core of the virus. The addition of 260 trimers of VP6 on top of the VP2 layer produces double-layered particles (DLPs). The outermost layer is made by 780 copies of the glycoprotein VP7 arranged in trimers, which form a smooth surface layer from which 60 spikes composed of trimers of VP4 protrude to form the characteristic, infectious, triple-layered particles (TLPs) [37].

During or shortly after cell entry, the infecting TLP loses the external protein layer and is converted to a DLP. Once in the cytoplasm, the DLP, which is transcriptionally active, begins the synthesis of viral mRNAs that direct the synthesis of six structural proteins (VP1 to VP4, VP6, VP7) and six nonstructural proteins (NSP1 to NSP6). In addition to their function as mRNAs, the viral transcripts also serve as RNA templates for the synthesis of negative-strand RNAs to form the dsRNA genomic segments. The newly synthesized viral proteins are recruited to viroplasms, electrodense cytoplasmic structures, where the viral genome replicates and double-layered replication intermediate (RI) particles assemble. The DLPs newly formed in the viroplasms mature by budding into the lumen of the endoplasmic reticulum (ER) through the ER membrane, which is modified by the viral glycoproteins VP7 and NSP4. During this process, mediated by the interaction of VP6 with NSP4, the DLPs acquire a transient lipid envelope that is subsequently lost to yield mature infectious TLPs. Finally, in MA104 cells, the virus is released into the medium by cell lysis, whereas in Caco-2 cells, the virus exits through a non-lytic mechanism that is not well characterized [37] (Fig. 2.1).

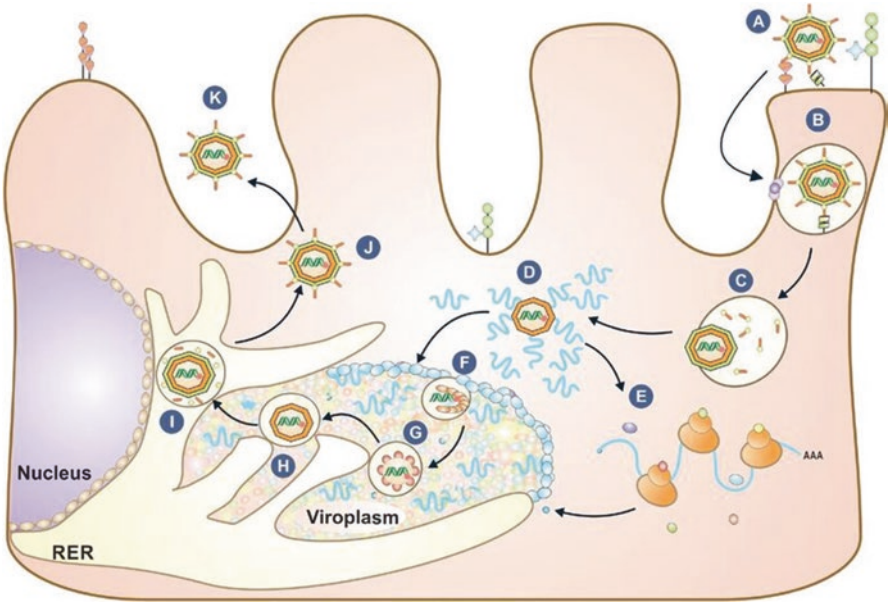

Fig. 2.1 Rotavirus replicative cycle. The virus replication cycle starts with the binding of the virus to the cell surface **A** and its internalization by endocytosis **B**. Inside the cell, the outer protein layer is shed **C**, and the double-layered particle becomes transcriptionally active **D**, giving rise to 11 RNA transcripts that encode 12 viral proteins. **E** Once a critical mass of viral protein is accumulated, the mRNA transcripts also serve as templates for the synthesis of the genomic double-stranded RNA (dsRNA), which occurs in replication intermediate particles within electrodense structures called viroplasms that are composed of viral proteins, viral RNA, and some cellular proteins **F**–**H**. Newly synthesized single- and double-layered particles assemble concurrently with genome replication, and **I** the double-layered particles then bud through a NSP4- and VP7-modified endoplasmic reticulum membrane into the lumen of the Rough Endoplasmic Reticulum (RER), where the final maturation of the virus particle takes place. **J**, **K**, Triple-layered, infectious particles exit the cell either by lysis or through a non-lytic process, depending on the cell line

Considering the purpose of this book, we describe in this chapter aspects of rotavirus biology where significant contributions by researchers working in Latin America have been made, with emphasis in our own work. This manuscript does not pretend to be a comprehensive review of the area, and we apologize to the colleagues we do not cite because of length restrictions.

2 Rotavirus Cell Entry

Among our principal contributions to the field of rotavirus is the characterization of the early events of virus–cell infection. Our research group has described the existence of at least four distinct interactions between the virus and host cell-surface molecules that mediate the attachment of the virus particle to the cell membrane and its subsequent entry into the cell. We have identified cell receptors and co-receptors,