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Current Topics in Tropical Emerging Diseases and Travel Medicine

Edited by Alfonso J. Rodriguez-Morales

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Notice

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Meet the editor

Dr. Alfonso J. Rodriguez-Morales received his Doctor of Medicine (MD) degree from the Universidad Central de Venezuela, Caracas, and his Master of Science (MSc) degree in Protozoology from the Universidad de Los Andes, Trujillo, Venezuela. He received his Diploma in Tropical Medicine and Hygiene (DTM&H) from the Universidad Peruana Cayetano Heredia, Lima, Peru, and University of Alabama at Birmingham, Birmingham, Alabama, USA. He is a fellow of the Royal Society for Tropical Medicine and Hygiene (FRSTMH), London, UK, a fellow of the Faculty of Travel Medicine (FFTM) of the Royal College of Physicians and Surgeons of Glasgow (RCPSG), Glasgow, Scotland, UK, and a fellow of the American College of Epidemiology (FACE), USA. He is also a candidate for Doctor in Parasitology (PhD) at the Universidad Central de Venezuela. He is a Doctor of Sciences *honoris causa* (HonDSc), Universidad Privada Franz Tamayo (UniFranz), Cochabamba, Bolivia. Prof. Rodriguez-Morales is the president of the Travel Medicine Committee, Pan American ID Association. He is the secretary of the Colombian ID Association, a senior researcher of the Colciencias (2015–2019), and a professor in the Universidad Tecnológica de Pereira, Pereira, Risaralda, Colombia (H index = 26).

Contents

Preface

Tropical emerging diseases pose a significant risk for the circulation of old and new pathogens in areas previously unknown, which implies the possibility of new morbidities and mortalities and new consequences for naïve populations. Globalization, migration and travel [1–5], as well climate change and variability [6–10], are key factors for tropical diseases, and represent the need for integration of tropical medicine, travel medicine and epidemiology in the understanding of such complex situations. Among these, neglected tropical diseases (NTDs) are relevant especially in the more vulnerable populations [11–13]. According to the World Health Organization (WHO), NTDs constitute a diverse group of communicable diseases that prevail in tropical and subtropical conditions in 149 countries. These affect more than one billion people and cost developing economies billions of dollars every year; a significant burden in multiple terms. Populations living in poverty [14,15], without adequate sanitation and in close contact with infectious vectors and domestic animals and livestock, are those worst affected. Leprosy and Chagas are examples of diseases in which new advances and evidence are relevant for NTDs deserving further studies [16].

Recently, viruses transmitted by vectors (arboviruses) affecting not only people living in the tropics but also travelers and migrating populations, such as dengue, chikungunya, Zika, Mayaro, and encephalitis, among others, have been causing epidemics, and emerging and reemerging in multiple regions of the world, as occurred in the Americas (2013–2017) with chikungunya and Zika [17].

However, in the tropics, old conditions, such as the human immunodeficiency virus (HIV), Ebola virus [18–20], and arenaviruses, continue to cause morbidity and mortality among developing countries in Africa, Asia, and America.

Keeping these issues in mind, this book includes different topics regarding research and clinical topics related to those relevant tropical emerging infectious diseases, including their implications for public health and travel medicine. This book has been organized into three major sections: I. Neglected Tropical Diseases; II. Arboviral Diseases; and III. Other Viral Diseases in the Tropics. Section I includes topics related to leprosy, Chagas disease and other NTDs with impact on kidney function. Section II includes experiences on arboviral diseases in Asia and Europe, such as dengue. Section III discusses HIV and Ebola in Africa, as well as arenaviruses.

The commissioning of this book by InTechOpen editorial has been related in part to my long commitment to vector-borne, zoonotic and tropical diseases, being involved as Co-Chair of the Working Group on Zoonoses of the International Society for Chemotherapy (WGZ-ISC), as well in Colombia at the Committee on Tropical Medicine, Zoonoses and Travel Medicine of the Colombian Association of Infectious Diseases (Asociación Colombiana de Infectología,

ACIN) and more importantly as the Chair of the Colombian Collaborative Network of Research on Zika (Red Colombiana de Colaboración en Zika)(RECOLZIKA), since January 2016.

I have been involved in vector-borne diseases (VBDs) for the last two decades, including malaria, leishmaniasis, Chagas disease as well as dengue, and since 2014 with chikungunya and emerging arboviruses such as Zika and Mayaro. After moving from Venezuela to Colombia in 2011, I have been involved in research of VBDs in Risaralda, such as malaria and leishmaniasis (still prevalent in the area), where we still keep working on these important tropical diseases. Part of all this is a clear reflection of the work impulse at the Research Group Infection Public Health and Infection (classified A1 by Colciencias) of the Faculty of Health Sciences of the Universidad Tecnológica de Pereira, directed by Dr. Guillermo Javier Lagos-Grisales, who is not just a partner, but a colleague and mainly a friend and extreme believer in our work in vector-borne and zoonotic diseases. However, I must recognize also the beginning of a significant collaboration after a meeting in Cartagena in 2013, during the Colombian Congress of Infectious Diseases, where I met Dr. Wilmer Ernesto Villamil-Gómez from Sincelejo, Sucre, Colombia, also part of the former Committee of Zoonoses and Hemorrhagic Fevers of the Colombian Association of Infectious Diseases (Asociación Colombiana de Infectología, ACIN) (now called Committee of Zoonoses and Tropical Medicine), who became my most important collaborator on arboviruses, including Zika. In addition to that, since 2002 I have been involved in tropical medicine and travel medicine, participating in multiple studies on malaria, Chagas disease, leishmaniasis, toxocariasis, giardiasis, and other intestinal parasitoses, tuberculosis and HIV. Currently in Colombia, we continue to study most of them, including now their assessment in internally displaced populations.

Following the same philosophy as we did on my seven previous books with InTechOpen, *Current Topics in Tropical Medicine* [21], *Current Topics in Public Health* [22], *Current Topics in Echinococcosis* [23], *Current Topics in Chikungunya* [24], *Current Topics in Malaria* [25], *Current Topics in Giardiasis* [26], and *Current Topics in Zika* [27], this book does not intend to be an exhaustive compilation and this first edition has included not just multiple different topics but also a wide geographical participation from many countries of different regions of the world. Its online availability through the InTechOpen website, as well the possibility to upload the complete book or their chapters from personal websites and institutions' repositories, allows it to reach a wider audience. Continuing with the series of *Current Topics* books, we are planning to develop other projects such as *Current Topics in Zoonoses*.

I would like to give a very special thanks to InTechOpen (for the fourth time), and particularly to Maja Bozicevic, Irina Stefanic, Markus Mattila, Romina Rovan and Nina Kalinic (Author Service Managers), for the opportunity to edit this interesting and important book, as well for their constant support.

I want to take the opportunity as always to dedicate this book to my beloved family (Aurora, Alfonso José, Alejandro, and Andrea, the neurologist), and also to my friends and my undergraduate and postgraduate students of health sciences in Colombia, Venezuela, and around Latin America. Also it is time to say thanks to my colleagues at the Working Group on Zoonoses, International Society for Chemotherapy and the Committee on Zoonoses, Tropical Medicine and Travel Medicine (formerly the Committee on Zoonoses and Haemorrhagic Fevers) of the Colombian Infectious Diseases Society (ACIN) and a large list of members of RECOLZIKA (www.RECOLZIKA.org). Special thanks again to my friend and colleague Dr. Guillermo J.

Lagos-Grisales, MD, MPH. Members of our research group and incubator consist of young and enthusiastic medical students and some veterinary medical students as well as young medical doctors, who are pursuing significant improvements in the understanding of the epidemiology of zoonotic, vector-borne, parasitic and in general, infectious diseases in our country with international projection. The year 2018 was a complex year, but still productive for this recognized group, which was classified in 2017 by the national agency of science, Colciencias, with the highest rank "A1", which is positioning as a leader in infectious diseases epidemiology research in the coffee-triangle region and in the country.

Finally, I hope our readers enjoy this publication as much as I did reading the chapters of *Current Topics in Tropical Emerging Diseases and Travel Medicine.*

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Section 1

Neglected Tropical Diseases

Chapter 1

Leprosy: The Ancient and Stubborn Disease

Prasetyadi Mawardi

Additional information is available at the end of the chapter

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Abstract

Leprosy can be caused by an infection of *Mycobacterium leprae* commonly acquired through contact with an infected person. Clinical presentation depends on the patient's immune status at the time of infection and during the course of disease. Leprosy is associated with disability and marginalization. The Global Leprosy Strategy 2016–2020 released in April 2016 underscored its goal of "accelerating towards a leprosy free-world." Today's leprosy differs from the leprosy of the past, but yet there are still many things that are not immediately known, so it is still a broad socioeconomic challenge for scientists to solve. Leprosy has low pathogenicity, only a small proportion of infected people develop signs of the disease. If leprosy is not diagnosed and treated in the early stages, further progress of the disease is determined by the strength of the patient's immune response. Various clinical signs can be known during the early phase of leprosy, defined as indeterminate phase, so that it is difficult to diagnose the disease. Multidrug therapy (MDT) was recommended as the standard treatment. The morbidity report of leprosy will be important in epidemiology because it is based on real events and not based on estimate.

Keywords: leprosy, multidrug therapy, *Mycobacterium leprae*

1. Introduction

Leprosy or Hansen disease is caused by an infection of *Mycobacterium leprae,* an acid-fast, rod-shaped bacillus, usually acquired through contact with an infected person. However, not every person exposed to an infected contact will develop leprosy [1]. *M. leprae* multiplies slowly, and the incubation period of the disease, on average, is 5 years. In some cases, symptoms may occur within 1 year but can also take as long as 20 years to occur. Clinical presentation depends on the patients' immune status at the time of infection and during the course of disease. Leprosy is associated with disability and marginalization [2].

Fourteen countries reported more than 1000 new cases, of which three countries—India, Brazil, and Indonesia—account for more than 80% of all the cases in the world [2]. The World Health Organization (WHO) reported that there were approximately 213,899 newly diagnosed patients in 2014 (a detection rate of 3.0/100,000 population), with 94% of LPs located in only 13 countries, one of which was Indonesia [3]. Indonesia's Ministry of Health reported 16,131 newly diagnosed cases of leprosy in 2014. On the other hand, the Department of Health found 498 cases of pauci-bacillary leprosy and 3337 cases of multi-bacillary leprosy [2]; the highest number of which were found in the Jember district. ***The Global Leprosy Strategy*** 2016–2020 released in April 2016 underscored its goal of "accelerating towards a leprosy-free world" and its commitment to an approach based on the principles of initiating action, ensuring accountability, and promoting inclusion [4]. The Global Leprosy 2016–2020 aims to detect early leprosy and prompt treatment to prevent disability and reduce transmission of infection in the community. The proportion of G2D cases (2 degree disability) among newly diagnosed patients and G2D levels in a population indicates the efficiency of early leprosy detection. They also showed indirectly the level of awareness of early signs of leprosy, access to leprosy services, and the skills of health care staff in diagnosing leprosy. This strategy is designed to achieve the long-term goal of a "leprosy free world," which refers to situations where societies are free of morbidity, disability, and social consequences due to leprosy [3]. Since 1980 era, Leprosy remains a problem in public health in Indonesia. The program implemented in Indonesia reduced the prevalence to 17,539 cases in 2000. The prevalence was 86% decreased in a 15-year period. It has been noted that a significant increase in leprosy control is due to the large-scale promotion of leprosy prevention and multidrug therapy (MDT) in more than 5600 primary health centers in Indonesia [5].

2. Epidemiology

Leprosy was first described in 600 BC and was recognized in the ancient civilization of China, Egypt, and India. The global prevalence of leprosy has decreased with the widespread use of effective therapy. More than 5 million cases were documented in 1985 and fewer than 300,000 cases 20 years later. Leprosy is spread by person to person contact. Although the most important route is unclear, it is believed that *M. leprae* is spread either through the inhalation of infectious aerosols or through skin contact with respiratory secretions and wound exudates. Numerous *M. leprae* are found in the nasal secretions of patients with lepromatous leprosy. *M. leprae* cannot grow in cell free cultures. Thus laboratory confirmation of leprosy requires histopathologic finding consistent with the clinical disease and either skin test reactivity to lepromin in tuberculoid leprosy or observation of acid fast bacteria in the lesion of patients with lepromatous leprosy [6].

The post millennium development goals have begun in 2015. Achieving the Millennium Development Goals (MDGs) as targets for global development needs to be evaluated. New ongoing and sustainable targets are important for the elimination of neglected tropical diseases (NTD) in Indonesia. This review illustrates the NTD situation in Indonesia and highlights issues under NTD transmission. A multidisciplinary approach is a promising strategy to help marginalized people [7].

Over the past 20 years, more than 16 million leprosy patients have been treated. The prevalence rate of the disease has declined by 99%: from 21.1 cases per 10,000 people in 1983 to 0.2 cases per 10,000 people by 2015. According to official reports received from 138 countries from all WHO regions, the global prevalence of leprosy by the end of 2015 was 176,176 cases (0.18 cases per 10,000 people). The number of new cases reported globally over the last 3 years is as follows: 2015: 211,973 (0.21 new cases per 10,000 people), 2014: 213,899 new cases, and 2013: 215,656 new cases (**Figures 1–4**; **Table 1**) [8].

As can be seen from the above table, only three countries reported more than 10,000 cases in 2015: **India, Brazil, and Indonesia**. With 127,326 new cases, India accounted for 60% of the global new cases; Brazil reported 26.395 new cases, representing 13% of the global new cases; and Indonesia reported 17,202 new cases, 8% of the global case load. In 2016, WHO has launched "Global Leprosy Strategy 2016–2020: Accelerate towards a leprosy-free world"-aimed at reviving leprosy control efforts and to avoid disability, especially among children affected by disease in endemic countries. This strategy emphasizes the need for ongoing expertise and increases the number of skilled leprosy staff, increases the participation of affected people in leprosy services, and reduces visible abnormalities—also called G2D defects—as well as the stigma associated with the disease. The targets of the new global strategy to be met by 2020 are [8]:

1. Without a disability among new pediatric patients.
2. The level of class-2 disability is less than 1 case per 1 million people.
3. Zero countries with laws that allow discrimination on the basis of leprosy.

Figure 1. Madarosis on facial region in Lucio phenomenon's patient.

Figure 2. Infiltrate and atrophic on auricularis dextra region in Lucio phenomenon's patient.

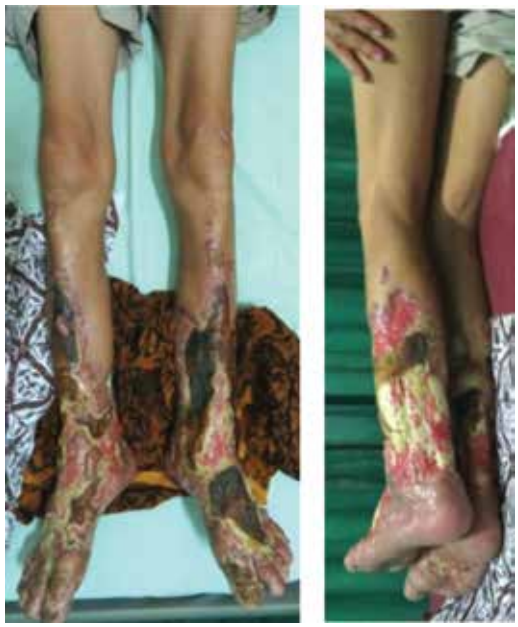

Figure 3. Ulcer on lower extremities in Lucio phenomenon's patient.

In some areas in Indonesia, leprosy is still prevalent even though the infectious disease is no longer a mystery and can be prevented and treated by adopting a clean and healthy lifestyle. Cases of leprosy continue to surface in some areas, and people who were afflicted with the disease but have been cured continue to face the stigma and discrimination. Health workers

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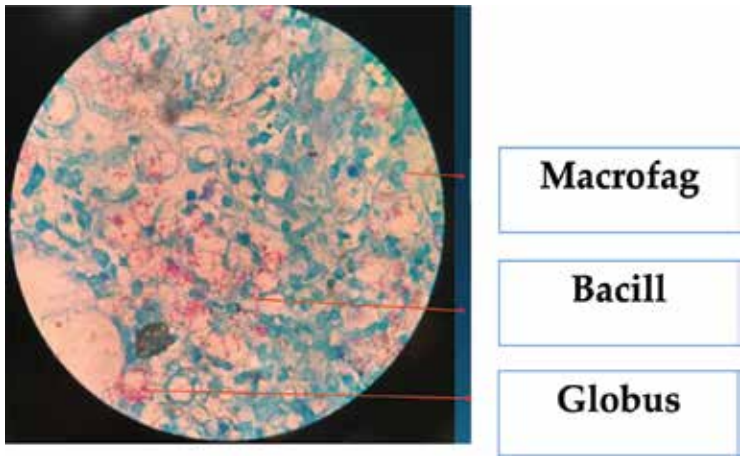

Figure 4. Histopathological examination with Wade Faraco staining in Lucio phenomenon's patient showed multiple bacilli commonly in lepromatous leprosy.

Country	Number of new cases detected					
	2010	**2011**	**2012**	**2013**	**2014**	**2015**
Bangladesh	3848	3970	3688	3141	3622	3976
Brazil	34,894	33,955	33,303	31,044	31,064	26,395
Democratic Republic of the Congo	5049	3949	3607	3744	3272	4237
Ethiopia	4430	NR	3776	4374	3758	3970
India	126,800	127,295	134,752	126,913	125,785	127,326
Indonesia	17,012	20,023	18,994	16,856	17,025	17,202
Madagascar	1520	1577	1474	1569	1617	1487
Myanmar	2936	3082	3013	2950	2877	2571
Nepal	3118	3184	3492	3225	3046	2751
Nigeria	3913	3623	3805	3385	2983	2892
Philippines	2041	1818	2150	1729	1655	1617
Sri Lanka	2027	2178	2191	1990	2157	1977
Mozambique	1207	1097	758	NR	NR	1335
United Republic of Tanzania	2349	2288	2528	2005	1947	2256
Total (%)	**211,144 (92)**	**208,039 (92)**	**217,531 (93)**	**202,925 (94)**	**200,808 (94)**	**199,992 (95)**
Global total	**228,474**	**226,626**	**232,857**	**215,656**	**213,899**	**210,758**

Table 1. New case detection trends in countries that reported >1000 new cases in the past 5 years.

encounter difficulties in reaching out to people living with leprosy to offer treatment. Although Indonesia has managed to reach a stage close to eliminating leprosy, the country has not been able to completely eradicate the disease. In some areas, such as Banten, West

Kalimantan, South Kalimantan, and East Java, leprosy remains prevalent. Tangerang District Health apparatuses in Banten province recently conducted a leprosy census and found that as many as 397 patients with leprosy were in need of serious attention. Leprosy remains a largely neglected disease, especially in the rural areas of the country, where little is known about it, and many suffer from the stigma and lack of knowledge surrounding the disease. East Java provincial Health Office Chief Dr. Harsono remarked in Surabaya that the local government would continue to take steps to reduce the number of leprosy patients and hoped that the figure will come down to less than one patient per 10,000 population. Leprosy has been around since the beginning of time, often surrounded by terrifying, negative stigma, and tales of leprosy patients being shunned as outcasts [9]. The 5-year strategy was launched previously: Strategy "The last impetus for eliminating leprosy as a public health problem" (2000–2005) aimed at eliminating leprosy as a public health problem at the country level. This is data-making and the general public, communications, and campaigns. All countries with a population of one million or more have achieved leprosy eradication as a public health problem at the national level [10].

Two successive strategies—the "Global strategy to further reduce leprosy burden and maintain leprosy control activities" (Period of planning stage: 2006–2010) and "Improved global strategy to further reduce the burden of disease due to leprosy" (Period of planning stage: 2011–2015)—maintaining an emphasis on reducing disease burden by focusing on sustainability through integration. They have moved from the target of "elimination" in terms of the prevalence of disease to the target, which emphasizes the decline in the number of new cases with G2D to promote early detection and reduction of transmission [11].

Leprosy has been linked to stigma throughout history [10]. Stigma manifestations, including self-stigma, social exclusion, and discrimination, although now more subtle with less exile, remain a reality for many affected people [12]. To help leprosy services become more responsive to problems surrounding leprosy-related stigma and reduce its impact, it is necessary to understand the stigma from the perspective of affected people and their family members. Also the views of key people in society, such as neighbors, teachers, religious leaders, and health workers, should be considered. The "human face of leprosy" edited by Gokhale and Sohoni in 1999 has emphasized the need for such stories [13].

3. Risk factor

Factors to consider are pathogenesis of germs, mode of transmission, environmental conditions, and genetic variants associated with susceptibility, immune change, and the possibility of reservoirs outside humans. Today's leprosy differs with environmental conditions, genetic variants associated with susceptibility, immune change, and the possibility of reservoirs outside humans. Today's leprosy differs from the leprosy of the past, but yet there are still many things that are not immediately known, so it is still a broad socioeconomic and challenge for scientists to solve. Research by Bakker et al. found that the risk factors of leprosy in Indonesia

are genetic, household size, and gender. People living in households with more than seven members had a risk of 3.1 [9, 10, 12].

4. Pathogenesis of leprosy

Leprosy onset is insidious. It is about the nerves, skin, and eyes. It can also attack the mucosa (mouth, nose, and pharynx), testes, kidneys, smooth/smooth muscle, reticulo-endothelial system, and vascular endothelium. Germs enter the body usually through the respiratory system. It has low pathogenicity, and only a small percentage of infected people show signs of the disease. Although infected, most of the population do not develop the disease. Upon entering the body, the bacilli will migrate to the neural network and enter Schwann cells. Bacteria can also be found in macrophages, muscle cells, and endothelial cells of the blood vessels. After entering Schwann/macrophage cells, the fate of the bacteria depends on the resistance of the infected individual to the infecting organism. Bacilli began to proliferate slowly (about 12–14 days for one bacteria split into two) inside the cell, released from the crushed cell and into the unaffected cells. Until this stage, people remain free from signs and symptoms of leprosy. When bacilli proliferate, the bacterial load increases in the body, and the infection is recognized by the immunological system. Lymphocytes and histiocytes (macrophages) invade infected tissue. At this stage, clinical manifestations may appear as nerve involvement with impaired sensation or skin patches. If not diagnosed and treated in the early stages, further progression of the disease is determined by the strength of the patient's immune response [14].

In people with strong cell-mediated immunity (CMI), granuloma formation occurs in skin nerves, and cutaneous nerve is enlarged and damaged. Often only a few infiltrated nerves are inflamed, but the inflammation inside the epineurium causes compression and destruction of the unmyelinated sensory and autonomous fibers. Myelinated motor fibers are affected last to produce motor damage. Severe inflammation can cause necrosis in the nerve. Clinical manifestations of sensory loss occur when nearly 30% of sensory fibers are destroyed. A good CMI manages to limit disease to nerve Schwann cells that result in pure leprosy. *M. leprae* can escape from nerves to adjacent skin all the time and causes classic skin lesions. Areas of the skin with relatively higher temperatures such as axilla, groin, perineum, and hairy scalp are usually spared. But in people with immature cell-cell immunity, the bacilli that enter Schwann cells multiply uncontrollably and destroy the nerves. Also, the bacilli released by infected and destroyed cells are swallowed by histiocytes. Histiocytes with bacilli in it become wandering macrophages. Bacilli multiply in this macrophage and run into other tissues through blood, lymph, or tissue fluid [14].

The modulation of lipid metabolism and reprogramming of mature Schwann cells have been suggested as a mechanism used by *M. leprae* to spread disease. New markers associated with local disease, dissemination, or occurrence leprosy reaction events include human interferon, CD163, microRNA-21, NOD2, galectin-3, and toll-like receptors. The role of keratinocytes other than macrophages is interesting to understand in the pathogenesis of leprosy. Adaptive

immune reports focus on the role of regulatory T cells and cytokines secreted by T helper cells in leprosy. Finally, a newly identified species named *M. lepromatosis* has been detected in patients with severe leprosy and erythema nodosum leprosum [8].

5. Clinical appearances

Based on two types of extreme immune responses, two polar forms (tuberculoid at one end and lepromatous in the other) of the clinical presentation of the disease occur. The disease may present with a clinical picture representing severity, anywhere in the continuous/variable spectrum between these two polar forms (see Appendix No. IV for Ridley's general overview and Jopling Hardness classification on leprosy, in the form of a continuous spectrum and Appendix III for differential diagnosis leprosy). People with a "good" CMI response develop a milder and localized form of disease (tuberculoid) with fewer bacterial loads, whereas people with weak or absent CMI develop a widespread spread of disease (lepromatous) with a high bacterial load.

The transmission of leprosy exactly is still understood. The human being is the main reservoir of leprosy infection, although by history, transmission through African green monkeys and Armadillos has been reported [15]. Other dissemination routes were suspected, but their role in the transmission of leprosy was not clearly defined [16]. *M. leprae* has a tropism for the skin and Schwann cells of the peripheral nerves. The primary sign appears as sensory neuritis, but in untreated patients seeking medical treatment at a late phase with severe motor impairment. Plantar ulcers, lytic bone lesions and ulnar nerve paralysis or lagophthalmos are frequent complications in leprosy patients [16].

Leprosy diagnosis remain based clinical findings and easy make for health workers to treat patients. Various clinical signs can be known during the early phase of leprosy, defined as indeterminate phase, so that it is difficult to diagnose the disease.

6. Immunology

M. leprae is an acid-alcohol-fast, Gram-positive obligate intracellular bacillus that shows tropism for cell and endothelial system and peripheral nervous system (Schwann cell). The leprosy bacillus has a predilection for macrophages, collecting intracellular globus. The bacteria never cultured in vitro but have been grown in the foot pads of 9-banded armadillos. Predisposed to infect cold areas of the body surface such as the skin, nasal mucosa, and peripheral nerves, the best temperature for *M. leprae* grows is 27 and 30°C. The efficacy of this pathogen within a narrow ecological niche is primarily explained by the properties conferred by two structural elements: the capsule and the cell wall [17]. The capsule is composed of two lipids, phthiocerol dimycoserosate and a phenolic glycolipid-1, which is the main target of humoral immune response, like immunoglobulin M-mediated [18]. Another important component of the cell wall is lipoarabinomannan, which is an antigen for the macrophage.

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M. leprae has a predilection for Schwann cells, for specific binding to the G domain of the laminin alpha-2 chain, which is expressed specifically in the basal lamina of peripheral nerves [19]. Development of the disease depends on the immune status of patients. The role of genetics, associated with a susceptibility locus at chromosome 10p13, near the mannose receptor on the surface macrophages, is important in the phagocytosis. The other role of major histocompatibility complex (class II HLA) genes at chromosome 6 has been implicated in the clinical type of patient leprosy. The lepromatous form affects the skin and peripheral nerve, causing well-defined infiltrated plaques that are annular or ovoid form. Immunohistochemical findings in skin biopsies show mainly interleukins 4 and 10. The immune response to *M. leprae* is variable and gives rise to spontaneously changing clinical manifestations that may present as type 1 or 2 leprosy reactions [17].

The steps in the transmission of the disease are not entirely clear. However, it is acceptable that the mycobacterium reservoir is exclusively human, and it is most often transmitted through the spread of basil aerosol. In many cases, the infection appears to occur during childhood, with an incubation period ranging from 6 months to several years. Rarely, very long incubation periods of up to 40 years have been reported, although infection rates in adults with close contact with infected individuals (e.g. spouse) are as low as 5% even in long-term investigations [20].

Leprosy usually begins as an indeterminate form that can heal spontaneously, remain unchanged for long periods, or progress to a more severe form. About 95% of contact with the bacillus will result in spontaneous resolution without the development of clinical symptoms. This initial indefinite form can produce patches or macules of the skin, which is not clear with little hypopigmentation. In parallel, such patches may coincide with hypoesthesia of the corresponding skin nerve. If the disease develops, tuberculoid leprosy can develop as long as the host immune response is preserved enough. At this stage, rapid skin sensation loss due to severe neurological damage can occur, as well as local paralysis, loss of sweat and sebaceous glands, and hair loss. The skin shows macular lesions with significant hypopigmentation; peripheral nerves are infiltrated and may present as thick subcutaneous bundles. Secondary symptoms include bruised skin under hypoesthesia due to local external damage and superinfection with poor healing ulcers [21].

The lepromatous stage occurs in individuals with poor immune reactions. Clinically, this is the most severe form and can lead to mutilation. Skin lesions may appear as macules, papules, or plaques with hypopigmentation. The most affected areas are the ears, the central face, the fingers, and toes, but the distal extremities, such as the extensor surfaces of the thighs and forearms, can also be affected. Severe skin infiltrations in the peri-nasal and periorbital areas lead to "facies leonine" or lion face, associated with loss of eyelashes and lateral eyebrows ("facies leprosy"). Often, blindness can occur when the eye is exposed. Osseous resorption of the nostrils and destruction of the bridge of the nose result in severe facial mutilation. The *Mycobacterium leprae* affected throat can cause a distinctive hoarseness. In fact, all other body areas can also be affected, leading to varying clinical features. The fourth stage, called the borderline stage, also exists, which is somewhat intermediate between tuberculoid and lepromatous stages in clinical symptoms. Considering the various clinical symptoms, especially the early stages and stages of minor illness, leprosy can easily be confused with various other diseases. This is often important for historical

reconciliation, since evidence previously interpreted as supporting the diagnosis of "leprosy" should be carefully considered. In contrast, typical skeletal mutilation in the form of severe leprosy leaves a distinctive footprint of disease that may be identified in historic relics with high levels of certainty [21].

More advanced leprosy presentations have been reported and classified as tuberculoid leprosy and lepromatous leprosy. Many other clinical presentations, known as intermediate or borderline leprosy, have been identified and classified among the two types. The Ridley and Jopling System (RJ) defines five clinical presentations of leprosy: polar tuberculoid leprosy (TT), borderline tuberculoid leprosy (BT), borderline-borderline leprosy (BB), borderline lepromatous leprosy (BL), and polar lepromatous leprosy (LL) [22].

But according WHO classification, leprosy is divided into two major groups: pauci-bacillary subtype and multi-bacillary subtype. The WHO system is based on the quantity of skin lesions and the number of bacilli on skin smear. Skin smears are made by squeezing a fold of skin and making a shallow slit in the skin with a scalpel [23]. The two main categories of the WHO classification system are: (1) pauci-bacillary (PB) leprosy: ≤5 skin lesion with no bacilli on skin smear and (2) multi-bacillary (MB) leprosy: ≥6 skin lesion and may have bacilli on skin smears.

The Ridley-Jopling classification system is based on the histopathology of skin lesions and essentially represents a spectrum of disease. The spectrum of leprosy classification is not static. For example, in some cases, untreated TT can progress into LL, given a long enough time and the proper immunologic environment. There are the two classification systems that are mutually exclusive. Indeterminate and tuberculoid leprosy (TT) are commonly referred to as PB leprosy, while BB and LL are commonly referred to as MB leprosy [23, 24]. This was confirmed by the WHO Expert Committee on Leprosy at the seventh meeting in 1997, which defined a case of leprosy as follows: A case of leprosy is someone who has one or more of the following features and who still needs to complete a complete treatment: skin lesions hypopigmentation or redness with a definite loss of sensation, peripheral nerve involvement, as shown by neural thickening accompanied by loss of sensation, as well as positive skin-smears for acid-fast bacilli [24].

The following recommendations are based on the evidence just described:

a. Approximately 70% of leprosy patients can be diagnosed using a single mark of anesthetic skin patch, and this leprosy sign should be taught as widely as possible.

b. 30% of all patients, including many MB patients, do not show with this sign, and health care workers should be taught to suspect and refer other possible cases.

c. The referral of a suspect who has no anesthetic symptoms is given to a person with a higher experience who has been taught peripheral nerves should be straightforward. Palpation of only two nerves (ulnar and common peroneal) may allow diagnosis of as many as 90% of patients with neural enlargement.

d. Classification should be based on the number of skin lesions: PB < 5 patches; MB > 5 patches.

e. Skin smear on new case samples can provide quality control. Research into laboratory tests (e.g., serological or skin tests) that may be useful in the field in identifying *M. leprae* infection, diagnosing active disease, and classifying leprosy cases should be continued [25].

Leprosy patients have been traditionally classified based on the number and type of skin lesions into various clinical groups. However, according to current WHO guidelines, only the number of skin lesions determines the length of therapy patients received for leprosy. Various studies have reported and highlighted the differences between clinical and skin classification and nerve biopsy findings of leprosy patients [26]. The WHO classification (1988) for leprosy control program has differences with the 1998 WHO classification. The patients are categorized into PB and MB leprosy depending upon whether the slit skin smears demonstrate any bacilli or not. Earlier, the cases with a bacterial index of 2 or less had been categorized as PB, but later on, for feasibility and operational difficulties, all the patients with demonstrable bacilli in slit skin smear without any reference to bacterial index were to be categorized as MB, whereas 1998 WHO Classification for leprosy control program was based on the total number of leprosy lesions in the patient. This is a corollary to the fact that PB patients have good immunity and present with only limited number of lesions. Furthermore, the single lesions leprosy was also segregated on the ground that this can be treated with the limited amount of chemotherapy. On the practical side, the WHO expert committee's conclusions about new classification based on the number of lesions are translated as [27]:

1. Pauci-bacillary single lesion leprosy (SLPB)
2. Pauci-bacillary leprosy (2–5 skin lesions)
3. Multi-bacillary leprosy six or more skin lesions and also all smear positive cases

7. Pauci-bacillary

Pauci-bacillary leprosy is found in people with good CMI. The disease remains localized to produce single or little skin lesions with or without peripheral nerve involvement. The skin lesion may be macular (flat) or papule (slightly raised) and plaque. People with strong immune responses are capable of destroying large amounts of normal organisms and skin normally most of them exhibit negative skin examinations.

8. Multi-bacillary

Multi-bacillary leprosy is found in people with poor CMI. Bacilli multiply and spread more widely resulting in a common disease: usually accompanied by widespread lesions in the skin, nerves, and at lower levels in other organs such as the eyes, respiratory mucosa, testes, and reticuloendothelial system in men and usually the central nervous system and the upper reproductive system in women. The skin lesions may be multiple (borderline) or uncountable (lepromatous). Lepromatous lesions may be symmetrical and unclear bilateral macules or

diffuse infiltration, which may develop into plaque and nodule formation. In addition, there may be nasal bleeding and edema on both legs. If the patient does not receive treatment, the pauci-bacillary form of leprosy can be downgraded to the multi-bacillary form (from tuberculoid to lepromatous) through the borderline spectrum.

9. Leprosy reactions

A major problem in the management of leprosy patients is the occurrence of the leprosy reactions, which are consequences of the dynamic nature of the immune response to leprosy bacteria *(M. leprae)* that may occur before, during, or following the completion of multidrug therapy (MDT). Reactions in leprosy constitute the main complications of the disease, which can lead to serious consequences like nerve damage and deformities. Leprosy reaction is immunologically mediated episodes of acute or subacute inflammation, which interrupts the relatively uneventful usual chronic course of diseases affecting the skin, nerves, mucous membrane, and/or other sites. Reaction may occur in any type of leprosy expect the indeterminate type. Unless promptly and adequately treated, it can result in deformity and disability. Three types of reactions recognized are classified as: (1) type 1 reaction (T1R), (2) type 2 reaction (T2R) or erythema nodosum leprosum (ENL), and (3) the Lucio phenomenon [28]. Type 1 LR (T1R) and type 2 LR (T2R) are the main causes of nerve damage and permanent disability. The LR immune-pathogenesis is currently an important research focus, as it can provide relevant targets and goals for early detection and control of this episode [29].

9.1. Type 1 reaction

Type 1 reaction is associated with sudden alteration of cell-mediated immunity associated with a shift in the patient's position in the leprosy spectrum that is usually observed in borderline spectrum of the disease except very rare reports in lepromatous leprosy. If there is increase in the immunity, the shift is from borderline spectrum toward the tuberculoid pole and is called **upgrading** or **reversal reaction**. On the other hand, if there is sudden shift toward the lepromatous pole with reduction of immunity, it is called as downgrading reaction. These acute inflammatory events may accentuate the chronic course of the disease in the total clinical spectrum of the disease, usually in Borderline leprosy (BT, BB, and BL) and rare in LL. Clinical symptoms may present with complain of burning, stinging sensations in the skin lesions. They may have aches and pains in the extremities and loss of strength and/or sensory perception. Sign manifestations increased inflammation become erythematous swollen and may be tender looking like erysipelas, edema of extremities or face frequently accompanied by nerve involvement, rapid swelling with severe pain/tenderness (neuritis), and sometimes loss of nerve function [28].

9.2. Type 2 reaction

Type 2 reaction is an immune complex syndrome (antigen–antibody reaction involvement complement). It is an example of type III hypersensitivity reaction by Coombs and Gell classification.

T2R occurs mostly in lepromatous (LL) and sometimes in borderline lepromatous leprosy (LL), which occurs mostly during the course of antileprosy treatment. A few cases present for the first time with features of reactions before leprosy is diagnosed and treatment started. In one third of the cases, pain and swelling in the joints precede or are a component of other constitutional symptoms. In cutaneous onset, there may be appearance of skin lesions in the form of maculopapular, popular, nodular, or plaque type lesions before appearance of constitutional signs and symptoms. Fever, joint pain and constitutional sign and symptom, and skin lesion appear together. T2R without ENL is possible that the manifestations of the reactions may not be confined to the skin, and the patients may develop neuritis or systemic involvement or both, depending upon the target organs where immune complexes deposition occurs [28].

10. Lucio phenomenon

Lucio phenomenon is a special type of reactions observed in uniformly diffuse shinny infiltrative nonnodular form of LL, which is chiefly encountered in Mexicans. Its unique feature is that it is seen only in untreated cases. The etio-pathogenesis is less well understood. *M. leprae* are found unusually in large numbers in the endothelial cells of superficial blood vessels, and this finding may be responsible for the serious vascular complications seen during the reactive phase. There is marked vasculitis and thrombosis of the superficial and deep vessel, resulting in hemorrhage and infarctions of the skin. Clinical manifestations begin with slightly indurated red-bluish plaques on the skin with an erythematous halo, sometimes larger inflamed bullous lesions, which burst leaving a deep ulcer with jagged edges. The lesion takes about 3 weeks to develop an ulcer from the initial lesion, and it heals slowly and secondary cellulitis may complicate. Patients remain afebrile [28].

11. Management by community approach

Findings on the meaning of leprosy show confusion about the concept of leprosy and the lack of knowledge about the disease, its causes, and the mode of transmission. In addition, the views and perceptions of leprosy that have been internalized or newly acquired cause fear. The perception that leprosy is a highly contagious disease that can be transmitted by touching the same objects that have been touched by lepers is very worrying. Therefore, increasing knowledge about leprosy in affected people, community members, and health workers remains an important goal for leprosy services, and although that is not the only answer to stigma, it is an important prerequisite. Some challenges arise in relation to seeking care, recognizing the symptoms, making the right diagnosis, and sharing the diagnosis with the patient and the treatment. What is visible is the strength and influence of leprosy workers and therefore the destructive impact of their stigmatization behavior on the people they care for. White calls this the "iatrogenic stigma" or stigma produced by meeting patients with health care workers [30]. Multidrug therapy (MDT) was recommended as the standard treatment. The recommended treatment duration for MB is 12 months; otherwise, the duration for PB is

6 months. In 1997, WHO recommended treating patients with SLPB with single-dose regimen contain of 600 mg rifampicin, 400 mg of ofloxacin, and 100 mg of minocycline [16].

Corticosteroids should be given to patients experiencing leprosy reactions due to their ability as anti-inflammatory and immunomodulatory. Duration of treatment >12 weeks is recommended only if the patient is supervised by a specialist. Treatment duration is different for leprosy type 1 and type 2 reactions [31]. The morbidity report becomes very important in epidemiology because it is based on real events and not based on estimates or estimates. In addition, morbidity recording and reporting can be determined from changes in the incidence and prevalence of the disease until the results can be used for the planning and management of health problems. Full support from the Government is needed to carry out continuous activities in preventing the spread of leprosy [10].

12. Conclusion

Leprosy is still a public health problem in Indonesia. Although from year to year its prevalence decreased, new cases were still found in various populations and communities that did not belong to the risk group. Hard work is required to achieve the 2020 goal. Active case discovery is absolutely necessary for all layers of health workers, both in primary, secondary, and tertiary care. Government support both cross-program and cross-sectoral will help to reduce morbidity of leprosy. Public health centers are required to be more pro-active in sensitizing problems within their communities.

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Chapter 2

Chagas Disease in the Yucatan Peninsula, Mexico

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Additional information is available at the end of the chapter

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Abstract

American trypanosomiasis or Chagas disease is caused by the protozoan *Trypanosoma cruzi*, which affects a wide variety of hosts including the man, until now treatment options or vaccines developed are not enough to control or prevent infected cases. The main way of transmission is vectorial, through insects of the *Reduviidae* family, as well by congenital transmission, blood/organ transplants or oral transmission. Chagas disease are considered as endemic in many areas due to the presence and lack of control of insect vectors. Many touristic places in Latin America are located in endemic areas; however, there is a nonexistence of knowledge by touristic service providers about the theme. For that reason, there is a latent risk that tourists who come to vacation in endemic areas are exposed get the infection. The risk factors are well identified, and this allows that well-defined prevention strategies can be established in order to avoid the presentation of cases in visitors to the tourist zones. This chapter aimed to describe the situation of Chagas disease in touristic areas of the Caribbean of America Latina as and to provide a brief review of information that allows visitors to know about the epidemiology and potential risks of this infection.

Keywords: American trypanosomiasis, tourism, endemic regions, vector transmission, Mexico

1. Introduction

Nowadays, any destination can be reached from any other place around the world in 36 h of travel or less. This 36-h window fits well within the incubation period of most diseases,

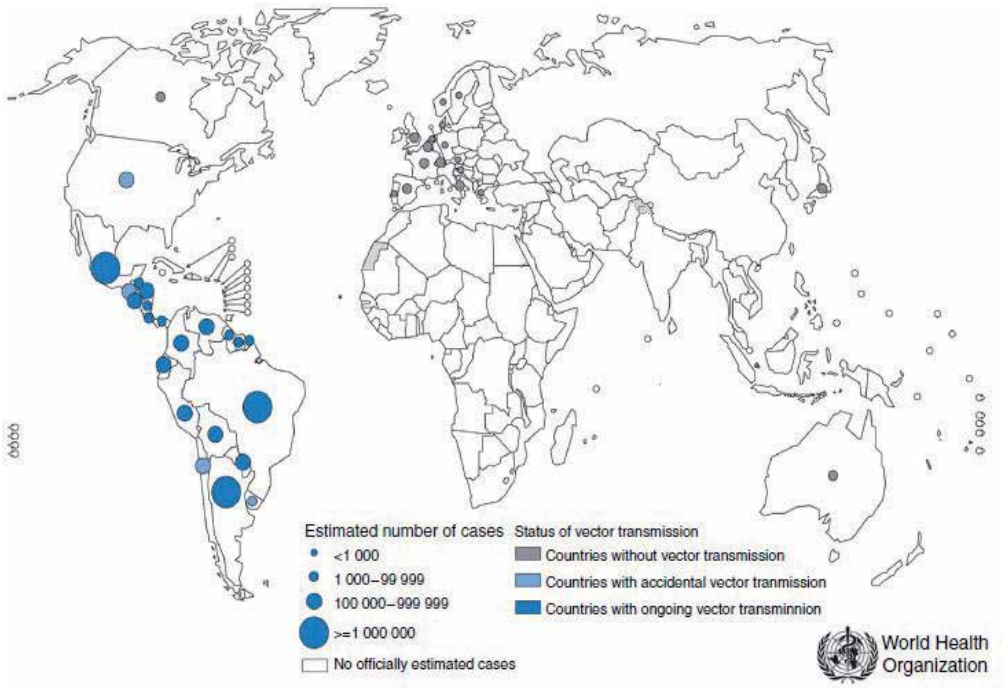

Figure 1. Distribution of cases of *Trypanosoma cruzi* infection, based on official estimates and status of vector transmission, 2006–2009 [4].

affording ample opportunity for the unrecognized pathogen movement from place to place and for rapid global spread of microbial agents [1].

There are nearly 20 million seropositive persons with Chagas disease (CD) in the Americas, and 90 million persons, or about one-fifth of Latin America's population, are at risk of contract *Trypanosoma cruzi* (*T. cruzi*) infection [2]. The protozoan, known as the causative agent of CD, a neglected tropical disease, extends through North, Central, and South America, affecting 21 countries [3], with over eight million people across Mexico and Central and South America. In the United States, around 300,000 to over 1 million people are infected, mostly because of traveling and migration to Chagas disease-endemic regions (**Figure 1**) [5]. This disease has caused more deaths from parasitic disorders than from any other parasitic disease in Latin America and was responsible for the third highest number of parasitic infections in the world following malaria and schistosomiasis [6].

2. Presence of Chagas disease in the Yucatan Peninsula

Chagas disease transmission has been reported in the Yucatan Peninsula as early as 1941, but current information on its incidence is probably underestimated. Mexico is a leader in Latin American tourism, and the Yucatan Peninsula is one of the most important tourism destinations in Mexico; it has emerged as a major tourist destination for archeology, weddings and romance, rest, parties (spring breakers), ecotourism, diving in subterraneous rivers in caves and cenotes, over the past several decades. Tourism numbers have increased during 2017 over 1,652,653 arrivals each year, that is, over 0.6% from previous years. As in January 2017,

the arrival of foreign air-coming visitors who reside in the United States represented 52.5% of all foreign arrivals, followed by Canada as 18.7% and other Latin American and Caribbean countries as Argentina and Colombia with 3.3 and 2.3% of total visitors, respectively [7].

Dumonteil and Goubrière [8] constructed a risk map for the natural transmission of Chagas disease in the Yucatan peninsula of Mexico, using geographic information systems to design predictive models of reduviid abundance (*T. dimidiata*) and infection rates. They described that there were (a) greater numbers of *T. dimidiata* in warmer and drier climates of the Yucatan, including coastal tourist areas; (b) higher reduviid infection rates in areas with lower temperatures and higher precipitation rates, specifically rain forests; and (c) greater reduviid abundance in areas of "perturbed" vegetation, defined in their study as agricultural fields and pastures.

In a study conducted by Reyes-Novelo [9], houses of a rural community in Yucatan were studied to identify intra or peridomiciliary infestation with *T. dimidiate*. It was found that 76.5% of the houses were infested, of those 27.7% colonized and 75% had infected triatomines, indicating that both habitats may contribute as sources of *T. dimidiata* house infestation.

2.1. Risk of travel

Travelers not only put themselves at risk by visiting areas where diseases are emerging, but they also run the risk of exposure to disease they do not usually encounter at home. After exposure, they may not manifest symptoms due to incubation period, until they return to their countries, risking the potential for spread of microbes to new areas [1]. Chagas disease is an autochthonous disease of many countries of the western hemisphere, travelers, including immigrants have been considered a potential source of introduction of diseases, and CD can pose increasing communicable disease risks to travelers in endemic regions of the Americas, including beachside resort regions and interior rural regions [2, 10].

The touristic areas in the Yucatan peninsula include coasts, colonial cities and Mayan ruins, most of them located in perturbed forested areas, and they are among the most scenic and popular destinations for historians, archeologists, ecotourists and cruise line passengers. The urban areas in the Yucatan Peninsula had/have a big rate of growing due to the migration even from other states of Mexico (caused by the insecurity levels in other states of the country) as well the presence of many other citizens coming mainly from Venezuela and Argentina. Therefore, many areas from the Yucatan Peninsula are still high-risk regions for Chagas disease and are the most frequently visited areas throughout the year by travelers, including Quintana Roo (where Cancun and Playa del Carmen are located) Campeche and Yucatan [8].

Human seroprevalence has been reported since the 1970s with a 11.2–18.8% prevalence infection, and more recent further studies have shown a prevalence in Mayan communities ranging from 1 to 4%, being similar in the city, over 0.6 and 1.7% in blood donors [11, 12].

Over the years, *T. cruzi* seroprevalence has been reported in several ways, and many population groups, which help us, have a general panorama of the situation represented in the Yucatan Peninsula. The few studies conducted in the region show the urban and rural prevalence that has changed among years, not differing much from each other, pointing out Chagas Disease its present, especially in cardiopathic patients, pregnant women and any population group, even domestic animals, such as cats and dogs, which humans generally coexist with. Seroprevalence found in diverse studies is shown in **Table 1**.

Study group	Area development	Seroprevalence (%)	Reference
Pregnant women	Rural	4.4	Gamboa-Leon et al. [11]
Mothers and newborns	Urban	0.8–1.2	Gamboa Leon et al. [13]
General	Rural	11.2	Zavala-Veláquez [12]
Pregnant women	Rural and urban	0.8	Sosa-Estrani et al. [14]
General	Rural and urban	3.7	Nouvellet et al. [15]
General	Rural	2.3	Monteon et al. [16]
Cardiopathics	Urban	15	Alducin-Tellez et al. [17]
General	Urban	8	Jiménez-Coello et al. [18]
Cardiopathics	Rural	4.8	Monteón et al. [19]
Other factors			
Dogs	Rural and urban	14.76	López-Céspedes et al. [20]
Dogs	Rural	29.9	Carrillo-Peraza et al. [21]
Cats	Urban	8.6	Jiménez-Coello et al. [22]

Data are obtained from [11–22].

Table 1. Prevalence reported in the Yucatan Peninsula.

A big number of tourists from non-endemic countries can become infected through the skin or mucous contact with the stool or urine of infected bloodsucking insects of the triatomine species when they visited endemic areas, were exposed, and almost never had previous knowledge about the pathogen or the Chagas disease. The acute phase is usually not diagnosed because it is characterized by an influenza-like illness acutely in adults. However, as *T. cruzi* can be presented in the blood of infected individuals, decades after infection took place; the infection can be also transmitted through blood transfusion and organ transplant, which is considered the second most common mode of transmission for *T. cruzi*. Indeterminate and chronic infections may be reactivated by immunosuppression, particularly human immunodeficiency virus (HIV) infection or acquired immunodeficiency syndrome (AIDS), and by pregnancy [23, 24].

On the acute infection, symptoms are suggestive of a nonspecific febrile illness, and these may occur between 1 and 4 weeks after infection; there also are severe forms that include myocarditis or meningoencephalitis, chagomas are indurated areas of erythema and swelling at the site of parasite penetration. Romaña's sign is the classic sign of acute Chagas disease; it is characterized by painless edema of the palpebrae and periocular tissues that appear when the conjunctiva was the parasites route of entry; not all patients with acute Chagas disease present with Romaña's sign (**Figure 2**) [26].

Immunocompromised travelers, such as advanced aged and people with chronic diseases, need to be concerned by their travel physicians of the potential risk for Chagas disease transmission by American Triatomines, due to coasts, colonial cities and ancient archeological ruins from Latin America are high endemic regions among the most popular touristic destination, these patients must be advised about the behavioral risk factors of the disease and take the appropriate steps to reduce risk [2, 27].

Figure 2. Romaña's sign and characteristic sign of acute Chagas disease [25].

Muñoz-Vilches et al. [28] recommended that tourists who travel for long periods through the tropics (endemic areas)—like the Yucatan Peninsula—are at risk for acquiring these endemic tropical diseases and should be screened similar to patients born in these areas, especially when they are symptomatic. Animals can also increase the risk of infection, acting as intermediate hosts of infection; the prevalence of stray dogs nearby plays an important role in CD prevalence of endemic places, such as Yucatan [29].

2.2. Presence of vectors

Natural landscapes of the Yucatan Peninsula (YP) have been modified since the expansion of Mayan groups at least until 900 A.D., and subsequently the Spanish conquest, mainly in the north and east region between Yucatan and Quintana Roo state [30].

Mexico is located in both the neotropical and Nearctic regions, having different demography, climates and vegetation, with over 19 vector species, Yucatan has the *Triatoma dimidiata* as its most abundant and epidemiologically relevant vector (**Figure 3**), although other triatomines are present and sporadically collected even in anthropic habitats: *Panstrongylus rufotuberculatus, Eratyrus cuspidatus, Triatoma nitida, Triatoma hegneri* with 88% of the population exposed to at least one of this competent vector species [31].

Figure 3. *Triatoma dimidiata* [25].

Previous reports show that there is a heterogenic distribution of the vector, identifying the north of the peninsula as the highest bug abundance area and a greater risk of natural transmission [8].

T. cruzi reduviids are hardy and ubiquitous vectors of Chagas disease among wild and domestic animals and humans in a variety of transmission cycles, from dense rain forest to packed cities and beaches [2]. The vector adapts quickly to houses, gardens and parks in the city and can spread quickly when urbanization is introduced in rural areas. The blood of dogs may be an important source of triatomine bugs and acts as sentinels of domestic and peridomestic vector-mediated transmission [32].

T. cruzi reduviids are recognized to be sylvatic, periurban and now urban agents of transmission cycles of Chagas disease; in the sylvatic cycle, the vector becomes infected with *T. cruzi* as either nymphs (the five juvenile stages or instars) or adults during rainy seasons by feeding on many nonspecific wild animal hosts. As humans begin to deforest and develop rural areas for agriculture, ranching, and housing, infected reduviids are attracted to more stable, peridomestic environments. Most often, the pathogenic agents are within the vector's saliva injected directly into the skin, but the metacyclic trypomastigotes of American trypanosomiasis, in contrast, are passed not in the saliva, but in the feces of their triatomine vectors. The host is infected when these protozoa are scratched or rubbed into the wound or mucus membranes. Triatomine bugs of tropical and subtropical Central and South America serve as vectors for the agent of Chagas Disease, and these burden people with more than their bites, as with bedbugs, conceal themselves during the day, often in thatched roofs and porous walls of logs and sticks, and glide down from roof crevices and cracked walls seeking blood from almost any available warm-blooded host at night, passing the infectious forms in their feces while feeding painlessly [33]. In heavily infested areas, residents may suffer dozens of bites each night, with individual adult bugs drawing up to 4 g of blood per meal (**Figure 4**).

2.3. Preventive recommendations

Travelers in endemic regions are wise to select accommodations with solid ceilings or roofs, no thatched palmetto or palm fronds which are the natural canopy homes for reduviids and with smooth walls without crevices. Cabanas and lodges that provide accommodations in rural or isolated settings for large numbers of tourists are also perfect accommodations for reduviid families, who will both blood-feed and breed while hiding in such dwellings, some of which may be very comfortable and elegant [34].

As clinicians usually consider, evaluate and recommend prophylactic treatments, vaccines and other travel-related issues, they must offer appropriate guidance on preventing vector borne illnesses they might encounter and their arthropods, focusing on their destinations, accommodations and the risks accompanying their planned activities.

Diaz [2] and Carter et al. [26] mentioned that the best preventive strategies of travelers to the Americas should be directed at (1) information about *T. cruzi* endemic areas and about the transmission risks of Chagas disease, (2) avoid residence in poorly constructed dwellings, (3) be advised to take precautions while engaging in outdoor activities, and (4) a recommendation for sleeping under pyrethroid-impregnated insect nets (taking care of tucking in the edges to

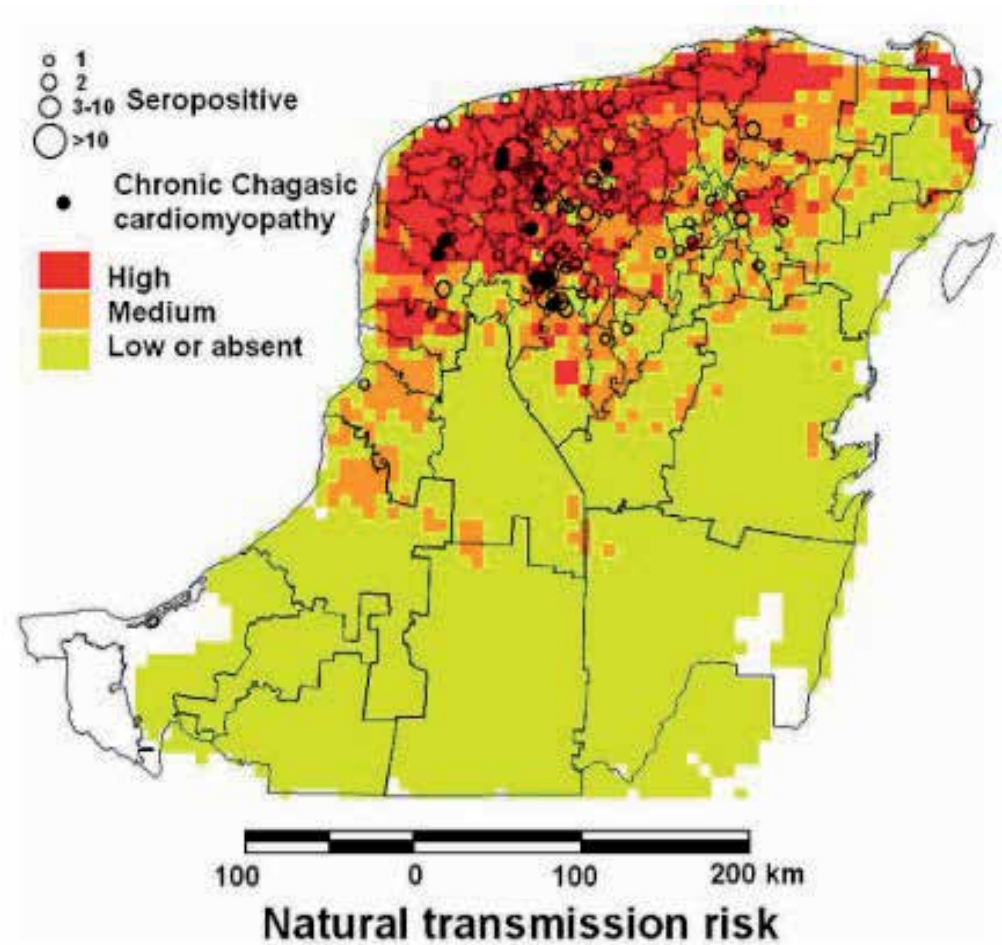

Figure 4. Natural transmission risk map for Chagas disease. The risk of natural transmission of Chagas disease, defined as proportional to the abundance of infected *Triatoma dimidiata* [25].

provide a physical barrier to the triatomine bugs). However, pyrethroid impregnated nets are not definitive or efficient, enough to avoid the insect nearby; also the exposition to chemical agents is not recommendable, mainly if their effect over the triatomines is not adequate.

Infection prevention requires travelers to avoid sleeping in hovels and mud dwells and checking in their hotel rooms and potential places where the triatomine vector could be hidden, using bed nets, and avoid potentially contaminated fruit or cane juices from street vendors [35]. Packing efficient insect repellents and properly applying them, if not purchasing them on the destination is an option, always keeping in mind that formulations based on DEET remain as the best available repellents, other methods as clothing cleaning with permethrin should be considered, clothing is more protective if it has a tight weave and it is loose and baggy. Window screens, bed nets and air conditioning can markedly reduce annoyance and risk from those biting insects that live or venture indoors [36].

The riskier blood or organ donors are those who come from areas where the prevalence of infection is high; there is also an increased risk in places with a great number of immigrants from Mexico, Chile, El Salvador, Colombia, Bolivia, and other Latin American countries. The most effective form of prevention is to avoid blood use from donors of countries where Chagas disease is endemic, or performing serological screening for *T. cruzi* exclusively on donors from these countries [37].

This lack of knowledge and awareness of Chagas disease and its relationship with triatomines can be considered a major barrier for vector control, as it likely results in communities having limited interest in and motivation for eliminating triatomines [38].

Concrete recommendations for preventive actions are needed for the national and international visitors from endemic and non-endemic countries, focusing on vector control, spreading information and screening in health procedures, all of this should be following a structure as listed:

1. Tourism providers and local government from traveler's country of origin must inform about the Chagas disease in this area, its changing nature of ecology, of where no formal measures to control or eradicate it have been taken.
2. Protect yourself and your pets from the vector, as they are also warm-blooded hosts and sentinels of this disease, they should be required to have a good insecticide or repellent.
3. Hotels and resorts should also have their own vector control plan, as they do with mosquitoes and other insects, keeping their green areas clean and not having palm roofs or other cracks and holes that could lead to unwanted triatomines.
4. Touristic areas such as ruins, colonial cities and other attractions in endemic zones must be fumigated regularly, as a control for the vector, which could be easily attacked.
5. If receiving medical attention, particularly blood transfusions after an accident, labor or any other conditions, screening for Chagas Disease is important, due to lots of donors could have been previously exposed, have history of CD or the receiver is a female on reproductive age, with a higher risk of vertical transmission.

Providing acute information and taking the corresponding recommendations could allow reduce the travel-associated risks of Chagas disease infections in this *T cruzi* endemic region from southern of Mexico, and where the CD which is now extended and widely stablished as well in urban and rural areas.

3. Conclusions

Chagas disease is as a neglected tropical disease that must be considered by people willing to travel to endemic zones, such as the Yucatan Peninsula. Travelers must be aware of the potential risk that exist in the areas they will be interacting in, such as public or resort beaches, archeological places, parks, rural areas and even yards; it is crucial for everyone traveling to correctly identify the vector, and to use properly insecticides to prevent the

triatomines bite and probably infection. However, information about this and other vector transmitted diseases must be displayed in the touristic areas and written information given to everyone upon arriving, and tourists should take preventive measures such as a good quality repellent, checking window screens, holes or other places where the reduviids could hide and request bed nets that could be offered by the hosting accommodations, including hotels, archeological sites and ecotourism locations. Other measures can be also taken, as reduction of the stray dog population, limiting contact with them, and green areas fumigation for vector control; even when staying in previously fumigated areas, insecticides should be used, especially in exterior areas, when walking and even resting on the beach, but one of the most important things is to correctly identify the reduviid vector, so travelers can stay away from them.

Latin American immigrants represent an important segment in worldwide population; therefore, they must consider risks when arriving from their native countries and never lose sight of other forms of infection blood transmission possibilities, especially in immune-depressed patients and vertical transmission that is taking more importance nowadays.

Conflict of interest

The authors declare no conflict of interests.

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Chapter 3

Neglected Tropical Diseases with an Impact on Kidney Function

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Additional information is available at the end of the chapter

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Abstract

Neglected tropical diseases are a group of infectious diseases caused by infectious and parasitic agents that occur in a large part of the world affecting millions of people and can complicate matters with serious organ damage. The kidneys can be affected in many of these diseases, including Chagas disease, dengue, leishmaniasis, leprosy, and schistosomiasis. In this chapter, we describe the mechanisms by which the kidneys are damaged in the setting of these diseases, the clinical manifestations, and the current available treatment options. We also describe the recent novel biomarkers that are under investigation for the early diagnosis of kidney injury in the course of these diseases and the future perspectives.

Keywords: tropical diseases, neglected diseases, kidney diseases, acute kidney injury

1. Introduction

The World Health Organization (WHO) lists a group of communicable diseases that currently affects more than one billion people in more than 100 countries and cost billions of dollars every year [1]. These diseases have the common characteristics of affecting predominantly poor people living in precarious conditions, with close contact with vectors and domestic

animals, and also facing difficulties to access health-care facilities. The 2018 WHO list of neglected tropical diseases includes Buruli ulcer, Chagas disease, dengue, chikungunya, dracunculiasis, echinococcosis, foodborne trematodiases, human African trypanosomiasis, leishmaniasis, leprosy, onchocerciasis, rabies, scabies and other ectoparasites, schistosomiasis, soil-transmitted helminthiases, snakebite envenoming, taeniasis/cysticercosis, trachoma, yaws, lymphatic filariasis, mycetoma, chromoblastomycosis, and other deep mycoses [1]. The kidneys are frequently affected in these diseases, and in some cases, the complications increase the risk of death. In this chapter, we discuss the main neglected tropical diseases, which complicate matters with kidney involvement.

2. Chagas disease

Chagas disease (or American trypanosomiasis) is the infection caused by the flagellated protozoan *Trypanosoma cruzi* and transmitted by insects of the family Triatominae, the *Triatoma infestans*, known as "kissing bugs" (in Brazil: "barbeiro"; in Argentina, Bolivia, Chile, and Paraguay: "vinchuca"; in Colombia: "pito"; in Venezuela: "chipo"; and in Central America: "chinche") [2]. The transmission occurs through the inoculation of *T. cruzi* in the blood of the host, through the insect stool left next to the site of the bites. The infection can also occur through the ingestion of the infected insects (contaminated food), blood transfusion, organ donation, or vertical transmission [3]. Kidney involvement in Chagas disease is seldom described and rarely studied [4].

The disease is endemic in 21 Latin American countries, and the highest prevalence is registered in Bolivia, Argentina, Paraguay, Ecuador, El Salvador, and Guatemala [3]. In Brazil, there was a significant decrease in incidence due to the vector control program, and now the disease is predominantly seen as chronic cases with cardiac or gastrointestinal manifestations [5, 6].

Clinical manifestations of Chagas disease can be divided into acute and chronic phase [3, 4]. The acute phase manifests with fever, signs of inflammation at the inoculation site, unilateral palpebral edema (Romaña sign), lymphadenopathy, and hepatosplenomegaly [3]. Severe acute Chagas infection is rare and occurs in less than 5% of the cases, manifesting with acute myocarditis, pericardial effusion, and meningoencephalitis [3]. The chronic phase develops in 10–30% of infected individuals and is characterized by heart disease and gastrointestinal disease (megaesophagus and megacolon) [7, 8].

T. cruzi has the ability to act as a parasite to a great variety of cells, including kidney cells [4]. There is evidence of functional and structural kidney abnormalities after *T. cruzi* infection, associated with reduction in renal blood flow, proximal tubular damage, and inflammatory interstitial infiltrate. An observation is made of an increased production of pro-inflammatory cytokines and nitric oxide, as well as renal function loss associated with high parasitic load [9–12]. In the acute phase, the renal lesion is related to cardiovascular dysfunction, due to a transitory decrease in renal blood flow, which was demonstrated in experimental models [11, 13]. Glomerulonephritis is reported in *T. cruzi* infection and also demonstrated in

experimental studies [14, 15]. The pathophysiology of kidney involvement in Chagas disease is associated with immunological process and includes autoantibodies production [16, 17]. There are reports that in the chronic phase of the disease, glomerular deposits of IgM occur at early stages, with intense inflammatory response, resulting in immune complex formation, which leads to glomerulonephritis [9]. The exact mechanism by which *T. cruzi* causes kidney disease is still to be elucidated [4].

Treatment of Chagas disease is based on antitrypanosomal drugs, which is indicated for all acute and congenital cases, reactivated cases, and chronic disease in individuals <18 years [3]. Specific treatment is currently recommended even for patients with the chronic forms of the disease, despite achieving lower cure rates than in acute phase [6]. The main available drugs are benznidazole (dose for adults: 5 mg/kg/day; for children: 5–10 mg/kg/day) and nifurtimox (dose for adults: 8–10 mg/kg/day; for children: 15 mg/kg/day), for 60 days.

3. Dengue and other endemic arbovirosis

Dengue is the most common arbovirosis in the world, there are more than 2 billion people living in endemic areas [18], and it is the second most common disease transmitted by mosquitoes, following malaria [19]. Tropical countries face repeated outbreaks of the disease. In Brazil, the most recent outbreaks have affected millions of people [20, 21]. In recent years, other arbovirosis has caused epidemics in the Caribbean region and Latin America: chikungunya fever and Zika virus infection, and renal involvement has already been reported [22–27].

Clinical manifestations vary according to patients' age, and the incubation period varies from 3 to 14 days. The majority of patients are asymptomatic or have a mild disease characterized by fever, headache, myalgia, arthralgia, retroorbital pain, and maculopapular rash, which has been known as dengue fever [19, 28]. Severe forms of the disease, characterized by coagulation disturbances, increased vascular permeability, and hemorrhagic manifestations, are classically known as dengue hemorrhagic fever and can evolve to dengue shock syndrome [19]. There is evidence that severe cases of dengue are increasing, complicating with organ damage, including kidney involvement [14, 18].

Renal abnormalities in dengue include hydroelectrolyte disturbances, acute kidney injury (AKI), and, less frequently, glomerulonephritis, rhabdomyolysis, and hemolytic uremic syndrome [18, 29–31]. AKI seems to be more frequent in adults than in children with dengue and is associated with hypotension, sepsis, multiple organ dysfunction, and use of vasoactive drugs [30–37]. Direct kidney damage caused by dengue virus is described through cytopathic effect of viral proteins on glomerular and tubular cells, associated with immunological mechanisms mediated by viral antigens deposited on glomerular structures and tissue damage by immune complex deposition [18, 31, 38]. Other factors that mediate renal lesions in Dengue are hemodynamic instability, hemolysis, rhabdomyolysis, and nephrotoxic drug use. Autopsy studies show evidence of acute tubular necrosis, predominantly in the proximal tubules, hemorrhage, and edema, more accentuated in the medullary region [29, 38]. Dengue-associated AKI usually has a favorable outcome, with recovery in around 2 weeks [18]. Among patients

with chronic kidney disease, dengue is associated with high mortality [18]. Among renal transplant patients, dengue fever also presents favorable outcomes and does not significantly affect the allograft function [39].

Chikungunya infection is another arbovirosis that has been responsible for large epidemic in recent years, causing self-limited disease in most cases, with predominant symptoms being fever and arthralgias [22, 23], but renal involvement has been reported in fatal cases [22, 24]. A series of 10 autopsies from Colombia evidenced that renal involvement is frequent, with serum creatinine at admission varying from 1.2 to 8.9 mg/dl (median 2.8 mg/dl), and the main histopathological findings were glomerular involvement (glomerular edema, nephrosclerosis, and membranoproliferative glomerulonephritis) and acute tubular necrosis [22, 24]. Similar to what is seen in dengue infection, transplanted patients who acquire Chikungunya infection seem to have a milder disease, with less severe arthralgia, and this could be due to immunosuppressant drug effects [25]. The acute viral infection also seems not to significantly affect the renal allograft [25].

Zika is another arbovirosis that usually causes a mild infection, which is predominated by fever, arthralgias (milder than in dengue and chikungunya), and a typical rash with pruritus, which was brought to the attention of the Public Health authorities in 2015—the outbreak occurred in Brazil affecting a large number of people and causing neurologic disturbances in newborns, including microcephaly [26, 27]. Renal involvement has been recently described in experimental studies, which demonstrated that the Zika virus has the potential to infect different types of kidney cells, including podocytes, endothelial and mesangial cells, and the virus can also be detected in the urine, which is an evidence of the kidneys as reservoirs of the virus and a potential source for the transmission [26, 27]. These findings also suggest that patients infected with Zika virus are at risk of developing glomerulonephritis, as expected in the other arbovirosis.

There is no specific treatment for dengue, as well as for the other arbovirosis, so it consists in supportive measures and depends on clinical manifestations [40]. Treatment basis include hydration (oral or venous, depending on disease severity), resting and symptomatic medication (antipyretics, analgesics), and avoiding the use of anti-inflammatory drugs. Correction of metabolic and electrolyte disturbances is crucial and if instituted early can prevent complications [18]. Dengue vaccines are under research and the available ones present good results in individuals with previous exposure to the virus [41].

4. Leishmaniasis

Leishmaniasis is the tropical disease caused by the parasites from the genus *Leishmania*, transmitted by mosquitoes *Phlebotomus* and *Lutzomyia*, with the majority of cases concentrated in Latin America, South East Asia, East Africa, and, less frequently, Europe [42, 43]. There are 12–15 million infected people in the world, and 350 million are at risk [43]. Approximately, 1.5 –2 million new cases of leishmaniasis are registered in the world each year [43], and 90% of cases are concentrated in India, Ethiopia, Somalia, Sudan, and Brazil [44–46].

The disease can be asymptomatic or manifest as a localized disease in the skin (cutaneous leishmaniasis) or affect different organs (visceral leishmaniasis, also known as kala-azar or black fever). Incubation period can vary from few weeks to years, and the main symptoms and signs include insidious low-grade fever, pallor, splenomegaly, hepatomegaly, fatigue, weakness, hyporexia, and weight loss [42, 47]. In endemic areas, such as northeast of Brazil, the most affected individuals with visceral leishmaniasis are children [28]. Laboratory abnormalities are common and are typically characterized by pancytopenia, associated with hyperglobulinemia and hypoalbuminemia [28].

Kidney involvement in visceral leishmaniasis has been studied in both cutaneous form of leishmaniasis (American cutaneous leishmaniasis), in which tubular dysfunction predominates [48], and visceral leishmaniasis, in which renal lesions seem to be more frequent and severe [49]. Immunological mechanisms seem to be the key components of kidney injury in leishmaniasis, with immune complex deposition, T cell activation, and inflammatory process, leading to glomerulonephritis and interstitial nephritis [49–51]. Clinically, renal involvement in visceral leishmaniasis can manifest with acute kidney injury (AKI), mild-to-moderate proteinuria, hematuria, leukocyturia, hydroelectrolyte disturbances, and tubular defects—urinary concentration and acidification defects, which can persist even after specific treatment [52–54]. Glomerular disease in leishmaniasis is characterized to be mesangial proliferative, with immune complex deposition, and can complicate with rapidly progressive glomerulonephritis [55, 56]. Microalbuminuria is the most frequent renal abnormality found in patients with visceral leishmaniasis (around 40%), evidencing important glomerular filtration barrier defect caused by the infection process [57, 58]. AKI has also been reported in a considerable number of patients (more than 30%); it seems to be more frequent and severe in adults and is associated with hypokalemia, leukopenia, and use of amphotericin B [53, 59]. AKI was also associated with hyponatremia, hypoalbuminemia, hyperglobulinemia, and secondary infections in children with visceral leishmaniasis [60]. New biomarkers are under investigation aiming to detect early kidney injury in tropical diseases, including leishmaniasis. The most studied by now are the neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and monocyte chemotactic protein-1 (MCP-1) [61–63]. NGAL has been associated with AKI in visceral leishmaniasis, presenting good accuracy for the early diagnosis of AKI, and is a promisor tool for the identification of patients at risk of developing severe disease [64].

Treatment consists of anti-Leishmania drugs, and the standard treatment is pentavalent antimonials [42, 43]. The most commonly used drug is meglumine, but the biggest problem is its toxicity (cardiotoxicity). The second choice is amphotericin B, which has nephrotoxicity as the main adverse effect [42, 43].

5. Leprosy

Leprosy is a chronic disease caused by the *Mycobacterium leprae*, an acid-fast bacillus, characterized to be incapacitating and to cause organ failure in some cases, including kidney disease

[49, 65, 66]. The disease is highly contagious, but few people develop the disease once there is natural resistance to the bacillus [65]. This is one of the oldest diseases known to affect humankind, with reports in the Bible [67].

There are reports of leprosy in 138 countries, with world prevalence of 0.2 cases per 10,000 inhabitants in 2015 [68]. The number of new cases in this same year was 211,973 cases, evidencing continued transmission (2.9 cases per 100,000 inhabitants). India and Brazil are responsible for the highest majority of cases in the world [68].

The spectrum of manifestations depends on the host's immunity and response to M. leprae, and the incubation period is long, going from 6 months to 10–20 years [65, 66]. During the course of the disease, there are "reactional states," in which the immune system reacts against the bacilli, exacerbating the clinical manifestations. The disease can be classified as paucibacillary and multibacillary, according to the number of skin lesions and bacilli found in skin smears [65, 66]. Besides affecting the skin, leprosy characterizes to cause peripheral nerve damage [65] and can complicate with systemic involvement, including nephropathy [49].

Kidney involvement in leprosy has been described for the first time a long time ago in autopsy studies and includes different types of glomerular lesions, such as epithelioid granulomas, and also Hansen's bacillus in renal parenchyma [49]. It is found in all forms of the disease but is more frequent in multibacillary forms [69, 70]. The most common leprosy-associated glomerular disease is mesangial proliferative glomerulonephritis and other histologic types are also found: acute proliferative glomerulonephritis, membranous glomerulonephritis, rapidly progressive glomerulonephritis, and others. Chronic tubulointerstitial nephritis and amyloidosis have also been described in the course of leprosy, leading to chronic kidney disease [71, 72]. The mechanics by which *M. leprae* infection leads to kidney disease are not completely understood [49], and it is probably associated with immunological phenomena. Complement reduction and immune complex deposition in glomerular basement membrane have been described [73–75]. Oxidative stress also plays a role in leprosy nephropathy. There is evidence of subclinical kidney damage even in patients with controlled infection. The levels of urinary MCP-1 are increased in leprosy patients and are associated with microalbuminuria levels and amount of bacilli of skin smears [76]. Renal tubular disturbances have also been described in leprosy, including concentration and acidification defects [77]. AKI can also occur in leprosy and seems to be associated to glomerulonephritis complications or secondary to the use of nephrotoxic drugs, mainly anti-inflammatory drugs and rifampicin, which are used in leprosy therapeutic scheme. Leprosy nephropathy seems to have changed in recent years, becoming a milder disease, characterized by micro-hematuria and mild proteinuria, which tends to recover after specific treatment [78].

Treatment consists of specific multi-drug therapy, reactional state treatment, prevention of disabilities, and psychosocial support. Specific therapy includes rifampicin and dapsone for paucibacillary patients (for 6 months) and rifampicin, dapsone, and clofazimine for multibacillary patients (for 12 months). Corticosteroids may be used for reactional states. Leprosy-associated end-stage kidney disease may require renal replacement therapy, which includes dialysis or transplantation, and immunosuppression for renal transplanted patients seems not to alter the response to leprosy-specific treatment [49].

6. Schistosomiasis

Schistosomiasis is the parasitic disease caused by organisms of the genus *Schistosoma*, which affects more than 200 million people in the world and is endemic in some tropical areas of the globe [79, 80]. It is the second most frequent parasitic disease, after malaria, and is still an important infection-associated cause of death [81].

The definitive host is the men, where the adult parasite reproduces and eliminates its eggs, which contaminated water resources [79]. In nature, disease transmission depends on the presence of snails from the family *Planorbidae* and genus *Biomphalaria*, and the men acquire the infection when the cercariae penetrate through the skin. The presence of the parasite in the human organism is relatively harmless, and the manifestations depend on immune response to different stages of the parasite in the body [82]. The disease can manifest as an acute syndrome, also known as Katayama syndrome, which is more common among travelers or immigrants in endemic areas with no immunity to *Schistosoma* and is characterized by fever, malaise, myalgia, headache, and abdominal pain, which can last from 2 to 10 weeks [80]. Depending on the patient's immune system response, the disease can progress to the chronic forms, in which predominate the intestinal form, in the case of *S. mansoni*, *S. japonicum* and *S. mekongi* infections. Patients with poor immune regulation develop severe fibrosis and hepatosplenic disease with periportal fibrosis [80]. The species *S. haematobium*, which occurs in some parts of Africa, affects the urogenital system, manifesting mainly by hematuria [80–82]. Other symptoms described in the infection by *S. haematobium* include hematospermia and inflammation in other structures in men—epididymis, testicles, spermatic cord and prostate, and women—hypertrophic/ulcerative lesions of the vulva, vagina, and cervix [82].

Kidney involvement in schistosomiasis is described and predominates in the form of glomerulonephritis [79]. In schistosomiasis, there are circulating antigens of adult parasite, and its eggs can be found in different organs, including the kidneys [79]. Acute kidney injury (AKI) is also described, with high prevalence in endemic areas, affecting as high as more than 40% of patients with the chronic forms of the disease [79]. Schistosomal glomerulonephritis is classically associated with the hepatosplenic form and ranges from asymptomatic disease to nephrotic syndrome [79]. Renal histopathological analysis evidences immune complex deposition, with schistosomal antigens in the glomerular basement membrane, as well as the presence of schistosomal eggs and granulomas. The mechanism of these lesions includes immunological phenomena, with response directed against parasite's antigens and immune complex formation and deposition in the glomeruli [83–86]. There is also evidence of polyclonal B-lymphocytes activation [87], but it is not sufficient to induce the process of nephritis, so more recent studies suggest the participation of autoimmune mechanisms [88]. The most common pattern of glomerular disease in schistosomiasis is mesangial proliferative and membranoproliferative glomerulonephritis [89, 90]. There are deposits of IgM, IgG, and C3 [91]. Amyloidosis has also been described [79, 92] but is less frequent, although it is always a possible pattern of kidney disease in any infectious disease with chronic evolution. Schistosomal glomerulonephritis can be classified in five types: mesangial proliferative glomerulonephritis, membranoproliferative glomerulonephritis, focal and segmental glomerulosclerosis, exudative glomerulonephritis, and amyloidosis [91]. Granuloma formation in the kidney is also a possible but rare complication of schistosomiasis [92]. Tubular

dysfunction also occurs in schistosomiasis, mainly urinary concentration dysfunction, which can be found in as high as 85% of patients with the hepatosplenic form in endemic areas [93]. Even in patients infected with *S. mansoni*, without clinically significant disease, there is evidence of renal inflammation, which was shown through increased urinary levels of MCP-1 [94].

Treatment is based on specific drugs, the first choice being praziquantel [80, 82], which has action against all *Schistosoma* species and provides cure rates above 80% [82]. The recommended dose is 40 mg/kg for *S. haematobium* and *S. mansoni*, and 60 mg/kg for *S. japonicum* [82].

Table 1 summarizes the main aspects of kidney involvement in the neglected tropical diseases discussed in this chapter.

Figure 1 illustrates the general pathophysiology of kidney involvement in tropical infectious and parasitic diseases.

Disease	AKI	CKD	Histopathological features	Novel biomarkers
Chagas	Yes	Probable, associated with cardiac involvement and heart failure	Deposits of IgM, IgG, C3, mesangial deposits, renal infarction, tubulointerstitial damage, renal infarction	—
Dengue	Yes	Rare	Deposits of IgM, IgG, C3, and less frequently IgA, acute tubular necrosis, glomerulonephritis	—
Leishmaniasis	Yes, associated with drugs, mainly amphotericin B	Possible, associated with amyloidosis and other complications	Deposits of IgM, IgG, C3, different types of glomerulonephritis, most common: mesangial and membranoproliferative, tubulointerstitial lesions, amyloidosis, tubular dysfunction	NGAL, early predictor of AKI, associated with microalbuminuria
Leprosy	Yes	Possible, associated with amyloidosis and other complications	Deposits of IgM, IgG, C3, different types of glomerulonephritis, mainly mesangial and membranoproliferative, but also rapidly progressive glomerulonephritis, tubulointerstitial nephritis, amyloidosis, tubular dysfunction	MCP-1, associated with renal inflammation
Schistosomiasis	Yes	Not common	Deposits of IgM, IgG, C3, different types of glomerulonephritis, mainly mesangial and membranoproliferative, amyloidosis, tubular dysfunction	MCP-1, associated with renal inflammation

AKI: acute kidney injury; CKD: chronic kidney disease; NGAL: neutrophil gelatinase-associated lipocalin; MCP-1: monocyte chemoattractant protein-1.

Table 1. Kidney involvement in neglected tropical diseases.

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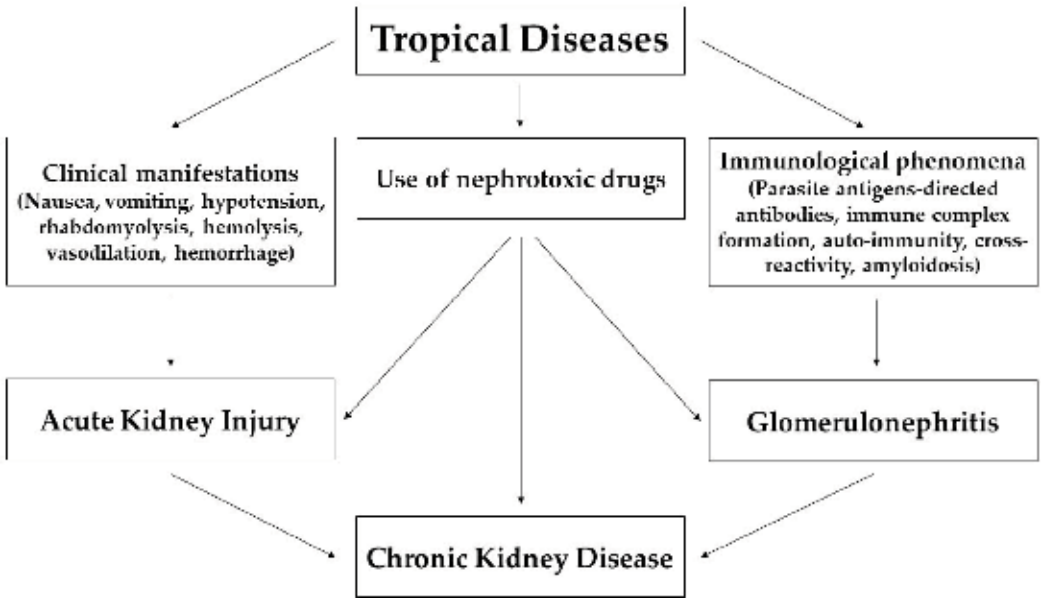

Figure 1. General pathophysiology of kidney involvement in tropical infectious and parasitic diseases.

7. Conclusion

Neglected tropical diseases represent a global public health problem, affecting billions of people and requiring billions of dollars to treat and control. Authorities should have in mind that the key approach to these diseases, including kidney disease itself, is prevention, which is far from ideal, mainly in the developing world. Governments should also have in mind that the main determinant factors for the perpetuation of the neglected tropical diseases in the world in the sum of poor living conditions (lack of sanitation, potable water, piped water, garbage collection, education, domestic animal control, disease-vector control, environmental problems, lack of urbanization plans, and many others), and they have responsibility for the control of these diseases [95]. Many complications can arise from these diseases, and one of the most frequent and severe is kidney disease. The main pathophysiologic factors include immunological phenomena and hemodynamic abnormalities. Endemic diseases, such as dengue, chikungunya, and Zika virus infection, which have affected millions of people in the last few years, are having increasing evidences of kidney involvement in severe cases. In the majority of cases, kidney involvement in these diseases develops as a complication when the patient has poor or delayed access to health care. Research is being conducted to better control these diseases, including vaccine development. Concerning tropical diseases-associated kidney injury, the current "hot-topic" research is to detect this complication through novel biomarkers in order to provide an early and more specific management aiming to avoid or stop renal function loss and then prevent permanent renal insufficiency. Physicians attending patients with tropical diseases should always evaluate renal function, once renal involvement is frequent, and renal recovery is possible if adequate management is provided.

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Conflict of interest

The authors declare there are no conflicts of interest regarding this work.

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Section 2

Arboviral Diseases

Chapter 4

Mosquito-Borne Diseases and 'One Health': The Northwestern Italian Experience

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Additional information is available at the end of the chapter

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Abstract

In Italy, the surveillance of Mosquito-Borne Diseases (MBDs) is regulated by two national preparedness plans: (1) for West Nile and Usutu viruses, integrating human and veterinary surveillance in order to early detect viruses circulation and to quickly apply control measures aimed at reducing the risk of transmission through blood and blood components and (2) for Arbovirosis transmitted by *Aedes* mosquitoes, mainly Chikungunya, Dengue and Zika viruses, based on surveillance of both imported and autochthonous human cases. This chapter reports the results of the application of these National Plans in Northwestern Italy and their impact for human health. In detail, we present the coordinated activities enforced in Piemonte and Liguria Regions, as a good example of the 'One Health approach' to control MBDs and prevent human transmission.

Keywords: mosquito-borne diseases, Italy, one health

1. Introduction

Mosquito-borne diseases (MBDs) are dangerously increasing in prevalence, geographical distribution and severity, representing a worldwide emerging threat for both humans and animals. Of great concern are the infections caused by viruses belonging to the *Flavivirus*

genus. This genus includes viruses considered endemic in Italy as West Nile Virus (WNV) and Usutu virus (USUV) transmitted by *Culex* sp. and viruses as Dengue virus (DENV) and Zika virus (ZIKV), transmitted by *Aedes* sp., not endemic in Italy but with the potential to spread to new areas where the mosquito vector is present.

Furthermore, in the last 10 years (2007/2017), two autochthonous Chikungunya virus outbreaks (CHIKV_ *Alphavirus* genus, family Togaviridae transmitted by *Aedes* sp.) with hundreds of cases due to an imported case followed by local vectorial transmission occurred in Italy, showing the impact that this virus could have even in a non-endemic country [1, 2].

Given the complex epidemiology of MBDs, Public Health Authorities are encouraging the interaction between multiple disciplines to implement an effective early warning.

WNV and USUV are antigenically close flavivirus with similar enzootic birds-mosquitoes transmission cycle where co-circulation has been reported in several studies [3–6].

WNV can be occasionally transmitted, through mosquito bites, to vertebrates other than birds as humans and horses that are considered dead-end hosts. Human infection through blood transfusion and solid organ transplantation is also demonstrated [7].

WNV risk for human health is well recognized: the majority (*80%) of infections in humans are asymptomatic, and symptomatic infections are mostly characterized by a mild, self-limiting febrile illness. WNV neuroinvasive disease develops in <1% of WNV-infected persons [8].

USUV is generally correlated with high mortality rates in its bird reservoirs. Mosquitoes infected with USUV can incidentally transmit the virus to other vertebrates, including humans, which can result in neuroinvasive disease [9].

In Italy, an increasing number of outbreaks of West Nile disease, with occurrences of human cases, have been reported since 2008, mainly in the North East part of the Country.

WNV lineage 1 (Lin1) was the only strain detected until 2011 when, for the first time, the presence of both WNV Lin1 and WNV lineage 2 (Lin2) was demonstrated. Since 2013, WNV Lin2 was the main strain detected, and a west bound spread of the virus started [8].

USUV has been detected in Italy since 2007 in mosquitoes, birds and humans [10–13].

Although characterized by a lower pathogenicity than WNV, USUV pathogenic potential for human is not completely characterized, and knowledge about this crucial aspect is constantly evolving [14–16]. In 2009, two cases of encephalitis in immunocompromised patients were reported in Italy [10, 12, 17], and additional USUV neuroinvasive infections in humans were described in Croatia in 2013 even in one healthy patient [18].

Finally, retrospective analyses to monitor the spread and to evaluate the role for public health of USUV in an endemic area conducted in the city of Modena showed a high seroprevalence in humans with or without neurologic impairments, underlying that USUV infection in humans should not be considered a sporadic event [16].

DENV and ZIKV are characterized by a human-to-mosquito-to-human cycle of transmission.

DENV is the most prevalent arthropod-borne viral disease in tropical and subtropical countries. The disease manifestations range from an influenza-like disease known as dengue fever

to a severe, sometimes fatal disease characterized by haemorrhage and shock, known as dengue haemorrhagic fever/dengue shock syndrome [19].

The classic clinical picture of ZIKV infection resembles that of dengue fever and is manifested by fever, headache, arthralgia, myalgia, conjunctivitis and maculopapular rash. Recently, a possible association between ZIKV infection in pregnancy and fetal malformations has been hypothesized [20].

Up to date, all the DENV and ZIKV human cases reported in Italy have been related only with returning travellers from endemic countries and not associated with transmission through local potentially competent vectors [21].

However, if vectors are present, infected returning travellers could initiate a local virus transmission as in the CHIKV outbreak occurred in the Emilia-Romagna Region, Italy, in 2007 [22].

CHIKV is characterized by a human-to-mosquito-to-human cycle of transmission.

Clinical onset is abrupt, with high fever, headache, back pain, myalgia and arthralgia; the latter can be intense, affecting mainly the extremities (ankles, wrists, phalanges) but also the large joints. Skin involvement is present in about 40–50% of cases and consists of a pruriginous maculopapular rash predominating on the thorax. The symptoms generally resolve within 7–10 days, except for joint stiffness and pain: up to 12% of patients still have chronic arthralgia, 3 years after onset of the illness [23].

Italy experienced two CHIKV autochthonous outbreaks, in Emilia Romagna Region in 2007 and in Lazio Region in 2017, due to returning infected travellers during the vectors' season with hundreds of cases.

Given the complex epidemiology of MBDs, determined by the interaction between pathogens, hosts, vectors and ecosystem, the cooperation of multiple disciplines (veterinarians, epidemiologists, entomologists, biologists and doctors) is needed for an effective early warning, surveillance and control [24]. A deep knowledge is required to better perform the surveillance plans: viruses with different cycles need different surveillance approaches. The syndromic surveillance on human cases could be considered more adequate to detect the introduction of new viruses in which humans are the reservoir. However, the entomological surveillance can be a valuable additional tool, considering that the presence of risk factors as competent vectors and suitable climatic conditions significantly increases the potential risk for the possible autochthonous transmission of MBDs.

1.1. West Nile virus national surveillance programme: origin and evolution towards an integrated system

Among MBDs, since almost 20 years, surveillance activities and control measures against WNV represent a deal both in human and in veterinary medicine, due to its complicated transmission cycle, the zoonotic potential and the risk of human-to-human transmission mainly through blood transfusion and organ transplantation.

In Italy, the WNV circulation was firstly detected in the late summer of 1998, in horses that displayed neurological clinical symptoms in a wetland area located in Toscana Region (West-central Italy); no cases of human encephalitis were reported [25].

After this outbreak, a national veterinary surveillance plan was implemented in 2001 under the coordination of the Italian Ministry of Health (MoH) and of the National Reference Centre for Exotic Diseases of Animals (CEntro Studi Malattie Esotiche; CESME), with the aim to early detect new incursions of WNV. Throughout the whole Italian territory, 15 risk wetland areas were identified, considering the presence of migrating birds as a possible risk of virus introduction. During the transmission season, active surveillance was conducted by the periodic serological screening of sentinel-chicken flocks and sentinel horses, in order to detect antibodies against WNV. Moreover, passive surveillance was conducted by virological screening of carcasses of dead birds and by clinical surveillance of neurological signs in equines all over the year [26].

In 2008, after 10 years of disappearance, a large WNV epidemic affected regions in the Northeast of Italy surrounding the Po river Delta. The virus was identified in mosquitoes, birds, horses and humans.

Following these events, the surveillance system was updated with the aim to detect as early as possible the WNV circulation. Three epidemiological areas were identified with different surveillance approach (**Figure 1**) [27]:

1. Area with Virus Circulation (AVC): areas of Northern Italy with WNV circulation during 2008. The aim of surveillance in this area was to evaluate WNV spread. Active surveillance was conducted by virological testing on synanthropic birds and/or by serological testing on sentinel-chicken flocks or free-range poultry flocks throughout the epidemiological season; entomological surveillance was enforced in 10 horse stables during the whole year with monthly mosquito trapping, in order to evaluate the role of mosquitoes as WNV vectors;
2. Surveillance Zone (SZ): areas surrounding the AVC for an extension of 20 km. The aim of surveillance in this area was to monitor WNV spread over the AVC. Active surveillance was conducted by virological testing on synanthropic and/or by serological testing on chickens or poultry flocks as enforced in AVC and by recruitment of sentinel horses for repeated serological tests in May, August and September in order to detect seroconversion against WNV. Entomological surveillance was enforced in three horse stables with monthly mosquito trapping throughout the year, in order to evaluate the role of mosquitoes as WNV vectors;
3. Areas at Risk (AR): 14 wetlands inherited from the previous surveillance plan. The aim of surveillance in this area was to detect WNV new introduction by migration routes. Active surveillance was conducted on synanthropic and chickens or poultry flocks as enforced as in AVC, and the same testing of sentinel horses as in SZ; entomological surveillance was enforced in one horse sentinel stable by monthly mosquito trapping during the epidemic season, in order to evaluate the composition of mosquito fauna.

Passive surveillance was carried throughout the year in the whole Italian area by the detection of neurological signs in equines and increased mortality among wild birds.

Since 2008, WNV has expanded to a wide geographical area in Europe and the Mediterranean basin, with an increasing number of outbreaks, including several human cases of West Nile Neuroinvasive Disease (WNND).

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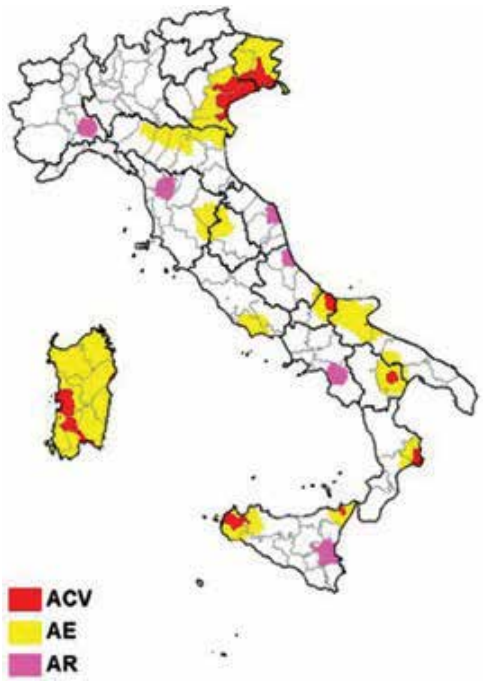

Figure 1. Map of Italy with WNV surveillance areas.

In Northern Italy, the virus has become endemic, progressively extending the range from the East to the West in the Po river valley area. The establishment of overwintering cycle was due to the presence of the main mosquito vector *Culex pipiens* at high density and the evidence of virus transmission in resident wild bird species susceptible to WNV infection [28].

In 2010, the MoH published a National Plan for WNND Human Surveillance in Italy, which refer to the WNV veterinary Surveillance Plan. This was the first step towards the adoption of a comprehensive human and veterinary (animals and vectors) surveillance system against WNV in Italy.

The national definition of WNND suspected human case included every patient presenting with fever ≥38.5°C and neurological symptoms: encephalitis, meningitis or acute polyradiculoneuritis (Guillain-Barré syndrome) or acute flaccid paralysis. Every suspect case of WNV infection was promptly reported to the Public Health Department and laboratory investigated. Clinical suspected cases of WNND or West Nile Fever (WNF) were classified as confirmed cases if they fulfilled at least one of the following laboratory criteria: (1) isolation of WNV from blood or cerebrospinal fluid (CSF), (2)the presence of IgM antibodies in CSF by ELISA, (3) the detection of WNV RNA by RT-PCR in blood and/or CSF and (4) the detection of increased levels of IgM and IgG antibodies against WNV by neutralization testing [29].

Human or veterinary WNV confirmed cases have to be notified by the Regional authorities to the national level, both to the MoH and to the National Blood Center (NBC).

In line with the EU directive for blood safety (Directive 2004/33/EC, Annex III), from 2009 to 2014, the NBC implemented, as a preventive measure, a 28-day deferral period for blood

donor, leaving areas with ongoing transmission of WNV and WNV Nucleic Acid Test (NAT), testing of all donations (peripheral blood, bone marrow and cord blood stem cell donations) generally from July to November [30] and coming from Provinces in which human cases of WNND had been reported the previous year.

The NAT screening approach changed in 2015 for five Northern Italian Regions (Piemonte, Lombardy, Friuli Venezia Giulia, Veneto and Emilia-Romagna) concerned by WNV circulation in previous years. These regions guaranteed an active standardized integrated entomologic and veterinary WNV surveillance from June to October. In addition to the notification of human cases, the date detection of WNV in mosquitoes and wild birds or detection of WNV-IgM antibodies in horses was introduced as new trigger criteria for the implementation of WNV NAT testing for the screening of blood donors [31].

Since 2009, a total of 103 WNV positive samples in blood donors have been reported in Northern Italy (Veneto, Lombardia and Emilia-Romagna Regions).

Comprehensive epidemiological, virological and entomological surveillance system is crucial for the timely detection of the spread of WNV, implementation of control measures and prevention of virus transmission in humans. Both national WNV veterinary and human surveillance plan have undergone further revisions year by year, in order to adapt to the seasonal evolution of the epidemiological scenario.

In 2016, the MoH has released a National WNV veterinary-entomological-human integrated Surveillance Plan. According to 'One-Health' approach, real-time cross-sectorial collaboration by Public and Veterinary Health Institutions is crucial to timely achieve and share information in WNV surveillance as a key to improve the management of WNV outbreaks and mitigate the risk of human transmission.

The aim of the programme was to early detect WNV circulation, reducing the risk of infection in the human populations. The steps to achieve this goal are as follows:

1. Veterinary surveillance: the Italian territory was subdivided into two distinct epidemiological territories:

   a. Endemic areas, where WNV was detected in the previous 2 years (mainly Regions of the Po river Valley, Sicilia e Sardegna islands): reinforcement of the virological screening in migratory and resident wild birds, mainly belonging to *Corvidae* genus. Alternatively, serological testing of rural poultry or sentinel chickens groups.

   b. Non-endemic areas (the remaining Italian Regions): serological testing of horses sera, in order to detect WNV-specific IgM early antibodies (recent infection).

2. Entomological surveillance: active mosquitoes fortnightly trapping during the epidemic season. Sampling sites selection was made according to risk factors for WNV cycle establishment: proximity to wetland areas, the presence of hosts, previous outbreaks. Moreover, a standardized approach was suggested, mapping provinces into equal quadrants (20 x 20 km), with at least one mosquito trap per quadrant. The aim was to establish seasonal mosquito patterns and detect WNV in mosquito pools by virological screening.

3. Human surveillance: active identification of all possible, probable and confirmed WNV infection human cases, including WNND.

The integrated plan was updated in 2017, including activities to control USUV and assess the risk for public health (MoH) [32]. Indeed, in the last years in Europe and northern Italy, the co-circulation of WNV and USUV was largely demonstrated [4–5, 33]. The two viruses seem to show biological cycle similarities, but while the WNV risks for human health are well recognized, knowledge about the medical importance of USUV is not fully understood and the transmission risk throughout blood transfusion has been recently suggested [34, 35].

This underlined the need to put in place surveillance measures to detect USUV activity and to assess the risk for public health. As in northern Italy, USUV shows a substantial geographic overlap with WNV circulation, surveillance was enforced in WNV endemic areas, by routine differential diagnosis of all cases of WNV-positive test (serological/virological).

2. Human MBDs national surveillance plan

After the CHIKV outbreak in 2007 in Italy, a national plan on imported and autochthonous human vector-borne disease (including CHIKV, ZIKA, DENV and WNV) has been implemented and annually updated on the basis of the epidemiological changes-based evidences.

The plan has been annually revised to 2017 with minor changes except for the exclusion of WNV and USUV in 2016/2017 from the human plan, due to the birth of the WNV/USUV National integrated Plan.

Here, we summarize the main concepts.

Epidemiological surveillance of human cases.

The main objectives of human surveillance are the following:

- to monitor imported cases in Italy, in particular in areas where there are competent mosquitoes, for the assessment of the risk of possible autochthonous transmission of the virus;
- the early identification of outbreaks and the monitoring of local transmission in order to implement control measures (prevention and response activities);
- to prevent accidental transmission through blood or organs transfusion and to identify other potential transmission pathways (e.g. sexual).

The human surveillance is carried out throughout the year. However, during the period of a major vector activity (June–October), the surveillance system will have to be strengthened (in terms of timeliness and sensitivity) in mosquito-infested areas, to allow the identification of cases, for the immediate adoption of the necessary control measures (in relation to entomological surveillance) and to reduce the risk of transmission.

Therefore, from the beginning of June to the end of October, particular attention must be paid to:

- early identification of suspected cases (symptomatic cases returning from an endemic Country);
- identification of people with compatible clinical symptoms but who did not travel to endemic countries, to early detect autochthonous outbreaks (two or more cases occurred within 30 days in a restricted area).

The plan defines, in a given area, three situations with different risk levels depending on vectors presence and density and occurrence of human cases:

Area A: the vector is present/absence of human cases; Area B: the vector is present/one or more imported human cases; Area C: the vector is present/isolated autochthonous cases/outbreaks.

For each area, the actions to carry out during the vector season and the rest of the year are defined:

Area A

December–May and November

- no activities

June to October

- monitoring and treatment protocols, if already existing, following Regional or national legislation;
- prevention activities: health education, methods of vector control including the elimination or management of breeding sites, larviciding with insecticides, the use of biological agents avoiding the application of adulticides.

Area B

December–March

- no activities

April–May and November

In the presence of human cases (probable and confirmed) **depending on the seasonal climatic conditions**, the following activities must be activated:

- activation or enhancement of entomological surveillance around the house of the patient for at least 2 weeks from reporting;
- treatments on private and public land, in an area within a radius of 200 m around the positive house;
- elimination of breeding sites;

- adulticide treatment (1 cycle);
- treatment of non-removable breeding sites with larvicidal products;
- information to the inhabitants about preventive measures to apply to avoid contact with vectors;
- follow-up for the week following the alert.

Area C

December–March

- no activities

April–May and November

In the presence of human cases (probable and confirmed) **regardless of the seasonal climatic conditions**, the following activities must be activated:

- activation or enhancement of entomological surveillance around the house of the patient for all the vector season;
- treatments on private and public land, in an area within a radius of 200 m around the positive house;
- elimination of breeding sites;
- adulticide treatment (1 cycle);
- treatment of non-removable breeding sites with larvicidal products;
- information to the inhabitants about preventive measures to apply to avoid contact with vectors;
- replication of all interventions in case of rain or poor efficacy of the first treatment cycle;
- in case of outbreak, repeat the disinfestation protocol after the first week;
- follow-up for all the vector season.

Human cases are notified by Regional and local authorities to the MoH and to the Istituto Superiore di Sanità (ISS, national public health institute) using a specific password-protected web-based system, which permits to report probable and confirmed cases, adding available epidemiological (including the province of exposure), clinical and laboratory information. The web-based system is accessible also to the National Blood Center (NBC) and to the National Transplant Center (NTC), which in cases of WNV human cases will implement precautionary measures on blood donation and transplant activities.

Measures for human cases

In order to reduce the disease spread, the home isolation of the case is recommended up to the exclusion of the disease and, in any case, not beyond the time course of viremia. The adoption of protective measures against vector bites is essential to interrupt the transmission cycle.

Other family members and people should use general precautions for parenterally transmitted diseases, such as:

- wash hands with soap and water, before and after assisting the patient, and, in any case, after removing the gloves if used;
- use gloves, not sterile, if contact is made with the patient's blood;
- do not use sharp products used for patient care or assistance.

Risk communication

Risk communication, training, information and health education have a decisive role in obtaining people collaboration.

Since there are currently no vaccines and/or therapies for the prevention and treatment of diseases as Chikungunya and Dengue (authorized only in some endemic countries) and Zika, the most effective prevention is to reduce people exposure to mosquito bites.

Currently, therefore, the key message is: 'Protect yourself from mosquito bites' which includes both the active control of the vector (use of insecticides, reduction of breeding sites) and adoption of individual protection measures (clothes, mosquito nets, repellents and also preventive measures to avoid sexual transmission, e.g. for Zika virus).

Risk communication to travellers going to or returning from endemic areas is of primary importance:

1. People travelling to endemic areas or endemic countries should inform about the circulation of ongoing epidemics (consulting ECDC and WHO sites and the Safe Travel website of the Ministry of Foreign Affairs and International Cooperation) and protect themselves from mosquito bites.
2. People travelling from endemic areas or endemic countries who develop suspected symptoms within 2 weeks of returning home should seek medical attention.

3. Materials and methods

3.1. West Nile virus integrated surveillance system in Northwestern Italy

Up to 2011, according to the national WNV surveillance programme, just a small area in Piemonte, defined as a 'risk area', in the Provinces of Alessandria and Asti, had to be monitored by entomological surveillance and checking for WNV seroconversion in horses residing in the area. No active surveillance activities were planned in Liguria. Even if any animal nor human cases had not been reported before, geographical and environmental features were conducive to maintaining competent vectors for MBDs.

Since 2011, supplementary surveillance activities have been put in place to control MBDs, with particular attention to WNV.

3.1.1. Area under surveillance: Piemonte and Liguria regions

The study area includes Piemonte and Liguria regions, in Northwestern Italy (**Figure 2**).

Piemonte region is the second largest Italian Region by geographical area (25.402 km^2) and comprises eight Provinces. It is surrounded on three sides by the Alps. About 41% of the Region is mountainous (prealpine and alpine) and 59% is hilly or flat. It is crossed from the West to the East by the Po river and bounded to the East by the Ticino river. Surrounding highly urbanized areas are intensive agriculture farmlands; they cover approximately 960,000 hectares where cereals and forage are predominantly cultivated; extensive rice fields dominate the landscape in the northeast part of the Region.

Liguria is one of Italian smallest Regions. It is divided into four Provinces. It is a narrow strip of land (5.416 km^2), highly urbanized, overlooking the Mediterranean Sea: the ring of Maritime Alps and Ligurian Apennines beyond the narrow strip of coast descends almost immediately to a considerable marine depth. It represents an important touristic and commercial area.

Characteristic rocky coasts and seascape attract many travellers to spend the holidays in the most famous tourist resorts along the Italian Riviera. The Port of her capital City Genova, with a trade volume of 58.6 million tonnes, is the first port of Italy. The invasive alien tiger mosquito (*Aedes albopictus*), which is an important vector of viral MBDs, was firstly detected in Italy in the city of Genova in 1990.

Piemonte and Liguria Region neighbours on areas where WNV is historically endemic: Camargue and Var in Southeastern France [36] to the West, Lombardy and Emilia Romagna Regions in Italy to the East.

Figure 2. Map of Italy with regional boundaries. Piedmont and Liguria in the upper left, in white colour.

In both Regions, the climate is warm temperate, and socioeconomic and geographic features described earlier provide ideal habitats for potential mosquito vector species for MBDs.

3.1.2. Entomological surveillance on mosquitoes

Entomological surveillance was activated from July to the end of October through adult mosquito collection with fortnight samplings. After every daily catch, mosquitoes were collected from each trap, and then they were transported in refrigerated boxes to the laboratories. Adult females were counted and identified to the species level, using a stereomicroscope and morphological standard classification keys [37]. After identification, mosquitoes were pooled in groups of up to 100 specimens each, sorted by species, date and site of collection, frozen, and then stored at −80°C for virological investigation.

3.1.2.1. Piemonte region

Prior to 2011, Piemonte had no a widespread entomological surveillance system for public health purposes. A local mosquito-fighting programme was carried out since 1997 for nuisance issues, only in eastern areas where rice is extensively cultivated nearby human settlements [38].

Besides the active surveillance in the 'risk area' established by the WNV National Surveillance Plan, a Region-wide systematic mosquito collection system has been carried out during seasonal mosquito activity since 2011 to gather entomological and virological data to support the Regional MDBs monitoring programme.

The Region was mapped into 73 equal quadrants (20 km x 20 km) as a proxy for geographical area, where the quadrant was defined as the epidemiological unit.

Trapping sites were selected according to risk factors of mosquito spread and the epidemiological cycle for MBDs: proximity to wetland zones, the presence of hosts, commercial areas and touristic routes (ports, airports and freight terminals), different land use and habitat features. At least one trap was placed in each quadrant at an elevation below 600 m. a.s.l., because most anthropophilic mosquito species, potential vectors of MBD, generally inhabit areas below 600 m. a.s.l. in Italy [39].

All trapping sites were mapped by latitude and longitude on a geographical positioning system (**Figure 3**).

Two types of traps were employed to collect adult mosquitoes:

- CDC traps baited with dry ice as a source of carbon dioxide (CO_2) (approximately 0.5 kg/trap): they attract adult females searching for a blood meal and are routinely used as the most common sampling method in WNV surveillance programmes in many Regions in the world. Each trap worked for a minimum of 12-h periods from sunset to sunrise.

- BG sentinel traps baited with BG-Lure and CO_2 (approximately 0.5 kg/trap): the BG-Lure attractant contains a combination of substances found on human skin that strongly attract anthropophilic mosquitoes (mainly belonging to the *Aedes* genus). Nevertheless, with the addition of CO_2, they are adapted to the collection of *Culex* mosquito species [40]. They were

located in sampling stations considered at a higher risk level for the introduction of exotic species and exotic pathogens. Each trap worked for a 24-h period.

3.1.2.2. Liguria region

Prior to 2011, no entomological surveillance activities were conducted in Liguria Region.

Since 2011, a Regional entomologic surveillance plan was carried out during seasonal mosquito activity with the goals to gather entomological and virological data and early detection of the introduction of invasive vectors and MDBs.

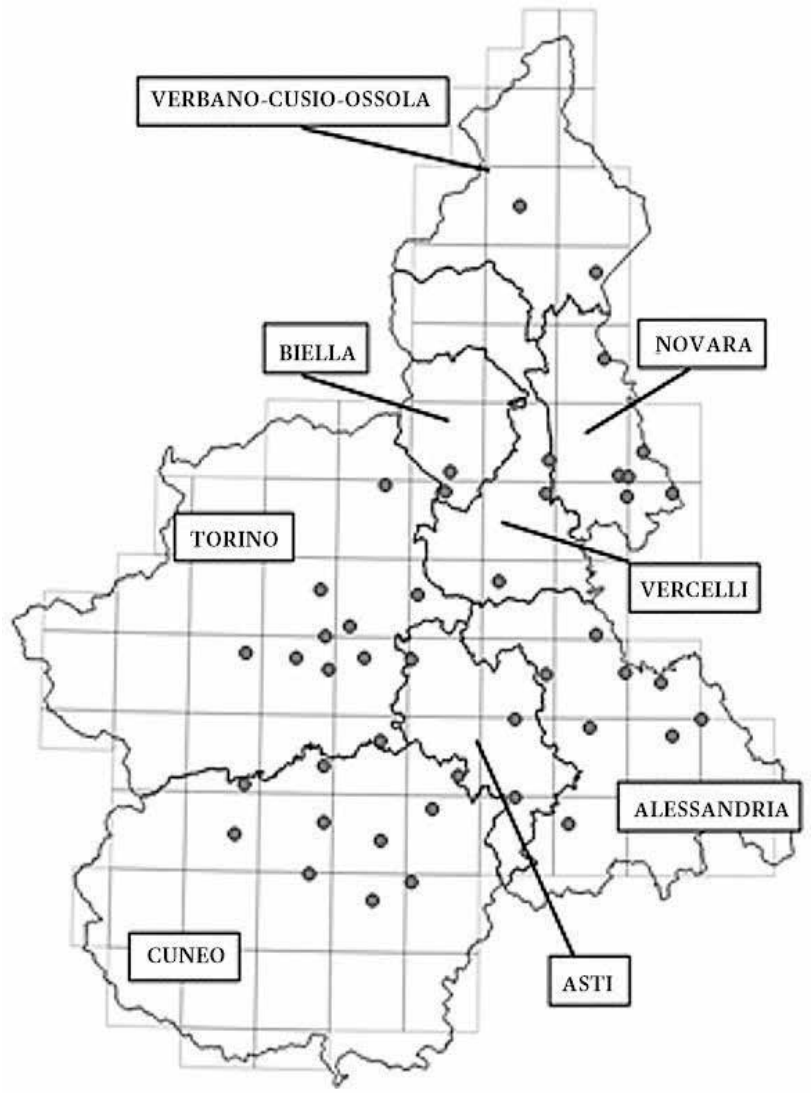

Figure 3. Map of Piemonte region with the grid of 20 x 20 km quadrants. Grey dots represent trapping sites. The eight provinces are named in uppercase letters in textboxes.

As the Regional territory displays characteristic feature of a narrow hilly coastal strip, trapping sites were located along the coastline. Locations were selected according to risk factors for the introduction and spread of exotic invasive mosquitoes and MBDs: habitat features, proximity to commercial areas and touristic routes (ports, airports and freight terminals) and presence of hosts.

The surveillance system has undergone further updates over time, increasing the number of trapping sites in order to best adapt to the evolving epidemiological scenario of the Region.

Particular attention was paid to the city of Genova, due to the presence of several risk factors of importing invasive mosquitoes and viral diseases: the port, the airport and the largest ethnic Latin America Ecuadorian community outside Ecuador itself. In the last decade, South America recorded changes in spatio-temporal distribution of infectious diseases, including MBDs due to globalization and climate changes [41, 42].

All trapping sites were mapped by latitude and longitude on a geographical positioning system (**Figure 4**).

Three types of traps were employed to collect adult mosquitoes:

- CDC traps baited with CO_2 and BG sentinel traps baited with BG-Lure and CO_2, with the same rules as in Piemonte Region;
- gravid traps baited with hay infusion added with yeast (almost 2.5 l): these traps attract *Culex* females that have blood fed and are ready to lay their eggs [43] in sites containing water high in organic matter, increasing together the likelihood of collecting infected mosquitoes. Each trap worked for a 24-h period.

3.1.3. Virological surveillance on mosquitoes in Piemonte and Liguria regions

Virological surveillance was performed since 2011 in Piemonte and 2013 in Liguria, focusing on mosquitoes collected from a selection of traps, updated every year, according to risk-based factors. In 2016 and 2017, mosquitoes collected at least from a trap in each quadrant (20 × 20) were analysed. Pools were homogenized in phosphate-buffered saline (600 μl if pool of <30 mosquitoes or 1200 μl if pool of >30 mosquitoes) in a 2-ml microtube with round copper beads.

Total RNA was extracted from mosquito samples according to the RNeasy Mini kit (Qiagen) manufacturer's instructions, with an *automated QIAcube protocol*.

All pool were analysed by a TaqMan® One-Step RT-PCR protocol distinctive for WNV Lineage 1 and Lineage 2 with WN-LCV-F1 and WN-LCV-R1 primers [44] and a TaqMan® One-Step RT-PCR protocol for USUV with USU F and USU R primers [45].

On positive pools, two traditional RT-PCRs for the amplification of WNV [46] and USUV [47] were carried out. Amplicons of the expected size (408 and 425 bp, respectively) were sequenced using the Big Dye Terminator kit v 3.1 (Lifetecnologies) and run on a ABI3130 Genetic Analyzer (Applied Biosystems). The related sequences were employed to perform a basic local alignment search tool (BLAST) in the GenBank library to confirm the specificity of positive reaction and to estimate the degree of identity of detected strains.

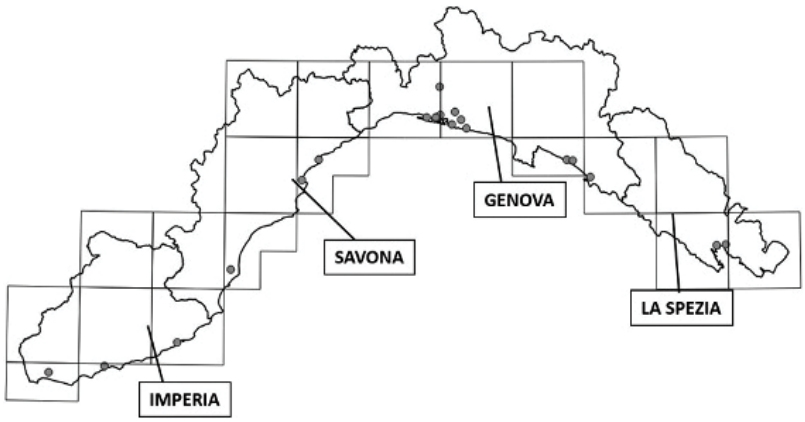

Figure 4. Map of Liguria region with the grid of 20 x 20 km quadrants. Grey dots represent trapping sites. The four provinces are named in uppercase letters in textboxes.

All WNV and USUV positive pools were sent to the National Reference Centre for Animal Exotic Diseases (CESME, Teramo) for confirmation, sequencing and determination of Lineage.

In addition, *A. albopictus* pools collected in sites considered at a higher risk level for the introduction of exotic invasive species and exotic pathogens (annually revaluated) were tested using a panflavivirus end-point RT-PCR, targeting the conserved Region of the NS5 gene sequences.

The SuperScript_ III Platinum_ One-Step qRT-PCR Kit (Thermo Fisher Scientific) was used to detect other flaviviruses of extreme importance, such as Dengue or Zika as previously described by Scaramozzino et al. [48].

3.1.4. Veterinary surveillance

According to WNV National Surveillance system [32], a passive clinical surveillance of all neurological signs in equine, and monitoring of wild birds found dead have been carried out in the study area. Necropsy of dead animals and virological molecular testing by real-time RT-PCR was performed on EDTA whole blood (live or dead animals) or on pooled samples of target organs (brain, heart, spleen and kidney) (dead animals). All samples were analysed by a TaqMan® One-Step RT-PCR protocol distinctive for WNV Lineage 1 and Lineage 2 with WN-LCV-F1 and WN-LCV-R1 primers [44] and a TaqMan® One-Step RT-PCR protocol for USUV with USU F and USU R primers [45].

Active surveillance activities have been updated according to the epidemiologic scenario of the previous year in each region.

3.1.4.1. Piemonte region

In 2009, the WNV National Surveillance plan introduced serological screening of horses in a small wetland area in southeast Piemonte Region. The area named 'Garzaia di Marengo' was classified as at risk for WNV introduction due to the presence of waterfowls, including species of migratory birds.

A fixed number of seronegative unvaccinated equines sentinel (28 animals: 10% of prevalence, 95% confidence level) was selected in order to uniformly cover the study area. Horses were sampled three times during the transmission season: immediately before (month of May), at seasonal peak (last week of August) and at the end of the transmission season (last week of September), with the aim to monitor the raising of WNV-specific IgM antibodies that are related to recent infection by commercial competitive ELISA. Positive samples were confirmed by seroneutralization assay (SN) and IgM ELISA at the CESME. Seroconversion was confirmed if SN titre was at least 1:10 and there was evidence of IgM antibodies [49].

Since 2014, the sentinel horses system in 'Garzaia di Marengo' was replaced by a one-shot random sampling of horses in order to detect IgM antibodies suggestive of WNV early infection. The number of animals to be tested for each Province during the transmission season was assigned based on the provincial equine census, as recorded in National Livestock Database [50].

Since 2015, after the detection of WNV circulation in mosquito pools and in horses in Piemonte Region, veterinary surveillance procedures were revised, and the Region has been considered as WNV Endemic Area by the National Surveillance programme.

Serological testing on horses was suspended while active virological surveillance on synanthropic non-migratory birds was implemented. From April to November, synanthropic birds (magpies—*Pica pica* and hooded crows—*Corvus cornix*), in the framework of the Regional control programme against pest birds for cultivated land protection, were weekly captured by Larsen traps and then killed or directly shot by trained hunters. Samples of organs (brain, heart and kidney) of each bird were pooled and tested for WNV and USUV.

All samples were analysed by a TaqMan® One-Step RT-PCR protocol distinctive for WNV Lineage 1 and Lineage 2 with WN-LCV-F1 and WN-LCV-R1 primers [44] and a TaqMan® One-Step RT-PCR protocol for USUV with USU F and USU R primers [45].

In 2017, the control programme against synanthropic birds was not activated in Torino Province. Then, as alternatively recommended by the National Surveillance Plan, a specific serological monitoring activity was enforced on rural poultry, with the aim to detect IgG antibodies by commercial ELISA test.

3.1.4.2. Liguria region

Since 2014, Liguria Region was included in WNV National Surveillance plan by serological random sampling of horses in order to detect IgM antibodies suggestive of WNV early infection. The number of animals to be tested for each Province during the transmission season was assigned based on the provincial equine census, as recorded in National Livestock Database [50].

3.2. Human surveillance West Nile virus and others MBDs in Piemonte region and Liguria region

Human surveillance activities against MBDs in Piemonte Region are regulated by both the National Surveillance Plan of imported and autochthonous MBDs (CHIKV, DENV and ZIKV), annually published by MoH and by National Integrated Surveillance Programme against WNV and USUV.

Local Health Authorities must establish an active surveillance system against human MBDs during the transmission season. Furthermore, passive surveillance has to be set up during the whole year, requesting physicians to report all possible, probable and confirmed cases according to national case definition [32]. Surveillance against Zika virus infection includes investigation on neurological disorders such as Guillain-Barrè syndrome in adults and microcephaly in infants.

Every case confirmed by the Regional and National Reference Laboratories has to be subjected to epidemiological investigation with the aim to establish any local viral circulation. Moreover, during the transmission season, measures for vector control have to be implemented promptly.

According to the blood directive, if veterinary or human cases of WNV are detected, immediate WNV NAT screening of all blood and haematopoietic stem cell donations of solid organ donations is introduced in affected areas (provinces). Every suspected case of Chikungunya or Zika virus has to be temporarily deferred from donations for 28 days starting to recovery [51].

In addition to surveillance actions, another important item of the MBDs surveillance is the information activity to raise awareness on population on the links between mosquitoes and the diseases they can transmit.

Information campaigns focused on personal protection measures against mosquito bites, especially for pregnant women, and mosquito fighting were conducted in Piemonte and Liguria since 2014 due to WNV spread in Northern Italy and in 2016 due the Zika virus emergency in South America.

Training courses have been organized for public health physicians and veterinarians in order to early detect any MBDs clinical suspected case. Advertising as poster and leaflets was shared in hospitals, offices of health authorities and travel medicine centres.

4. Results

4.1. Entomological and virological surveillance on mosquitoes

4.1.1. Piemonte region

Since 2011, a total of 111.676 adult mosquitoes, divided in 4620 pools, were identified and analysed.

The most abundant species detected was *C. pipiens*, mainly collected by CDC traps followed by *Ochlerotatus caspius*, *Anopheles maculipennis* s.l. and *A. albopictus.*

Details of collected species for years are shown in **Table 1**.

USUV was reported since 2011 [52]. After the first detection, it was found every year in field-collected mosquitoes (*Cx. pipiens*), confirming the establishment of the virus in the Region.

WNV Lin2 was detected for the first time in 2014, in Alessandria Province, in two *C. pipiens* pools (August 27, 2014; September 10, 2015).

Mosquito species	Total	
	N	Pool
Ae. albopictus	8692	875
An. maculipennis s.l.	12,126	502
An. plumbeus	74	38
Ae. vexans	4074	288
Ae cinereus	1	1
Cx. hortensis	4	2
Cx. pipiens	50,660	1672
Cx. territans	16	2
Cx. theileri	34	10
Cs.annulata	6	3
Cx.modestus	1621	100
Cs.subochrea	14	6
Cs. longiareolata	5	4
Culiseta sp	2	2
Oc.caspius	34,085	1053
Oc.geniculatus	198	51
Oc.cantans	56	8
nd	8	3
Total	111,676	4620

Table 1. Total amount of mosquitoes collected and tested in Piemonte region since 2011, sorted by species and number.

In 2015, WNV Lin 2 was detected in six pools of *Cx. pipiens*. They were collected with CDC dry ice-baited traps from July in four provinces: Novara (July 29, 2015, and August 26, 2015), Alessandria (August 6, 2015), Vercelli (August 21/26, 2015) and Torino (September 23, 2015).

In 2016 and 2017 in Alessandria and Novara provinces, respectively, positive *Cx. pipiens* pools were found. No other *flavivirus* of medical interest was detected [53, 54].

4.1.2. Liguria region

Since 2011, a total of 33,244 adult mosquitoes were collected.

Virological investigations were performed since 2013 on 23,050 adult mosquitoes, split in 1255 pools (**Table 2**).

The most abundant species detected was *C. pipiens*, mainly collected by gravid traps followed by *A. albopictus* collected in Bg-Sentinel. Since 2015, in the city of Genova, adult female *A. koreicus* specimens were detected [55]. Pool collected in 2015 and 2016 was

analysed with bio-molecular assays to confirm the species identity. Since 2017, considering the most abundant number of adults trapped, *A. koreicus* specimens were also screened for flavivirus.

In 2014, WNV and USUV were detected for the first time in the area. A WNV-positive pool was collected by gravid traps at Genova airport on September 29. Actually, this is the only report of WNV circulation in the Region.

USUV was detected in a *C. pipiens* pool in La Spezia Province by gravid traps on September 23.

Unlike WNV, in the following years, USUV continued to circulate in the Region, and it was annually reported in mosquitoes. No other flavivirus of medical interest was detected [5].

Mosquito species	**Total**	
	N	**Pool**
Ae. albopictus	9054	493
Ae. koreicus	26	15
Aedes spp	92	11
An. claviger	4	4
An. maculipennis s.l.	3	3
An. plumbeus	10	8
Anopheles. spp	1	1
Cq. richiardii	19	3
Cx. hortensis	5	5
Cx. impudicus	21	4
Cx. mimeticus	1	1
Cx. pipiens	13,191	536
Culex spp	184	34
Cx. territans	2	2
Cx. theileri	3	3
Cs. annulata	9	9
Cs. longiareolata	381	99
Culiseta sp.	2	2
Oc. caspius	35	15
Oc. geniculatus	1	1
Oc. mariae	2	2
Ochlerotatus spp	4	4
Total	23,050	1255

Table 2. Total amount of mosquitoes collected and tested in Liguria region since 2013, sorted by species and number.

4.2. Veterinary surveillance

4.2.1. Piemonte region

Since 2009, in the framework of passive surveillance, 35 suspected equine clinical cases were notified: three in 2014, 24 in 2015, six in 2016 and two in 2017.

Nine of the suspected cases were confirmed: six in 2015 (Alessandria Province), one in 2016 (Vercelli province) and two in 2017 (Asti and Cuneo Provinces).

Within the passive surveillance on wild birds that were dead, from 2009 no WNV infection cases were detected.

The active surveillance from 2009 to 2013 on 28 sentinel horses selected in the risk area sited in 'Garzaia di Marengo', annually tested three times during the transmission season, did not detect any seroconversion. In 2014 and 2015, a total of 1819 equines sentinel were screened by ELISA IgM serological test. The only seropositive horse detected (2014) lived in Alessandria Province, within 4 km of radius from the first WNV-positive mosquito pool.

Since 2016, the Surveillance Plan was selectively targeted on the active surveillance of wild, synanthropic and rural birds, and then sentinel horses system was dropped out.

A total of 2451 wild birds were tested by RT-PCR for WNV, mainly corvids species: 14 corvids were confirmed WNV infected, in 3 out of 8 Piemonte Provinces (Alessandria, Torino and Vercelli).

In 2017, the serological monitoring activity on rural poultry in Torino Province allowed to detect 17 WNV positive hens; 4 animals out 17 tested SN positive both for WNV and for USUV by the CESME, providing demonstration of co-circulation of both viruses in the same areas.

4.2.2. Liguria region

Since 2009, in the framework of passive surveillance, seven suspected clinical cases were notified: none was confirmed by laboratory investigation.

A total of 97 wild birds found dead were virologically tested by RT-PCR for WNV. In 2013, the necropsy of an Eurasian hobby (*Falco subbuteo*), a long-range migratory species, found dead at the end of spring in Imperia Province, accidentally allowed to identify WNV Lin2. This report has not been considered a significant proof of virus circulation, because the infection was probably contracted in Africa. This is to date the only WNV virological positivity in birds in Liguria region.

By active surveillance, since 2014, 611 horses were screened by ELISA IgM serological test: no positivity was detected.

4.3. Human surveillance of West Nile virus and others MBDs in Piemonte and Liguria region

Every WNV or MBDs suspect case was promptly reported to the Regional Public Health Authority. For Piemonte Region, biological samples were transmitted to the Laboratory of Microbiology and Virology, within the Regional Reference Centre for Infectious Diseases of

the Amedeo di Savoia Hospital, Torino. From 2015 to date, four WNND autochthonous cases were detected: one fatal case in 2015 in Torino Province, one fatal case in 2016 in Novara Province and two fully recovered cases in 2017 in Asti province [56].

For Liguria Region, the Regional Reference Centre for MBDs diagnosis is located at the Institute of Hygiene of the Policlinico San Martino Hospital, Genova.

Notified human cases of CHIKV, DENV and ZIKV since 2011 in Piemonte and Liguria Regions are displayed in **Table 3** (all imported cases).

As WNV surveillance involved both Veterinary and Human Public Health Regional Authorities, specific flow charts were prepared and shared among working group with the aim to coordinate the notification of viral circulation triggers and the implementation of control measures. Hierarchy of actions is shown in **Figure 5**.

Arbovirosis human surveillance		**Piemonte**	**Liguria**
2011	CHIK	0	0
	DEN	1	0
	ZIK	0	0
2012	CHIK	0	0
	DEN	5	0
	ZIK	0	0
2013	CHIK	0	0
	DEN	12	0
	ZIK	0	0
2014	CHIK	1	0
	DEN	5	1
	ZIK	0	0
2015	CHIK	1	0
	DEN	12	0
	ZIK	0	0
2016	CHIK	2	0
	DEN	14	0
	ZIK	14	1
2017	CHIK	1	*
	DEN	18	*
	ZIK	2	*

*data not available.

Table 3. Notification of Chikungunya (CHIK), dengue (DEN) and ZIKA (ZIK) human cases in Piemonte and Liguria regions from 2011 to 2017 (all imported cases).

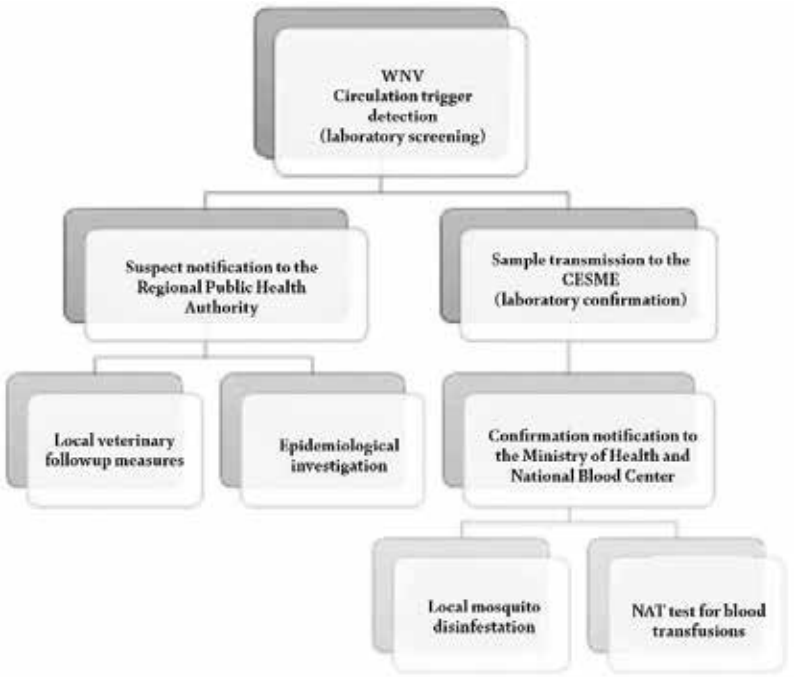

Figure 5. Flow chart for the management of WNV viral circulation trigger event in Piemonte and Liguria regions, since 2015.

During transmission season, following the detection of WNV viral trigger by the national Integrated Surveillance Plan, the NAT test was progressively introduced throughout affected Provinces of Piemonte Region. According to National legislation, it was stopped on November 30.

In 2014 and 2015, only Alessandria province was involved in blood screening.

In 2016, four (Alessandria, Novara, Torino and Vercelli) of the eight Provinces in the Piemonte Region were involved in NAT testing of blood donors.

In 2017, blood screening was enforced in Novara, Vercelli, Cuneo and Asti Provinces.

Since 2014, a total of 99,882 blood bags were screened by NAT (5172 in 2014; 38,623 in 2015; 35,812 in 2016 and 20,275 in 2017). No infected blood bags were detected.

5. Conclusions

MBDs have complicated transmission cycles, involving different reservoirs, competent vectors and environmental and climatic features that influence their epidemiology. Therefore, surveillance methods have to be planned according to these aspects.

In Italy, WNV and USUV are considered endemic in several Regions, whereas CHIKV, DENV and ZIKV are essentially related to infected travellers returning from endemic countries [57].

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Two National Surveillance Plans regulate MDBs management in Italy: the first regarding WNV and USUV, which integrates human and veterinary surveillance and the second regarding MBDs specifically transmitted by *Aedes* mosquitoes, mainly CHIKV, DENV and ZIKV, based on the surveillance of both imported and autochthonous human cases.

In Piemonte and Liguria Regions (Northwestern Italy), a WNV multisectoral task force, including representatives of Public and Animal Health and Vector Control, was established in 2011 and then strengthened annually according to epidemiological findings. Since 2013, the working group cooperates with experts of four neighbouring Regions, sharing integrated WNV and USUV surveillance guidelines (human, entomologic and veterinary) throughout the whole Po river valley area. The main goal of this standardization has been the reduction of the transmission risk through blood transfusions, quickly implementing local preventive measures when the virus (in animals, vectors or humans) is detected in a specific Province. This allows to definitely decrease the risk of human transmission and consequently results in a significant reduction of health-care costs [58].

The active virological surveillance on mosquitoes and birds is considered an important tool to early detect virus circulation in a specific area, since it has been shown that the virus in mosquitoes and birds appears much earlier than the occurrence of clinical cases in dead-end hosts (humans and equines).

Passive surveillance of clinical cases in horses also can be considered a useful tool for the detection of WNV activity, but it will be less sensitive, and a positivity regarding the proof that the cycle of the virus is ended must be considered. Being dead-end hosts, the probability of infection is similar for horses and humans; then, an early detection tool especially in an endemic area cannot be considered. Furthermore, considering that in affected countries, vaccinations of horses are progressively adopted, it is estimated that surveillance in equidae will gradually become irrelevant [59].

The entomological surveillance can be a valuable additional tool also for the surveillance of MBDs caused by non-endemic viruses (CHIKV, DENV and ZIKV) in which humans are the reservoirs. Even if the syndromic surveillance on human cases is considered the most adequate approach to detect the introduction of these viruses, the presence of risk factors, as competent vectors and suitable climatic conditions, can significantly increase the potential risk for the local transmission in vectors, as happened in Emilia Romagna (2007) and Lazio (2017).

Indeed, the selection of the most suitable mosquito-trapping method and the identification of areas with major risks of introduction of exotic mosquitoes and pathogens are crucial, and surveillance should be planned in response to a recognized risk and carried out to support subsequent actions. In Piemonte and Liguria, the entomological surveillance in such sites (ports, international airports, international connection points and hospitals) revealed the presence of *A. albopictus*, competent vector of CHIKV, DENV and ZIKV, but no positive mosquito was found. In Liguria region, Genova city, this risk-based surveillance allowed to detect in 2015 the introduction of *A. koreicus*, an Asiatic mosquito which has become invasive in Europe in the recent years, proven to be an effective transmitter of Japanese encephalitis virus, *Dirofilaria immitis* and CHIKV [60]. While the origin of these specimens remains unknown, the presence of *A. koreicus* in such an important commercial and tourism hub is worrisome, as it might strongly accelerate the species' spread in Italy and in the rest of Europe, as already happened with *A. albopictus*.

Given the long-distance passive transfer of infected mosquito by trades, or movements of infected tourist and business travellers, particularly during the vectors season and the favourable transmission period, the potential risk of introduction and spread of emerging MBDs exists [61].

In conclusion, the creation of a regional working group composed by public and animal health authorities, together with the authorities in charge of vector surveillance and control that regularly share information, is a crucial point towards the achievement of an integrated surveillance. The approach adopted in Northwestern Italy has been demonstrated a key point to promptly implement control measures and save resources, reducing the risk of MBDs human transmission.

The periodical evaluation of planned actions and their updating according to the evolving epidemiological scenario is of paramount importance for the prevention of the diseases and the maintenance of both human and animal health.

Further cost–benefit evaluations, including an accurate estimation of indirect costs, are needed to improve the knowledge of the economic context of MBDs and its mitigation, allowing to better target the Public Health response.

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Conflict of interest

The authors declare that no competing financial interests exist.

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Chapter 5

Indonesia Dengue Fever: Status, Vulnerability, and Challenges

Budi Haryanto

Additional information is available at the end of the chapter

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Abstract

In Indonesia, the incidence rate (IR) of dengue fever reported increase almost in every year since the first cases were found in 1968, from 0.05 to ~35–40 per 100,000 population in 2013, with superimposed epidemics demonstrating a similar increasing trend with the highest epidemic occurring in 2010 (IR 85.7). Most currently, about 80% of regencies/cities had been infected and posed as very high vulnerability of spreading the disease. Increased incidence of dengue fever is associated with the increase of rainfall and temperature in particular years. Up to the year of 2038, a climate model of Meteorological, Climatological, and Geophysical Agency shows increasing trend of rainfall and temperature. Along with its unsuccessful of Indonesia dengue fever control program will lead challenges to reduce dengue fever endemic in the future. Revitalization of dengue disease control program in every single stage with close monitoring implementation is urgently needed. Socialization, community capacity building, and participation could also be a joint sectoral action to enhance the dengue fever control program.

Keywords: Indonesia dengue fever, climate vulnerability, eradication challenges

1. Introduction

Dengue fever is a mosquito-borne disease caused by any one of four closely related dengue viruses (DenV-1, DenV-2, DenV-3, and DenV-4). Dengue fever is transmitted by the bite of an *Aedes* mosquito infected with a dengue virus. The female mosquito becomes infected when it bites a person with dengue virus in their blood both indoors and outdoors during the daytime (from dawn to dusk). *Aedes aegypti* is particularly involved, as it prefers to lay its eggs in artificial water containers, to live in close proximity to humans, and to feed on people rather than other vertebrates.

Dengue infection is the most rapidly spreading mosquito-borne viral disease in the world. Infections are most commonly acquired in the urban environment. In recent decades, the expansion of villages, towns, and cities in the areas in which it is common and the increased mobility of people have increased the number of epidemics and circulating viruses. Dengue fever, which was once confined to Southeast Asia, has now spread to Southern China, countries in the Pacific Ocean and America, and might pose a threat to Europe. In the last 50 years, dengue virus infections had expanded to many other countries with significant increasing cases [1] up to 2.5 billion people living in endemic countries where about 1.8 billion (more than 70%) in Southeast Asia and the Western Pacific Region [1–4]. About 50 million dengue infections occur every year [2, 3], and approximately 500,000 patients are hospitalized of whom dominated by children [2–7]. The increasing incidence and geographical spread of dengue virus were more likely driven by demographic and societal changes such as population growth, urbanization, and modern transportation [8]. The traveler movement also contributed to the risk of contracting dengue disease from nonendemic countries to endemic dengue areas to nonendemic regions where competent mosquito vectors are currently found [9–12].

Indonesia, with 257.5 million inhabitants and 17,500 islands spread across the equator, poses as the largest archipelago country in the world [13], comprising 3.1 million km^2 of ocean (62% of the total area) with a coastline of 81,000 km and approximately 2 million km^2 of land (38% of the total area). Its tropical climate and subsequent relative high humidity makes Indonesia favorable conditions for vector-borne disease transmission. The increasing trend of dengue infections over the current decades putting Indonesia as one of endemic area for dengue fever and tread both the people as well as travelers visiting the archipelago [14]. Its burden is a result of a constant ground of established infections in the past period, combined with epidemics of emerging infectious diseases (EID) [15]. This chapter describes the dengue fever status or situation in Indonesia, its vulnerability among population, the future challenges, and the disease prevention and control.

2. Dengue fever status in Indonesia

Indonesia is reported as the second largest with dengue fever cases among 30 endemic countries. The number of cases of dengue fever is most prevalent in the provinces of East Java, West Java, and Central Java. However, there are a number of provinces that are vulnerable with its high incidence rate of dengue fever. In 1968, the first 58 dengue cases were reported in Indonesia from the city of Jakarta (DKI Jakarta) and Surabaya (East Java) [16–19]. Since then, the sharp increasing numbers of cases and spreading to many other geographical locations have been reported [16, 17, 20–25]. The epidemiology of dengue fever in Indonesia has been described mostly in the form of case series, reporting on single outbreaks, or clinical and virological studies in confined geographical locations and selected years [26].

A study in 2014 reported that the annual dengue fever incidence increased from 0.05/100,000 in 1968 to ~35–40/100,000 in 2013. The highest epidemic occurred in 2010 with the incidence of 85.7/100,000 population. The data revealed declining of case fatality rate (CFR) from 41% in 1968 to 0.73% in 2013. Dengue cases increased among ages during the observation period up to 1998 with the highest incidence of aged 5–14 years. From 1999 onward, the trend of dengue incidence increased among those aged 15 years or over. This study indicates incidence of

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dengue fever increased rapidly over the past 45 years in Indonesia with peak incidence shifting from young children to older age groups [27].

The threat of dengue fever among children was emphasized clearly on a recently published study among 3194 children aged 1 through 18 years who lived in 30 different urban neighborhoods. Children blood samples were drawn for antibodies to dengue, an indication that someone has been infected with the virus in the past, and found that 69.4% of all children tested positive for dengue antibodies. Among the age groups, positive antibodies found 33.8% at the group of 1–4 year olds, 65.4% at the group of 5–9 year olds, 83.1% at the group of 10–14 year olds, and 89% at the group of 15–18 year olds. The first time to become infected with dengue was at the age of 4.8 years as the median, and in addition, 13.1% of children on average get their first dengue infection each year. It was also found that the more people in a household who had been diagnosed with dengue since a child's birth, the more likely the child were to test positive for dengue antibodies [28].

The incidence rate (IR) for every 100,000 population in seven provinces were found over 100 or are prone to dengue cases. The seven provinces are Bali (484), East Kalimantan (306), DKI Jakarta (198.7), DI Yogyakarta (167.9), North Kalimantan (158.3), Southeast Sulawesi (123.3), and South Kalimantan (101.1). The lowest IR is achieved by Papua province (11.8) and West Kalimantan (12.1) (**Figure 1**). The whole of Indonesia is high (IR is 78.0). In general, the increasing number of dengue fever cases is more likely followed by the spread of the cities

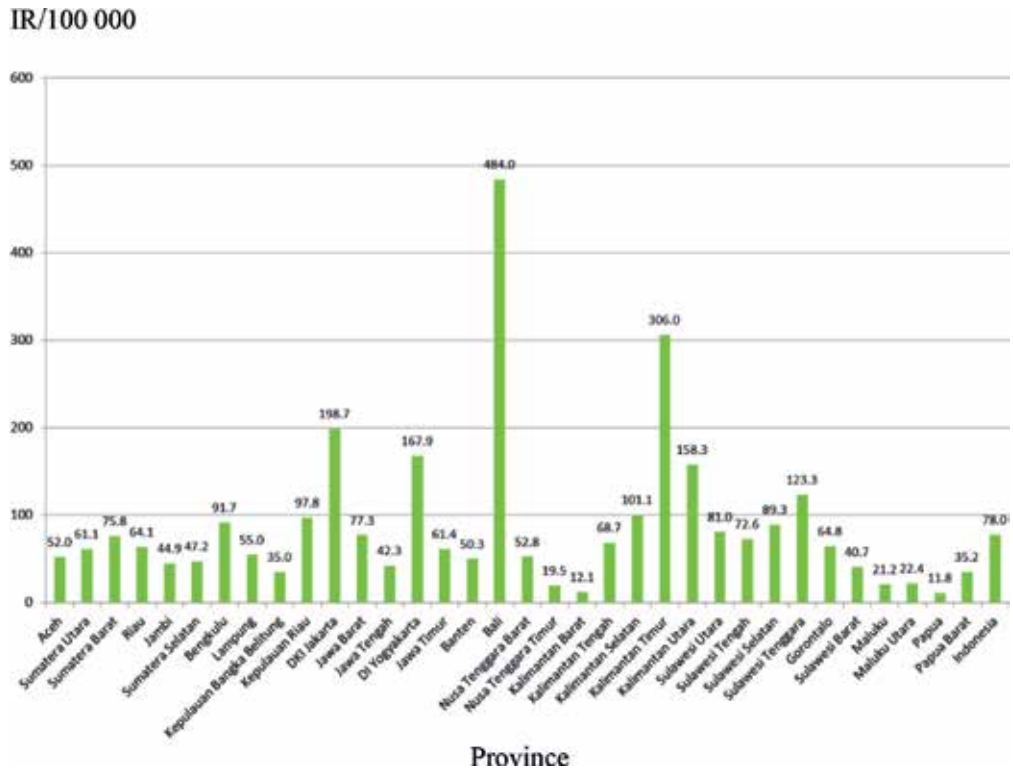

Figure 1. Incidence rate (IR) of dengue fever per 100,000 population by province in Indonesia 2016 (source: DG of CDC MOH 2017).

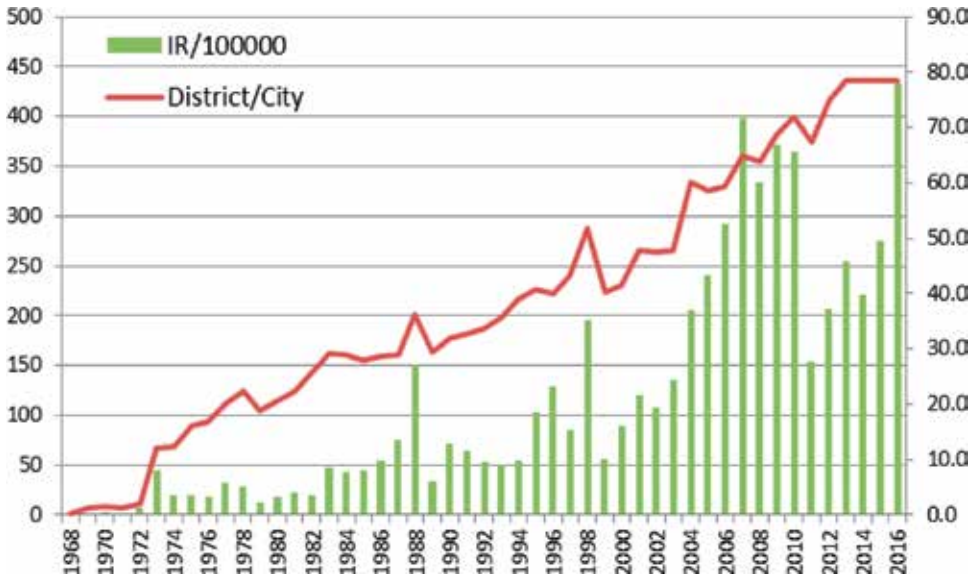

Figure 2. Incidence rate (IR) of dengue disease per 100,000 population and number of cities/districts infected in Indonesia 1968–2016.

and districts infected in all of 34 provinces in Indonesia (**Figure 2**). From the total of 497 cities and districts in Indonesia, about 80% have reported the dengue fever cases in 2017.

In the context of dengue fever mortality, as many as 1229 people died in 2015 from the disease caused by this dengue virus. Throughout the history of dengue fever in Indonesia, the highest death rate occurred when first time the disease was discovered in 1968 in Surabaya. Of the 58 people infected, 24 lives were lost. In 2016, the highest percentage of CFR was obtained in Maluku Province (6.0%), Gorontalo (6.1%), and West Papua (4.6%). Provinces with the lowest CFR were achieved by Papua (0%), DKI Jakarta (0.1%), and NTT (0.2%). In some provinces, dengue disease was an outbreak in 1998 and 2004 that caused 79,480 people and 800 more deaths. In subsequent years, there has been reported a decrease in the case of death but note that the number of cases continues to increase. In 2008, there were 137,469 cases and 1187 deaths. In 2009, there were 154,855 cases and 1384 deaths [29].

3. Dengue fever vulnerability

Studies on Indonesian vulnerability to climate change were mostly focused on mitigation aspects, such as water scarcity, reduction emission from deforestation and degradation (REDD), the forest conservations, disasters, land drought, floods, and others. Meanwhile, the vulnerability study on adaptation is still rare, especially to human health. In 2013, Research Center for Climate Change—University of Indonesia (RCCC-UI) initiated a study on vulnerability of dengue disease to climate change/variability in collaboration with the Directorate of Environmental Health of the Ministry of Health and supported by Indonesia Climate Change Trust Fund (ICCTF). The study involved 20 districts/cities in 5 provinces namely West Sumatra,

Jakarta, East Java, Bali, and Central Kalimantan which were selected based on the availability of monitoring station of the Indonesian Agency for Meteorology, Climatology, and Geophysics (BMKG). The dengue disease vulnerability components were generated based on bionomic mosquito and habitat, pathology dengue disease, and factors related to dengue disease occurrence. The exposure variables include land use (settlement, offices, business, schools, etc.) and population density. The sensitivity variables include breeding places and resting areas of *Aedes* mosquitoes, pupa and adult density, incidence of dengue fever, and population mobility. The adaptive capacity variables include availability of health services (number of hospitals, clinics, and public health centers), treatment management and skilled providers, implementation of dengue fever intervention program, community participation and involvement on dengue fever prevention program, and personal protection behavior. The Intergovernmental Panel Convention for Climate Change (IPCC) vulnerability analysis was implemented to gain the coping range index of DF for each city/district [31]. The coping range index (CRI) = 1 (blue) indicates the people vulnerability of having dengue fever is very low and located at quadrant between low exposure and sensitivity index and high adaptive capacity index; CRI = 2 (green) indicates the people vulnerability of having dengue fever is low and located at quadrant between high exposure and sensitivity index and high adaptive capacity index; CRI = 3 (yellow) indicates the people vulnerability of having dengue fever is medium and located at quadrant between medium exposure and sensitivity index and medium adaptive capacity index; CRI = 4 (brown) indicates the people vulnerability of having dengue fever is high and located at quadrant between low exposure and sensitivity index and low adaptive capacity index; and CRI = 5 (red) indicates the people vulnerability of having DF is very high and located at quadrant between high exposure and sensitivity index and low adaptive capacity index (**Figure 3**) [30].

A study of Research Center for Climate Change—Universitas Indonesia 2013–2014 reported that in almost all districts/municipalities under study (in 17 out of 20 regencies/cities) indicated a very serious vulnerability condition of very high coping range index (CRI) (red = 5) since 2005.

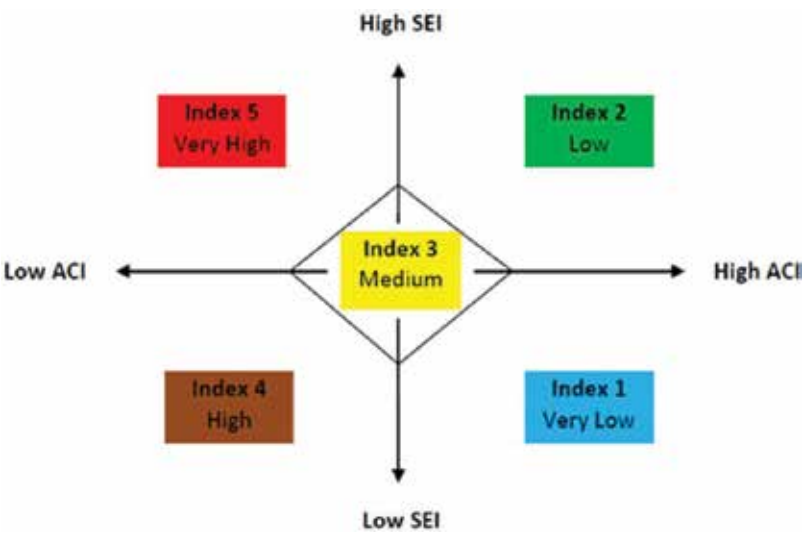

Figure 3. The coping range index (CRI) of dengue disease vulnerability.

Very high CRI was found in 75% of regencies/cities in West Sumatra province (City of Padang in 2005, 2007, 2008, 2009, and 2012; Padang Pariaman Regency in 2008, 2011, and 2012; and City of Padang Panjang in 2007 and 2008), all of regencies/cities in Bali province (City of Denpasar in 2006, 2009, and 2010; Jembrana Regency in 2007; City of Badung in 2007, 2009, and 2010), 80% of regencies/cities in East Java province (City of Surabaya in 2007, 2008, 2009, 2011, and 2012; Malang Regency in 2007, 2008, 2009, 2011, and 2012; City of Pasuruan in 2007, 2008, 2009, 2010, and 2011; Sumenep Regency in 2007, 2008, 2009, 2011, and 2012), all of cities in Jakarta province (City of Central Jakarta in 2005, 2006, 2007, 2008, 2009, and 2012; City of North Jakarta in the year 2006–2012), half of cities in Banten province (City of Tangerang in 2007–2012), all of regencies/cities in Central Kalimantan province (City of Palangkaraya in 2006, 2008, and 2012; Kotawaringin Barat Regency in 2005–2008 and in 2012; Kotawaringin Timur Regency in 2008, 2010, and 2011; Barito Utara Regency in 2008). High CRI (brown = 4) was also happened more often before and following the years of the very high CRIs occurrences in the regencies/cities [30]. Thus, this concluded that dengue fever is in the level of seriously vulnerable to people living in the regencies/cities under study in Indonesia. **Figures 4** and **5** show the dengue fever vulnerability among cities/districts in 2012 in the provinces of Jakarta/Banten, Bali, Central Kalimantan, and East Java.

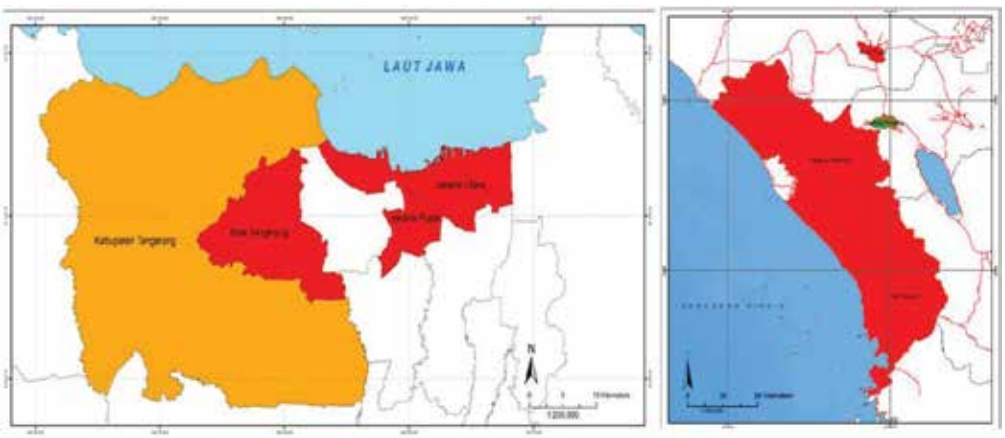

Figure 4. Map of CRI of dengue fever vulnerability in Jakarta/Banten and West Sumatra in 2012.

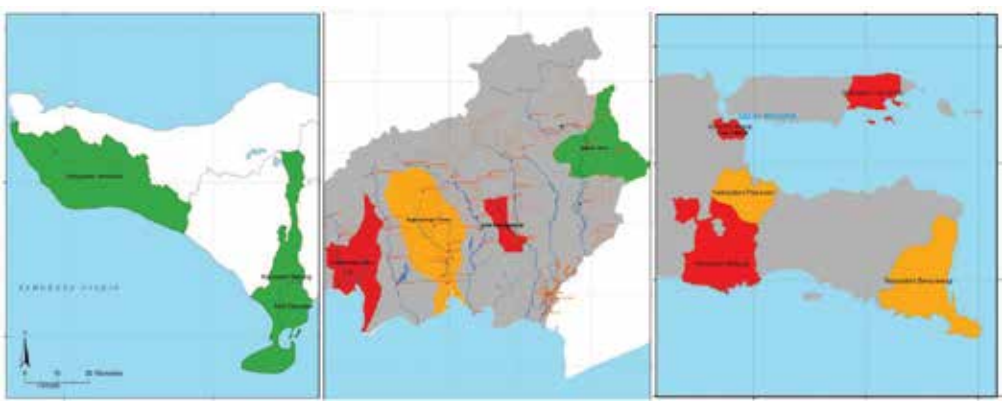

Figure 5. Map of CRI of dengue fever vulnerability in Bali, Central Kalimantan, and East Java in 2012.

4. Dengue fever challenges

Dengue emerged as a public health burden and has become increasingly important, with progressively longer and more cyclical epidemics of dengue including cases of dengue with alarm signs and severe dengue. In Indonesia, although some programs and control efforts have been performed, both the incidence and case fatality rate are still high and not showing significant changes. There are still some challenges that need to be handled, such as surveillance system, availability adequate laboratory, community knowledge, awareness, and involvement against dengue, many new cases reported from new city or district, high mobility of dengue fever's carrier, density of community in the city/district central, access to health centers, and the availability of drugs and vaccines.

Surveillance for this vector-borne viral disease remains largely passive and based on the hospital report which is the estimation of real cases still underreported. It was also reported that many health centers and clinics were without adequate laboratory support. This will lead increasing of referral activities to hospitals with the consequences of time spent and transportation challenges in rural areas. Some studies found about one-third adult population with sufficient knowledge about dengue fever and its fast spreading to other people. However, only about 17% of them aware and clearly know the way for prevention [31, 32]. In addition, the high number of dengue fever incidence can also be caused by increasing *Aedes aegypti* mosquito breeding places, mosquito habitat, more effective mode of transmission, more frequent dengue fever course, shorten dengue fever symptoms, access for dengue fever treatment. Home conditions such as governance and the layout of goods at home can also affect the high number of dengue fever incidence.

Indonesia is a country with a vast region, varied geographic and biodiversity, populated density, and characteristics of various populations. In the last decade, several new administration districts developed with the newly reporting and recording management systems. This will lead underreporting of dengue fever both from the passive surveillance and the number of real cases estimation. The increasing number of people and the area of dengue fever spread in Indonesia is due to the high population mobility, the development of urban areas, climate change, increasing population density, and changes in population distribution. Climate change causes changes in rainfall, temperature, humidity, and air direction thus affecting the breeding of *Aedes aegypti* mosquitoes.

The last and most important thing of the challenges is community participation. The participation of the community to participate consistently to keep the environment from dengue is still difficult. Various breakthroughs by government such as 3 M plus (draining, covering, burying or utilizing/recycling and all forms of prevention, such as to apply powder of larvae-killers in water tanks, to use mosquito repellents, to keep fish predators to consume mosquito larvae, etc.) movement, Jumantik (volunteer or student who periodically monitor *Aedes* larvae on water storages at home) and so have long been circulated. But people who forget and bored easily become a problem. For example, after some time, there was no extraordinary incident, the community considered it safe and careless, consequently when the case exploded, people just reacted [33].

Among other challenges, passive surveillance systems tend to underestimate the burden of communicable diseases such as dengue. By utilizing the data from the Indonesian surveillance

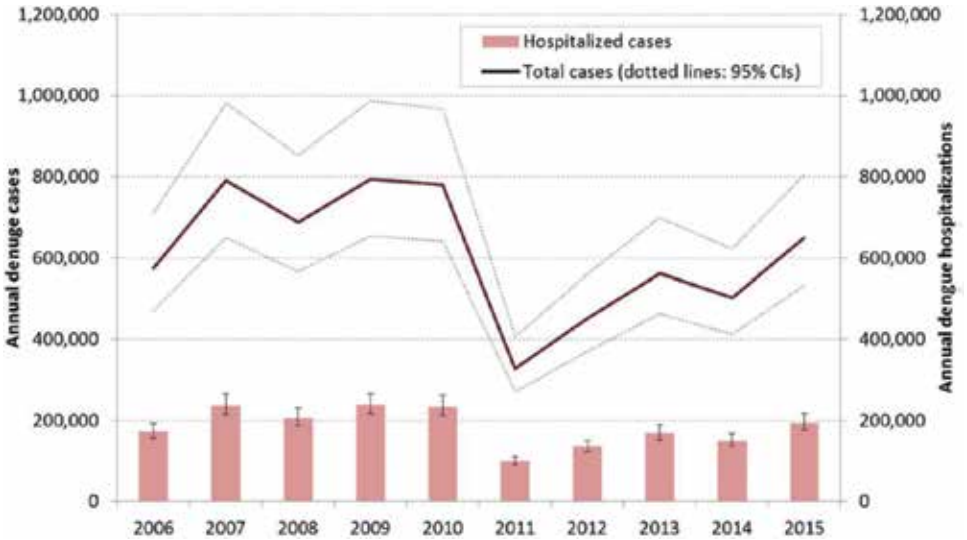

Figure 6. Estimated annual number of dengue cases and hospitalizations in Indonesia following adjustment of surveillance reports with EFs, and their 95% confidence intervals (CIs), 2006–2015.

system and associated health system parameters, a study to estimate the proportion of dengue was conducted by Delphi panel in 2017. The iterative estimation was generated by calculating the expansion factors (EF), the ratio of total and reported cases during the presentation of medical and epidemiological data and subsequent discussions. The data revealed that from all of symptomatic Indonesian dengue episodes, 57.8% enter healthcare facilities to seek treatment but only 39.3% of them are diagnosed as dengue. Furthermore, only 20.3% of them are subsequently reported in the surveillance system. Public sector found dominating occurrence of hospitalizations and followed by private sector for ambulatory episodes (~55%). Therefore, estimations gave an overall EF of 5.00; hospitalized EF of 1.66; and ambulatory EF of 34.01 which, when combined with passive surveillance data, equates to an annual average (2006–2015) of 612,005 dengue cases, and 183,297 hospitalizations (**Figure 6**.). The findings are lower than those similar estimations published elsewhere, perhaps due to case definitions, local clinical perceptions, and treatment-seeking behavior [34].

5. Dengue fever prevention and control

The goal of WHO Global Strategy is to reduce the burden of dengue. Its specific objectives are: (1) to reduce dengue mortality by at least 50% by 2020, (2) to reduce dengue morbidity by at least 25% by 2020, and (3) to estimate true burden of the disease by 2015 (the year 2010 is used as the baseline). The implementing strategy is expected to pave the way for reducing dengue morbidity and mortality nationwide through strengthening local and national capabilities, as well as regional coordination. National Dengue Control Program in Indonesia

is currently implementing WHO Global Strategy 2012–2020 that promotes coordination and collaboration among multisectoral partners, an integrated vector management approach and sustained control measures at all levels. Dengue is an ecological disease, therefore coordination and collaboration by all sectors within the government, communities, civil societies, private sectors, and media need to be strengthened. All sectors should harmonize the prevention, surveillance (entomological and epidemiological), and case management with the existing health systems, in order to make the program sustainable, cost-effective, and ecologically sound.

It has long been believed that preventing and reducing dengue virus transmission was very depended upon vectors control (*Aedes sp*.) or interrupt the human-vector contact. Activities to control transmission should target *Ae. aegypti* (the main vector) in the habitats of its adult stages as well as the immature. The high death toll from dengue fever demands people to stay alert to possible outbreaks of this disease in their neighborhoods [35]. Therefore, it is important for the community to collectively jointly create a healthy environment free of larvae to suppress the incidence of dengue disease. The prevention and control programs need to be undertaken with specific commitments from stakeholders from the top to the bottom levels. Currently, the Ministry of Health has launched a program of Nest Mosquito Eradication Program (PSN) through 3 M plus way.

6. Conclusion

Given the wide area in the tropical temperature, high population density in urban area, and various geographic and biodiversity, putting Indonesia as a natural potential for the habitat of dengue viruses. The number of dengue fever cases reported dramatically increases since it was firstly found in 1968 and spread out almost in 80% cities and districts in Indonesia in 2016. Many of those cities and districts were very vulnerable and putting million people at risk to the disease in 2012. Some challenges are still heading in the front of the prevention and control implementation actions. However, keeping spirit for struggling to combat dengue fever in Indonesia along with full commitment and involvement of community are urgently needed as well as to revitalize dengue disease eradication programs at every stage with close monitoring implementation.

In addition, technical guidance and increased skills of health officers are indispensable. Socialization of a hands-on program activities in particular and increased capacity and active participation of community on the action could be a joint action in preventing the increase in dengue disease associated to climate change.

Conflict of interest

The author declares no competing financial interests.

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Chapter 6

RNA Association, RNA Interference, and microRNA Pathways in Dengue Fever Virus-Host Interaction

Imran Shahid

Additional information is available at the end of the chapter

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Abstract

Dengue fever is a fatal vector transmitted disease and is one of the most significant health problems which have magnified its impact globally by afflicting 390 million people across 110 countries. The causative agent of this life-threatening disease is a positive single-stranded RNA arbovirus known as dengue virus (DENV), which uses *Aedes aegypti* mosquito as an intermediate host. It has been well demonstrated that virus evades mosquito's RNA interference (RNAi)-mediated antiviral defense and manipulates host microRNA (miRNA) profile to its own benefit. However, the exact mechanisms are still not exclusively elucidated. The molecular mechanisms which characterize the role of novel DENV-encoded small RNAs and other viral proteins in host miRNA modulation and evasion of RNA interference are still elusive. Furthermore, the possibility of small activating RNAs-(RNAa)-mediated activation in mosquitoes in conjunction with dengue virus genes is not fully explored. This book chapter pragmatically overviews intricate interplay between virus-host interactions, how virus invades host antivirus defense mechanisms, and possibly the potential emerging therapeutic role of RNA activation (RNAa) and RNAi for the infections, which can be cured by specific gene activation and gene silencing, respectively.

Keywords: dengue fever, dengue fever virus, *Aedes aegypti*, RNA interference, microRNAs, RNA association, host-virus interaction, DENV therapy

1. Introduction

Dengue virus (DENV) is a major arbovirus that uses *Aedes aegypti* mosquitoes as an intermediate host to be transmitted to human and is the causative agent of most serious mosquito-borne viral disease (i.e., dengue fever) afflicting around more than 390 million people worldwide [1]. Despite substantial efforts to control dengue virus vector, it is still emerging rapidly [2]. Therapeutic

options are limited as the available treatments are just supportive, and there is no specific therapy or approved vaccine available. DENV belongs to *Flaviviridae* family, which comprises lipid-enveloped, positive-sense single-stranded RNA viruses [3]. DENV is classified into four closely related serotypes based on antigen distinction and represented as DENV-1 to DENV-4. The length of DENV genome is 10.7 kilobases comprising a single open reading frame (ORF) flanked by highly conserved 5′ and 3′ nontranslated regions. Single ORF encodes a polyprotein of approximately 3391 amino acids, which is further processed by host (furin and signalase) and viral proteases (NS2B/NS3 protease complex) to generate three structural (C, prM, and E) and seven nonstructural genes (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [4, 5].

RNA interference (RNAi) is evolutionarily conserved phenomenon, constituting a major component of mosquito innate immune response to virus infections [6]. It has been demonstrated that dengue virus elicits RNAi response in *Ae. aegypti* but is unable to completely repress viral replication instead it may just modulate virus replication to maintain persistent viral infection to ensure long-term survival of infected mosquitoes [7]. It has been explored that DENV employs two different mechanisms to inhibit RNAi pathway, involving RNAi suppression protein NS4B and subgenomic flavivirus RNA (sfRNA). Dengue virus encoded nonstructural protein, NS4B, has been reported to suppress RNAi pathway in both human Huh-7 cells by the mechanism still not fully understood [8]. Furthermore, dengue virus infection produces 3′ NTR degradation product sfRNA that is thought to incapacitate RNAi pathway by inhibiting dicer-mediated cleavage of dsRNA [9]. Recently, it has been ferreted out that in Culex mosquitoes, West Nile virus (WNV) replication is repressed by cytokine-like secreted antiviral protein "vago" in a dicer-2-dependent manner [10]. However, the possible effect of vago protein against dengue virus replication in *Ae. aegypti* research area is not explored yet. Such evidence clearly indicates that dengue virus opts variety of mechanisms to evade mosquito immune response to successfully replicate in the mosquitoes.

Apart from small interference RNAs (siRNA) reputation to inhibit gene expression through a phenomenon called RNAi, the target gene can also be induced by a mechanism called RNA activation (RNAa). The term RNAa was first coined by Li, who named them after he surprisingly found up regulation of human E-cadherin, p21, and VEGF genes by synthetic dsRNAs designed to target promoters of these genes [11]. He further named those promoters targeting small RNAs as short activating RNAs (saRNAs). Recent studies in which authors evaluated RNAa of above-said genes in monkey, rat, and mouse found that RNAa is highly conserved among all mammals [12]. However, the term RNA activation is not necessarily limited to transcriptional activation by saRNA, targeting promoter region only, rather it should be used to describe all small RNA-mediated gene and epigenome activation mechanisms, including transcriptional activation by targeting 3′ terminal regions of genes with saRNAs [13], piwi-interacting RNA (piRNAs)-mediated epigenetic activation [14], and microRNA (miRNA)-mediated translation activation [15]. One of the key differences between RNAi and RNAa is their kinetics. RNAi effect is known to occur within a couple of hours and disappears 5–7 days following siRNA transfection, while RNAa effect does not appear until about 48 h posttransfection and lasts much longer (at least 2 weeks) [16]. This delay may be due to the more complex mechanism of RNAa involving nuclear access and chromatin restructuring as compared to RNAi [17].

microRNAs (miRNAs) represent one of the shortest functional classes of noncoding RNAs, depicting hottest spot of gene regulation research. They were first reported in 1993 in small round worms *Caenorhabditis elegans* [18]. Since then, miRNAs have been implicated in regulating all cellular pathways in eukaryotes ranging from development to oncogenesis. These 20–24 nucleotide-long noncoding RNAs have been demonstrated to be encoded by a diverse range of organisms including plants, humans, insects, and even viruses [19]. The miRNA shows a very high degree of evolutionary sequence conservation among species [20] proved by the presence of miRNA biogenesis complex in archaea and eubacteria [21]. Historically, these microRNAs were thought to regulate different gene expressions either by degradation of particular mRNA or inhibition of translation [22, 23]. However, recent studies have revealed that these small noncoding RNAs can also activate the target gene expression through mRNA stabilization [15, 24]. As the molecular biology, bioinformatics approaches and protocols of small RNA sequencing continue to grow and sophisticate, so is the number of miRNAs discovered in different species. It is becoming more and more apparent that miRNAs not only constitute a fundamental part of regulatory machinery but also may act as molecular decoys to interfere with the function of regulatory RNA-binding proteins [25]. miRNA is involved in fundamental physiological and pathological processes like cancer development and host-pathogen interactions [26–29]. The pathogens have evolved to use to host machinery by altering the expression level of host miRNAs for their benefit [30]. Furthermore, microorganism-encoded miRNAs have also been reported to vary the host gene expression to their advantage [30]. Despite recent advances, there are still significant gaps in our understanding of miRNAs role in host-pathogen interactions, which is probably due to lack of miRNA characterization.

This pragmatic book chapter overviews the possibility of RNAi suppressor activity of DENV in the context of different viral genes. By characterizing the role of different DENV genes in RNAi suppression, we will review to gather valuable lead into the mechanism that governs DENV RNAi evasion in mosquitoes and the viral genes that are responsible for it. We will also explicit the possibility of RNA activation (RNAa) in insect, especially mosquitos. It is interesting to characterize whether RNAa has any role in host pathogen interaction because, despite the difference in their mode of action, RNAa use same RNAi machinery to induce gene activation. Although very few virus-encoded miRNAs have been fully characterized, it is understandable that they can target both viral and host genes. The information will produce a better understanding of microRNAs role in DENV replication and further deeper insight into characterization of DENV-mediated differential expression of *Ae. aegypti* miRNAs.

2. Dengue virus genome organization

The dengue virus (DENV) genome comprises a single open reading frame (ORF) of approximately 10.7 kilobases flanked by 5′ and 3′ nontranslated regions (NTRs) [30]. The nontranslated regions contain highly conserved secondary structures and coding regions in ORF, which play integral roles in viral life cycle. The composition of secondary structures is based on the sequencing of DENV-2 (a southeast Asian strain) strain, which is similar to other

Structural proteins	Protein functions
C	Viral RNA packaging
prM	Prevention of premature fusion
E	Envelope glycoprotein, receptor binding and fusion
Non-structural proteins	**Protein functions**
NS1	Signal transduction
NS2B	Co-factor for NS3 serine protease
NS3	Serine protease/helicase; NTPase
NS4B	Inhibition of interferon signal transduction
NS5	RNA-dependent RNA polymerase (RdRP); methyltransferase
RNA elements	**Functions**
5' UTR	Viral RNA synthesis and non-canonical translation
3' UTR	Cap-dependent and non-canonical translation; viral RNA synthesis

Table 1. Functions of DENV genome structural and nonstructural proteins.

different viral strains and serotypes. Although most of the DENV proteins are with known functions but some remains to be explored (**Table 1**) [31].

3. Viral entry

DENV is mostly introduced in human by infected *Ae. aegypti* mosquitoes. After the introduction, DENV particularly targets mononuclear phagocyte lineage cells including the skin resistant Langerhans cells [31, 32]. However, in mosquitoes, DENV is thought to initially target midgut and then extends and replicate in other peripheral tissues [33]. DENV can infect a very diverse range of cell lines including human, mosquito, monkey, hamster, and murine cell linages. This suggests that either DENV uses a ubiquitous receptor or it uses multiple receptors for its entry [33]. In mosquito cells, different independent groups have reported many potential DENV receptors like heat shock protein 70 (HSP), R80, R60, and 45 kDa protein [33]. In human beings, heparan sulfate [34], Hsp90 [35], CD14 [36], GRP78/BiP [37], and a 37/67-kDa high-affinity laminin receptor have been reported [38]. DENV particles interact with human myeloid cells by C-type lectin receptors (CLR) including DC-specific intracellular adhesion molecule 3 (ICAM-3)-grabbing no integrin (DC-SIGN, CD209) [39, 40], mannose receptor (MR), and C-type lectin domain family 5, member A (CLEC5, MDL-1) [41].

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4. Viral replication

DENV like other flaviviruses, after adsorption to receptors, is endocytosed by cells in clathrin-dependent manner and then is further transported to endosomes [42]. However, according to one study, DENV can adopt clathrin-independent pathways as well to gain entry into mammalian cells in particular [43]. DENV nucleocapsid is released into cytosol after acidic pH triggered rearrangements of envelope protein in late endosomes leading to fusion of viral envelope and cellular endosome membrane [44, 45]. The nucleocapsid disassembles, and viral genome is translated by endoplasmic reticulum (ER)-located ribosomes generating numerous copies of viral proteins. NS5 and other viral nonstructural proteins along with various host proteins establish replication complexes [46]. At replication complex, the viral polymerase (NS5) transcribes negative viral RNA, which serves as a template for synthesis of subsequent positive viral RNA copies. Viral replication is regulated by 5′-3′ UTR sequences (upstream of AUG region) present in 5′-3′ UTRs, which are actively involved in the circularization of DENV RNA [47]. These newly synthesized positive DENV RNAs interact with core proteins to assemble into nucleocapsids [48]. The nucleocapsid buds into ER lumen, thereby getting enveloped in a membrane bilayer carrying the viral prM and E proteins [46]. From ER lumen, immature viral particles are transported through cellular secretary pathways, furin protease cleaves prM, and results in the formation of mature viral particles capable of infecting naïve cells [49].

5. Virus-host interactions

In nature, almost every organism engages in ecological or molecular interactions whether antagonistic or mutualistic with other organisms of different species to thrive and excel. These interactions are major drivers of diversification and adaptive evolution. Among these interactions, most fascinating examples are those involved in invertebrate susceptibility to pathogens [50]. According to a recent study, the susceptibility of *Ae. aegypti* to dengue virus has a genetic basis for genotype-by-genotype interactions [51]. It is evident by the fact that despite pathogenic nature of dengue virus in human, its interaction with *Ae. aegypti* mosquito is somehow mutualistic. DENV has somehow improvised to overcome mosquito defenses and manipulate its cellular machinery in a way still not well known that emancipates its replication without interfering too much with normal growth of mosquitos.

6. RNA interference (RNAi)

RNA interference (RNAi) is a major component of innate defense of invertebrates against pathogens. Most arboviruses cause persistent infections within their arthropod vectors. However, how viruses maintain persistent infection in the face of a robust RNAi response is still not fully understood. Many plants and animal viruses have evolved molecular mechanisms for subverting the host RNAi response. For example, Flock house virus (FHV) encodes B2 protein, which

directly binds to dsRNA to inhibit DCR2 activity, to inert siRNA pathway [52]. In another study, researchers have found that plasmid-expressed La Crosse virus (LACV) NSs protein successfully inhibits interferon (IFN) and RNAi pathway in mammalian cells and mice, while fails to show any RNAi-suppressive effect in transfected U4.4 cells and C6/36 cells infected with LACV [53]. A recent publication has revealed that DENV NS4b functions as an RNAi suppressor in human Huh-7 cells via inhibition of dsRNA processing by Dicer [8]. However, whether NS4b behaves similarly in mosquitoes has not been investigated, and to date, no RNAi suppressor activity has been described for an arbovirus protein during mosquito infection. Given that DENV successfully develops persistent infection in mosquitoes despite their RNAi response, the precise mechanism of RNAi evasion is definitely a significant point of interest.

7. RNA activation (RNAa)

RNA activation (RNAa) is one of the nascent phenomena around the scientific field. RNAa is considered to be a potent emerging therapeutic strategy for the disease that can be cured by particular gene activation [54]. However, most of the studies relating to RNAa research have been carried out in human cell line. Till date, proper mechanism of RNAa is still not clear. However, it has been demonstrated that it is mostly a nuclear event that leads to chromatin restructuring [17]. RNA activation is still in its infancy, so its exact mechanism is still elusive. Recently, Portnoy et al. has proposed a model for the promoter-directed-small-activating-RNA (saRNAs)-mediated RNA activation. In this model, authors proposed that an exogenously introduced saRNA or endogenously produced small noncoding RNA, (e.g., miRNA) is loaded into an Ago protein in the cytoplasm. The Ago protein processes the saRNA by discarding its passenger strand to form an active Ago-RNA complex. Ago-RNA complex enters the nucleus through active transport/passive diffusion cell division. Guide strand directs Ago-RNA complex to complementary genomic DNA sequences usually in promoter region or to noncoding RNA (ncRNA) sequences, which tethers to the DNA. After that, the Ago-RNA complex initiates a process that differs from RNAi, which alter chromatin structure and epigenetic states of target gene via two different potential mechanistic models. In model A, the saRNA guide strand leads the Ago protein to its DNA target by constituting an RNA–DNA duplex or triplex structure, while Ago protein then serves as a docking platform to attract histone-modifying activities, like opening of chromatin structure and active transcription. On the other hand, in model B, the RNA guide strand binds to cognate promoter transcripts making saRNA-RNA complex and then Ago protein recruits histone modifiers to introduce active chromatin marks on local chromosome, resulting in activation of transcription [17].

8. MicroRNAs (miRNAs)

As the molecular biology, bioinformatics approaches and protocols of small RNA sequencing continue to grow and sophisticate, so is the number of miRNAs discovered in different species. Virus-encoded miRNAs have been known to be crucial for the viral replication. miRNAs encoded by DNA viruses have been well studied in comparison with those by RNA viruses because RNA

viruses lack nuclear access [55]. However, recent discovery of noncanonical miRNA biogenesis pathways that utilize Argonaute (Ago) 2 for processing of pre-miRNA to mature miRNA has highlighted the fact that RNA viruses may opt similar pathway to generate functional miRNAs. Similar noncanonical pathways might be used to generate functional miRNAs derived from RNA viruses that replicate in the cytoplasm. Several examples up to now have been accumulated like cellular miRNAs, miR-124, is expressed from a cytoplasmic RNA virus, Sindbis virus (SINV) [56]. Several miRNAs were found to be produced by a retrovirus, Bovine leukemia virus (BLV), based on DNA polymerase III transcription *in vivo* as well as *in vitro* [57]. Recently, Hussain et al. have identified six DENV-encoded miRNA like small RNAs in which one of miRNAs directly targets DENV NS1 gene during late infection to autoregulate its replication. Functional analysis has revealed that viral miRNAs can target both cellular and viral mRNAs to regulate viral replication leading to a successful infection [58]. Although, very few virus-encoded miRNAs have been fully characterized, it is understandable that they can target both viral and host genes.

Flaviviruses are well reported to manipulate host miRNA machinery to facilitate their replication. It has been well reported that DENV induce differential expression of miRNAs in human peripheral blood mononuclear cells (PBMCs) [59]. It has been previously reported that miR-124a, -128a, -218, and -let-7c may be important for neurological symptoms caused by a chimeric tickborne encephalitis/dengue virus [60]. Additional studies have shown that miR-122 and miR-142 of the host cells are involved in restricting dengue virus [61, 62]. However, most of the above work has been done in human. Very little is known about the differential miRNA expression of *Ae. aegypti* influenced by DENV infection, leaving a significant gap in our understanding of DENV-*Aedes aegypti* interaction. Although a recent study has shown aberrant host small RNA (sRNA) profiles in *Ae. aegypti* during DENV infection, it mainly focused on piRNAs [63] leaving a significant gap in understanding the differential expression of *Ae. aegypti* miRNAs during DENV replication.

9. miRNA biogenesis

9.1. miRNAs genomic arrangement and transcription

miRNAs are usually encoded by different regions of genome ranging from coding as well as noncoding [64]. Seventy percent of mammalian miRNAs are the intron products of protein coding genes, while rest comes from the noncoding transcription units. miRNAs have been found to be originated from both senses and antisense strands of DNA [65]. Studies have shown that about 30% of *C. elegans* miRNA genes are on the antisense strand overlapping protein coding region [66]. In Drosophila too, many miRNAs are originated from antisense strand overlapping protein coding region [67]. Multiple miRNAs can be transcribed as one long transcript named clusters [18].

Majority of miRNAs are transcribed by RNA polymerase II; however, some miRNAs usually located near Alu repeats are transcribed by RNA polymerase III, from independent genes having their own promoter or represent introns of protein-coding genes [18]. Nearly, all the independent miRNA promoters have particular features of Pol II promoters, including initiator elements and TATA boxes [18].

9.1.1. Canonical pathway of miRNA biogenesis

So far, a vast majority of reported miRNAs are produced through an RNase III enzyme-controlled canonical pathway. Transcription of primary transcript (i.e., pri-miRNA) harboring one or several stem loop structures by RNA polymerase II or otherwise RNA polymerase III kick start the biogenesis process in nucleus [68, 69]. pri-miRNAs are then further processed at stem loop sites by two RNase III proteins Drosha and DGCR8/Pasha (DiGeorge critical region 8) to produce about 70 nucleotide pre-miRNA [70]. This pre-miRNA is then exported to cytoplasm with the help of exportin-5 and RanGTP proteins [71]. Once in cytoplasm, pre-miRNA is chopped down into 22 nucleotide miRNA duplexes. Apart from above-mentioned proteins, many other proteins also work as cofactors to influence the outcome of miRNA biogenesis [65, 72].

9.1.2. Noncanonical pathway of miRNA biogenesis

Although, it was thought earlier that there is only one universal mechanism of all mature miRNAs biogenesis; however, multiple recent discoveries have led us to the conclusion that there may be several other miRNA biogenesis pathways as well. Biogenesis of quite a few miRNAs has been demonstrated to be not only Drosha independent, but also Dicer dependent [73]. It has been reported that in Drosophila and *C. elegans* along with some other vertebrates, mature miRNAs called "mirtrons" can be produced after splicing from intron hairpins independently of Drosha processing [74–76]. Recent studies have revealed that mature functional miRNAs can arise from highly conserved small nucleolar RNA (snoRNAs) in human and *Giardia lamblia* [77, 78] as well as DicerII-processed endogenous siRNAs mostly derived from transposable elements and load into AgoI in Drosophila [76]. Furthermore, researchers have identified miRNAs derived from transfer RNA (tRNA) in mouse embryonic stem cells [79].

Recent studies have led to the discovery of other types of miRNA noncanonical biogenesis pathway that is Dicer independent. One prime example of this is miR-451 biogenesis, which is Dicer independent as pre miR-451 has ~18 nucleotide stem duplex that is too short for Dicer activity. However, pre miR-451 requires AgoII for its maturation [80]. A few examples of other types of noncanonical biogenesis come from virus-encoded miRNAs. For example, several miRNAs are produced by a retrovirus, Bovine leukemia virus (BLV), based on DNA polymerase III transcription *in vivo* as well as *in vitro* [57]. The products of these transcripts were too small to be recognized by Drosha; therefore, these were directly processed by Dicer-1 to mature miRNAs.

10. Mode of action of miRNAs

After the production of mature miRNA by either canonical or noncanonical pathway, the resulting mature miRNA duplex is loaded into miRNA inducing silencing complex (miRISC) having Ago as core component [81]. After degradation of one strand, other strands of duplex, representing mature miRNA, then guide miRISC complex to target mRNA to determine its

fate [82]. The stability of the base pairs at the ends of the strands of miRNA duplex usually determines which strand will be degraded. Usually, less stable strand avoids degradation [82]. There are different modes of action of miRNAs that influence the expression of target gene ranging from mRNA degradation, translational repression to activation of transcription [16, 83, 84]. Whatever the outcome is, the general way of miRNA-mRNA interaction relies on the sufficient sequence complementarity between mRNA and 5′ seed region (usually 2–8 nucleotides) of miRNA [85]. Perfect complementarity between mRNA and miRNA is likely to downregulate the expression of target gene through its mRNA degradation by endonuclease activity of miRISC complex. This type of miRNA-mediated gene regulation is mostly evident in plants [86]. However, in animals, there is mostly imperfect complementarity between mRNA and miRNA, which usually leads to translational repression of target gene [86]. Interestingly, there are solid experimental evidences that miRNAs are also involved in upregulation of target genes as well. One of its well-characterized examples is host miR-122-mediated upregulation of HCV RNA replication by stabilizing HCV RNA and preventing its decay [87].

11. Virus-encoded miRNAs

As most viruses need host genes to facilitate their replication, one cannot rule out the possibility of viruses encoding miRNAs in order to manipulate host gene expression or keep viral copy number under check to ensure persistent infection. Possible benefits of virus-encoded miRNAs are that they usually do not elicit immune response, require less coding ability, and they have ability to constantly evolve to target new transcripts. Till date, more than 200 viral miRNAs have been identified. Most of the well-characterized viral-encoded miRNA comes from DNA viruses as compared to RNA viruses, with herpes viruses as a major contributor having average copy number of more than 10/genome [58]. Different recent studies have identified novel microRNAs from different RNA viruses. Bovine leukemia virus (BLV), a retrovirus, also encodes microRNAs [57]. Hussain et al. has reported microRNA like small RNAs in both West Nile virus and Dengue virus [88]. These viral-encoded miRNAs are produced through canonical as well as noncanonical biogenesis pathways. Based on their function, viral encoded miRNAs can be classified into two broad classes:

i. Virus-encoded miRNA regulating host genes

ii. Virus-encoded miRNAs regulating virus copy number

Important functions of viral miRNAs, which target host genes, include promoting cell survival through downregulation of apoptotic factors, thus promoting infected cells survival and proliferation, as well as modulating the immune response of the host cell [89]. It is becoming increasingly evident that one mechanism the viruses have evolved to facilitate regulatory control over their hosts is by generating transcripts, which outmatch cellular miRNAs. Interestingly, Human Kaposi's sarcoma-associated herpes virus (KSHV) and chicken oncogenic Marek's disease virus (MDV) transcribe miR-K12-11 and miR-M4, respectively; these miRNAs appear to be an ortholog of miR-155 to identical seed region [90, 91]. miR-155 has been reported to be involved in many malignancies [92], so exploitation of its targets by

viruses may contribute to viral oncogenesis. Recently, it has been reported that a flavivirus named West Nile virus encode an miRNA KUN-miR-1 that specifically induces the expression of GATA4 in *Ae. albopictus* that in turn facilitates the WNV replication [88].

Interestingly, virus-encoded miRNAs can regulate their own replication as well their own benefit. For example, simian virus 40 (SV40)-encoded miRNA miR-S1, expressed during late infection, has been shown to downregulate viral T-antigen, which is crucial to evade cytotoxic T cell response [93]. Another example is HvAV-miR-1 encoded by *Heliothis virescens* ascovirus (HvAV), an insect with dsDNA virus that downregulate viral replication by targeting viral DNA polymerase I [94]. Recently, it has been reported that Dengue virus (DENV)-encoded miRNA like viral small RNA DENV-vsRNA-5 plays an important role in the autoregulation of DENV replication by directly targeting dengue virus NS1 protein during late infection [30].

12. miRNA role in host-viral interactions

Regulative control of miRNAs over gene expression in every organism is the core reason why pathogens try to hijack them. This takeover may include disruption of miRNA biogenesis pathway, inhibiting a specific host miRNA that hinders pathogen propagation or differential miRNA expression. In case of human, it has been well documented that miR-146 and miR-155 are involved in upregulating immune function in response to various bacterial infections including *H. pylori* [95] and *Salmonella enterica* [96]. Many viruses encode miRNAs, having the potential to not only the viral genomes, which encode them, but also to target host transcripts, which facilitate their replication [97, 98]. Functions of viral miRNA targeting host genes include cell survival through downregulation of apoptotic factors as well as modulation of host immune response [89]. One example is upregulation of GATA4 by WNV-encoded miRNA, which has been found to be very important in virus replication in mosquito cells [88]. Recently, it has been demonstrated that viruses have evolved to encode miRNAs that mimic host miRNAs. Kaposi's sarcoma-associated herpes virus (KSHV) encoded miR-K12-11 that is an ortholog of human miR-155 sharing same seed region homology that will likely be able to regulate all targets of miR-155 [90].

12.1. miRNA role in *Aedes aegypti* with an insect-specific flavivirus

Previous studies demonstrated that mosquito miRNAs could be affected by different viral infections. However, those studies were restricted to pathogenic viruses, mainly restricted to the role of differently expressed miRNAs with limited characterization. Limited knowledge is available about the modulation of host miRNAs with respect to insect-specific flaviviruses (ISFs, e.g., Palm Creek virus (PCV)). ISFs share the similar genome organization with other flaviviruses; however, their rate of vertical transmission between insect hosts is higher than other vertebrate-infecting flaviviruses. Although it is still unclear that why ISFs fail to replicate in mammalian cells, it is assumed that ISFs potentially lack to antagonize the host interferon system. Similarly, ISFs express a high adaptation level in insect hosts, which may further limit their replication in vertebrate hosts. Recently, Lee et al. explicated the potential role

of miRNAs in Palm Creek virus (PCV) infection using the *Ae. aegypti* mosquito model [99]. They proposed differently expressed miRNAs after combining small-RNA sequencing and bioinformatics tools although the results hardly predicted the potential involvement of PCV infection to alter host miRNAs. They revealed that only one miRNA (i.e., aae-miR-2940-5p) out of 101 reported miRNAs of *Ae. aegypti* that had significantly altered expression over the course of PCV infection. The level of aae-miR-2940-5p was induced within 2 days p.i. and suppressed at 12 days p.i.; however, a different pattern of miRNA screening was demonstrated in *Ae. aegypti* Aa20 cell line without displaying any significant change upon PCV infection. Further *in vitro* miRNA inhibition experiments while using aae-miR-2940-5p inhibitors demonstrated that this miRNA did not directly impact on PCV replication and has no significant role in PCV-*Ae. aegypti* interaction. This variable response to virus infection in cell lines and host mosquito might be due to tissue tropism of the virus. At mRNA target level, the study reported the inconsistent expression level of MetP with miR-2940-5p expression although previously it was reported a potential target of aae-miR-2940-5p with positive interaction. It means that there was no correlation of MetP expression to PCV infection because silencing the gene did not significantly affect the virus replication. The pair MetP--aae-miR-2940-5p also positively regulate West Nile virus (WNV) virion production where aae-miR-2940-5p was selectively downregulated upon WNV infection in infected cells. The MetP human ortholog like M41 ftsh, YME1L, plays an important role as an antiapoptotic factor. It is assumed that the induction of aae-miR-2940-5p in PCV-infected cells during infection boosts MetP transcript levels and thus protects infected cells undergoing apoptosis under stress responses. However, the MetP physiological role is still uncertain in insects and requires further investigation [99].

13. RNA interference in *Aedes aegypti* with an insect-specific flavivirus

RNA interference is considered a vital antiviral defense response in mosquitoes as lot of studies reported the production of viral-specific small RNAs for different viruses. However, several reports also confirm the establishment of persistent viral infection only in mosquitoes either infected by pathogenic mosquito-borne viruses or insect-specific viruses that produce these small RNAs. While doing so, viral-derived DNA (vDNA) is produced by reverse transcription during persistent infection and may survive in extrachromosomal or integrated forms. Such vDNAs increase RNAi-mediated antiviral response in mosquitoes to boost mosquito tolerance to arbovirus infection while establishing persistent infection. In one study, Lee et al. detected PCV-specific 21 nucleotide small-RNAs in mosquitoes at different time points of infection (2, 6, and 12 days p.i.) as a potential indicator of active viral replication in the mosquitoes [99]. These findings complied with other studies where the researchers demonstrated the similar phenomenon for other pathogenic mosquito-borne flaviviruses (e.g., DENV) and insect-specific flaviviruses (e.g., cell fusing agent viruses). Their presence as hot spots suggests that either those are the potential targets of Dicer-2, or are more stable or can be reverse-transcribed into vDNA. All these might be the potential cause to enhance RNAi antiviral response. However, the authors are also afraid of bias, which could be due to

technical issues such as library preparation. In addition to RNAi, piRNAs (piwi RNAs) have been reported in mosquitoes and knockdown of piRNAs-related specific proteins indicates their endogenous antiviral activity instead of well-established exogenous RNAi activities in mosquitoes. Similarly, virus-specific piRNAs with typical A_{10} bias in sense RNA and U_1 bias in antisense RNA have been reported to be produce by Bunya viruses and alphaviruses. However, the piRNAs expressed by dengue virus and cell fusing agent virus have only A_{10} bias in sense RNA. Interestingly, the PCV-specific small RNAs do not show either A10 or U1 bias features. In this scenario, it is uncertain that either they are piRNAs or just viral degradation products. This situation is very similar seen in other flavivirus-specific piRNAs, which map to very small number of sequences in the genome where the presence of one copy of genome indicates more specific targeting than random RNA degradation. The production of virus small RNAs and piRNAs through vDNA synthesis is also documented in mosquitoes or in their derived cell lines. It is worth mentioning to note the contribution of vDNA in the production of small RNAs of virus and piRNAs in mosquitoes, although insect-specific viruses like PCV establish persistent infection in their hosts [99].

14. Long intergenic noncoding RNAs (lincRNAs) and their association with DENV-host interaction

Considering as another important class of regulatory RNAs, lincRNAs (sometimes known as dark matter) have various biological functions including genomic imprinting and cell differentiation specifically in host-pathogen interaction [100]. A growing number of evidences also reflect their possible role in gene regulation either epigenetics or nonepigenetics. However, their role in immune cell differentiation and activation is poorly understood, but the recent discoveries show their potential role in defense system as well as rapid responses to various stimuli and stress factors. Some studies also predict their active role to enhance viral replication or decrease antiviral immunity. It was also depicted that some lincRNAs also interact with other noncoding RNAs like miRNAs. The *Ae. aegypti*-linked lincRNAs are shorter in length (approximately 3000 nucleotide bases) than their protein-coding genes. Similarly, their GC content was also lower (mean:40.1%) than their protein-coding gene sequences (mean:47.8%). However, the AT enrichment or lower GC content is a typical characteristic of lincRNAs and congruent with other lincRNAs in other species [100].

Etebari et al. identified lincRNAs in dengue fever vector *Ae. aegypti* and demonstrated their potential role in host antiviral defense [100]. They evaluated lincRNA's expression in DENV serotype 2 (DENV-2) and *Wolbachia*-infected and noninfected adult mosquitoes as well as in Aa20 cells. The findings revealed the increased number of host lincRNAs under the circumstances of DENV-2 infection, some of which inhibit viral replication in mosquito cells. Furthermore, the silencing of only one lincRNA_1317 by RNA interference enhanced the viral replication in host cells, which clearly indicates their possible role in host antiviral defense. The lincRNA_1317 suppression was confirmed by reverse transcription quantitative polymerase chain reaction (RT-qPCR). It was found that the lincRNA_1317 expression was increased substantially upon the progression of infection, indicating the possible role of this

lincRNA in antiviral defense. The findings might be consistent as the highly overexpressed lincRNA_1317 expression (2.33 fold) was reported in *Wolbachia*-infected mosquitoes as compared to noninfected [100].

The authors also described lincRNAs potential involvement in mosquito-pathogen interaction by determining its association with host-endogenous small RNAs and its direct interaction with DENV-2 infection. lincRNA_1317 was not found to be located in any of the known piRNA clusters; however, no differences were found in the mapping pattern and mapped read length distribution when reads from DENV-infected and noninfected small RNA libraries were mapped to lincRNA_1317. Gene silencing like role of piRNAs on lincRNA_1317 transcriptome was also speculated. However, a little information is available about piRNA-mediated lincRNA although some recent studies predict piRNA-mediated degradation of lincRNAs in mouse's late spermatocytes [100].

The researchers also tested the hypothesis that *Ae. aegypti* lincRNA_1317 response to microbial challenge could be due to cross regulation between miRNAs and the lincRNAs. For this purpose, the normalized minimum free energy (mfe) of hybridization for each *Ae. aegypti* miRNA and lincRNA_1317 was calculated by using RNAhybrid core script. The basic objective was to identify *Ae. aegypti* miRNA recognition elements on lincRNA_1317. The results were quite interesting as binding sites enrichment for a few miRNAs with more than two recognition elements were detected on lincRNA_1317 (e.g., more than four recognition sites for miR-278-5p and miR-252-3p were predicted on lincRNA_1317). Furthermore, some hot spots for miRNA recognition sites on lincRNA_1317 were also identified, which may facilitate multiple miRNAs to bind the same regions. microRNAs contain the capability to shake lincRNA stability by targeting their transcripts similar to targeted mRNAs. Similarly, lincRNAs possessing multiple recognition sites might act as a competitive inhibitor of miRNA by eliminating them to bind their genuine targets by sequestration. The mfe values of hybridization for miRNA-lincRNA recognition sites could be a strong predictor of a binding event between two; however, further investigations and trials are still needed to validate this interface [100].

15. IsomiRs and their impact on miRNAs in dengue fever vector *Ae. aegypti*

microRNAs may exist in various lengths and sequence variations, which are known as isomiRs [101]. Earlier, those were considered as sequencing errors; however, some studies predicted to be physiologically relevant and posttranscriptionally modified miRNA variants. IsomiRs may express affinities for different targets than their canonical miRNA counterparts. Furthermore, nucleotide heterogeneity could be found at both ends of miRNA sequence in the form of nucleotide substitutions despite the variations are more frequent at 3′ end. The molecular biology, genome structure, and epigenetics of isomiRs are still poorly understood, and the mechanisms of their biogenesis are also considered very complex and even cell-type specific. Some variations in miRNA sequences are supposed to be the product of template variations, which might be brought by the exonuclease activity of Drosha and Dicer [101].

It is a well-documented fact that miRNAs may play a vital role to modulate the capability of vectors to propagate the infection for widely damaging arboviruses (e.g., dengue virus) [101]. It has been demonstrated that miRNA could be modified in mosquitoes upon the induction of DENV infection. As genetic variations in isomiR prevalence have significant impact on gene regulation, a clear understanding of the role of isomiR profile of mosquitoes in DENV and other arboviruses infection propagation is urgently needed. Etebari et al. in one study found this altered posttranscriptional modification role of miRNAs in *Ae. aegypti* mosquitoes after DENV infection in comparison with uninfected mosquitoes. For this purpose, they utilized already published RNA-seq data from 2-, 4-, and 9-day DENV-infected and uninfected mosquitoes. The findings showed significant variations in miRNA prevalence in response to dengue virus infection although the effects were not ubiquitous, and no remarkable alteration in overall pattern of isomiR expression was noted upon DENV infection. They calculated the exact/all read count ratio as an index for isomiR production rate for all known *Ae. aegypti* miRNAs. DENV-infected mosquitoes increased the isomiR production of 3 miRNAs (miR-2c, miR-210, and miR-34) with notable impact on two miRNAs (miR-276 and miR-10) with less read count by DENV infection. The data also demonstrated that 3 isomiRs of miR-34-5p were also significantly altered by dengue virus infection. Collectively, those alterations might have net benefit to determine the mosquito's role upon DENV infection propagation; however, potential biological significances of these modifications are still unclear except to infer that some evolutionary function of miRNAs. Similarly, it is also ambiguous that why some specific isomiRs potentially modify more in response to DENV infection in *Ae. aegypti*. Furthermore, it was also noted that one particular miRNA with significant increase in one specific isomiR variants upon DENV infection also contained a common variant in at least one other isomiR as well. The authors speculated that establishment and persistence of DENV infection might cause significant changes in activity levels of some enzymes involved in the production of isomiRs, which ultimately modify the isomiR production frequencies of some specific miRNAs. The isomiR production power against different *Ae. aegypti* miRNAs was also demonstrated differently as observed by the read count. The "true" miRNAs were considered as those which had an exact match to the canonical sequence (already reported and available in miRbase), while the "false" ones were classified as those which significantly differed to that reported in miRbase. The authors explicated that in *Ae. aegypti*, most abundant miRNA sequences matched to true miRNAs were just 45%, while 55% miRNAs produced by *Ae. aegypti* miRNAs were false miRNAs, and interestingly, this overall trend was not altered by DENV infection. Although the findings are striking and indicate that little variations in miRNA sequences may significantly impact target affinities in DENV-host interaction as well as in infection propagation, still extensive studies are required in the field to validate such hypothesis [101].

16. Conclusions

An over-growing number of reports and continuous publication of journals and books illustrate an intricate interplay between virus-host interactions in dengue fever virus infection. Similarly, an increase in morbidity and mortality rate with DENV infection and still unavailability of standard care to treat the infection have diverted the DENV-research to explore new paradigms in treatment and to map/identify cellular/molecular pathways to better understanding of disease progression in infected vectors, either mosquitoes or humans. Small interference

RNA and microRNA as anti-mRNA-based treatment strategies with strong evidences are evolutionary, but unfortunately still not a successful and reliable treatment line in viral infections with certain issues of drug delivery and long-term therapeutic effects. The involvement of lincRNAs and variations in miRNA epigenetic profile in disease-specific arboviruses (e.g., dengue virus) during infection also indicate complex genome interactions between virus and hosts. RNA association as a novel therapeutic approach to treat infection by gene activation, to find out some specific modulators of gene regulation in viral replication as well as in disease progression is still in an explanatory phase specifically for disease-specific or insect-specific flaviviruses. In conjunction, the molecular approaches to find out disease progression pathways in disease-specific arboviruses are still investigative and antiviral treatment approaches in the form of siRNAs, microRNAs as well as RNA association still need to be explored fully before their practical implementations in diagnostics and as some antiviral therapeutics.

Conflict of interest

The author declare that there is no conflict of interest.

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Section 3

Other Viral Diseases in the Tropics

Chapter 7

HIV/AIDS in a Community of Western Cameroon

Sevidzem Silas Lendzele

Additional information is available at the end of the chapter

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Abstract

This chapter aims at raising awareness on the prevailing HIV/AIDS situation in a community of West Cameroon. Overall prevalence was 5.21%. Males were more infected than females and individuals ≥ 36 years old recorded highest prevalence. There was no significant difference in prevalence with profession, analysis based on marital status revealed that unmarried were more infected as compared to married, based on the motif of test, those who made the test because of sickness were infected than those who did for pregnancy purpose. The year interval [2014-2016] recorded highest prevalence as compared to other year-intervals; usage of condom in sexual practice for prevention in such individuals showed low prevalence as compared to individuals who did not consider such a prevention option. HIV/AIDS prevails in the Fondonera Community of west region and serious sensitization on its occurrence/level is of vital importance to prevent future infections.

Keywords: HIV/AIDS, West Cameroon

1. Introduction

HIV/AIDS remains a threat to rural development rather than simply a health issue. HIV-AIDS have claimed the lives of an estimated 310,000 [220,000–400,000] adults and children in 2016, 21% fewer than the estimated 390,000 [300,000–480,000] who died due to AIDS-related causes in 2010 in Western and Central Africa [1]. The prevalence of this disease in the African continent is country- dependent. For Cameroon, the UNAIDS estimate data on the occurrence of HIV/AIDS says 58% of the infected population are aware of their status and 38% are on treatment [1]. It is tragic that youths in Cameroon like in order African countries still have blurred information and misconception about HIV transmission and prevention [2]. Human work

force can be hampered by several pathologies such as cancer, lung diseases, malaria, gastritis, tuberculosis, HIV/AIDS and others, but since many rural African communities rely on oxen for traction services in agriculture, a pathology such as African Animal Trypanosomosis (AAT) has also contributed in hindering agricultural production [3]. Agriculture remains the backbone of Cameroon's economy, employing 70% of its work force while providing 42% of its gross domestic product (GDP) and 30% of its export revenue. Such agro-activities take place in villages where they still exist enough surface area for it like the case of our study area. From the report of Manu et al. [4], 53.1 and 19.5% among other causes of food shortages have been caused by sickness and drought, respectively. Fondonera is a poor rural community found in the west region of Cameroon with a greater fraction of its population relying on agriculture as a source of livelihood. It was noticed that about 12.2% of patients coming for HIV test were farmers, and 79.2% were pregnant women advised to carry out the test through Antenatal Clinic (ANC) checkups. This indicates the capability of this disease in reducing agricultural output in this community as reported by Saliu and Adejoh [5] that the quantity and quality of labor input is strongly determined by state of health of individuals of that community.

It has been estimated that most vulnerable and affected groups to HIV in Cameroon include sex workers, truck drivers, mobile populations and military personnel; young people (15–29 years old) are also highly affected. Urbanization is associated with higher levels of HIV infection than rural residents [6], many engage in risky business (prostitution) to meet up with their needs as well as those of their children [7] living with their parents in the village or with them in town.

Protected sex is a critical element in a comprehensive, effective and sustainable approach to HIV and other sexually transmitted diseases (STDs) prevention and treatment. It was confirmed in Uganda that the use of condom coupled with increase delay in the age of first sexual intercourse and the reduction of sexual partners was an important factor in the decline of HIV prevalence in the 1990s [8].

Rural women especially in village settings still live in a world where they are expected to be submissive to men and where it is unacceptable for a woman to say no to unwanted and unprotected sex [9], and this makes it difficult for women to have a say when it comes to negotiating safer sex. Certain religions as well as social norms in many SSA contexts permit (and even encourage) men to get several wives, engage in sex with multiple partners, favor sex with younger partners, and dominate sexual decision-making [10].

In Africa, marriage is a social obligation and a woman's status in society is judged based on it [11]. Sex is considered as a marital duty to which no woman should withdraw herself from the moment when her husband wishes and even when she has doubts about her husband's sexual life [12]. Against the backdrop of such expectations, women often feel powerless to protect themselves against HIV infection and unintended pregnancies. Economic realities enable men to monopolize the sources of income. In addition, in certain village communities in Cameroon, men have the possibility of opening plantations and getting married to several women who are expected to give birth to several children who

will add to the labor force. This permits men to use their money to get any kind of young girl they want, exposing themselves and their family to the disease through such promiscuous habits.

HIV test is one of the important tests carried out in pregnant women during ANC checkups; this is to avoid mother to child transmission (MTCT), which is another means of transmission of the disease apart from sexual intercourse. However, many villages in SSA lack health units and many pregnant women end up giving birth at home through local means, making it dangerous for both the woman and the child who has not received the necessary follow-up before delivery and obviously does not know her HIV status. Knowledge of HIV status is crucial in order that pregnant women access the appropriate treatment and care for themselves and their unborn infants [13]. The study area of this present study has one health unit offering health services to about 24 villages, and this community relies on agriculture as their main economic activity. This study seeks to determine the demographic factors and vulnerable groups associated to HIV infection in order to raise awareness about the existence/level of the disease so as to meet with the millennium development goal of Cameroon. This millennium development goal is to halve, between 1990 and 2015, the population of people who suffer from hunger through combatting HIV/AIDS, malaria, and other diseases.

2. Materials and methods

2.1. Study area

Fondonera was our study area; it is situated 30 km from the town of Dschang, to the extreme south east of Menoua Division, west region of Cameroon. This area is bordered to the North by Fongodeng, southwards by Foguetafou Village in the Sanzo community, eastwards by Fossong Wentcheng community and westwards by Fontem in the Lebialem Division. The name of this area is colloquially known as Ndoung'lah following the Bamileke tradition (meaning summit of villages). This area suspends on a mountain at altitude between 800 and 1700 m asl with surface area of 120 km^2 with an estimated population of about 21,000 inhabitants. The climate here is equatorial type, characterized by a long rainy season and short dry season and vegetation here is forest. Agriculture is the main activity of the natives of this community, with cash crops such as coffee, cocoa, cassava, cocoyam, plantain, banana and pepper and others. This community is made up of 24 villages and they all seek for health services in the lone Nguiango health center.

2.2. Study design

A retrospective study was carried out by studying hospital consultation and laboratory registers from November 27, 2008 to November 20, 2015, a prospective study commenced in December 1, 2015 to February 27, 2016. A prospective study was carried out in collaboration

with consulting/counseling senior nurse and laboratory technician. In addition, questionnaires were administered and group discussions were organized. Home visits were also made to know the conditions of individuals living with the disease while collecting vital information. All patients coming to carry out the test were considered, but note was taken when studying past data to ensure that the same kit previously used for diagnosis was the same with that presently used for diagnosis. Diagnosis was supervised by a senior researcher to ensure that protocol for testing using the test kit was in accordance with manufacturer's instructions. Confidentiality of test results following the test was confidential, and only a code was designated for each test and not the patient's identity.

2.3. HIV testing

Rapid diagnostic tests using standard commercially sourced 'Determine' and Uni-Gold test kits were used to determine the HIV status of individuals who come for the test. 'Determine 'HIV rapid test kit (www.who.int/diagnostics laboratory) used with whole blood, serum or plasma) as pre-test. Uni-Gold test kits (The trinity Biotech Uni-Gold™ HIV test) are kits that pick or react only with HIV in blood sample and was used for confirmation. The protocol for the usage of the above kits was as outlined by Olusi and Abe [14]. Storage conditions and protocols according to manufacturers of kits were strictly followed.

2.4. Ethical consideration

An authorization was given by the Chief medical officer at the Dschang health district. Based on the fact that we were working on hospital registers in collaboration with laboratory technicians and nurses following instructions of the head of health unit on patients showing up for the test, ethical clearance was not required since we were not recruiting individuals for HIV screening. All clinical investigations were conducted according to the Declaration of Helsinki principles.

2.5. Data analysis

Data were analyzed using the SPSS statistical software of version 22.0, graphs and pie chart were constructed using MS excel software of version 2010. Chi-square test was used to compare HIV prevalence with, sex, age cohort, years of screening, marital status, motif of test and profession.

3. Results

From November 27, 2008 to February 22, 2016, 221 individuals showed up for HIV screening to know their status, 91.4% of them were farmers and this revealed how agriculturally dependent this population is.

Sex	N	I	P (I/N × 100)%	$\chi 2$	*df*	P-value
Male	21	3	14.3			
Female	200	8	4.0	4.251	1	0.039
Total	221	11	18.3			

N = number sampled, I = number infected, P = prevalence, $\chi 2$ = Chi-square, df = degree of freedom, P-value is level of significance (P < 0.05).

Table 1. HIV prevalence with sex.

Age	N	I	P (I/N × 100)%	$\chi 2$	*df*	P-value
14–24	73	5	6.8			
25–35	104	5	4.8			
>36	44	1	11.1	3.096	3	0.377
Total	221	11	22.7			

N = number sampled, I = number infected, P = prevalence, $\chi 2$ = Chi-square, df = degree of freedom, P-value is level of significance (P < 0.05).

Table 2. Prevalence with age cohorts.

Prevalence with sex revealed that male (14.3%) were more infected than female (4.0%) with a statistical significant difference ($\chi^2 = 4.251$, $df = 1$, P = 0.039) (**Table 1**). Prevalence recorded with respect to age showed that the highest cases were signaled in individuals of ages ≥36, followed by 14–24 and lastly by 25–35 years, even though such discrepancies in prevalence existed with age, there was no significant difference ($\chi^2 = 3.096$, $df = 3$, P = 0.377), recorded with age cohorts (**Table 2**).

Evolution of the disease in this area since 2008 till date was monitored. Prevalence based on the year of screening showed that the years between 2014 and 2016 (30.0%), recorded highest infected and 2012–2013 (4.1%) presented the least number of cases. Statistically, there was a significant difference ($\chi^2 = 27.373$, $df = 8$, P = 0.002) in HIV prevalence with years of testing (**Table 3**).

Prevalence based on profession showed that traders (20.0%) presented the highest prevalence, followed by farmers (14.8%) while students and teachers had zero prevalence, despite the difference in HIV prevalence registered in various occupations, there still existed no statistical significant difference ($\chi^2 = 9.531$, $df = 6$, P = 0.146) (**Table 4**). The high HIV prevalence recorded by farmers in this community is an indicator of a possible decrease in agricultural work force in this agriculture-dependent community if serious measures are not taken to prevent spread of the disease among farmers. Traders recorded the highest infection rate among others, and this is due to their high mobility rates exposing them to high risks of contracting the disease.

Year	N	I	P (I/N × 100)%	$\chi 2$	*df*	P-value
2008–2009	12	1	14.3			
2010–2011	44	5	21.2			
2012–2013	93	2	4.1			
2014–2016	72	3	30.0	27.373	8	0.002
Total	221	11	69.6			

N = number sampled, I = number infected, P = prevalence, $\chi 2$ = Chi-square, df = degree of freedom, P-value is level of significance (P < 0.05).

Table 3. Prevalence of HIV with year of testing.

Profession	N	I	P (I/N × 100)%	$\chi 2$	*df*	P-value
Farmer	27	4	14.8			
House wife	175	6	3.4			
Student	12	0	0.0			
Teacher	2	0	0.0			
Trader	5	1	20.0	9.531	6	0.146
Total	221	11	38.2			

N = number sampled, I = number infected, P = prevalence, $\chi 2$ = Chi-square, df = degree of freedom, P-value is level of significance (P < 0.05).

Table 4. Prevalence based on profession.

Motif	N	I	P (I/N × 100)%	$\chi 2$	*df*	P-value
Pregnancy	166	5	3.0			
Sick	55	6	10.9	5.44	1	0.020
Total	221	11	13.9			

N = number sampled, I = number infected, P = prevalence, $\chi 2$ = Chi-square, df = degree of freedom, P-value is level of significance (P < 0.05).

Table 5. Prevalence based on reason of test.

Apparently, there are two reasons why people in this study area go in for HIV screening, one being pregnancy and the other is sickness for both men and women. From data recorded, pregnant women frequently showed up for this test than those who choose to make HIV test when they come to consult because they are sick. From the prevalence results, those who diagnosed because they were sick (10.9%) as reason recorded the highest number of cases as compared to women who did for pregnancy reasons (3.0%), with a statistical significant difference ($\chi^2 = 5.44$, $df = 1$, P = 0.020) (**Table 5**).

Marital status	N	I	P (I/N × 100)%	$\chi 2$	*df*	P-value
Single	25	4	16.0			
Married	196	7	3.6	7.241	1	0.007
Total	221	11	19.6			

N = number sampled, I = number infected, P = prevalence, $\chi 2$ = Chi-square, df = degree of freedom, P-value is level of significance ($P < 0.05$).

Table 6. Prevalence based on marital status.

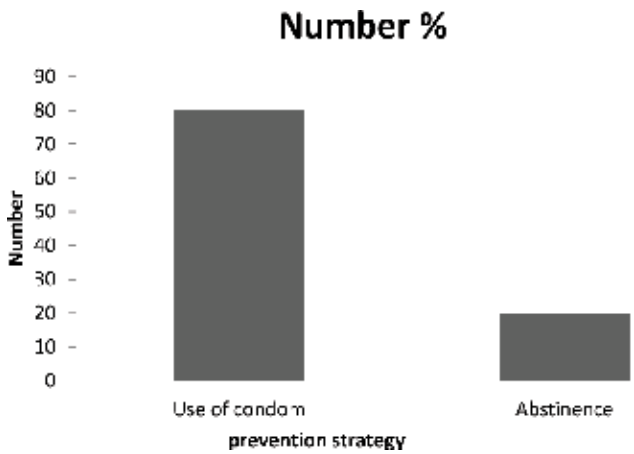

Figure 1. HIV prevention strategies.

Prevalence based on marital status indicated that single (unmarried) (16.0%) recorded high cases of the disease than their married counterparts (3.6%), with a statistical significant difference ($\chi^2 = 7.421$, $df = 1$, $P = 0.007$) (**Table 6**). It was observed that married people showed up for the test than single persons.

The most frequent control measure for sexually transmitted diseases (STDs) in this community is the use of condoms. During community visits, it was discovered that 100% of shops sold condom, and when shop sellers were interviewed on which age group frequently purchased it, the response was students in secondary and high schools and rarely parents. From the results of our group discussions and questionnaires analysis, we realized that 80% of adolescent population used condom for safe sex as a preventive tool from STDs while 20% preferred abstinence (**Figure 1**). A further analysis of the effect of condom usage by youths of this village as a prevention option for HIV showed that 60% of individuals who came for screening and were diagnosed/confirmed positive did not practice safe sex (use condom); meanwhile, the other fraction who practiced safe sex with condom recorded 38% HIV-AIDS prevalence (**Figure 2**).

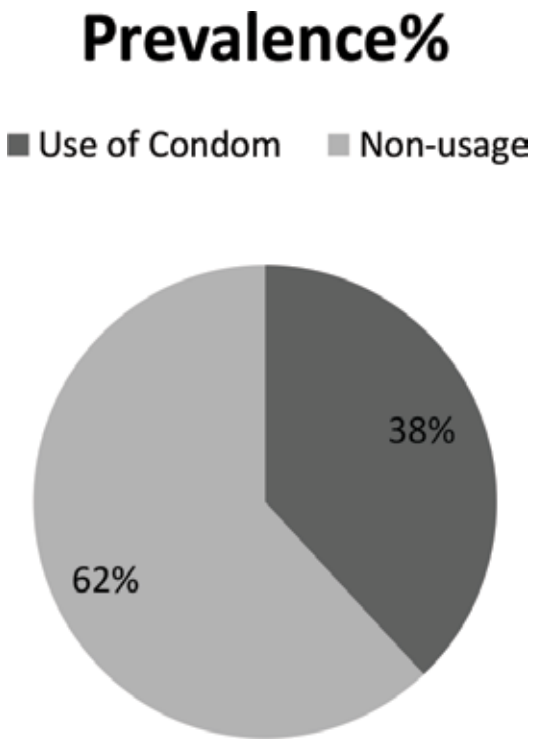

Figure 2. Sexual practice based on the use and nonusage of condom for safe sex.

4. Discussion

An overall prevalence of 5.21% was recorded in the Fondonera community of west region of Cameroon, which is greater than 2.8% reported by the Demographic and Health Survey and Multiple Indicators Cluster Survey (DHS-MICS) in 2011 for this same region. The number sampled was small as compared to other studies because people in this village rarely go to the hospital even for routine checkups; hence, the data collected represent the actual number of individuals who willingly demanded for the test. From our results, 91.4% of individuals coming for HIV screening are farmers, and this proves that a greater population of the indigenes of this area are farmers. Females (especially pregnant women and those carrying out pre-marital tests) regularly come for consultation as well as for HIV testing than men in this area. Prevalence of the disease with sex indicates that males were more infected than females; this projects a poor quality and quantity of work force and food security as they contribute more for the family upkeep as well as to agricultural work force than women. According to the DHS-MICS [15, 16], women were most infected than men, and this same report was made in a rural community known as Noni in the North West region of Cameroon by Manu et al. [4]. HIV prevalence was the highest in individuals of ages ≥36 years, this result is similar to the HIV prevalence curve for age plotted by DHS-MICS [16] which indicated peak prevalence in ages between 35 and 39 years and above. From community visit observation, 90%

of individuals from 35 years and above living in this village did not go to school and 95% of youths below 30 years have at least attended primary school. Lack of education among parents in this village has led to their unawareness of the transmission and prevention of the disease as well as lack of parental doctrine about the disease to their children. Parents in this community are equally high consumers of alcohol (beer or palm wine) which renders them senseless, exposing them to risky behaviors and disease. The years between 2014 till date recorded the highest HIV-positive cases in this community. This can be justified by referring to some socio-economic reasons which involve relocation of youths from cities during festive periods into this community and introduction of risky habits brought from the town, exposing the community to more danger. In addition, the opening up of the Santchou-Fondonera road has increased accessibility by indigenes of this village and visitors based in towns to frequently visit this area as compared to past years. From our frequency table analysis, 75.1% of individuals coming for HIV test constitute pregnant women and only 24.9% carry out the test because they are sick. It is clear from these figures that HIV test is not a priority of sick patients in this village and they prefer routine tests like stool, typhoid, and malaria. The high screening percentage for pregnant women is because HIV test is obligatory for them through ANC teachings. Even though a greater fraction consulting is made of pregnant women, they rather recorded low (3.0%) prevalence as compared to 10% in cases testing for sick reasons. This low prevalence in pregnant women in this community is still epidemiologically significant because a seropositive pregnant woman being mainly married have a far-reaching implication to the family as well as the socio-economic life of the people [17]. Based on the marital status and the frequency of consultations, free persons (single, divorced, widows, and widowers) recorded an 11.3% testing frequency as compared to 88.7% in married persons. From the prevalence results, free persons were infected than married, and this finding is contrary to that of Manu et al. [4] who reported that married were more infected than unmarried. It is logical that free persons have multiple sex partners to a greater extent than married people in a village setting like our study community. Such risky habits expose those free individual to HIV infection than those legally married; therefore, the present result was expectant. Fondonera community is an agriculture-dominated area with a greater population of indigenes resident in the villages of this community being farmers. This is portrayed from the global hospital statistics of patients consulting yearly according occupation, with 91.4% of them consulting as farmers. It was interesting even though vexing to know that farmers had the second highest consultation frequency after traders than any other occupation with no association. This finding is similar to that of Nyambi et al. [7] who reported that there is no association of HIV infection with occupation of participants in rural areas of Cameroon. Traders were highly infected because their mobility is the highest hence confirming the risk of mobile populations in the contraction of the disease. This finding is similar to that of Njukeng et al. [17] who reported highest cases with traders. The consequences of high farmer infection in this rural agriculture-dependent society can be deduced from the report of Gillespie and Kadiyala [18] in Rwanda who said that 60–80% reduction rates witnessed in farm labor are due to illness and death of infected households. It was noted from respondents about prevention strategies that 80% of them used condom for safe sex and only 20% preferred abstinence to the use of condoms. A further analysis was made on HIV prevalence among users and nonusers of condom as prevention strategy, and it revealed that 62% of infected cases did not use condom during sex

and only 38% of infected were aware of the necessity of condom in protection against STDs. It was reported by shop sellers during interviews that most of their customers are students and teachers who reported zero HIV prevalence as compared to parents who had high cases. This finding is in consonance with the report of Zacharie and Barthelemy [19] who reported that abstinence was the best option by participants to control the spread of the disease followed by use of condoms. From the abovementioned findings, it can be deduced that even though condom users recorded low prevalence than noncondom uses, condom still failed because it did not give a 100% protection from the virus hence the existence of positive cases with users.

5. Conclusion

From the 221 who tested to know their HIV status since 2008 till 2016, 11 of them were confirmed positive with an overall prevalence of 5.21%. Demographic information revealed that sex, marital status and years of testing showed association with HIV prevalence, but age, reason and profession showed no association with HIV prevalence in the Fondonera community. This community is dominated by farmers, and farmers recorded the highest prevalence which is a threat to agricultural production and food security in this poor rural community. As a prevention strategy, condom will not be 100% protective against the virus and should not be indispensible, but abstinence will be the best knockout option which can only be transmitted through stringent sensitization. Positive cases are encouraged to follow-up their antiretroviral treatment and avoid spread of the disease to other vulnerable groups.

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Conflict of interest

The authors declare that they have no competing interests.

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Chapter 8

Ebola Virus Disease: Progress So Far in the Management of the Disease

Godwill Azeh Engwa

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.79053

Abstract

Ebola virus disease is one of the most deadly emerging infectious diseases in the world which causes severe haemorrhagic fever, with a mortality rate of 50–90%. Following the largest outbreak in West Africa in 2014 which was the most deadly of all time challenging global health, so much concern has been tilted towards the management of the disease. Some of the major global challenges that prolonged and escalated the gravity of the 2014 outbreak were the lack of prompt, reliable and affordable diagnostic tools, but most importantly no specific treatment and vaccines were available to manage the infection. Though certain non-licensed experimental drugs as well as vaccines were introduced during the 2014 outbreak that contributed towards the control of the epidemic, their efficacy was yet to be confirmed in randomized trials. Presently, a few rapid diagnostic test kits have been approved by FDA and WHO. Also, several experimental drugs and vaccines are undergoing randomized clinical trials with a few currently at phase III. Thus, it is our hope that most of these drugs and vaccines will be available in future to better manage re-emerging Ebola infections or outbreaks.

Keywords: Ebola virus disease, haemorrhagic fever, rapid diagnostic test, specific treatment, vaccine, outbreak

1. Introduction

Ebola haemorrhagic fever is very deadly viral disease which originated in the Democratic Republic of Congo (DRC), a formerly known as Zaire with the first outbreak in 1976 [1]. Since then this virus has spread across the globe but more prominent in the African continent. This virus causes fatal haemorrhagic fever to humans as well as non-humans including monkeys, gorillas, chimpanzees, etc. [2]. Though a definitive host is yet to be confirmed, this virus is

believed to be transmitted by bats being the primary reservoir mostly through body fluid or contact to humans and other primates. The virus can penetrate mucosal membrane to infect various cells in the body including macrophages, monocytes, dendritic cells, etc. and spread into the circulatory and lymphatic systems damaging blood vessels leading to haemorrhage. Though no approved standard treatment is available for the disease, it has been managed with certain antiretrovirals and supportive treatments such as rehydrating solutions for maintaining fluid and electrolyte balance as well as treatments against secondary infections. Thus, the availability of a standard approved drug is one of main concern in recent time following the 2014 fatal outbreak. More so, since this disease is sporadic and usually emerges as an outbreak, effective control is usually difficult as diagnosing viraemia is usually challenging. Also, no existing vaccine is available against the disease. Following the introduction of experimental drugs and vaccines during the 2014 West-Africa Ebola outbreak which contributed to its control [3], it has been of interest to know the progress so far and efforts that are been laid to ensure that these drugs and vaccines are licensed in future. Hence, this chapter will focus on the progress towards the provision of diagnostic tools, and availability of specific treatments and vaccines. However, an understanding of the aetiology and pathogenesis of Ebola virus disease is necessary so as to expose possible drug targets as well as various vaccine candidates for a better management of the disease.

2. The origin and epidemiology of Ebola virus disease

This viral disease emerged around 1976 with the first outbreak in the northern part of DRC, formerly known as Zaire which affected about 318 individuals with a mortality rate of 88% [1]. Almost at the same period and year, another outbreak emerged in southern Sudan with 284 cases killing about 150 individuals (53%) [4]. In 1976, a case of the infection of the Sudan virus was identified in England but there was no casualty [5]. The following year in 1977, a case was reported in DRC which the individual died. By 1979, there was reoccurrence of an outbreak in Southern Sudan which affected 34 individual with a 65% mortality rate. The disease was absent for about 2 decades before re-emerging in Gabon and Ivory Coast in 1994, with a mortality rate of 60% in Gabon [6]. The disease reoccurred in Gabon in 1996 and 1997 [6], and again in DRC in 1995 [7]. In 2000, an outbreak was reported in Uganda with over 80% mortality while the following year, another outbreak was reported in Gabon with 53% and 82% mortality rate respectively [8]. Since then, there has been reoccurrence of outbreaks in DRC between 2001 and 2008 [9] and in Uganda between 2007 and 2012 [10]. In 2012, there was another outbreak in DRC with a fatality rate of about 40% [11]. In 2014, the largest outbreak was recorded in DRC [12] and West Africa [13] that spanned across various countries including United State, Spain, Mali, Senegal and Nigeria [14].

It should also be noted that some non-human primate infections have also occurred. The first to be observed was in the United States between 1989 and 1990 [15] followed by another outbreak in Italy in 1992 which were related to the importation of monkeys from the Philippines [16]. In 2008, cases of respiratory and porcine reproductive syndrome in sows and piglets caused by the Ebola virus were observed in China and Philippines with high mortality. Animal farmer workers who were in contact with the virus became infected but the infection was asymptomatic and no casualties were recorded [17].

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3. The 2014 West African Ebola outbreak

The first trace of the infection was in Guinea in December 2013 which subsequently spread to Liberia and Sierra Leone. In 2014, the world recorded the largest Ebola virus outbreak in West Africa particularly in Sierra Leone, Guinea, and Liberia, with over 7178 infected cases 3338 deaths amounting to a mortality rate of 51% as of 1st October, 2014 [18]. Also, the disease spread to other countries including Nigeria and USA. [18]. In August 2014, the epidemic was declared by the World Health Organization (WHO) as a public health emergency of international concern [19]. By September 2014, the fatality rate of infected individuals was about 70.8% in Liberia, Guinea, and Sierra Leone. About 20 cases were report in Nigeria which originated from a traveller from Liberia in July 2014 with a fatality rate of 45.5%. As of 23rd October, 2014, about 450 health care personnel were known to be infected with Ebola virus of which 244 died [20]. In October 2014, two imported cases with one death, as well as two locally acquired cases from health care workers were identified in the United States. By November 2014, a cumulative total of 20,000 Ebola cases were reported in the West Africa outbreak including 5740 cases from Guinea, 9890 from Liberia and 5000 from Sierra Leone [20] killing over 11,300 individuals within the course of 2 years.

4. The 2018 Ebola outbreak in DRC

Recently, an Ebola outbreak has emerged in DRC with the first cases detected on May 8, 2018 at the Bikoro zone, a remote rural region and the virus has spread to Mbandaka, an urban area which inhabits more than 1 million people. Mbandaka is about an hour's flight from Congo's capital Kinshasa, thus, there is more concern about the possibility of the virus to spread to other larger populations. As of May 21, 2018, about 46 cases of haemorrhagic fever had been recorded. Among these cases, 21 have been confirmed as Ebola infected, 21 are probable cases of the virus while 4 are only suspected to be related to the epidemic. Twenty-six deaths have already been reported [21].

5. Ebola virus species

Ebola haemorrhagic disease is caused by Ebola virus which is an RNA virus in nature. It is a virus that belongs to mononegavirales order, Filoviridae and the genus *Ebola*. There exist five species of this virus which include the following [22, 23].

i. Zaire Ebola virus (EBOV): Previously known as the Zaire virus (ZEBOV) was the first to be identified following the first outbreak in DRC in 1976. It has recorded the highest mortality rate of approximately 83% over 27 years.

ii. Sudan Ebola virus (SUDV): This virus emerged in 1976 in Southern Sudan as the second outbreak almost simultaneously with the Zaire outbreak. It presents an average fatality rate of 53% since 1976 to 2001.

iii. Reston Ebola virus (RESTV): It was discovered in 1989 during an outbreak of simian haemorrhagic fever virus (SHFV) in Reston USA which infected non-human primates. It has also been identified in Pennsylvania, Texas and Siena, Italy.

iv. Côte d'Ivoire Ebola virus: Also referred to as Taï Forest Ebola virus (TAFV), it was first discovered in chimpanzees from the Taï Forest in Ivory Coast in 1994

v. Bundibugyo Ebola virus (BDBV): This virus species was discovered in 2007 in Uganda following the outbreak in Bundibugyo District which recorded 39 deaths with a mortality rate of 34%.

6. Ebola virus morphology

The genus Ebola are negative-sense, single stranded RNA viruses which are non-segmented belonging to the Filoviridae family. The negative-sense RNA genome is approximately 19 kb in size but varies among the various Ebola species and it is encapsulated in a lipid membrane used for the formation of new particles on the surfaces of their host cells [24, 25]. The core of the virus constitutes the genomic RNA surrounded by nucleoproteins (NP). The Ebola genome consists of seven genes that codes viral proteins (VPs) each of which differs in function [26]. Among these proteins, VP24 which constitutes the main matrix protein is the most abundant virion protein. VP30 is involved in the activation of RNA transcription while VP35 is involved in viral RNA synthesis. VP35 is also attributed to be responsible for varying degrees of virulence among different strains of Ebola virus. VP40 is also a matrix protein of the negative stranded RNA and its roles is to assemble the lipid envelop of the virus by linking the nucleocapsid to the surrounding membrane. The virus also contains a transmembrane glycoprotein (GP) which is responsible for the formation of virion spikes which facilitates viral entry into cells. A section of this glycoprotein (GP1 and GP2) are responsible for immunosuppression

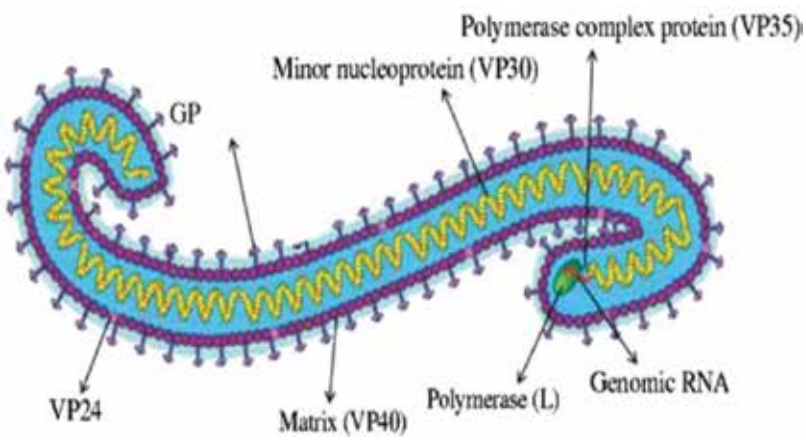

Figure 1. Morphological representation of the Ebola virus showing the various proteins: VP, virion protein; and GP, glycoprotein [28].

permitting the virus to evade the immune system as they show very high homology with immunosuppressive protein found in oncogenic retroviruses [27]. There is also an RNA-dependent RNA polymerase known as the L protein which catalyzes transcription. The morphology of Ebola virus is shown in **Figure 1**.

7. Transmission and transmission dynamics

Ebola haemorrhagic fever is a zoonotic borne disease believed to be transmitted from rodents and bats as primary reservoirs. It has been noticed that bats are usually present at the sites of several outbreaks in large numbers and Ebola virus antibodies have been found in fruit bats [29] though the virus has not been isolated from these animals. It is believed that this infection is asymptomatic in bats and can be transmitted to chimpanzees, gorillas, monkeys, other mammals and humans. These transmissions may be due to direct contact with the reservoir species (**Figure 2**). In humans, transmission from infected persons to health humans is through direct contact with body fluids or secretions such as saliva, stool, urine, semen, and blood [30]. The virus has been shown to persist for up to 7 weeks in semen after recovery of infected individuals from the illness suggesting sexual intercourse as probable means of transmission. Also, contact of broken skin or mucous membranes with items such as clothing, bed linen, or used needles are possible means of transmission [30].

Health workers are another category of persons exposed to the infection following their care for Ebola infected patients as contact with used equipment, gloves and other clinical materials can promote transmission. Health workers or other individuals can become infected if they get in contact with dead bodies of infected subjects. In all, it has been concluded that Ebola transmission is only by means of contact as there has been no evidence of transmission from

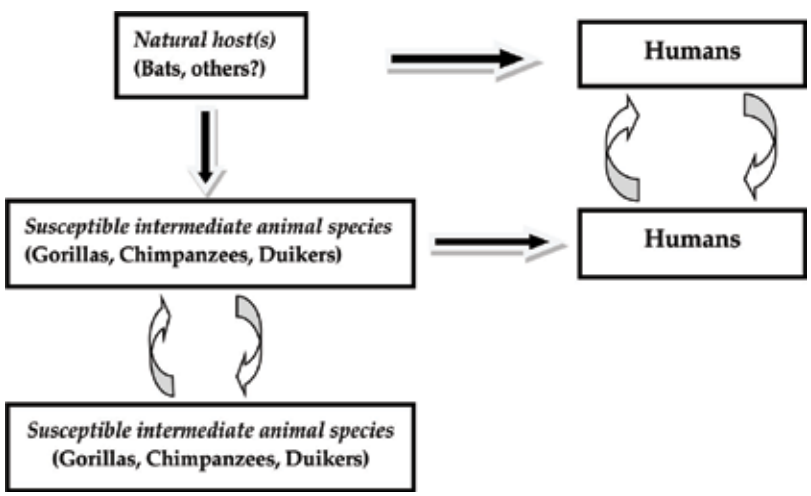

Figure 2. Transmission of Ebola virus. Source: http://www.cdc.gov/vhf/ebolaresources/virus-ecology/.html.

Levels of risk of transmission	Type of contact
Very low or no recognized risk	Casual contact with a feverish, ambulant, self-caring patient. Examples: sharing a sitting area or public transportation; receptionist tasks.
Low risk	Close face-to-face contact with a feverish and ambulant patient. Example: physical examination, measuring temperature and blood pressures.
Moderate risk	Close face-to-face contact without appropriate personal protective equipment (including eye protection) with a patient who is coughing or vomiting, has nose bleeds or who has diarrhoea.
High risk	Percutaneous, needle stick or mucosal exposure to virus-contaminated blood, bodily fluids, tissues or laboratory specimens in severely ill or known positive patients.

Table 1. Risk of Ebola virus transmission and its association with the level of contact.

human to human via the respiratory route [31]. The type of contact among individuals can influence the risk of transmission of Ebola virus disease as shown in **Table 1** [32].

8. Pathogenesis of the disease

Though the natural reservoir of Ebola virus is unknown, bats are the main primary reservoirs along with some non-human primates such as monkeys, baboons, chimpanzees and gorillas known to transmit the virus to humans through contact with the animals or their body fluids such as sweat, blood, urine, and other secretions or other infectious objects. Ebola virus can persist on objects in a dried state for several hours and can persist in body fluids for several days [28, 33]. Once humans get in contact with the virus, it enters the body through mucous membranes following abrasions or cuts and adheres to cell membranes. Following attachment on cell membrane, it penetrates, uncoating its membrane and replicates it RNA which then expresses its constituent proteins and reassembled to a matured virus that is release from the cell. It gets into the circulatory system infecting monocytes, dendritic cells and macrophages and subsequently spread in the lymphatic system infecting lymphocytes and other organs such as the spleen, liver, kidney, etc. [34]. This has been confirmed in *in vitro* studies where macrophages largely infected by Ebola virus produce high amounts of viral particles which are delivered to various organs such as the lymph nodes, liver, spleen, endothelium, adrenal gland, kidney and pancreas [35, 36].

The infected cells as well as lymphocytes becomes destroyed releasing inflammatory substances such as interleukins (interleukin-2 and -10), interferons (interferons-alpha and -gamma), tumour necrosis factor etc. which destroys the vascular and endothelial system increasing vascular permeability. Peripheral smears of infected persons have shown atypical or death lymphocytes which are suggested to result from apoptosis triggered by inflammatory mediators released from viral infected target cells and/or from viral glycoprotein secretions [36].

The destruction of the microvascular tissues changes vascular permeability, cause cellular necrosis and activate clotting factors thereby leading to coagulopathy, hypotensive shock and possibly death [37]. Also, impairment of endothelial and platelet cells alter fluid and electrolyte balance disrupting the body's homeostasis [38]. Virus induced shock is due to elevated

increase in nitric oxide production which leads to damage of the vascular system altering the blood pressure [39]. Also, these hypotensive shock may also result from platelet-derived agents such as thrombin following the damaged of endothelial cells. Theses clotting factors can disseminate into various organs causing intravascular coagulation [40].

One of the possible mechanisms the virus is able to persist in the body is through its ability to evade the immune system by destroying immune cells such as lymphocytes, natural killer cells, phagocytes as well as impairment of the action of dendritic cells [41].

9. Clinical manifestation

Once an individual gets infected with Ebola virus, it can persist in the body for a few days with no clinical manifestation. Thus, the incubation period ranges from 2 to 21 days with an average between 4 and 10 days. After this incubation period, an acute infection emerges which starts to portray clinical manifestations. The illness commences with symptoms of flu-like syndrome which includes a sudden onset of high fever, chills and myalgia. This early infection can affect the gastrointestinal system causing anorexia, vomiting, nausea, diarrhoea, abdominal pain, as well as the respiratory system causing cough, chest pain and dyspnea. Also, the vascular system can be affected leading to hypotension, oedema as well as neurologic system causing headache and coma [42, 43]. Though the periodical manifestation of Ebola virus varies among individuals, generally, these clinical features can be categorized into four phases as suggested by Suresh and Dashrath [44].

Phase 1 - Influenza-like syndrome: The onset of the infection commences with non-specific signs or symptoms such as high fever, nausea, headache, sore throat, arthralgia, and myalgia.

Phase 2 - Acute phase: A persistent acute fever emerges along with headache and intense fatigue within 1–6 days which is not responsive to antibiotics or antimalarial drugs. This is usually followed by gastrointestinal obstructions such as abdominal pain, diarrhoea, vomiting, etc.

Phase 3 - Pseudo-remission: After the acute phase, a false recovery phase emerges by days 7–8 where the patient feels better showing some signs of recovery such as gain of appetite. In some patients, this phase may eventually lead to total recovery and survival of the disease.

Phase 4 - Aggravation phase: By day 9, the health status gets worsen in most individuals presenting respiratory disorders such as cough, dyspnea, hiccups, throat and chest pain as well as cardiovascular distress and hypovolemic shock. Also, rask may develop on the skin as well as petechiae.

During the infection, laboratory investigations show high levels of aminotransferase, and marked lymphocytopenia, and thrombocytopenia in patients' blood [45]. More so, bleeding usually occurs in the gastrointestinal tract and may be expressed as petechiae, melena, conjunctival haemorrhage, easy bruising, haematuria, or intraperitoneal bleeding. Also, mucous membrane bleeding as well as excessive clot formation and failure of venipuncture sites are evident during infection. Progression of these symptoms over a period of time may lead to dehydration, confusion, stupor, hypotension and failure of multiple organs culminating to

fulminant shock and eventually death which occurs between the 6 and 16 days of illness [46, 47]. However, a few patients may survive and recovery from the infection gradually presenting arthralgia and fatigue.

10. Ebola virus identification

One of the primary means of management of Ebola disease is to promptly diagnose the virus. Hence, early laboratory diagnosis for the confirmation of suspected individuals with haemorrhagic fever is paramount for the implementation of appropriate control measures. Reverse transcription-Polymerase chain reaction (RT-PCR) and viral isolation on Vero cells are definitive diagnostic methods for the detection of Ebola virus infections [48, 49]. Also, serological diagnosis based on enzyme-linked immunosorbent assays (ELISAs) to detect immunoglobulins such as IgG and IgM specific to Ebola virus antigens are effective diagnostic tools [50, 51]. Viral nucleic acid and antigen can be detected in blood as early as 3 days after symptoms starts to manifest and can be identified by the above diagnostic tools [52]. Prior to laboratory diagnosis, clinicians should examine patients for vital signs and symptoms such as high fever, severe headache, muscle pain, bleeding, bloody diarrhoea, blood in urine, vomiting, abdominal pain, diarrhoea, or unexplained haemorrhage, etc. in suspected individuals [53]. In addition to primary diagnosis of Ebola virus, secondary diagnosis, particularly in infected patients such as the presence of atypical lymphocytes, leucopenia (as low as 1000 cells/L), thrombocytopenia (50,000–100,000 cells/L), elevated aspartate aminotransferase and alanine aminotransferase level, prothrombin and partial thromboplastin time are necessary to manage related complications that may arise due to the infection [54]. The various diagnostic tests for Ebola are summarized in **Table 2**.

One of the major challenges in the diagnosis of Ebola virus is that the infection presents similar signs and symptoms to that of common diseases such as flu, malaria, yellow fever, typhoid, meningococcal meningitis and other bacterial and viral infections [24, 30]. As such, clinical diagnosis is usually not sufficient for routine screening especially during outbreaks where the infection rate is high. More so, definitive diagnosis by RT-PCR and viral culture are usually not

Time line of infection	Primary diagnostic tests	Secondary diagnostic tests
Within a few days after symptoms begin	Immunohistochemistry testing Rapid diagnostic test Antigen-capture ELISA testing IgM ELISA RT-PCR Virus isolation on Vero cells	Atypical lymphocytes Leucocytes Thrombocytes Aspartate aminotransferase, alanine aminotransferase prothrombin and thromboplastin
Later stage of disease course or after recovery	Rapid diagnostic test IgM and IgG antibodies	
Deceased patients	Rapid diagnostic test Immunohistochemistry testing RT-PCR Virus isolation on Vero cells	

Table 2. Ebola diagnostic test.

feasible in Africa as most local health settings lack or do not have sufficient or adequate laboratory facilities for such molecular techniques. Another major challenge is the high cost for the molecular diagnostic tests (RT-PCR, ELISA). The cost per sample may cost between $50 to $100 which may not be affordable by majority of individuals in developing countries especially in endemic areas including West Africa, Sub-Saharan Africa and central East Africa. More so, though these molecular diagnostic tools are very reliable, analyses take about 2–6 h, which is too long for such acute infection. As such, there is considerable need for rapid diagnostic tests which can take just a few minutes for detection. Also, cell culture on vero E6 African monkey kidney cells which is a traditional gold standard test requires biosafety level 4 (BSL-4) containment, thus, restricts its use for routine diagnosis. More so, the test can last for up to 5 days from the moment of viral inoculation to microscopic visualization [55].

An immunochromatographic assay may be suitable for such effective and prompt diagnosis. In 1995, a colorimetric assay was developed by Dr. Sherif Zaki of the CDC for the identification of Ebola virus in skin biopsies preserved in formalin [56]. However in recent years, this diagnostic tool is not readily available.

10.1. Rapid diagnostic test for Ebola virus disease

Following the 2014, outbreak in West Africa, several field trials on rapid diagnostic tests (RDTs) are ongoing and a few RDT kits have been approved by U.S. Food and drug Administration (FDA) and WHO on Emergency Use Authorization (EUA) status. These RDT kits are lateral flow immunoassays (LFIs) which basically detects viral protein antigens circulating in blood. Three of the recently approved RDTs include ReEBOV Antigen Rapid Test kit, OraQuick Ebola Rapid Antigen Test and SD Q Line Ebola Zaire Ag test [57].

The ReEBOV Antigen Rapid Test kit by Corgenix, Inc. was the first LFI for EVD to receive emergency use authorization (EUA) status from both FDA and WHO) [58, 59]. This chromatographic dipstick immunoassay kit is a RDT that detects the Ebola virus VP40 matrix protein of three species which includes EBOV, SUDV, and BDBV in whole blood, plasma or serum. Following a finger prick, a drop of blood is applied directly unto the nitrocellulose test strip. The nitrocellulose strip is then deepened into a tube containing reaction buffer which initiates the movement of the sample along the test strip by capillary action. The presence of VP40 in the sample leads to the formation of an immune complex between the VP40 matrix protein antigen and gold-labelled anti-antibodies against VP40 which is subsequently deposited along the strip boundary of anti-VP40 producing a pink-red line that is visible between 15 to 25 min after the analysis. Validation study for the performance of ReEBOV RDT conducted in Sierra Leone on venipuncture blood showed a 100% sensitivity and 92% specificity when compared with results obtained from RealStar Filovirus Screen RT-PCR kit by Altona Diagnostics [58].

The second RDT kit that has received approval from WHO and FDA on EUA status is the OraQuick Ebola Rapid Antigen Test manufactured by OraSure Technologies, Inc. [60, 61]. Just like the ReEBOV Antigen Rapid Test kit, this RDT kit detects VP40 matrix protein of EBOV, SUDV, and BDBV species with similar assay procedure. In addition to the use of whole blood, this kit also makes use of cadaveric oral fluid which is collected using an oral mucosa swab for the detection of Ebola virus. Similarly as ReEBOV RDT, the presence of Ebola virus antigens is

visibly detected following immune complex between viral proteins and gold-labelled antibodies bound along the test line in less than 30 minutes. Validation of the test performance of the OraQuick RDT based on a retrospective study in Sierra Leone as reported by WHO showed the OraQuick RDT with a 84% sensitivity and 98% specificity compared to clinical real-time RT-PCR testing [60].

SD Q Line Ebola Zaire Ag test by SD Biosensor, Inc. is the third and most recent RDT kit to be approved by WHO on EUA status [62]. Unlike ReEBOV and OraQuick RDTs which detects only VP40 in EBOV, SUDV, and BDBV species, SD Q Line Ebola Zaire Ag test is a chromatographic deep stick test that simultaneously detects GP, NP, and VP40 antigens of EBOV in whole blood, serum or plasma. In this test, the presence of the three antigens in the sample forms complex with their specific gold-labelled mouse monoclonal antibodies at three different test boundaries at which visible lines are seen. Thus, three drops of sample are added to a sample port on the assay device and visualized at 20–30 min. The presence of at least any of the three test lines is interpreted as positive result. A WHO validation study in Sierra Leone using a total of 446 specimens including 100 fresh venous whole blood and 346 frozen plasma showed SD Q Line Ebola Zaire Ag test with 84.9% sensitivity and 99.7% specificity when compared to the RealStar Filovirus Screen RT-PCR kit 1.0 as gold standard [62].

In a nutshell, these RDT kits are very effective in diagnosis Ebola virus and useful for field settings especially during outbreaks as results can be obtained within a very short time without the use of any electronic equipment and does not require refrigeration for storage. The approval of these RDTs is of major importance for public health management of the disease as prompt diagnosis especially in the field following Ebola outbreaks is key to effective treatment.

Recently, a new immunochromatographic strip and a smartphone reader based on Sudan virus (SUDV) glycoprotein monoplex which detects and semiquantifies Ebola-specific IgG antibodies in human survivors has been developed [63, 64]. When the point-of-care test was tested in freshly collected patient samples including 90 SUDV survivors and 31 non-infected controls in Uganda, it showed a sensitivity of 100% and a specificity of 98% compared to standard enzyme-linked immunosorbent assay (ELISA) of whole Ebola antigen [64]. More so, a multiplex test which simultaneous detects antibodies against three recombinant SUDV proteins has also been developed. A pilot study involving 15 survivors and 5 non-infected controls showed sensitivity and specificity of 100% compared to standard ELISA [64]. Also, another multiplex subtype assay for the identification of three Ebola species: BDBV, SUDV, and EBOV based on recombinant viral glycoproteins has been developed [64]. The advantage of this multiplex viral species test is that it could differentiate the host's immunity to specific viral species and also identify cross-reactive immunity in infected patients.

11. Therapeutic interventions

Till date, there is no precise treatment for Ebola virus disease which constitutes one of the major draw backs in its management. Treatments available for Ebola virus infection are basically supportive and symptomatic remedies for dehydration, maintenance of oxygen

saturation and blood pressure, replenishment of nutrients, antivirals as well as antibiotics for concomitant infections [65]. Administration of sufficient fluids by oral or intravenous route serve to maintain circulatory stability and replenish electrolytes and fluids lost during the infection. A broad-spectrum of antibiotics are used to manage potential concomitant bacterial infections; antimalarials are used for the treatment of malaria while antiretrovirals are used to inhibit viral replication. Antipyretics and analgesics are frequently used for the control of fever or body temperature and pain respectively. Also, specific drugs could be administered for the control of organ failure.

With no specific treatment against the disease, considerable efforts in research have been ongoing for the identification of possible drug candidates for therapeutic interventions. One of such clinical investigation was conducted in 1995 during the Ebola epidemic in Kikwit where blood of improving patients was transfused to eight Ebola patients as a means of passive immunization. Among the eight patients, seven of them successfully survived the infection while only one patient died [66]. However, subsequent *in vitro* assays showed antibodies not to have neutralizing action against Ebola virus. As such, clinical investigation based on passive immunization has not been conducted in subsequent outbreaks. Furthermore, *in vitro* assays showed monoclonal antibodies against the GP of Ebola virus to exhibit defensive and healing properties in mice but were unable to protect non- human primates [67, 68]. Also, immunoglobulins which were raised in goat and had undergone pre-clinical test on laboratory animals were administered to infected scientists with Ebola haemorrhagic fever during an outbreak showed some degree of protection against the disease. Thus, these immunoglobulins were suggested to be beneficial as an emergency cure for individuals inadvertently infected with Ebola virus [69]. More so, a series of nucleoside analogue inhibitors for carbocyclic 3 deazaadenosine and S-adenosylhomocysteine hydrolase were shown to avert death in infected mice by inhibiting Ebola virus replication [70].

With such preliminary studies showing immunoglobulins to have protective effects against the infection, several researches have dueled on this aspect as well as other targets and several clinical trials have been ongoing to assess some potential drug candidates. The main classes of drugs which are being evaluated for potential therapeutic effect against Ebola virus infection include monoclonal antibodies such as ZMapp, nucleoside analogues, RNA inhibitor based (TKM-Ebola) agents, positively charged phosphorodiamidate morpholino oligomers as well as antisense-based (AVI-7537) drugs [71]. Among these drug candidates, ZMapp is one of the most promising therapeutic interventions against Ebola virus disease that affects viral replication inhibiting its expression.

ZMapp is an experimental drug by Mapp Biopharmaceutical, Inc., which comprises of a combination of 3 monoclonal humanized murine antibodies produced in mice infected with Ebola virus and subsequently generated in tobacco plants [71, 72]. *In vivo* pre-clinical animal study showed 43% of infected mice treated with Zmapp to survived infection [73]. Though pre-clinical studies had exhibited therapeutic effect of Zmapp against Ebola virus, the experimental drug came into the lamplight when two US citizens who were health workers in Liberia during the 2014 West-Africa outbreak became infected and were successfully treated with ZMapp in Atlanta USA. Following this success, the drug was then used as an experimental treatment in the 2014 West-Africa Ebola outbreak and several patients survived and recovered

from the infection [74]. Though this experimental drug was helpful during the outbreak, its therapeutic efficacy remained inconclusive since no randomized controlled clinical trial had been conducted as of 2014 [74, 75].

Another hopeful candidate drug that act by preventing viral replication is favipiravir (T-705), a pyrazinecarboxamide derivative which has shown to be effective against EBOV in *in vitro* and *in vivo* studies [76]. Also, a promising experimental drug for Ebola virus infection is BCX4430. This drug possesses antiviral activity for marburg, yellow and Ebola fever and it is also being tested for its ability to inhibit target enzymes in Ebola virus. BCX4430 has been shown to be effective in infected animals if the treatment was administered within 48 h after the infection [77]. Other therapeutic candidate drugs include RNA polymerase inhibitors as well as small interfering RNA nano particles that act as protein synthesis inhibitors. Studies in Ebola infected guinea pigs and non-human primate models showed small interfering RNAs agents and gene-silencing drugs to protect against Ebola infections [78].

One of the major challenges for the availability of treatment against Ebola virus disease is the inconsistency and sporadic nature of the virus which has limited clinical trials in humans. In as much as several drug candidates have emerged and have been effective in pre-clinical studies, without clinical trials in humans, there is no guarantee that these experimental drugs can effectively treat infected patients. It is relevant for such trials to be conducted even though it remains difficult as the disease usually emerges periodically as outbreaks. Even though these challenges are limiting, efforts in identifying other potential drugs targets should be encouraged with emphasis on the key viral surface proteins as well as nucleoproteins involved in viral replication and pathogenesis.

12. Vaccine candidates undergoing trials

Vaccines are one of the most effective means of managing viral infections especially for recurrent infections. This suggests that a vaccine for Ebola virus fever will be important for the management of the disease. Till date despite the several recurrent outbreaks of Ebola haemorrhagic fever, no licensed vaccine is available. However, several clinical trials are ongoing in Europe, United States, and West Africa, with preliminary findings on efficacy, and safety becoming available. These vaccine candidates are categorized as replication incompetent or non-replicative and replication competent vaccines [79]. Some of these vaccine candidates are summarized below.

12.1. Recombinant adenovirus based vaccines

Adenoviruses are generally non-enveloped, double-stranded DNA viruses isolated from mammalian species. Following the deletion of the E1 region in their genome which renders the virus non-replicative, this property makes them suitable as recombinant vectors [80, 81]. Several Ebola vaccines that have been developed make use of a variety of recombinant Adenovirus serotypes which includes the human serotypes such as Ad26 and Ad35 and the

chimpanzee Adenovirus serotypes; Ad3, Ad7 and Ad62 [82]. Recombinant Adenovirus 5 (rAd5) was the first recombinant Ebola vaccine to show protection to Non-human primates against the EBOV virus but required a period of over 6 months to attain complete immunization [83]. A double-blinded, placebo-controlled phase I clinical trial in 2010 showed rAd5 vaccine encoding the envelope GP from EBOV and SEBOV 1976 strain to be safe and immunogenic [84]. Following the 2014 West Africa outbreak, another phase 1 clinical trial was conducted with another rAd5 vaccine which encoded the envelope GP of EBOV 2014 strain. The findings showed that the vaccine was immunogenic and safe at high dose of immunization [85]. Studies with other recombinant adenovirus vaccines such as rAd26 and rAd35 have them to be immunogenic by stimulating T-cell responses of CD4+ and CD8+ as well as increase cytokine (TNF/IFN-γ) secretion. Recently, rAd26 vaccine expressing the full-length GP of EBOV is currently undergoing phase III trials [86].

ChAd3-EBOV defined as chimpanzee adenovirus serotype 3 encoding the monovalent Zaire strain of Ebola virus glycoprotein is a genetically modified non-replicative vaccine candidate produced by GlaxoSmithKline in collaboration with the National Institutes of Health, USA. In 2014, five phase 1 trials of ChAd3 conducted in Europe, North America, and Africa confirmed the vaccine to be immunogenic and safe [87]. As a result, Phase II and III trials were initiated in Sierra Leone, Liberia, and Guinea in 2015 [88, 89].

12.2. Recombinant Vesicular Stomatitis Virus

A recombinant Vesicular Stomatitis Virus (rVSV**)** was the first replicating Ebola virus vaccine developed in 2005. This vaccine was shown to provide 100% protection in non-human primates eliciting both humoral and cellular immune responses against lethal EBOV challenged animals [90]. Since then, eight human phase I trials of rVSV-EBOV vaccine has been conducted across Europe, North America and Africa. A phase III trial involving 7651 individuals to evaluate the efficacy of rVSV-ZEBOV showed a 100% vaccine efficacy after 6 days of vaccination [91]. These findings have shown that rVSV confers protection against Ebola between 6 and 21 days after vaccination [92].

Other potential vaccine candidates [35] which have initiated phase I clinical trials in 2015 include; EBOV GP Vaccine which is a recombinant nanoparticle vaccine using adjuvant Matrix-M. It is the first Ebola vaccine candidate based on the 2014 Guinea Ebola strain genetic sequence. DNA-EBOV is a multiagent filovirus DNA vaccine delivered into the body through intramuscular electroporation. Recombinant rabies EBOV is a chemically killed inactivated rabies virus virions containing EBOV glycoprotein.

Other forms of vaccine candidates include virus-like particle vaccines (VLPs). VLPs are produced by expressing certain viral proteins that mimics the conformation of natural Ebola virus in cells without any viral genetic material. EBOV VLPs have been produced by simultaneously expressing NP, GP, and VP40 proteins of EBOV in 293T cells. These particles when administered three times to NHPs in combination with Ribi adjuvant protected against EBOV [93, 94].

Following the recent Ebola outbreak in DRC, an experimental Ebola vaccine (rVSV-ZEBOV) developed by Merck, a German pharmaceutical company which is not yet licensed but was

effective during the catastrophic Ebola epidemic of 2014 has been approved by WHO for vaccination. According to Peter Salama, WHO's deputy director-general for emergency, preparedness and response, 8000 individuals are expected to be vaccinated, thus, about 8000 dose are required with 4000 doses already deployed to DRC [95].

13. Conclusion

Following the devastating 2014 West Africa Ebola outbreak, more concerted efforts have been put in place to improve on the management of the disease. More especially, three rapid diagnostic kits which includes ReEBOV, OraQuick and SD Q Line Ebola Zaire Ag RDTs have been approved by FDA and WHO on EUA status to overcome the urgency of prompt diagnosis during outbreaks and make routine screening cost-effective and available in the field as well as in local health settings. More so, several experimental drugs such as ZMapp, favipiravir, BCX4430, etc. and vaccines such as rAd5, rAd26, rAd35, ChAd3, rVSV, etc. are undergoing randomized clinical trials across the world with a few currently at phase III. Recently, an experimental rVSV-ZEBOV Ebola vaccine which was effective at the 2014 West Africa outbreak is been deployed for the present 2018 Ebola outbreak in DRC. Concerted effort is therefore needed from regularly bodies such as FDA, international organization such as WHO, UN, etc., pharmaceutical companies, as well as stakeholders to make available funds for research to improve on the existing experimental drugs and vaccine candidates as well as rapid diagnostic tools. Thus, it is our hope that most of these experimental drugs and vaccines will be available in future to help control the disease and better manage re-emerging Ebola infections or outbreaks across the world.

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Chapter 9

A Reemerging Lassa Virus: Aspects of Its Structure, Replication, Pathogenicity and Diagnosis

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Additional information is available at the end of the chapter

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Abstract

Lassa virus is a linear, bisegmented, single-stranded RNA virus, which belong to the *Arenaviridae* family that causes viral hemorrhagic fever transmitted by rats. The virus is endemic in West African countries, which may be due to its zoonotic nature. Lassa virus infection occurs through contact with the vector *Mastomys natalensis* or infected humans and can lead to wide symptoms from a mild infection to Lassa fever and to a severe fatal viral hemorrhagic fever, which include delayed cellular immunity resulting to fulminant viremia. The virus replicates through a strategy known as the Ambisense, where two RNA strands code for genes in both the sense and antisense direction that is rapid and demonstrate temporal control in replication. Different diagnostic tests for the virus are available, which range from viral culture to serological and molecular diagnostic tests. There is an urgent need to develop drugs and vaccines against the virus because the World Health Organization (WHO) has identified Lassa virus as one of the viruses that is likely to cause a future epidemic, although a research is ongoing to evaluate Lassa virus vaccine immunogenicity in the CBA/J-ML29 mouse model. This chapter gives an overview on the structure, replication cycle, pathogenesis, and diagnosis of the virus.

Keywords: Lassa virus, Lassa fever, replication, pathogenesis, diagnosis

1. Introduction

Lassa virus (LASV) is first described in the 1950s [1] but not identified until 1969 in Jos, Nigeria [2, 3]. The virus causes Lassa fever that is hemorrhagic in nature, which is severe and fatal. It affects 2–3 million people annually [4, 5] and has been known to be endemic in Benin Republic in 2014, Ghana in 2011, Guinea, Liberia, and Mali in 2009, Sierra Leone, and Nigeria [3, 4, 6], but probably exists in other West African countries as well [4].

It is a reemerging virus with a select agent, which requires Biosafety Level 4-equivalent containment [7]. It is endemic in West African countries including Sierra Leone, the Republic of Guinea, Nigeria, and Liberia, where cases of the infection is between 300,000 and 500,000 yearly resulting in 5000 deaths annually [4, 8, 9]. About 80% infected with the virus are asymptomatic and 1 in 5 infection results in severe disease, where the virus affects several organs such as the liver, spleen, and kidneys [10]. The virus is harbored by the multimammate rats of the genus *Mastomys* and transmitted to Mans through primary aerosols of the rat's urine, close contact with urine, feces, saliva, or ingestion of contaminated foods of the rat [11]. LASV is also spread through contaminated hospital equipment but interestingly, it cannot be contracted by humans to humans only via bodily fluids contacts [12]. Findings have reported the presence of the virus in seminal fluids up to 3 months after infection of the virus. Research to show that Lassa virus can be gotten via sexual intercourse has not been reported but there are speculations that LASV might possibly be used for bioterrorism, so it is now being studied at greater lengths [13, 14].

Due to the variability of the clinical course of the disease, detection of the disease in affected patients has been challenging. When presence of the virus is confirmed in a locality, quick isolation of infected patients, good infection prevention and control practices, and rigorous contact tracing can help halt epidemicity [15].

2. Aspect of Lassa virus structure

Lassa fever virus (LASV) is a member of the family Arenaviridae ("*Arena*" means "*Sand*" in Latin root) [16, 17]. The virus is single-stranded, bisegmented, ambisense RNA virus. Arenaviruses are classified as segmented negative-sense RNA (nsRNA) viruses and are phylogenetically closely related to other segmented nsRNA viruses belonging to the family *Bunyaviridae* and *Orthomyxoviridae* [9, 18]. The three virus families share similar characteristics of the intracellular replication cycle. It is round, oval, or pleomorphic, 110–130 nm in diameter, and has a protective envelop. LASV differ from 10 to 19 kilobases and contains two RNA species, which are the big and small units [11, 19]. The two genes at different point do not cross exist in every unit [20, 21]. The genetic material that encodes nucleoprotein is 1710 nucleotides in length and the protein has 569 amino acids and that of glycoprotein is 1473 nucleotides long. The viral agent has four lineages, which differ in strain by 27% in respect to their nucleotides and 15% in respect to amino acids [22]. In the virus, the 3′ half of the genetic material has a negative polarity and the 5′ half is of a positive polarity that makes few virus proteins to encode in virus-complementary subgenomic mRNA species, while other viral proteins are encoded in virus-sense subgenomic mRNA sequences. The replication strategy (Ambisense) of the virus is somewhat seldom among viruses and thus distributed among groups of the Arenaviridae [15, 18].

The virus envelope is gotten when new particles bud off through the plasma membrane of the host cell and it carries club-shaped surface projections that are about 10 nm long [4, 19]. Sandy-appearing granules that resemble ribosomes are found within the unstructured interior

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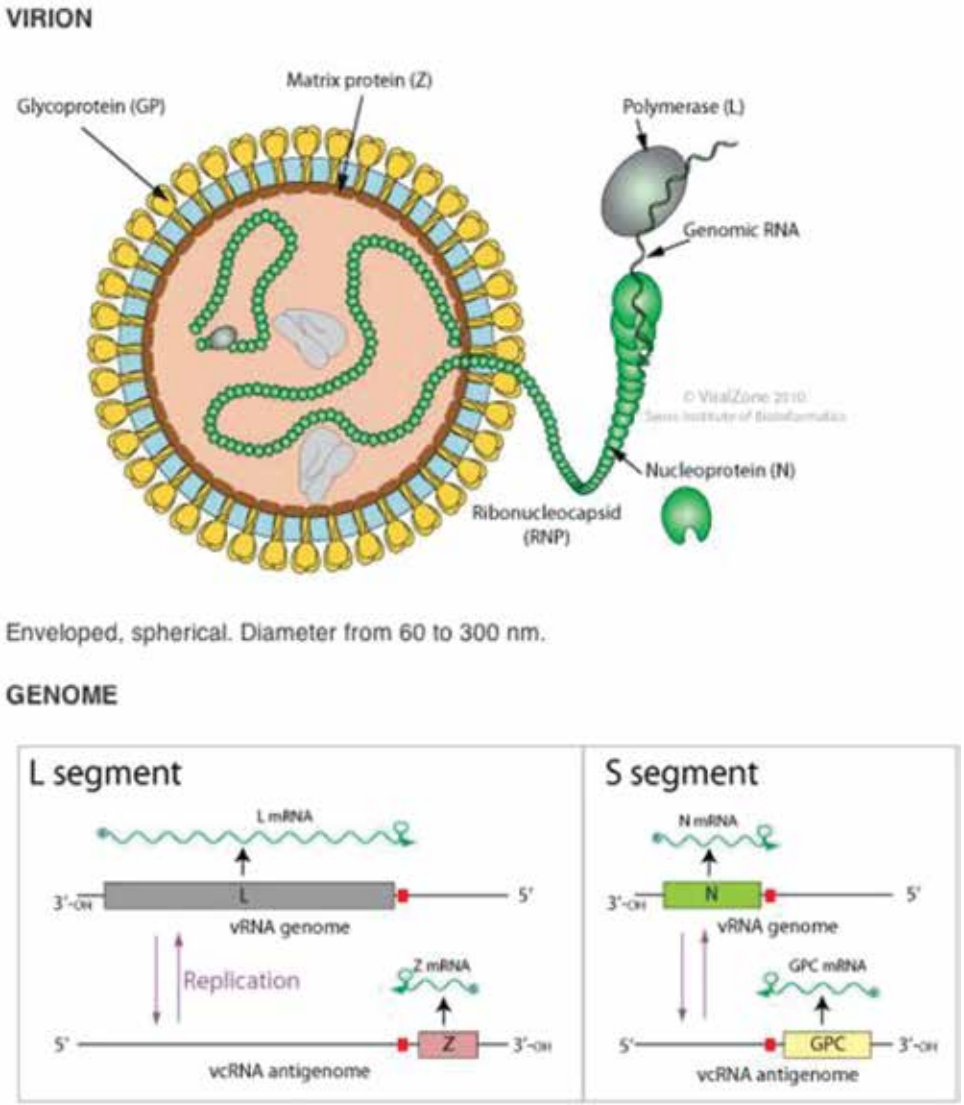

Figure 1. The Virion and genome having the L and S segments [15, 17, 21, 23].

of new viruses. However, these RNAs do not seem to have a required function in virus replication cycle and new work by the Viral Hemorrhagic Fever Consortium [23] sheds suspicion on whether these sandy granules are indeed host ribosomes (**Figure 1**).

3. How Lassa virus replicates

Lassa virus adopts a replication strategy known as "*Ambisense*," which is very rapid and demonstrates the replication of temporal control [24]. The initial stage of transcription of mRNA

copies of the negative sense gene, which makes sufficient deposition of virus proteins for the next stage of the replication process. Subsequently, L and NP proteins are translated from the mRNA. Copies of the viral complementary RNA (vcRNA) are made from the positive sense gene. Negative-sense progeny are produced by templates of RNA copies while mRNA is synthesized from it. The mRNAs produced from vcRNA are later translated to synthesize the Z and GP proteins. The temporal controls enhance the production of proteins spikes lastly, and therefore, delay the recognition by host immune system [25].

The virus enters into the host cell by means of the cell-surface receptor (alpha-dystroglycan alpha-DG), which is a versatile receptor for proteins of the extracellular matrix [26]. Receptor recognition is based on a specific sugar modification of alpha-DG by a group of glycosyltransferases known as the LARGE proteins. Specific variants of the genes encoding these proteins appear to be under positive selection in West Africa, where the virus is endemic [26]. Enveloped viruses makes use of clathrin-coated pits mostly to enter cells and bind to their receptors in a pH dependent way while Lymphocytic choriomeningitis and Lassa virus makes use of the endocytotic pathway independent of caveolin, clathrin, dynamin, and actin. The viruses are quickly delivered once within the cell to endosomes via vesicular trafficking albeit, which is extensively independent of the tiny GTPases Rab5 and Rab7. pH-dependent membrane binding happens on contact with the endosome, which is mediated by the enveloped glycoprotein, and at the acidic pH, the endosome fuses the lysosome protein LAMP1 that yields in membrane bind and endosome escape [19].

NP is the most dominant viral protein in virions and infected cells, which consists the main structural component of the viral ribonucleoprotein (RNP), which plays an important role in the RNA synthesis of the virus. Subsequently, the function required to assist replication of the virus, at least two viral proteins (NP and Z), have been proposed to modulate the cell response of the host to infection. Experimental data has showed that NP has a role in virus-induced inhibition of type I IFN signaling [27]. This role has been mapped to the C-terminal domain of NP with a folding that camouflages the DEDDH family of 3′-5′ exoribonucleases. The little RING finger protein Z is the arenavirus counterpart of the matrix (M) protein of other negative sense RNA viruses. The Z protein of lymphocytic choriomeningitis virus (LCMV) interacts with the promyelocytic leukemia (PML) protein as well as the eukaryotic translation initiation factor 4E (eIF4E) in infected cells and has been observed to function in the noncytopathic nature of LCMV infection and repression of cap-dependent translation [22, 28, 29]. Functional assays reported the exonuclease activity of LASV NP that has been proposed as important for its type I IFN counteracting functions [29].

The replication cycle of Lassa virus is similar to the Old World arenaviruses. It was reported that virus internalization is limited upon cholesterol depletion. Dystroglycan, which is later cleaved into alpha-dystroglycan and beta-dystroglycan is initially expressed in most cells to mature tissues, which provides molecular bridge between the actin-based cytoskeleton and extracellular matrix (ECM) [26, 30]. After the viral agent enters the cell by alpha-dystroglycan-mediated endocytosis, low-pH domain enhances pH-dependent membrane bind and releases ribonucleoprotein of the virus (RNP) complex into the cytoplasm. The enzymatic machinery for RNA synthesis in arenaviruses is housed within a single L polymerase protein. This 250–450 kDa

protein utilizes viral RNA templates that consist of genomic RNA encapsidated by the viral nucleocapsid protein NP and comprises viral ribonucleoprotein [21, 30]. L polymerase of arenaviruses contains the SDD motif feature of all RNA-dependent RNA polymerases (RdRp) [10]. When infected, the viral agent RNP is inserted into the cytoplasm of the host cell, the L polymerase associated with the viral RNP starts transcription from the genome promoter located at the 3′-end of each genomic RNA segment, L and S. The 5′ and 3′ terminal 19 nt viral promoter regions of both RNA segments required for the recognition and binding by the viral polymerase exhibit a high degree of conservation among the arenaviruses. The genome segments have a high complementary 5′- and 3′-ends (19 nt) have been predicted to form panhandle structures [10, 11]. Transcription stops at the distal side of the stem-loop (SL) structure within the intergenomic region (IGR). The L polymerase adopts a replicase mode and moves across the IGR to create a full-length complementary antigenomic RNA (agRNA) that serves as a template for mRNAs synthesis of viral genes encoded in genomic orientation, GPC and Z, from the S and L segments, respectively, and for the synthesis of full-length genomic RNA (gRNA) [13, 18]. This SL structure has been discovered to stabilize the 3′-termini of the viral mRNAs [10]. The primary transcription leads in the mRNA synthesis of viral genes, which is encoded in antigenomic orientation, NP and L polymerase, from the S and L segments, respectively. The viral agent uses a cap snatching strategy to acquire the cap structures of cellular mRNAs. Cap snatching is facilitated by the endonuclease activity of the L polymerase that is co-factored by the cap binding activity of NP. Therefore, LASV synthesizes capped nonpolyadenylated mRNAs. Both gRNA and agRNA of the viral agent contain a nontemplate G residue at their 5′-ends. The proposed "prime and realign" mechanism includes the production of a $pppG_PC_{OH}$ dinucleotide primer from the CG nucleotides at positions +2 and +3 of the 3′-end genome promoter sequence, that is, then realigned such that its 3′-terminal C_{OH} is opposite the genome 3′-terminal G residue, and the realigned $pppG_PC_{OH}$ then acts as a primer for a complementary RNA strand production. The matrix protein Z is not part of the viral genome transcription and replication but shows a dose-dependent inhibitory effect on viral RNA production. This inhibitory effect of Z has been proposed for Old and New World arenaviruses [27].

4. An aspect of Lassa virus pathogenicity

The Lassa virus is well-known to cause Lassa fever [31]. Its symptoms include flu-like illness characterized by fever, general body weakness, cough, tonsillitis, headache, and gastrointestinal disorders. Hemorrhagic manifestations are other features of Lassa fever, which include vascular permeability [10].

The virus pathogenesis is still unclear, but it has been shown that the virus chiefly target the antigen-presenting cells (mainly dendritic cells) and endothelial cells [32]. Lassa virus infects most tissues in the human body when gained entry. It starts with the mucosa, intestine, lungs, and urinary system, and then moves to the vascular system. There are findings that the viral agent can prevent a host's innate immune system by NP activity [33]. Usually, when a microbe penetrates a host, the innate defense system detects the pathogen-associated molecular patterns (PAMPs) and aggravates the response of the immune system. One of the

mechanisms identifies double-stranded RNA that is only produced by negative-sense viral agents [34]. In the cytoplasm, dsRNA receptors, such as melanoma differentiation-associated gene 5 (MDA-5) and retinoic acid-inducible gene I (RIG-I), detects dsRNAs and facilitates signaling pathways that results in the translocation of interferon regulatory factor 3 (IRF-3) and other transcription factors to the nuclear material [9]. Translocated transcription factors enhance expression of interferons **α** and **β**, and secreted interferons facilitate antiviral responses including adaptive immunity. NP encoded in the viral agent is important in the replication and transcription of the virus, but it also stops host innate IFN response by inhibiting translocation of IRF-3. NP of the virus is reported to have an exonuclease activity to only dsRNAs [12]. Double-stranded RNA exonuclease activity of the NP leads to counteract IFN responses by digesting the PAMP that leads to the evasion of host immune responses.

The recent understanding of the pathogenesis of the viral fever does not involve the chain of functions that take place during development of the disease state and leads to mortality of severed ill patients [35]. The high death and truly dramatic course of the disease state, the pathological findings do not give the bench that would explain the mechanism of disease progression and the cause of mortality by the viral agent [5, 8]. Development of the cellular immune response failure, which would control dissemination of LASV is indicated by high serum titers of the virus, together with dispersed replication in tissues and lack of neutralizing antibodies that could lead to the fatal Lassa fever development [6, 36]. Patients check physically after fever onset usually depicts facial oedema, bilateral conjunctival hemorrhages, purulent pharyngitis, and abdominal disorders [5]. Pathological changes physically may include pulmonary oedema, ascites, pleural effusions, and hemorrhagic signs in the gastrointestinal mucosa while examination under the microscope reveals splenic necrosis, hepatocellular necrosis, adrenocortical necrosis and apoptosis, mild mononuclear interstitial myocarditis without myocardial fiber necrosis, alveolar oedema with capillary blockage and mild interstitial pneumonitis, lymph nodal sinus histiocytosis with mitoses, gastrointestinal mucosal petechiae, renal tubular injury, lymph nodal sinus histiocytosis with mitoses, and interstitial nephri. More often, lesions of Lassa fever in man happen in the hepatic cells [5, 8]. There are four major characteristic hepatitis of LASV, which is derived:

i. Focal cytoplasmic degeneration of hepatocytes related to phagocytosed apoptotic fragments.

ii. Distribution of multifocal hepatocellular necrosis randomly.

iii. Monocytic reaction to necrotic hepatocytes.

iv. Hepatocellular mitoses.

The physical impacts do not happen uniformly in all cases, rather in some instances can be observed simultaneously.

The virus fever is not associated with coagulation dysfunction, for example, decrease in the coagulation factors and disseminated intravascular coagulation (DIC) have been revealed in infected subjects. More so, moderate thrombocytopenia with importantly damaged functionality of thrombocytes is reported in severe Lassa fever subjects [7, 36]. One significant mechanism involved in the pathogenesis of Lassa fever is infection-triggered induction of uncontrolled cytokine expression, which looks like what is seen in sepsis [9]. In this subject

that died from hemorrhagic shock and multi-organ failure, the proinflammatory cytokines, tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ) rises to extremely high level just before death. In a related study, no increase of both cytokine levels was reported in the checked fatal cases of the virus fever, and it is suggestive that the levels of IFN-γ and TNF-α are either elevated only in a fraction of patients or during a limited period that would involve frequent sampling for assay [12, 34].

Virus-induced immunosuppression may be involved in a severe Lassa fever pathogenesis where the LASV infection fails to trigger macrophages (MP) and monocyte-derived dendritic cells (DC) of human. Human-infected DC with the naturally nonpathogenic mopeia virus, induces stronger CD4 and CD8 T-cell responses when compared with those infected with LASV [5, 8]. Infected DC fail to secrete proinflammatory cytokines, do not upregulate costimulatory molecules, such as CD40, CD80, and CD86, and poorly induce proliferation of T cells. Downregulation of immune responses due to infection by LASV has been depicted in vitro, and it is also in consonance with findings of clinical reports demonstrating that the virus fever fatal outcome relates with low levels interleukin (IL) 8 and IFN inducible protein 10 (IP-10) in the system [14].

5. Aspect of Lassa virus diagnosis

Different diagnostic tests in the laboratory are carried out in order to check the presence of an infection and assess its course and complications. The unavailability of lab tests can compromise diagnosis confidence. The most disturbing factor is the presence of febrile illnesses in Africa that mimics the Lassa fever, such as typhoid fever especially for manifestations of nonspecific Lassa fever [31]. In illness with abdominal pain, in countries where the virus is epidemic, Lassa fever may be misdiagnosed as intussusception and appendicitis that leads to delay in treatments with the antiviral drug (ribavirin) [37]. In West Africa, where the virus is most prevalent, it is difficult for laboratory scientists to diagnose due to the absence of the right equipment to perform the tests [4].

The Federal Drug Administration (FDA) has not approved any widely validated laboratory test for the virus, but there are diagnostic tests, which have been able to provide definitive evidence of the presence of the virus [4, 14]. These tests include viral cultures, polymerase chain reaction (PCR) where the virus can be uncovered using reverse transcription PCR after first reverse transcribing the RNA of the virus into DNA, Enzyme Linked Immunosorbent Assay (ELISA) test, immunofluorescence test, and plaque neutralization. But, immunofluorescence tests give less definitive presence evidence of the viral presence. Other laboratory reports in the virus fever include thrombocytopenia, lymphopenia, and elevated aspartate aminotransferase levels in the blood. The viral agent can occasionally be present in cerebrospinal fluid [38, 39].

The following diagnostic methods are briefly discussed below.

5.1. Viral culturing

Viral isolation in cell culture remains the "gold standard" for the diagnosis of Lassa fever given the challenges in diagnosing the virus due to mutations [38], although RT-PCR and

immunoassays are commonly used assays for a clinically actionable diagnosis in recent time [7, 40]. Viral culturing is carried out by the inoculation of suspected samples containing the virus in Vero E6 cells in incubator at 37°C. A positive result may yield a cell cytopathic effect (CPE) [41], while, a second method of detection, such as viral antigen detection, RT-PCR, or electron microscopy, should be used as confirmatory for the presence of the virus. There is always viremia presence at the time of presentation to medical care and reduces after 6 days of illness in subjects, who survived the infection and there may be persistence presence until death in fatal cases [42]. The viral agent can also be cultured from throat swabs, blood, urine, and cerebrospinal fluid specimens from subjects [4]. The detection of virus in urine and throat swabs can be inconsistent in subjects with serum viremia. Positivity of the viral culture may exists in organ specimens such as spleen, liver, lung, heart, kidney, and placenta at post mortem in fatal infections cases [4].

Culturing of viruses allows for the identification, which is genetically independent of variabilities between types and further typing of the viral agent, if desired is achieved [38]. This technique also guarantee the quantification of viremia that might provide further viral typing data, as viremia with 10^3 50% tissue culture infective doses ($TCID_{50}$)/ml has a fatality odds ratio of 3.7 as regards to viremia with less than 10^3 $TCID_{50}$/ml. The method is neither fast, taking at least several days to yield results, and it is not widely available due to the need for BSL-4 precautions to handle live viral samples that limits its utility for the early diagnosis of the viral infection [31].

5.2. Rapid immunogenic tests

Rapid immunogenic tests are attractive alternatives to the technical requirements and high specificity of the PCR methods especially in LASV endemic areas [14, 37]. The antibody/antigen binding is usually less specific than primer/probe hybridization, leaving for greater flexibility in identifying diverse viral agents. Detection of antigens relies on specific antibodies usage against Lassa virus components to detect viral antigens in blood samples [14]. Nonspecific Lassa virus antigens with polyclonal antibodies are detected with initial assays, whereas more current ELISAs target the Lassa virus nucleoprotein antigen [31, 38]. A diagnosis based on the detection of the relatively conserved Lassa nucleoprotein antigen could reduce the differences in test efficacy between genetically diverse virus types and comparison to DNA-based techniques [43]. Antigenemia-increased levels have been identified in fatal cases of the fever when compared with nonfatal cases. The short time of antigenemia enhances the detection of the virus antigen more specific to acute infection with the virus when compared to detection with antibody tests. The antigen of the virus nucleoprotein can be detected in subjects with the virus in the first week of illness and wanes during the second week in temporal association with the increase in detectable immunoglobulins [42]. Antigen detection tests could identify Lassa fever earlier during illness than antibody tests, as antibodies often may not be detectable until the second week of illness [43, 44]. The virus antigen levels might become undetectable despite viremia persistence. It should be noted that, negative antigen test during an acute illness does not mean that the patient is free from the fever [44].

A lot of IgM and antigen capture ELISA methods have been invented with inactivated viral agent; but the protocol is restricted to BSL-4 capable machine [41]. Recombinant antigens

usage allows improved access and assay development. A lateral passage test for the virus nucleoprotein (ReLASV) is another type of diagnostic test, which may be used for point-of-care diagnosis [45]. Following initial development and testing efforts, this test received the CE mark in 2013, although approval by the US FDA has not been granted for the test. According to the product insert, the assay generates results in 15–25 minutes and has 85 and 99% sensitivity and specificity, respectively using confirmed Lassa virus-positive blood specimens [4].

In real sense, a diagnostic test would not only identify Lassa virus infection but would also check for other pathogens with similar clinical symptoms endemic in West Africa at the same time [39]. A transitioning Lassa and Ebola virus antigen- and IgM-based ELISAs onto a MAGPIX system has been described that uses individually labeled magnetic beads to identify multiple targets in a single test. This test has lower limits of detection for Lassa virus nucleoprotein and IgM than conventional ELISAs. Further development of multiplex MAGPIX assays, including testing for Lassa virus antigen and common endemic infections such as malarial infection, will assist with the diagnosis and clinical management of suspected cases of Lassa fever, especially in cases of coinfection with other pathogens where multiple therapeutic modalities may be indicated [38]. Patients on medical care dalliance following onset of the disease could affect negatively on the virus identification in a nucleoprotein detection test, and detection by viral-specific IgM might be more appropriate and reliable [37, 42]. IgG of the virus levels may increase later than IgM levels, with a mean time to detection of 25.6 days after symptom onset, although positive IgG titers have occasionally been detected presence in subjects with acute fever within the first few days of sickness onset [38]. IgM of the virus usually becomes noticeable and detectable in the second week of infections onset, although it could be detectable within 4 days of onset of illness in some subjects. Lack of an antibody response has been found in some fatal cases of Lassa fever.

5.3. Polymerase chain reaction (PCR) methods

Real-time RT-PCR is a commonly used diagnostic technique for infectious agents due to the high specificity and sensitivity and has become a gold standard clinically for Lassa fever identification [37, 46, 47]. Automated coupled specimen processing and 96-well plate thermocyclers, large samples can be evaluated fast and cheaply. The methods could detect viral agent for early illness and a longer time when compared to culturing of virus and might be carried out on samples that are inactivated chemically [4, 48]. Cycle threshold figures usage with quantitative rt-PCR can help with estimates of viremia using the right positive-control equipment for generation of standard curve [49]. Based on the virus strain and primers used, the 95% probability limit of detection estimates with RT-PCR vary from 1237 to 4290 RNA copies/ml [49]. With highly diverse viral agent such as Lassa virus, molecular diversity can be a problem for such assay, as even a single nucleotide variant in one of the primers can have a significant negative effect on the sensitivity of the assay depending on the location of the nucleotide variant [50–52].

The availability of additional sample testing and sequencing data has made mismatches identified using established assays, necessitating assay redesign to enhance performance. Multiplex panels to simultaneously detect a multitude of viruses that could produce hemorrhagic fever syndromes, including Lassa and Ebola viruses, using RT-PCR alone or in synergy with either enzyme hybridization or ligase detection reactions have also been produced [53, 54].

6. Conclusion

The Lassa virus is one of several viruses that are likely to cause a future epidemic as reported by World Health Organization [4, 43, 44]. The appropriate diagnosis of Lassa fever will likely involve a combination of a clinically compatible observation along with serological and molecular diagnostic assays [5]. There is a need for urgent research and development of new diagnostic tests, vaccines, and drugs [7]. Future directions of research for diagnostics in the viral infection are improvement of assays to enhance detection of different viral strains genetically, validation of assays to depict variations in viral lineages and regions, creation of point-of-care detection and field validation, and expansion of multiplex assays content to differentiate the virus fever from other fevers with alike clinical features. Research on the virus vaccine immunogenicity in the CBA/J-ML29 mouse subjects is ongoing. Until now, no licensed vaccine for humans against the virus exists [45].

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Tropical emerging diseases pose a significant risk for the circulation of old and new pathogens in areas previously unknown, also implying the possibility of new morbidities and mortalities and new consequences for naïve populations. Globalization, migration and travel are key factors for tropical diseases, and represent the need for integration of tropical medicine, travel medicine and epidemiology in the understanding of such complex situations. Neglected tropical diseases such as leprosy or Chagas disease, arboviral diseases, HIV, Ebola, and arenaviral infections are just a few examples. This book tries to update significant epidemiological and clinical research in many aspects with a multinational perspective.

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