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Preface

Leishmaniasis is an ancient disease, and indeed some lesions suggesting Old World cutaneous leishmaniasis were already described in tablets of the library of King Ashurbanipal from seventh century BC, while others were probably derived from earlier texts dating 1500 to 2500 BC.

Leishmaniasis is a worldwide, high-burden vector-borne disease with diverse clinical manifestations caused by protozoa belonging to the *Leishmania* genus. Different species of leishmaniasis have re-emerged in recent years with increasing global prevalence over a wide geographic range. A number of factors, including environmental, demographic and human behaviour, have contributed to the changing epidemiology of the disease and to its recent spread throughout the world.

The aim of this book is to update the readers interested in the different aspects of this ancient disease, included among the neglected tropical diseases by the World Health Organization (WHO) in 2010. Three years later, the 66th WHO General Assembly approved a resolution that represented an important milestone for the prevention, control, elimination and eradication of the disease.

In this book, various aspects are considered such as taxonomy of the different *Leishmania* species, vector biology, host immune response, immunopathological processes led by the parasite, diagnosis, clinical picture in both immunocompetent and immunocompromised patients, treatment of tegumentary and visceral forms and, finally, control perspectives.

Several eminent scientists in the field of parasitology have collaborated in the volume by providing an overview of topical issues on the different aspects of leishmaniasis.

In Chap. 1 Gradoni introduces the epidemiology of leishmaniasis, in order to help the reader to better understand the analyses of the more specialised chapters that will follow.

The chapter by Maurício is devoted to the rather complex taxonomy of the *Leishmania* genus, which includes at least 39 species that may differ highly not only in the ability to infect vertebrate hosts and vectors but also in the resulting clinical picture in humans. According to the author, as a result of recent molecular data and phylogenetic analyses, a simplification of *Leishmania* taxonomy could be possible by reducing the number of human-pathogenic species to six: *L. donovani*, *L. major*, *L. tropica* and *L. mexicana* within the sub-genus *L.* (*Leishmania*) and *L. braziliensis* and *L. guyanensis* within *L.* (*Viannia*). A consensus on *Leishmania* taxonomy

should be reached among different scientists, especially clinicians and researchers, so as to avoid confusion and facilitate ease of interpretation.

In the chapter on vectors, Dvorak, Shaw and Volf describe finely the species of sandflies (Diptera: Psychodidae, Phlebotominae) and focus on their geographical distribution in both the Old and New World. Particular attention is given to the various mechanisms adopted by the parasite in order to survive in the vector's gut, and in the immunomodulatory molecules of sandfly saliva, which play a crucial role for successful transmission and infection in the vertebrate host. Both the classical phlebotomine vectors and the non-phlebotomines such as midges (recently suspected to transmit infection in some geographical regions like Australia, Martinica or Thailand) may fulfil a significant role.

In the chapter on the reservoirs of the parasite, Maia, Dantas-Torres and Campino consider different aspects of zoonotic and anthroponotic transmission, focusing in particular on the large number of host species that may be responsible for zoonotic transmission in the different geographical areas. Future research should be concentrated on food sources, breeding season, movement and migration activities and longevity of the potential reservoir host(s).

The immune response against *Leishmania* is a multifactorial process comprehensive of several components having different roles in the transmission chain since the bite of the sandfly in its very early phase of the infection. The molecules present in the vector's saliva trigger the initial inflammatory response up to the onset of the disease, where immunopathological phenomena such as autoimmune reactions occur.

Lauthier and Korenaga describe the most important mechanisms of the host immune response to the parasite, which differ between cutaneous, mucocutaneous and visceral forms of leishmaniasis. A first line of intervention is represented by neutrophils, which can kill the parasite by means of different mechanisms including NETosis (death of the cell by the release of nuclear extracellular traps). Particular focus is placed on the regulation of immune response, involving both CD4+ and CD8+Th1 cells. On the other hand, Th2 polarisation is responsible for the exacerbation of the disease.

In Chap. 6 Rojelio considers the clinical aspects of leishmaniasis, which encompass a spectrum of signs and symptoms, from nodular or ulcerative lesions occurring in cutaneous leishmaniasis (in some cases evolving to mucocutaneous leishmaniasis) to disseminated syndromes known as diffuse cutaneous leishmaniasis to visceral leishmaniasis. These different evolutions depend mostly on the infecting *Leishmania* species but also on the immunological status of the host.

A diagnosis of leishmaniasis is arduous, as it reflects the complexity of the disease. However, Gramiccia and Di Muccio have been able to elucidate the most important laboratory tools that make the parasitological diagnosis essential, in order to correctly identify the causes of such an extremely multifaceted clinical picture. In addition to the classic microscopical, immunoparasitological and more advanced molecular analyses, the *in vitro* study of cellular immunity is promising, not only in visceral but also in cutaneous leishmaniasis. However, further studies in different

geographical regions are needed to achieve more accurate results and to confirm these data.

Begoña Monge-Maillo and Rogelio López-Vélez discuss the most recent acquisitions concerning the treatment of visceral leishmaniasis, by differentiating the different choices, which depend on the geographical region of the infection and on the immunological status of the host. In consideration of the development of resistance to the traditional antimonials, amphotericin B deoxycholate or lipid formulations of amphotericin B are indicated as first-line treatment for their efficacy and lower toxicity in different world areas, in particular in the Mediterranean region. However, in low-income countries, the possibility of using parenteral paromomycin should be encouraged. The promising orally administered miltefosine drug, used mostly in India, Pakistan and Bangladesh, has shown very good cure rates, but clinical failures are still relevant and probably depend on the increased development of parasite resistance.

Blum, Neumayr and Lockwood have reviewed the criteria for selecting a reliable method of treatment for tegumentary leishmaniasis, mainly determined by the infecting *Leishmania* species. Treatment options include systemic treatment with antileishmanial drugs, local topical treatment with antileishmanial ointments/creams, local intralesional injection of antileishmanial drugs and local physical treatment (cryotherapy, thermotherapy). The choice of systemic or local treatment depends on the species, size, number and location of the lesions but also changes in reason of possible comorbidities. Treatment suggestions for cutaneous leishmaniasis are presented for each species. The treatment of mucosal leishmaniasis and leishmaniasis in pregnant women, in children and in patients with immunosuppression is discussed separately.

According to Boelaert, Burza and Romero, the control of leishmaniasis depends on a limited number of key control strategies, the most important of which are early diagnosis and case management, and limit of transmission of the disease, at least in its anthroponotic form. However, sandfly control measures (insecticide spraying, insecticide-treated materials, environmental management and personal protection) are also useful to reduce transmission, especially when the procedures are conducted near human dwellings or are well addressed to vector exposure. In case of zoonotic transmission of the disease, reservoir hosts need to be controlled. Unfortunately, no human vaccine is yet available and those commercialised against canine leishmaniasis have not shown good efficacy in reducing the transmission to humans.

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1.1 Introduction

The leishmaniases are a group of human diseases caused by kinetoplastid protozoa of the genus *Leishmania*. Despite having a long history, dating back from more than 4500 years according to ancient descriptions (Akhoundi et al. 2016), leishmaniasis still ranks in the top three of the neglected tropical diseases caused by protozoa (Fenwick 2012). Why is that? Certainly not because of a negligible disease burden. A total of 98 countries and three "territories"—a United Nations definition for lands that do not possess full political sovereignty—in tropical, subtropical and temperate areas have reported endemic transmission (Alvar et al. 2012). With 350 million people considered at risk, some 1.3 million new cases of leishmaniasis are estimated to occur every year that means the ninth largest disease burden among infectious diseases and the second and fourth most common cause of death and disease, respectively, among tropical infections (Bern et al. 2008). Over the past 20 years, significant progress has been made for the improvement of diagnosis and treatment of the leishmaniases; these developments should have facilitated the implementation of sustainable national and regional control programs. However, we are a long way from it, and mortality and morbidity from leishmaniasis still show a worrying increasing trend worldwide.

There is not a single explanation of why the leishmaniases are largely ignored globally. Apart from the fact that they affect "the poorest of the poor" in most of their wide geographical range—a common denominator of neglected tropical diseases—the most probable reason for neglecting leishmaniasis, in the author's view, is because of its epidemiological and medical complexity. Crucial factors include (a) the wide genetic diversity of *Leishmania* parasites; (b) complex interactions

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among vertebrate and invertebrate natural hosts and their environment; (c) marked regional differences in clinical features; (d) and widely varying human-associated risk factors.

1.2 Epidemiological Complexity and Neglect of Leishmaniasis

At least 20 recognized *Leishmania* species are pathogenic to humans (Akhoundi et al. 2017), most of which can also be transmitted to and cause established infections in mammal species from at least eight orders. The increasing use of diagnostic molecular tools has considerably extended the list of mammals found positive for leishmanial genetic material, thereby making harder a clear distinction between dead-end and genuine reservoir hosts—the latter being mammal species capable of ensuring long-term maintenance of a *Leishmania* population and its taking up by a vector (Quinnell and Courtenay 2009).

All *Leishmania* members pathogenic to humans are transmitted by the bite of phlebotomine sand flies. Among over 900 species of sand fly recorded, about 100 are proven or suspected vectors of human leishmaniases; these include *Phlebotomus* species in the Old World and *Lutzomyia* species (*sensu* Young and Duncan 1994) in the New World (Maroli et al. 2013). According to the taxonomical revision proposed by Galati (2015), phlebotomines potentially implicated in Neotropical *Leishmania* transmission belong to 13 genera at least. The list of sand fly species found to harbour leishmanial DNA in nature has also increased considerably in the past few years. Assessing vectorial importance of a sand fly species in a specific endemic scenario can be a complex task as several biological, behavioural and ecological variables need to be considered (Ready 2013).

Clinical manifestations of human leishmaniasis are also largely diverse, although two main clinical forms are prevalent worldwide: visceral leishmaniasis (VL), a life-threatening condition that results from the dissemination of *Leishmania* in macrophage-rich tissues, and cutaneous leishmaniases (CL), a benign but often disfiguring skin condition which has a tendency towards spontaneous resolution. Other acute or chronic clinical forms may be less common or simply get unrecognized. They consist of primary conditions or sequelae of the main clinical forms and include localized leishmanial lymphadenopathy, localized mucosal leishmaniasis (such as laryngeal or lingual leishmaniasis), mucocutaneous leishmaniasis (MCL), diffuse and disseminated CL and post-kala-azar dermal leishmaniasis (PKDL). Asymptomatic human infections are increasingly recorded by means of modern diagnostics; however, their epidemiological role in different endemic settings has not been elucidated (Michel et al. 2011; Hirve et al. 2016).

As a consequence of such medical complexity, universal tools and guidelines for leishmaniasis case management—from clinical suspicion to post-therapy follow-up—are lacking or incomplete, and where available, they can only be applicable to regional contexts (see, for example, Gradoni et al. 2017). Case reporting to current surveillance systems, therefore, is inadequate so that accurate information on

leishmaniasis extent and distribution is incomplete or missing. Hence, leishmaniasis is not recognized and prioritized at the public health policy level, and its visibility is eventually not proportionate to its true burden.

Similarly, the complexity of eco-epidemiological interactions among reservoir hosts, phlebotomine vectors and their environment makes it difficult to implement national programs addressed to *Leishmania* transmission control. A schematic approach aimed at targeting epidemiological cycles with appropriate control measures was proposed by the World Health Organization (WHO) in 1990 and reiterated in 2010 (WHO 1990, 2010). Each pathogenic *Leishmania* agent associated with specific vectors and reservoir hosts (if known) and with predominant clinical manifestations in humans was mapped geographically and grouped into the so-called "nosogeographical entities." Eleven entities were listed in 1990, which increased to 15 in 2010. Some of them are labelled as "anthroponotic," thus providing an informative epidemiological tag within the entity definition, for example, "anthroponotic VL (AVL) caused by *L. donovani* in the Indian subcontinent" or "anthroponotic CL (ACL) caused by *L. tropica* in Old World countries." However the latter entity had to be limited to "densely populated settlements," since a zoonotic cycle of *L. tropica* was later demonstrated in rural/peri-urban settings of Middle East. Some entities are traditionally labelled as "zoonotic," such as "zoonotic CL (ZCL) caused by *L. major* in the Old World" or "zoonotic VL (ZVL) caused by *L. infantum* in the Old and New Worlds"; others are not, despite having an obvious zoonotic nature such as all the entities of tegumentary leishmaniasis in Latin America. Two or more nosogeographical entities can be endemic in one country (e.g. four entities are found in parts of the Maghreb area, North Africa), thus making the implementation of national control programs even more complex.

1.3 Determinants of Leishmaniasis Epidemiology

In 2010, the members of the WHO Expert Committee on the Control of Leishmaniases attempted to provide a consolidated view of the epidemiological determinants impacting on patterns and trends of human leishmaniasis worldwide (WHO 2010). A systematic list of such determinants is difficult to make, as several of them are deeply interlinked, for example, "poverty-migration-state of immunity." This section makes large use of the epidemiological definitions reported in the above WHO document.

First of all, it is important to note that unlike other neglected vector-borne diseases (e.g. American trypanosomiasis), leishmaniasis transmission occurs almost exclusively via vector. Even though congenital, blood transfusion or mechanical modes of transmission (e.g. syringe) have been reported, they are definitely rare events. Intrinsic tropism and virulence of *Leishmania* species, ecological characteristics of the transmission sites and widely varying human-associated risk factors (Desjeux 2001) are the main epidemiological determinants.

1.3.1 Age, Sex and Acquired Immunity

With some exceptions, parasites of the *L. donovani*/*L. infantum* complex exhibit predominant tropism to internal tissues and cause VL, whereas all other *Leishmania* species are predominantly dermotropic and cause tegumentary clinical manifestations in humans. In VL, the susceptibility of naive individuals varies greatly as not everyone exposed to transmission develops a typical fatal disease. In endemic areas, asymptomatic or subclinical cases outnumber far in excess clinical cases. The age range affected by clinical VL depends on the parasite species and the history of population exposure. Where the causative parasite is *L. infantum* and the disease incidence is elevated, VL patients tend to be younger (the most affected age group being 0–5 years) than in endemic foci of *L. donovani* in various sites from Asia and Africa (median age, 13–23 years).

By contrast, it seems that naive individuals are universally susceptible to clinical disease caused by the commonest agents of CL. The affected age range depends on the intensity of transmission ("force of infection") to which populations are exposed (Lysenko and Beljaev 1987).

The main entities of leishmaniasis are reported to affect more males than females. This may have different explanations, such as higher exposure of males to sand fly bites because of work or social activities; gender disparity in access to health care, especially in less developed countries; and sex-linked biological factors associated with natural immune responses to parasites. At least for VL, the latter could be the most probable, as a similar M:F ratio of about 1.5:1 was reported by active-case detection studies performed in quite different endemic settings of VL and social contexts, namely, two southern European countries endemic for *L. infantum* and a group of villages in Bihar state, India, endemic for *L. donovani* (Gradoni et al. 1996; Arce et al. 2013; Siddiqui et al. 2016).

In populations exposed to high levels of transmission over long periods, a large proportion of adults will have acquired immunity. In VL foci, this is indicated by the prevalence of positive leishmanian skin-test results, which typically rises with increasing age. Immunosuppressive conditions, either due to comorbidities (e.g. HIV infection) or therapies (e.g. organ transplantation or treatment of immunological disorders) may result in increased clinical susceptibility to primary infections or in the reactivation of latent infections.

In CL foci, the presence of old scars in exposed individuals is usually associated with the protection from newly acquired lesions. Actually, the deliberate syringe infection with *L. major* in covered parts of the body ("leishmanization") has been used for years as a vaccination against infections causing potential disfiguring lesions, and still it represents the gold standard for the development of modern *Leishmania* vaccines (Khamesipour et al. 2005). In general, acquired immunosuppressive conditions have a lower clinical impact in tegumentary forms of leishmaniases.

1.3.2 Human Behaviour

In AVL foci of Bangladesh, India and Nepal, the disease occurs in agricultural villages where houses are frequently built with mud walls and earthen floors. Cattle is kept close to human dwellings, and the sand fly vector *P. argentipes* feeds on both bovines and humans; consequently, sleeping outside or on the ground increases VL risk (Ranjan et al. 2005).

ZVL foci in the Mediterranean littoral and in Latin America consist of rural areas, villages and urban districts with vegetation, where many people have dogs, the domestic reservoir host for *L. infantum*. In both endemic scenarios, the location of other domestic animals close to the main habitation can be an additional risk factor because they attract sand flies and permit their resting. Transmission takes place micro-focally due to the opportunistic behaviour of *Phlebotomus* (*Larroussius*) species and *Lutzomyia longipalpis* in the respective endemic settings (Velo et al. 2017; de Araújo et al. 2013).

In Old World ZCL (*L. major*), the risk for infection is increased when agricultural projects are launched and irrigation systems are extended into arid or semi-desert areas. Usually, these man-made ecological changes are accompanied by the intrusion of large numbers of non-immune immigrants into an existing sylvatic cycle sustained by rodents and the vector *P. papatasi*. Transmission to humans is favoured by the practice of sleeping outside during the hot season.

Geographical range and risk factors for New World tegumentary diseases have expanded during the last decades. Traditionally, leishmaniases were predominantly occupational diseases related to specific activities such as rubber tapping, military operations, road construction and new agricultural development in the forest and other enzootic areas. Recent massive deforestation has led to a rapid increase in CL cases due to peridomestic, peri-urban and even urban transmission. Skin lesions caused by different *Leishmania* species are increasingly recorded in ecotourists who spend their holidays in nature reserves without appropriate protections against sand fly bites.

1.3.3 Poverty, Malnutrition and Migration

Poverty increases the risk for leishmaniasis by many mechanisms and can potentiate morbidity and mortality of the disease (Alvar et al. 2006). In endemic areas, poor housing and bad peridomestic sanitary conditions (cattle manure, garbage collection or open sewerage) are ecological factors favouring sand fly breeding and resting, resulting in high vector densities and easier access to human blood. Small rooms crowded with a large number of people may attract anthropophilic sand flies by providing a large biomass for blood meals and facilitate inter-human transmission in anthroponotic entities of leishmaniasis. Sylvatic cycles may approach poor dwellings at the periphery of new settlements in deforested areas or irrigated arid zones.

Poverty is often accompanied by poor nutritional status, including deficiency in protein energy, iron, vitamin A and zinc, which was shown to increase the likelihood that VL infections rapidly progress to fatal disease in animal models (Anstead et al. 2001). Protein-energy malnutrition has also been associated with an increased risk for MCL. Aggravation of clinical leishmaniasis is not only a consequence of immunological deficits and concurrent infections frequently associated with poverty but also of the lack of resources to pay for care or have access to remote health-care facilities.

Poverty-driven migration is a main cause of epidemics of both VL and CL worldwide. In East Africa, AVL outbreaks associated with repeated seasonal labour movements have occurred in Ethiopia, in a territory where the disease was previously absent or reported sporadically (Herrero et al. 2009). Drought and poverty have forced people to move from Darfur into VL-endemic areas of the Gedaref State in Sudan, resulting in a high number of fatal cases. Agricultural development and construction of infrastructures in North Africa and the Arabian peninsula have caused ZCL epidemics among non-immune young migrants. In the Andean countries, the massive migration of non-immune populations from the highland to the Amazonian basin resulted in one-fourth of the population with CL lesions within a few years (Desjeux 2001).

1.3.4 Military Operations and Population Displacement

Thousands of ZCL and ACL cases have occurred in soldiers from the United Kingdom, USA and other NATO military forces serving in Iraq and Afghanistan wars since the 2000s. In Colombia, military operations in the forest resulted in more than 45,000 cases of CL in soldiers between 2005 and 2010. In both military operations and civil war scenarios, however, civilians are those most affected by leishmaniasis because of the destruction of health-care infrastructures (lack of diagnosis, shortage of drugs and interruption of vector control activities) and forced displacement of populations. The latter caused one of the biggest epidemic of VL ever reported, with an impressive fatality rate (100,000 deaths) when a civil war in southern Sudan forced people to move into VL-endemic territories in the western Upper Nile. Originally, the disease was probably zoonotic and caused sporadic disease in that area, but the massive displacement of non-immune people gave origin to active anthroponotic transmission of *L. donovani* among 280,000 individuals in the 1980s and 1990s (Seaman et al. 1996). The migration of Afghan refugees into southern Pakistan is thought to have introduced ACL into areas where it had not previously been known. An investigation, however, found similar age-related pattern of ACL endemicity in both Afghan refugee camps and neighbouring Pakistani villages (Brooker et al. 2004). The ongoing Syrian war is having a dramatic impact on the incidence of CL among the population displaced internally (about 6.5 million) and in countries of Middle East and Africa (Turkey, Jordan, Lebanon, Iraq and Egypt) where some four million Syrians were forced to seek refuge. Before the war, the annual incidence of CL (including anthroponotic and zoonotic entities) within Syria

was estimated to be 23,000 cases (2004–2008). In 2012, 53,000 cases were reported, and in 2013, approximately 100,000 cases were estimated. In southeast Anatolia, Turkey, which is endemic for ACL, there is an indication of increased incidence of the disease among the Turkish population in relation with the entry of refugees from Syria. CL has emerged in Lebanon as well, although outbreaks have been largely contained to refugee populations (Du et al. 2016).

1.3.5 Human-Made Environmental Changes

Examples of environmental changes that can affect the incidence of leishmaniasis have been mentioned in previous sections. Most significant changes include urbanization and the incursion of agricultural farms and settlements into arid or forested natural areas, which may cause domestication and expansion of the natural transmission cycles. Latin American countries, particularly Brazil, have experienced epidemics of ZVL in the fast-growing peripheries of large- and medium-sized cities. In some epidemiological contexts, however, the destruction of natural habitats can reduce or even eliminate transmission of leishmaniasis, and such approach is commonly listed among the available tools for ZCL control. This is the case of the destruction of burrows and vegetation habitats of rodent population's natural reservoir of *L. major*, i.e. *Rhombomys opimus* in countries of Central Asia and *Psammomys obesus* in parts of northern Africa and Middle East. Rapid recolonization by rodents and vegetation is a common occurrence when environmental changes are not permanent because unsustainable economically; furthermore, a shift from sylvatic to synanthropic rodents acting as primary *L. major* reservoir (e.g. *Meriones*) may occur. In parts of Latin America, forest-free zones created for cattle breeding resulted in a buffer zone between the sylvatic cycle and human housing and reduced the transmission of CL. In other parts, however, deforestation appears to have led to an increase in the incidence of CL through a shift to a peridomestic transmission cycle.

A recent example of small-scale environmental change which caused to the largest community outbreak of leishmaniasis in Europe is that of Fuenlabrada, a city at the south of Madrid, in 2009–2012 (Arce et al. 2013). The area had long been known to be endemic for *L. infantum*, with sporadic human cases and a low prevalence in dogs. The outbreak peaked in 2011 and involved some 400 cases, of which one-third were VL and two-thirds CL. An investigation showed that most patients lived in proximity of a new green park afforested since 2005, enclosed by roads and other infrastructures recently built. A population of hares grew abnormally by colonizing the park in the absence of natural predators and was unable to migrate. A new sylvatic/peridomestic cycle of leishmaniasis was thus established, having hares as a major blood source for the local competent vector *P. perniciosus* and becoming an important reservoir of *L. infantum* (Molina et al. 2012). Interestingly, the susceptibility of wild hares to natural *L. infantum* infections was reported from Tarim Basin, China, in the same year the Fuenlabrada outbreak began (Liao et al. 2009).