The Montpellier *Leishmania* Collection, from a Laboratory Collection to a Biological Resource Center: A 39-Year-Long Story

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We report the development of a laboratory collection of *Leishmania* that was initiated in 1975 and, after 39 years, has become an international Biological Resource Center (BRC-Leish, Montpellier, France, BioBank No. BB-0033-00052), which includes 6353 strains belonging to 36 *Leishmania* taxa. This is a retrospective analysis of the technical and organizational changes that have been adopted over time to take into account the technological advances and related modifications in the collection management and quality system. The technical improvements concerned the culture and cryopreservation techniques, strain identification by isoenzymatic and molecular techniques, data computerization and quality management to meet the changes in international standards, and in the cryogenic and microbiological safety procedures. The BRC is working toward obtaining the NF-S 96-900 certification in the coming years. Our long-term expertise in *Leishmania* storage and typing and collection maintenance should encourage field epidemiologists and clinical practitioners in endemic countries to secure their own strain collection with the help of the French BRC-Leish.

Keywords: *Leishmania* collection, resources, Biological Resource Center

Introduction

LEISHMANIA ARE PARASITIC protozoa that infect many mammalian species, including humans, and are transmitted through the bite of an insect vector, the phlebotomine sandfly. The genus *Leishmania* includes about 40 different taxa, most of which commonly infect humans and cause leishmaniasis, a disease with different manifestations: visceral, cutaneous (localized or diffuse), and mucocutaneous.¹

The identification of *Leishmania* strains is a crucial step to determine the clinical type of leishmaniasis and establish the adequate treatment. Strain isolation and cryopreservation allow storing biological material for further basic (cell biology, genetic investigations) and applied research (epidemiology, population dynamics, clinical research, or therapeutic trials).

Our laboratory has a long experience in the isolation, identification, and cryopreservation of *Leishmania* parasites. Our first isolates were obtained from a human patient (MHOM/FR/71/LEM 36) in 1971 and an infected dog (MCAN/FR/72/LEM 35) in 1972. The first cryopreservation was performed in 1975.

For the last 39 years, our laboratory has been increasing and managing a collection, the wealth and diversity of which is presently unique in the world. The objective of this

article is to summarize the collection technical and organizational changes over time, describe its present state, and outline the future perspectives.

Collection Growth and Technical Improvements Between 1975 and 2014

During these 39 years, different technical items have been modified: culture and cryopreservation techniques, identification methods, collection management, and quality control. The result of all these changes is the transformation of a simple laboratory collection into an international Biological Resource Center for *Leishmania* (BRC-Leish).

Culture and cryopreservation techniques

Initially, the classical biphasic Novy-Mc Neal-Nicolle (NNN) medium was used both for parasite isolation and culture. It was prepared “in-house” and included rabbit blood collected from animals kept in the laboratory premises.² This progressively changed to comply with new safety regulations. Mainly, industrially controlled rabbit blood and polycarbonate tubes with an optical window for direct examination under an inverted microscope were introduced. Nevertheless, the NNN

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medium remains the reference medium for the primary isolation of strains. The culture protocol is unchanged from the beginning: cultures are subcultured once per week and five subcultures are required to conclude that a culture is negative.

For mass culture, brain-heart infusion agar supplemented with rabbit blood was used until 2009. It was then replaced by an axenic liquid medium (the semidefined medium SDM 79), supplemented with 10% fetal calf serum.

In the 1970s, sterility was assured by setting up the cultures between two Bunsen burners. Vertical laminar flow hoods were introduced in the 1980s, and since 2000, *Leishmania* parasites have been handled in containment level 2 or 3 laboratories, according to the class risk of the taxa handled.

For strain cryopreservation, a suspension of at least 3×10^6 promastigotes is mixed with 5% dimethyl sulfoxide, a cryoprotectant. Promastigote suspensions were initially stored in glass ampoules until 1994, and afterward in Nunc® cryogenic tubes. The cryopreservation procedure also changed over time: the manual technique with gradual descent in liquid nitrogen in a Biogel® apparatus was replaced in the 1980s by a Minicool® biological refrigerating apparatus with electronic programming.³ In the 1990s, the protocol was further simplified by a passage in isopropanol freezing boxes directly placed in a -80°C refrigerator for at least 4 hours, before immersion of the cryogenic tubes in liquid nitrogen.

Strain identification

From 1980 to 1997, strains were typed using exclusively the multilocus enzyme electrophoresis (MLEE) method. Since 1998, a molecular typing approach has been added.

MLEE was initially based on an 8-enzyme system, but in 1986, it was enlarged to 15 enzymes.^{4,5} Isoelectrofocusing was additionally employed as a technique of greater resolving power.⁶ Thus, currently starch gel electrophoresis is carried out according to Rioux et al.⁵ using the following 15 enzymatic systems: malate dehydrogenase, MDH, EC 1.1.1.37; malic enzyme, ME, EC 1.1.1.40; isocitrate dehydrogenase, ICD, EC 1.1.1.42; 6-phosphogluconate dehydrogenase, PGD, EC 1.1.1.44; glucose-6-phosphate dehydrogenase, G6PD, EC 1.1.1.49; glutamate dehydrogenase, GLUD, EC 1.4.1.3; NADH diaphorase, DIA, EC 1.6.2.2; purine nucleoside phosphorylase, NP₁, EC 2.4.2.1; purine nucleoside phosphorylase, NP₂, EC 2.4.2.*; glutamate-oxaloacetate transaminases, GOT₁ and GOT₂, EC 2.6.1.1; phosphoglucomutase, PGM, EC 5.4.2.2; fumarate hydratase, FH, EC 4.2.1.2; mannose phosphate isomerase, MPI, EC 5.3.1.8; and glucose phosphate isomerase, GPI, EC 5.3.1.9.

Molecular typing was initially based on sequencing part of the large subunit of the RNA-polymerase II gene. Since 2006, multilocus sequence typing of seven coding regions is carried out routinely. This approach was validated on all African and Eurasian species, by analyzing 222 strains of the collection.⁷ It was also compared to 4 other molecular identification systems using 74 strains that are representative of the different *Leishmania* species.⁸

Collection management

The collection management, schematically presented in Figure 1, concerns all the different steps required to add a strain to our stock, from its isolation/arrival to its cryopreservation. Briefly, after isolation, an information form that includes all the

available clinical and epidemiological data is completed for each strain. When the strain is cryopreserved, a specific LEM code (LEM for Laboratoire d'Ecologie Médicale, the former name of our laboratory) and a World Health Organization (WHO) code are attributed.⁹ The computerization of the whole process (including the stock management) started in 2004, and, in early 2006, a specific computer program (Modul-Bio) was developed and routinely used.

In 1984, the WHO recognized our collection and, consequently, our laboratory became an International Center for the Cryopreservation, Enzymatic Characterization, and Taxonomic Study of *Leishmania*.³

The first database, using dBASE II, included only little basic information. In 1995, a new database was required because of the huge increase in the number of strains (about 3000) and the amount of clinical, epidemiological, and taxonomic data collected for each strain. The newly developed database, using Microsoft ACCESS 95, allowed not only strain processing but also making various requests (geographical origin, clinical features, donor...). Since 1998, the molecular identification results were also included in the database.

In 2001, the Organization for Economic Cooperation and Development (OECD) proposed the concept of Biological Resource Center (BRC) (ISBN Biological Resource Centers: 92-64-18690-5, 2001, HOECD, Paris), and in 2004, defined the guidelines concerning their functioning, with the obligation of tracking all the different handling steps and ensuring the safety/integrity of the collections. To meet all these requirements, the Modul-Bio Company was asked to develop a new computerized management system for the whole collection and all the activities related to it.

This innovative application was developed using the ORACLE database assisted by the JAVA Enterprise Edition platform for application server and run through a server that allows the access from several guest workstations. This application provided security for the whole process, from the biological resource reception to its cryopreservation, through a bar code system (Fig. 1), and included the management of the cryostabitate stocks. To facilitate the distribution of the collection resources, an interactive catalogue was created in 2004 and is currently available on the internet.¹⁰

Following the definition of the new NF-S 96-900 standard (*Certification des Centres de Ressources Biologiques*) in 2008, the "National Infrastructure in Biology, Health and Agronomy" project (GIS-IBISA) was created, under the co-ordination of the Institut Pasteur, in Paris, with the objective of developing a modern and upgradeable management system that could comply with the specific requirements of this standard. Within this framework, a management system for the French Biological Resource Centers for Microorganisms (Laboratory Information Management System, BRC-Lims) was developed as a free software program in 2010. This system is now operating routinely in our center and in others from the beginning of 2013.¹¹ BRC-Lims allows monitoring the quality and activity indicators defined in our laboratory and easily managing all the stocks, such as strains, culture media, reagents, and laboratory material. The most innovative change in the system concerns the management of customers' requirements and biological resource orders. The functions included in this application allow meeting all the requirements of the NF-S 96-900 standard and its future changes, including those of the very rigorous ISO 9001-2008 standard, the worldwide reference.

FIG. 1. Diagram of the BRC-Leish workflow (*white rectangles*: technical processes; *diamond-shapes*: conditions defined based on a quality approach; *white ovals*: automated data management; *gray ovals*: human interventions). BR, biological resource; BRC-Leish, Biological Resource Center for *Leishmania*.

Quality management

In the current legal and scientific framework, the distribution of biological samples requires the work of a specialized unit. This service needs true technical expertise, a good knowledge of the current laws and regulations and of

the researchers' needs, and adapted logistics for the whole operation. The computerization of the entire process (described in the Collection management section) in 2004 and then the introduction of the BRC-Lims program and database fully meet the NF-S 96-900 quality requirements. Moreover, in 2009, the technical facilities dedicated to the

BRC-Leish were upgraded to comply with the recommendations of the WHO Laboratory Biosafety Manual.¹²

In 2012, the NF-S 96-900 standard was fully implemented concomitantly with the development of the new BRC-Lims database. The team in charge of the quality management system includes the BRC-Leish director and a biologist responsible for the quality management system, assisted by a technician. The first steps consisted of creation of a quality manual and a charter for all the concerned partners (i.e., staff, supporting institutions, and customers). According to the quality manual, the BRC-Leish director defines the quality policy and responsibility and the authority delegations. The BRC-Leish staff must sign a confidentiality commitment. All the documents required for the quality management system (standard operating procedures, operative modes, and validation methods) were written. Defined quality indicators allow the evaluation of the efficiency of the activity and defining the improvement objectives.

Strain catalogue

An online strain catalogue was created in 2004; it included 200 strains that were selected to reflect the geographical, epidemiological, and clinical diversity. Each strain is defined by a BRC-Leish strain code (LEM) and a WHO strain code and is accompanied by information on the host genus and species, clinical features (only for human strains), country where the infection occurred, year of isolation, strain identification, and person who isolated the strain. The inclusion of a strain in the database is submitted to the donor's agreement and the validation by the collection's curator. All these criteria need to be met before accepting a strain in the collection. Since its creation, the online catalogue has been regularly enriched and presently it includes 1500 strains. Specifically, it contains a sample of all described *Leishmania* taxa, samples from all the geographical areas where leishmaniasis is endemic and a large diversity of reservoir hosts, including mammals and phlebotomine sandflies.

To request a *Leishmania* strain, several steps must be followed as described in our website. The strain may be selected from the catalogue or with the curator's help. Indeed, strains not included in the catalogue can also be obtained for specific scientific purposes, after selection by the curator and the donor's agreement. All requests are submitted through an official e-mail or letter to BRC-Leish¹³ accompanied by an information form (available from our website¹⁰). A Material Technical Agreement, which regulates the conditions of the strain(s) distribution and the participants' commitments, must be signed for each request.

BRC safety procedures

Cryogenic safety procedures. The 6353 strains of the BRC-Leish are stored in cryogenic containers (one Espace 331[®], one Espace 151[®], five Arpège 140[®], and 16 GT40[®]; Air Liquide, Paris, France; and two LO 2200, Cryodiffusion, France), placed in a specific storage room, where a wooden slat walkway allows the personnel to safely reach the containers (Fig. 2). A technical room with a BRC-Lims terminal is annexed to the cryobank and the in-between door has a small window. A set of sensors measures the oxygen levels in both rooms and their alarms are connected with the security office of the University Hospital Center. The material and equip-

ment required in the event of anoxia are located near the cryobank entry door and antipanic bars at the doors facilitate a rapid exit in case of emergency.

The access to the cryobank is restricted only to the authorized personnel who have been specifically trained to recognize cryogenic risks. It is forbidden to work alone in the cryobank outside normal working hours.

Microbiological safety procedures. BRC-Leish is equipped with containment level 2 and 3 laboratories according to the international rules for handling biohazard group 2 and 3 pathogens, respectively. Most *Leishmania* species are handled in the level 2 laboratory, except *Leishmania donovani* and *Leishmania braziliensis* that must be handled in a containment level 3 laboratory. The access to these biosafety laboratories is restricted only to specifically trained and authorized personnel. Inside these laboratories, computer terminals allow tracing the whole handling process of each strain.

Knowledge transmission

BRC-Leish assisted in establishing other cryobanks and national *Leishmania* reference centers: Instituto de Salud Carlos III (Madrid, Spain), Istituto Superiore di Sanità (Roma, Italy), Pasteur Institute of Algeria (Algiers, Algeria), Laboratory of Parasitology-Mycology (Faculty of Pharmacy, Monastir, Tunisia) and Unidad de Parasitología, Facultad de Farmacia, Universitat de Barcelona (Barcelona, Spain).

The Present Collection

Since 1975, the inclusion of strains progressively increased each year to reach a peak in 1989 (388 strains). In the following years, about 200 strains per year were included in the collection. However, the large availability of molecular diagnosis and strain identification methods that do not require strain isolation has led, from 2008, to a slow decrease in the number of strains that are added every year (Fig. 3). In 2014, the collection contained 6353 strains in total.

The collection includes strains from the Old World (Africa, Asia, and Europe) ($n=5032$ strains, 82%) and the New World (the Americas) ($n=1108$, 18%) (Table 1 and Fig. 4A). The largest number of Old World strains originates from Europe (2356 strains). France is the most sampled country ($n=1283$), followed by Spain ($n=363$), Portugal ($n=293$), and Greece ($n=245$). Africa comes next with 1906 strains, particularly from the Maghreb area (Tunisia: $n=545$, Morocco: $n=536$, and Algeria: $n=325$). The collection also includes 770 strains from Asia, particularly from Syria ($n=143$) and Turkey ($n=106$). Most New World strains come from South America ($n=1012$), particularly from French Guiana ($n=442$). Few strains were isolated in Central ($n=93$) and North America ($n=3$).

The hosts from which the strains were isolated are mainly humans ($n=4469$, 70.4%), followed by canine ($n=1157$, 18.2%) and rodent reservoirs ($n=154$, 2.4%), as well as sandfly vectors ($n=358$, 5.6%) (Fig. 4B). Human strains were mainly isolated from patients with cutaneous leishmaniasis (CL; $n=2768$, 61.9%), followed by visceral leishmaniasis (VL; $n=1492$, 33.5%) and mucocutaneous leishmaniasis ($n=72$, 1.6%); nine strains corresponded to asymptomatic patients (0.2%) and the clinical outcome was unknown in 128 cases (2.8%) (Fig. 4C). Most human strains in the collection are primary isolates ($n=4256$, 96.16%). A

FIG. 2. Storage room of the *Leishmania* collection.

small number was obtained from simultaneous samples in a single patient ($n=30$) or successive samples in the same patient ($n=77$), or from primary isolations and relapses/reinfections ($n=63$); 10.7% of all human strains ($n=673$) were isolated in immunosuppressed patients (HIV and transplanted patients).

Strains originate from French (3880, 61.2%) or foreign laboratories (2463, 38.8%). The “non-French” strains come all from research laboratories, whereas the “French” strains originate from university hospitals ($n=1982$), research laboratories ($n=1555$), military hospitals ($n=282$), veterinary laboratories ($n=23$), dermatologists ($n=20$), and general hospitals ($n=19$). Some strains were obtained from experiments, including strain subcloning ($n=307$), and 10 were from unknown origin.

In summary, the collection includes 36 *Leishmania* taxa (Table 2) of which the most abundantly represented are *Leishmania infantum* ($n=2559$), followed by *Leishmania major* ($n=945$), *Leishmania tropica* ($n=497$), *Leishmania guyanensis* ($n=345$), *L. braziliensis* ($n=257$), and *L. donovani* ($n=191$). A few strains ($n=12$) correspond to hybrids (*L. infantum*/*L. major* and *L. braziliensis*/*L. guyanensis*). The collection includes also a few other Trypanosomatid taxa (*Trypanosoma*, *Leptomonas*, *Crithidia*, *Herpetomonas*, and *Endotrypanum*).

Collection certifications

The *Leishmania* collection (BRC-Leish, Montpellier, France, BioBank No. BB-0033-00052) is part of the French Network of Biological Resources Centers for Microorgan-

isms (FBRCMi) that includes 10 Microbiological Resources Centers. It is also affiliated to the World Data Center for Microorganisms, the World Federation of Culture Collections (WFCC), and the European Culture Collection Organization (ECCO). Finally, it is part of the French National Biobank Networking Infrastructure “BIOBANQUES” and MIRRI (Microbial Resource Research Infrastructure), a European Research Infrastructure Consortium project endorsed by the European Strategy Forum on Research Infrastructures (ESFRI).

Scientific Production Related to the Collection

Between 1980 and 2014, the scientific production related to the collection was based on the strain isoenzymatic identification and included epidemiologic and taxonomic studies that focused mainly on three interconnected topics: identification of a *Leishmania* species in an endemic focus, study of the endemic foci, and synthetic studies on different *Leishmania* foci. The introduction of molecular tools in 2006 allowed, in addition, performing genetic and population dynamics studies.

Concerning the first identification of a *Leishmania* species as a focus, several articles on Old World strains documented the presence of *L. donovani* in Djibouti,¹⁴ Cyprus,¹⁵ and Sri Lanka¹⁶; *L. major* in Mali¹⁷ and Cameroon¹⁸; *L. tropica* in Morocco¹⁹; and a *Leishmania* species close to *L. killicki* in Algeria.²⁰ Other research works reported the identification of New World species: *L. infantum* in Margarita Island, Venezuela²¹; *Leishmania amazonensis* in Suriname²²; *L. braziliensis* in French Guiana²³; and *Leishmania naiffi* outside Brazil.²⁴ Moreover, some new *Leishmania* taxa were identified: *L. killicki* in Tunisia,²⁵ *L. martiniquensis* in Martinique Island,²⁶ and *L. waltoni* in Dominican Republic.²⁷

During epidemiological investigations on leishmaniasis foci, mainly in the Old World, strain collections were obtained and identified by isoenzyme typing, leading to a better knowledge of the concerned taxa: *L. donovani* and *L. infantum* in Yemen²⁸ and in Sudan²⁹; *L. major* in Syria^{30,31}; *L. infantum* and *L. tropica* in Syria³²; *L. tropica* in Morocco^{33–35}; *L. donovani* in Sudan³⁶; *L. infantum* in Algeria³⁷ and Cataluña, Spain^{38–40}; *L. tropica* and *L. major* in Jordan⁴¹; *L. infantum*, *L. major*, and *L. killicki* in Tunisia^{42–46}; and *L. tropica* in Palestine.⁴⁷ In the New World, a *Leishmania enriettii* focus was specifically investigated in Brazil.⁴⁸

FIG. 3. Yearly number of *Leishmania* strains entered in the BRC-Leish collection (from 1975 to 2014).

TABLE 1. GEOGRAPHICAL ORIGIN OF THE STRAINS STORED AT BIOLOGICAL RESOURCE CENTER FOR *LEISHMANIA*, ACCORDING TO CONTINENTS AND COUNTRIES

Origin	No. of strains	Origin	No. of strains
Asia	770	Europe	2356
Afghanistan	30	Albania	4
Azerbaijan	16	Croatia	1
Bangladesh	1	Cyprus	75
China	16	France	1283
Ex-USSR	3	Germany	1
Georgia	4	Great Britain	3
India	49	Greece	245
Iran	41	Italy	71
Iraq	31	Malta	3
Israel	86	Monaco	8
Jordan	33	Portugal	293
Kazakhstan	9	Spain	363
Lebanon	9	Switzerland	1
Mongolia	3	Undefined origin	5
Pakistan	11	North America	3
Palestine	11	USA	3
Russia	2	Central America	93
Saudi Arabia	28	Belize	16
Sri Lanka	11	Costa Rica	16
Syria	143	Dominican Republic	5
Thailand	5	Guadeloupe	2
Turkey	106	Guatemala	8
Turkmenistan	37	Honduras	3
Ukraine	1	Martinique	10
Uzbekistan	34	Mexico	11
Yemen	46	Nicaragua	6
Undefined origin	4	Panama	13
Africa	1906	Trinidad	2
Algeria	325	Undefined origin	1
Burkina Faso	62	South America	1012
Cameroon	1	Argentina	6
Central African Republic	2	Bolivia	46
Chad	1	Brazil	146
Cote d'Ivoire	4	Colombia	161
Djibouti	5	Ecuador	99
Egypt	15	French Guiana (Fr)	442
Ethiopia	82	Paraguay	2
Kenya	23	Peru	41
Libya	5	Suriname	4
Mali	38	Venezuela	60
Mauritania	15	Undefined origin	5
Morocco	536		
Namibia	12	Undefined continent	213
Niger	2		
Senegal	50		
Sudan	172		
Tunisia	545	Total	6353
Zaire	5		
Undefined origin	6		

In some cases, strain identification allowed the discovery of newly emerging or reemerging foci, particularly in Israel,⁴⁹ Morocco,⁵⁰ and Crete island (Greece).⁵¹

The high richness of the collection allowed us to carry out various synthetic studies, based on large numbers of strains, such as the analysis of 712 *L. infantum* strains in Southern France⁵²; 213 *L. infantum* strains in Portugal⁵³; 100 *L. infantum* and *L. major* strains in Algeria⁵⁴; and 694 *L. infantum*, *L. major*, and *L. killicki* strains in Tunisia.⁵⁵ Moreover, two

FIG. 4. Diagrams of the *Leishmania* strain repartition according to their geographical origin (A), host from which they were isolated (B), and clinical features (for strains isolated from human patients) (C).

worldwide synthetic studies were based on 1048 *L. major* and *L. tropica* strains, including *L. killicki* and *Leishmania aethiopica* from Old World CL,⁵⁶ and 2277 *L. donovani* and *L. infantum* strains from Old World VL.⁵⁷

The HIV infection emergence in the 1980s led to an increase in the isolation of *Leishmania* strains in HIV-positive patients and the publication of several synthetic articles based on isoenzymatic typing of such strains.^{58–60}

The data obtained by isoenzymatic typing of *Leishmania* strains from 1981 onward have been used to generate phenetic and phylogenetic trees that allowed proposing a new *Leishmania* taxonomy.^{5,56,57,61–67}

Many genetic studies based on the collection resources analyzed the phylogeny and taxonomy of different species within the *Leishmania* genus,^{7,68–70} or the parasite population structure and dynamics.^{71–79}

TABLE 2. DIFFERENT *LEISHMANIA* SPECIES AT BIOLOGICAL RESOURCE CENTER FOR *LEISHMANIA*, WITH MENTION OF THE SUBGENUS (*SAUROLEISHMANIA*, *S.*, *LEISHMANIA*, *L.* AND *VIANNIA*, *V.*)

<i>Leishmania</i> (<i>S.</i>) <i>adleri</i> ^a	1	<i>Leishmania</i> <i>hoogstraali</i> ^b	1
<i>Leishmania</i> (<i>L.</i>) <i>aethiopica</i>	46	<i>Leishmania</i> (<i>L.</i>) <i>infantum</i>	2559
<i>Leishmania</i> (<i>L.</i>) <i>amazonensis</i>	44	<i>Leishmania</i> (<i>L.</i>) <i>killicki</i>	69
<i>Leishmania</i> (<i>L.</i>) <i>arabica</i>	5	<i>Leishmania</i> (<i>V.</i>) <i>lainsoni</i>	10
<i>Leishmania</i> (<i>L.</i>) <i>archibaldi</i>	51	<i>Leishmania</i> (<i>L.</i>) <i>major</i>	945
<i>Leishmania</i> (<i>L.</i>) <i>aristidesi</i>	1	<i>Leishmania</i> <i>martiniquensis</i> ^b	12
<i>Leishmania</i> <i>australiensis</i> ^b	1	<i>Leishmania</i> (<i>L.</i>) <i>mexicana</i>	39
<i>Leishmania</i> (<i>V.</i>) <i>braziliensis</i>	257	<i>Leishmania</i> (<i>V.</i>) <i>naiffi</i>	11
<i>Leishmania</i> (<i>L.</i>) <i>chagasi</i>	3	<i>Leishmania</i> (<i>V.</i>) <i>panamensis</i>	85
<i>Leishmania</i> (<i>V.</i>) <i>colombiensis</i>	2	<i>Leishmania</i> (<i>V.</i>) <i>peruviana</i>	9
<i>Leishmania</i> (<i>L.</i>) <i>deanei</i>	3	<i>Leishmania</i> (<i>L.</i>) <i>pifanoi</i>	3
<i>Leishmania</i> (<i>L.</i>) <i>donovani</i>	191	<i>Leishmania</i> (<i>V.</i>) <i>shawi</i>	1
<i>Leishmania</i> (<i>L.</i>) <i>enriettii</i>	4	<i>Leishmania</i> <i>siamensis</i> ^b	2
<i>Leishmania</i> (<i>V.</i>) <i>equatorensis</i>	1	<i>Leishmania</i> (<i>S.</i>) <i>tarentolae</i> ^a	17
<i>Leishmania</i> (<i>L.</i>) <i>garnhami</i>	1	<i>Leishmania</i> (<i>L.</i>) <i>tropica</i>	497
<i>Leishmania</i> (<i>L.</i>) <i>gerbilli</i>	5	<i>Leishmania</i> (<i>L.</i>) <i>turanica</i>	35
<i>Leishmania</i> (<i>V.</i>) <i>guyanensis</i>	345	<i>Leishmania</i> (<i>L.</i>) <i>waltoni</i>	5
<i>Leishmania</i> sp.	275		
<i>Leishmania</i>	1	<i>Leishmania</i> hybrids	12
(<i>Sauroleishmania</i>) <i>gymnodactyli</i> ^a			
<i>Leishmania</i> (<i>L.</i>) <i>hertigi</i>	1	Other trypanosomatids	27

^aSome authors consider *Sauroleishmania* as a genus.

^bNo subgenus attributed.

Finally, the scientific production generated by the study of the strains in our collection was evaluated by an external group that assessed the worldwide scientific production on leishmaniasis.⁸⁰ Based on the number of publications between 1961 and 2010, the Montpellier *Leishmania* group was ranked at the very top level of the most productive researchers on leishmaniasis. This study also highlighted the exceptional increase in our research output between 1981 and 2010 during the period of strong development of the scientific collaboration networks.

The Future

BRC-Leish provides a quantitatively and qualitatively exceptional collection of *Leishmania* strains. The strains' safe storage and distribution, as well as the protection of the depositors' rights comply with the most exacting international standards. The BRC-Leish facility is working toward obtaining the NF-S 96-900 certification in the coming years. Typing methods are also changing and mass spectrometry-based approaches are being developed for faster identification compared to DNA sequencing.⁸¹

Yet, the future of the collection remains subject to threats. Indeed, the generalization of *Leishmania* molecular typing directly from clinical samples, both for patient management and in epidemiological studies, is leading to a gradual reduction in the number of strains isolated from clinical settings or even from the field. This is a modern problem that concerns all microbiological collections. Indeed, a dynamic flux of incoming and distributed strains is essential for any microbiological collection.

Still, the expertise and knowledge of BRC-Leish (and of the associated French National Reference Centre for Leishmaniasis) remain a guarantee for many research groups worldwide. The storage traceability, security, and robustness

and the fact that strains are checked again using molecular typing before distribution, together with the importance and diversity of the collection, contribute to safeguarding the identity and quality of the strains cryopreserved and distributed by CRB-Leish. For these reasons, researchers widely use BRC-Leish knowing that they will obtain highly reliable parasitic material.

For example, the advent of high-throughput sequencing technologies now allows sequencing the genome of many strains, with enormous potential developments for epidemiology,⁸² population genetic, drug resistance,^{83,84} functional genomic,^{85,86} and molecular/cell biology studies.⁸⁷ These analyses require highly reliable parasite resources because of the high investment in terms of research time and money. BRC-Leish is involved in several of these projects and will inevitably continue to do so. Therefore, many samples from the collection will be sequenced, particularly those coming from a defined endemic focus where a thorough sampling was performed. Similarly, reliable samples and the accompanying data are required also for highly focused studies implying a long-term investment in one or two strains.

Finally, our long-term experience on parasite storage should encourage field epidemiologists and clinical practitioners in endemic countries to secure their own strain collection with the help of the French BRC-Leish.

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Author Disclosure Statement

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