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Original article

Epidemiology, clinical pattern and impact of species-specific molecular diagnosis on management of leishmaniasis in Belgium, 2010–2018: A retrospective study

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ABSTRACT

Background: Species-directed therapy of leishmaniasis has been recommended for travelers since 2014, but little is known about species distribution and treatment practices in non-endemic countries. We aimed to describe leishmaniasis cases in Belgium since species typing became available and evaluate its impact on patient management.

Method: Retrospective analysis of all patients diagnosed by PCR at our national reference laboratory from 2010 to 2018. Species were typed by *Hsp-70* sequencing.

Results: We identified 18 visceral leishmaniasis (VL) and 147 (muco)cutaneous leishmaniasis ((M)CL) cases. VL was exclusively due to *L. infantum* and consistently treated with liposomal amphotericin B, with four observed failures. (M)CL was caused by ten different species. Of 62 cases diagnosed and species typed after 2014 with timing information, 28 (45.2%) were treated before the species result was available. Therapy was not species-directed in 10/32(28.1%) of those treated after species identification. Patients treated according to the guidelines tended to have a favorable outcome more often than those who were not (36/44, 81.8% versus 8/19, 57.9%; p = 0.045).

Conclusions: In contrast to VL, various species caused (M)CL in our setting and species result was often not considered for treatment. Outcome tended to be better however when therapy was species-directed.

1. Introduction

Leishmaniasis is a vector-borne disease caused by intracellular protozoan parasites of more than 20 different *Leishmania* species. The parasite is transmitted by infected female sandflies (*Phlebotomus* spp. or *Lutzomyia* spp.). Leishmaniasis affects up to 12 million people worldwide with an estimate of two million new cases in almost 100 endemic countries annually [1,2]. The global number of cases is increasing due to urbanization, deforestation, drug resistance, improved diagnosis, but also because of failing health service coverage and vector control efforts in territories affected by armed conflict and people displacement [3,4].

The epidemiology of leishmaniasis is extremely complex and dynamic with species with large overlapping geographic areas. It is predominantly endemic in poor and rural areas of the Indian subcontinent, Central and South America, North and East Africa and the Middle East. Leishmaniasis is also endemic in Southern Europe (Mediterranean basin) [5]. Over the past two decades, leishmaniasis has increasingly been reported in non-endemic settings as an imported disease of international travelers, such as individuals visiting friends and relatives (VFR), military and immigrants [6].

The clinical range of leishmaniasis can vary from asymptomatic to fatal disease, and manifests with cutaneous, mucocutaneous or visceral

Abbreviations: VFR, visiting friends and relatives; ITMA, Institute of Tropical Medicine Antwerp; IDSA, infectious diseases society of America; IRB, institutional review board; LAmB, liposomal amphotericine B; IL Sb^v, Intralesional pentavalent antimonials.

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forms, depending on the causative (sub)species and the host's immune response [1]. Cutaneous leishmaniasis (CL) is the most common presentation. In order to harmonize therapy, CL was recently categorized into "simple" or "complex" according to expert opinion-based clinical criteria (e.g. size, number, anatomical location, signs of disseminated disease) [7,8]. Mucocutaneous leishmaniasis (MCL) is a rare disease manifested by destructive mucosal lesions after infection disseminates from the primary cutaneous lesion. Visceral leishmaniasis (VL) often presents with fever, hepatosplenomegaly, pancytopenia and cachexia. When left untreated, VL invariably leads to death [1].

Clinical presentation and prognosis are associated with different *Leishmania* species. For example, infection caused by *L. major* or *L. mexicana* causes limited CL lesions that frequently heal spontaneously within three months of onset [7]. In contrast, *L. tropica*, *L. infantum*, *L. braziliensis* and *L. guyanensis* species tend to cause more severe CL disease, and *L. braziliensis* is most commonly linked to MCL [9–11]. Life-threatening VL is almost exclusively caused by *L. donovani* and *L. infantum* species (the latter also named *L. chagasi* in South America) [1].

For decades, case management of leishmaniasis was based on clinical-epidemiological information [12]. VL, MCL and complex CL were treated with systemic therapies, while simple CL was mostly managed with local therapies or even simple wound care. Treatment was often based on the known geographic distribution of *Leishmania* species in the region of acquisition. Accumulating evidence indicates that the response to different available treatments may vary between species [8,13,14]. However, it is impossible to determine the causative species based solely on clinical and epidemiological information, particularly in non-endemic countries, or in areas where epidemiological data on species distribution is lacking or rapidly shifting [15]. Therefore, species typing by molecular methods and species-directed treatment has increasingly been promoted in recent international guidelines [7,8,16–18]. Species identification is expected to help harmonize the management of leishmaniasis in travelers, compared to the clinical-epidemiological approach. However, it is unclear to what extent a species-based approach has an impact on management in clinical practice.

The objective of this study was to describe the epidemiological, clinical, molecular, treatment and outcome characteristics of all PCR-confirmed leishmaniasis cases at the Institute of Tropical Medicine, Antwerp (ITMA), Belgium, over a 9-year period (2010–2018). In addition, we assessed whether the species identification had resulted in treatment modifications, after the species-directed guideline was released in 2014. We also challenged ITMA experts with a selection of CL cases extracted from the study cohort, but without information on species, in order to compare their suggested treatment decisions to guideline recommendations [8].

2. Methods

2.1. Study design and setting

This retrospective study was conducted in a cohort of confirmed leishmaniasis cases at the ITMA, which hosts the national reference laboratory for infectious and tropical diseases and a travel clinic with tropical diseases experts. External physicians also contributed to this cohort by sending tissue samples to the ITMA reference laboratory for leishmaniasis diagnostics.

Skin punch biopsies or bone marrow aspirations were obtained from patients with suspected (M)CL or VL, respectively. Upon arrival in the laboratory, microscopy was performed on the same day and PCR in batch once a week. A diagnostic real-time PCR targeting the 18S rRNA gene ('18S PCR') was used [19], and species typing was done by *Hsp70* sequencing on samples with positive PCR results [20]. Since 2012, species typing was performed on all 18S PCR positive samples, while from 2010 up to 2012 it was only done at physician's request. Typically, *Hsp70* species typing may take one to two weeks, and tends to be less successful for samples with high Ct-values in the diagnostic 18S PCR, and with DNA extracted from paraffin embedded tissues. During the study period, we used two guidelines for leishmaniasis treatment: a local adaptation of the Sanford guideline ("State of the art"-ITM guidelines for tropical and travel-related infections, edition 2010) that was not species-directed, until 2017 [21]. The second guideline, that promoted species-directed therapy based on the LeishMan group's recommendations [8], was used from 2014. Local and systemic therapies where administered either at the ITMA or in other hospitals, but most often under the supervision of ITMA experts.

2.2. Data collection

We retrieved all leishmaniasis cases diagnosed by PCR at ITMA between January 2010 and December 2018. The population consisted of patients who visited ITMA at least once ('internal cases'), and patients for whom only samples were sent to ITMA for diagnosis of leishmaniasis by referring physicians ('external cases'), and for whom ITMA expert advice was requested. For internal cases we extracted clinical and epidemiological data from the ITMA medical files. We directly contacted the attending physicians for external cases. Data was pseudo-anonymized and collected using an electronic data collection tool (KoBoToolbox).

In addition, we randomly selected 25 CL cases with complete data, pictures of lesions and *Leishmania* species identification from the study population. Withholding the species identification data, we provided detailed clinical information (age, country of exposure, immune status, de-identified pictures) to three ITMA leishmaniasis experts and gauged their therapeutic recommendations. These recommendations were then compared to species-directed guidelines, to assess the added value of *Leishmania* species identification to clinical management.

2.3. Definitions

CL cases were patients with PCR-confirmed leishmaniasis in skin lesions, and no signs of mucosal or visceral involvement. MCL cases had PCR-confirmed leishmaniasis and involvement of oral or nasal mucosa (with or without cutaneous involvement), but no signs of visceral involvement. VL cases were patients with PCR-positive bone marrow samples, associated with clinical features compatible with visceral involvement. We classified all cases into "New World" or "Old World" according to the most likely country of acquisition.

For categorization of CL cases as "simple" or "complex" we adapted (see Table A1) the classification of the Infectious Diseases Society of America (IDSA) and the LeishMan group guidelines [7,8], so that every patient could be classified as simple or complex (these two guidelines have some gaps, and are not fully congruent). Patients were considered as 'complex' if they met any of the criteria. We applied this classification to the clinical information at the first visit.

For this study, "Species-directed guidelines" were defined according to the 2014 LeishMan group therapeutic recommendations [7,8] (Table A2), for the participants evaluated after 2014 as well as for the 25 extracted cases submitted to the ITMA experts.

We assessed clinical outcomes based on patient records from their last visit. For patients who had multiple treatments, this was done using the last visit before starting the next treatment (outcome first treatment), as well as the last overall visit (final outcome). Favorable outcome was defined as either cure for VL and (M)CL cases (clinical resolution after therapy, without any evidence of relapse) or improvement for (M)CL cases (clinical improvement, but with residual signs of disease after therapy, i.e. "improving lesion"). Unfavorable outcomes included failure (no improvement for (M)CL or persistence of signs of active disease after first line therapy for VL cases), relapse (reappearance of clinical signs after healing), visceralization (development of VL-like symptoms following CL), dissemination (spreading of amastigotes in

unusual sites following (M)CL) or death. Need for second-line treatment either because of clinical non-response or toxicity was also considered as an unfavorable outcome.

Adverse events were defined as any recorded reaction or disability the patient experienced after having taken the drug.

2.4. Statistical analysis

Data was analyzed using R statistical software, version 3.6.1. Medians and interquartile ranges, and counts and percentages were used to describe the cohort. The association between outcome and treatment before or after species typing was analyzed using the chi-square test. Poisson linear regression was used to check the association between number of cases over time, using a value of 0.05 as a cutoff for significance.

2.5. Ethical approval

This study has been approved by the Institutional Review Board (IRB) of the ITM (reference: 1095/16) and according to the local rules for the other hospitals (either by additional ethical approval or by presumed consent). Of note, "presumed consent" has been in place at ITMA for more than ten years. Patients who do not specifically object automatically consent to use of their de-identified data for surveillance or research purposes.

3. Results

From 2010 to 2018, a total of 165 *Leishmania* cases were positive for *Leishmania* PCR at ITMA, of which 144 had CL, 18 had VL and 3 MCL. Microscopy was not possible for 42 patients (inadequate or too little sample); of the remaining patients, 53/123 (43.1%) were microscopy positive. The number of diagnoses of all *Leishmania* cases significantly increased over time (p = 0.002, intercept 1.1) (Fig. 1).

3.1. Epidemiological, clinical and outcome features of VL

Eighteen patients were diagnosed with VL (Table 1). Most of the VL cases were male, with a median age of 53 years old (range: 1–70). About half of the patients were tourists (44.4%), and all acquired the infection in the Old World, including Europe for 13 of them (Fig. 1). All cases were typed as *L. infantum*. The median duration of symptoms was 42 days. Immunosuppression associated with HIV infection, hematological malignancy or immunosuppressive therapy was present in 8/16 (50.0%) VL cases with available clinical information. Of these 16 cases, the 14 with known treatment were treated with liposomal amphotericin B (LAmB), with very varied dosages and schedules (minimum 18 mg/kg and maximum 63 mg/kg). Of the four who failed first line treatment, three were immunosuppressed (despite correct dosage (40 mg/kg in total) for two of them).

3.2. Epidemiological, clinical features, treatment, and outcomes of (M)CL

The 147 (M)CL patients' characteristics are described in detail in Table 2. The majority were male (66.7%) and the median age was 32 years (range: 2–86). The reasons for travel were tourism (20.4%); VFR (19.1%); living, working or studying abroad (19.1%); and immigration (15.6%). Most patients (68.7%) had Old World CL (Fig. 1), mainly from Morocco and Syria, followed by Spain, Tunisia, Italy, and Afghanistan. For New World CL, the most common places of infection were Ecuador, followed by Costa Rica, French Guyana, and Bolivia.

The median lesion size was 3 cm, the median number of lesions was

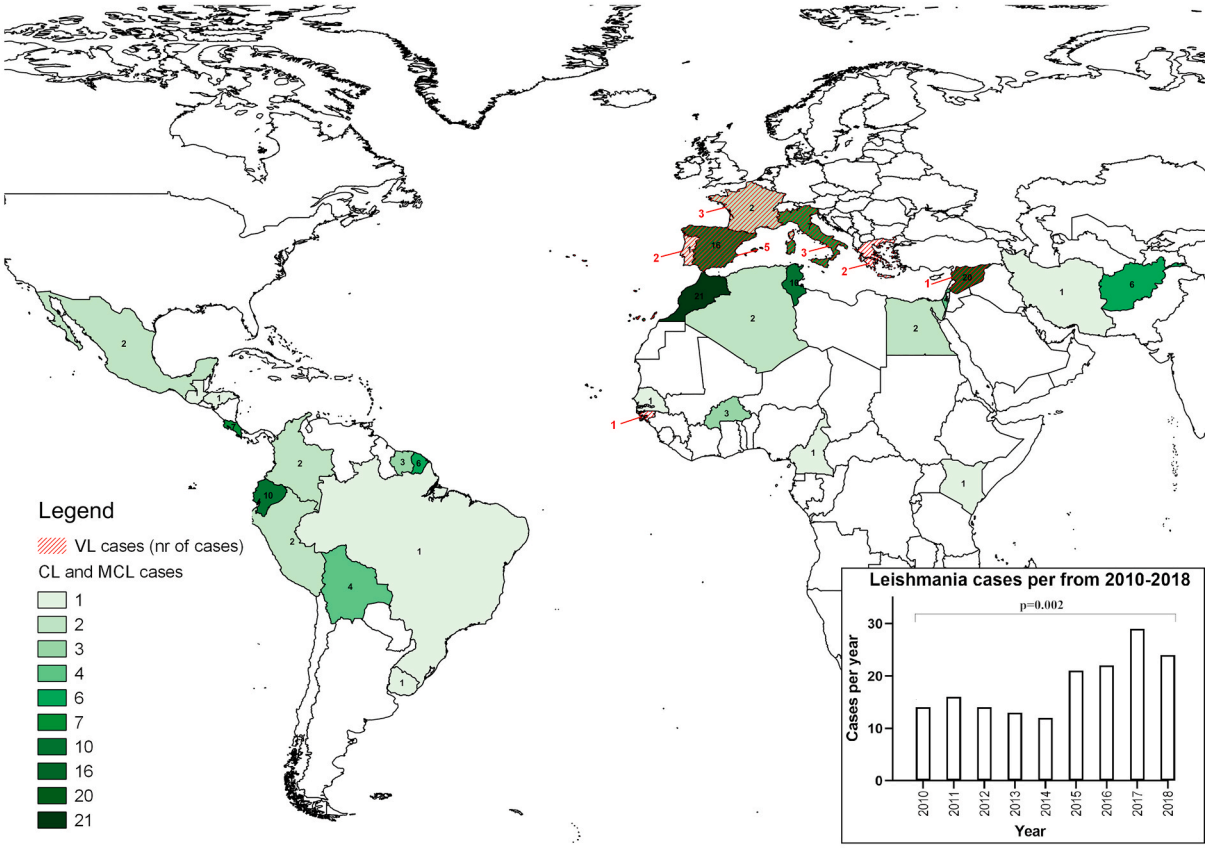

Fig. 1. Map showing the origin of infection for cutaneous/mucocutaneous (green) and visceral leishmaniasis (red) cases that were diagnosed between 2010 and 2018.

Table 1
Baseline characteristics, treatment and outcome of patients with visceral leishmaniasis.

	N = 18	%
Male	11	61.1
Median age in years (IQR)	53.0 (32.3–58.8)	
Old world	17/17	100.0
Reason of travel[a]		
Tourism	8	44.4
Visiting friends and relatives	5	27.8
Migrant	2	11.1
Living abroad	1	5.6
Unknown	3	16.7
Median duration of symptoms in days (IQR), *n = 15*	42 (15.3–70.8)	
Immunosuppression	8/16	50.0
Immunosuppressive therapy[b]	3	37.5[c]
HIV infection	2	25.0[c]
Hematological malignancy	2	25.0[c]
Chemotherapy for solid cancer	1	12.5[c]
Species		
Leishmania infantum	17	94.4
Typing not requested	1	5.6
Treatment		
Liposomal amphotericin B	14	77.8
Systemic antimonials	2	11.1
Unknown	2	11.1
Outcome after first treatment		
Cure	11	61.1
Improvement	1	5.6
Failure	1	5.6
Death	2	11.1
Relapse	1	5.6
Unknown	2	11.1

IQR: interquartile range.
[a] Multiple answers possible, sum of proportions equals more than 100%.
[b] Prednisolone for rheumatoid polyarthritis, fingolimod for multiple sclerosis, various immunosuppressive drugs for a combination of rheumatoid arthritis, vasculitis and cryoglobulinemia.

one, and the most common presentations were ulcers (44.2%) and nodules (34.7%). The anatomical locations most commonly affected were the limbs (59.9%), followed by the face (34.7%). The median duration of symptoms was 143 days (range: seven days to five years). 77 patients (52.4%) had simple CL, 58 (39.5%) complex CL and 12 (8.2%) could not be retrospectively classified. Patients were mainly classified as having complex CL (see criteria Table A1) because of lesions larger than 4 cm or located on sensitive areas. Ten CL cases were classified as complex because of underlying immunosuppression.

Most of the simple CL cases were treated with intralesional antimonials monotherapy (36/77, 46.8%). Other treatment options included cryotherapy (n = 1), surgical removal (n = 3), or simple wound care (n = 6). Azole monotherapy was also used in 13 patients (16.9%), for both New World and Old World CL, while azoles were used in combination with local treatment in seven patients (9.1%). Other systemic therapies were sometimes used for simple CL, such as systemic antimonials (n = 3), LAmB (n = 1), and pentamidine (n = 4).

For cases categorized as complex CL (n = 58), LAmB was most commonly used (17, 29.3%), followed by systemic antimonials (10, 17.2%), pentamidine (8, 13.8%) and azoles as monotherapy (3, 5.2%). Three cases were treated with a combination of systemic and local therapy. Miltefosine was administered to only one patient. Of note, eight complex CL cases were treated with local therapy only, without clear reason.

For systemic treatment, reported adverse events were more frequent for systemic antimonials (4/14,28.5%) and LAmB (5/18, 27.8%) than for pentamidine (2/12, 16.7%) and azoles (1/18, 5.5%). For local treatment, adverse events have only been reported with intralesional antimonials (5/41, 12.2%).

After the first course of treatment, out of the 122 CL/MCL cases with adequate follow-up data, 92 (75.4%) had a favorable outcome (40 cured and 52 with improvement). Two patients had visceralization/dissemination, two relapsed, two had treatments changed due to adverse events, and 26 failed to respond to treatment. Detailed information on the treatments and outcomes can be found in supplementary Table A3.

Table 2
Baseline characteristics, clinical features and species pattern of patients with cutaneous leishmaniasis and muco-cutaneous leishmaniasis.

	N = 147	%
Socio-demographic		
Male	98	66.7
Median age in years (IQR)	32 (21.5–50.5)	
Reason of travel[a]		
Tourism	30	20.4
Visiting friends and relatives	28	19.1
Living abroad[b]	28	19.1
Migration	23	15.6
Unknown	46	31.3
Characterization of the lesion(s)		
Classification		
Simple CL	77	52.4
Complex CL	58	39.5
Unclear/unknown classification	12	8.2
Median size of lesion in cm (IQR), *n = 115*	3.0 (1.8–4.0)	
Median number of lesions (IQR), *n = 138*	1.0 (1.0–2.0)	
Type of the main lesion[a]		
Ulcer	65	44.2
Nodule	51	34.7
Crustae	34	23.1
Papule	15	10.2
Unknown/Unclear	12	8.2
Site of lesion[a]		
Limbs	88	59.9
Face	51	34.7
Trunk	15	10.2
Other (corneas, throat, inner nose)	3	2.0
Unknown	7	4.8
Median duration of symptoms in days[c] (IQR), *n = 135*	143.0 (73.5–253.)	
Immunosuppression	11/141	7.8
Species typed		
Old world	**101**	**68.7**
L.donovani complex	46	31.3
L. infantum species	40	27.2
L. donovani species	1	0.7
Species undetermined	5	3.4
L. tropica	26	17.7
L. major	16	10.9
No complex or species typed	15	10.2
New world	**46**	**31.3**
L. guyanensis complex	24	16.3
L. guyanensis species	8	5.4
L. panamanensis species	4	2.7
Species undetermined[d]	12	8.2
L. braziliensis complex	15	10.2
L. braziliensis species	13	8.8
Species undetermined[d]	2	1.4
L. mexicana complex	6	4.1
L. mexicana species	1	0.7
L. amazonensis species	1	0.7
Species undetermined[d]	4	2.7
L.donovani complex	2	1.4
L. chagasi/infantum	2	1.4
No complex or species typed	1	0.7

CL: cutaneous leishmaniasis, IQR: interquartile range.
[a] Multiple answers possible, sum of proportions equals more than total.
[b] Includes extended stays for education or work purposes.
[c] Time in days between symptoms started and sample was sent.
[d] Typing to species level was not possible.

3.3. Species identification of (M)CL cases

Results of CL/MCL diagnosis (by PCR/microscopy) and species (by

hsp70 typing) were provided to the treating clinician within a median of three (IQR 2–7) and 17 (13–25.5) days respectively.

The species identified for Old World CL (n = 101) were mainly *L. infantum* (n = 40), *L. tropica* (n = 26), and *L. major* (n = 16). The 46 patients with New World CL were most commonly infected with *L. braziliensis* (n = 13), *L. guyanensis* (n = 8), and *L. panamensis* (n = 4). Out of the 147 cases, 23 (15.6%) skin specimens could only be typed up to the complex level, mostly the *L. guyanensis complex*, due to technical issues. No complex nor species information could be obtained for 16 cases (10.9%).

A detailed overview of the characteristics, treatment, and outcomes of the CL and MCL patients per species and per simple and complex case is shown in Table 3.

3.4. Impact of species determination on CL treatment and outcome

For sixty-two of 85 cases diagnosed after 2014 (*i.e.* introduction of species-directed recommendations), information on dates of treatment and species identification was available (see Fig A1 for flowchart). Antileishmanial treatment was initiated in 34 patients (54.8%) after species-identification and in the remaining 28 (45.2%) prior to species-identification. Among those 28 with treatment initiated prior to species typing, 17 (60.7%) had a favorable evolution, while treatment failure (n = 10) or relapse (n = 1) necessitated a therapy switch in 11 (39.3%). Only six had received a first-line regimen that complied with the guideline. Out of the five remaining, three got an "appropriate" treatment as second line.

Most importantly, outcomes were favorable more frequently in patients who were treated after species typing than in those who were treated empirically (27/31, 87.1% vs 15/25, 60.0%, p = 0.01). In addition, patients treated in line with the LeishMan guidelines (including 17 in whom therapy was initiated before species typing) tended to have favorable outcomes more often (36/44, 81.8%) than patients not treated accordingly (8/19, 57.9%), p = 0.045.

Table A4 describes the selection of 25 cases extracted from the dataset and submitted to ITMA experts. For nine cases (36.0%), at least one expert made a recommendation not in line with the species-directed LeishMan guideline (Table A2). This proportion was significantly higher (p = 0.031) for New World cases (5/7, 71.4%) than for Old World cases (4/17, 23.5%). In five of those nine cases (20.0% of the total), a systemic treatment was suggested while local therapy was recommended, or vice versa. For the remaining four, the systemic treatment proposed was not part of the recommendations. Non-concordance was mainly observed for *L. guyanensis* complex CL (n = 4) for which pentamidine was never offered. Also, for the different types of OW CL and *L. mexicana,* often (n = 10) local therapy was proposed while simple wound care could have been sufficient.

4. Discussion

This study described the clinical presentation and outcome of all PCR-confirmed leishmaniasis cases diagnosed in Belgium between 2010 and 2018, for which species typing was attempted. We observed that the number of cases has increased in the past decade, that CL accounted for most of them and that the majority of infections were acquired in the Old World. Consequently, *L. infantum*, *L. tropica* and *L. major* were the predominant species, mainly in VFR travelers and migrants. However, *L. guyanensis* and *L. braziliensis* were also commonly seen, almost exclusively in Belgian tourists.

In general, we noticed that species typing had little impact on the actual treatment practices. However, we observed that patients treated after species result was obtained had a better outcome and that patients who got a treatment in line with the guideline tended to evolve more favorably. Nevertheless, improved outcome might be due to some confounding factors we could not identify in this small retrospective series (for example, patients initially more severe could have been treated empirically more often, before species results).

Finally, when observing expert decisions based on clinical grounds only, we can consider that species identification would have been helpful to harmonize and optimize the management in a sizeable subset of cases, and may especially be useful for New World CL. Of note, a recent French study showed that prediction of the species based on history and clinical presentation was concordant with the final species identified in 96% of Old World CL, but prediction proved more difficult in New World CL, where 26% of cases could not be correctly predicted. However, our data, which focused on the subsequent therapeutic step, were less optimistic, since we found that in 23.5% of the Old World cases and 71.4% of the New World cases at least one expert made a recommendation not in line with the species-directed LeishMan guideline.

Our study had limitations inherent to the retrospective nature of our analysis. Indeed, some data were incomplete, in particular those related to treatment and outcome, or lacked sufficient detail for analysis. This prevented us from constructing a precise timeframe for the diagnosis and management of cases and, most importantly, from using well-established pre-defined outcomes. Also, we only included the cases for which a PCR had been requested and performed. Therefore, we may have missed an unknown number of cases diagnosed on clinical ground or by microscopy (and/or serology for VL) in Belgium. Third, for pragmatic reasons we adapted and simplified the LeishMan and IDSA "simple-complex" definitions for CL. This might somehow limit the comparisons with other published series, and also oversimplifies the clinical decision making that leads to the choice of treatment. Finally, challenging experts with "incomplete" cases is somewhat artificial, but we felt the current setup was the only ethical way to investigate the potential added value of species identification.

The predominance of CL cases in this series was expected [6], since this was observed in the most recent survey by the GeoSentinel Surveillance Network [15], as well as similar studies conducted in the UK, The Netherlands and Sweden [22–24]. Compared to the latter series, we observed more VL cases (all acquired in Southern Europe), although the reasons for this difference are unclear. There were also less soldiers than observed in The Netherlands and UK series, possibly related to a shorter stay of the Belgian Army in Afghanistan.

The treatment response of VL to LAmB, which was used consistently throughout our study since it is the first-choice therapy in Europe, was very satisfactory in the immunocompetent patients, but some failures occurred in immunosuppressed individuals, despite adequate LAmB dosage.

In contrast, we observed many more discrepancies for the treatment of CL/MCL. We were surprised by the high use of (long courses (up to 6 weeks) of) azole treatment in simple CL cases, while short-course local therapies may have been sufficient. In addition, evidence for azole efficacy is limited. It has been described in treatment of *L. major* infection in Saudi Arabia [25], but its efficacy was questioned in travelers [26]. Moreover, it is probable that simple wound care could have been implemented for several *L. mexicana* and *L. major* cases had the etiological species been known. Of course, patients' preference and treatment availability should also be considered in this retrospective evaluation, especially for a group of patients seeking care who may actively demand treatment. Finally, some long and complex treatments were also administered to *L. guyanensis* complex/species cases (including systemic glucantime, LAmB and azole), while evidence favors a rather simple pentamidine regimen.

The "species-targeted" approach has first been promoted in 2004 [27], and was published in detail in 2014 [7,8]. In 2013, a pioneering Swiss study retrospectively analyzed imported cases treated with this approach. It showed encouraging data: out of 61 cases, only 6 had treatment failure [28]. Despite this, our study highlighted that a species-directed approach is infrequently applied in the current Belgian practice. It appears that management is more often based on patient or physician preference, treatment availability or convenience rather than scientific evidence. This could be due to several reasons: the complexity

Table 3
Clinical, therapeutic and outcome features according to species for patients with cutaneous and mucocutaneous leishmaniasis (cases with complete treatment and outcome information, n = 141).

	Classification	Total N (%)	Main type of lesion (n, %)	Median size in cm (IQR) (if known)	First line treatments given[a] (if known). Treatments not in guidelines are marked in bold[b]	Favorable outcome[c]/ known outcome (%)
Old world species						
L. infantum species	Simple	21	Nodule (16, 76.2)	2.0 (1.9–3.0)	Simple wound care for 2	2/2 (100)
					IL Sb^v monotherapy for 11	10/11 (90.1)
					Other local treatment for 1	0/1 (0)
					Azoles monotherapy for 3	2/3 (66.7)
					Azoles + local treatment for 2	1/2 (50.0)
					Surgical removal for 2	1/2 (50.0)
	Complex	15	Ulcer (7, 46.7)	4.0 (2.0–4.0)	Abstention for 2	2/2 (100)
					IL Sb^v monotherapy for 1	1/1 (100)
					Sys Sb^v monotherapy for 2	1/2 (50.0)
					LAmB monotherapy for 6	5/6 (83.3)
					Azole monotherapy for 1	0/1 (0)
					Other drug(s)/combination for 2	0/2 (0)
	Unclear	1	Nodule	–	Simple wound care for 1	1/1 (100)
L. donovani species	Simple	1	Nodule	2.0	IL Sb^v for 1	1/1 (100)
L. donovani complex *(no species determined)*	Simple	4	Nodule (2, 50.0)	2.5 (1.6–3.3)	Simple wound care for 1	1/1 (100)
					Azoles + local treatment for 2	2/2 (100)
	Complex	1	Ulcer	1	LAmB for 1	1/1 (100)
L. tropica species	Simple	16	Crustae (7, 43.8)	1.5 (1.0–2.5)	Simple wound care for 1	1/1 (100)
					IL Sb^v for 7	7/7 (100)
					Azole monotherapy for 6	2/4 (50.0)
					Azole + local treatment for 1	0/0 (Unknown)
					Other drug(s)/combination for 1	0/0 (Unknown)
	Complex	9	Crustae (4, 44.4)	5.0 (2.0–5.5)	**IL Sb^v for 2**	2/2 (100)
					Other local treatment for 4	1/4 (25.0)
					Sys Sb^v monotherapy for 1	0/1 (0)
					LAmB for 2	2/2 (100)
	Unclear	1	Ulcer/Crust	–	**Azole monotherapy for 1**	0/1 (0)
L. major species	Simple	6	Ulcer/Crust (3, 50.0)	3.0 (2.6–3.8)	Simple wound care for 1	1/1 (100)
					IL Sb^v for 4	4/4 (100)
					Azole monotherapy for 1	1/1 (100)
	Complex	7	Ulcer/Nodule (4, 57.1)	5.0 (2.4–5.0)	**Simple wound care for 1**	1/1 (100)
					Sys Sb^v for 1	1/1 (100)
					LAmB for 4	2/2 (100)
					Other drug(s)/combination for 1	0/1 (0)
	Unclear	1	Ulcer	–	**Azole + local treatment for 1**	1/1 (100)
No complex/species typing	Simple	9	Ulcer (6, 66.7)	2.0 (1.5–2.5)	IL Sb^v for 6	4/6 (66.7)
					Sys Sb^v for 1	0/1 (0)
					Azole + local treatment for 2	2/2 (100)
	Complex	5	Ulcer/Nodule/ Crust (2, 40.0)	3.0 (1.0–5.0)	Sys Sb^v for 1	0/1 (0)
					LAmB for 1	0/0 (Unknown)
					Azole monotherapy for 1	1/1 (100)
					Azole + local treatment for 1	0/0 (Unknown)
New world species						
L. guyanensis species	Simple	4	Crust/Ulcer (2, 50.0)	2.3 (1.8–3.9)	**LAmB for 1**	0/0 (Unknown)
					Pentamidine for 2	1/2 (50.0)
					Azole monotherapy for 1	1/1 (100)
	Complex	3	Ulcer (3, 100)	5.5 (5.3–5.8)	Pentamidine for 3	3/3 (100)
	Unclear	1	Ulcer	–	**Azole monotherapy for 1**	1/1 (100)
L. panamensis species	Complex	4	Ulcer (3, 75.0)	4.5 (3.8–5.3)	**IL Sb^v for 2**	2/2 (100)
					Other local treatment for 1	0/1 (0)
					Pentamidine for 1	0/0 (Unknown)
L. guyanensis complex *(no species determined)*	Simple	5	Ulcer/Nodule/ Crust (2, 40.0)	4.0 (3.0–4.0)	IL Sb^v for 2	2/2 (100)
					Pentamidine for 2	2/2 (100)
					Azole monotherapy for 1	0/1 (0)
	Complex	7	Ulcer (5, 71.4)	4.0 (4.0–5.0)	Sys Sb^v for 2	1/1 (100)
					Pentamidine for 3	1/2 (50.0)
					Azole monotherapy for 1	0/1 (0)
					Other drug(s)/combination for 1	0/0 (Unknown)
L. braziliensis species	Simple	5	Ulcer (3, 60)	4.0 (1.0–4.0)	**Simple wound care for 1**	1/1 (100)
					IL Sb^v for 2	2/2 (100)
					Sys Sb^v for 1	1/1 (100)
	Complex	5	Ulcer (5, 100)	5.0 (4.5–5.3)	Sys Sb^v for 2	2/2 (100)
					LAmB for 3	1/2 (50.0)
	Unclear	2	Ulcer (1, 50)	NA	Sys Sb^v for 1	0/0 (Unknown)
L. braziliensis complex (no species determinedl)	Simple	1	Ulcer/Nodule	2.0	**Azole monotherapy for 1**	0/1 (0)
	Complex	1	Ulcer	6.0	Sys Sb^v for 1	1/1 (100)
L. amazonensis species	Simple	1	Ulcer	3.0	IL Sb^v for 1	1/1 (100)
L. mexicana species	Simple	1	Ulcer	1.0	IL Sb^v for 1	1/1 (100)
L. mexicana complex *(no species determined)*	Simple	1	Ulcer	2.0	IL Sb^v for 1	0/1 (0)
	Complex	1	Ulcer	6.0	Pentamidine for 1	0/0 (Unknown)
L. chagasi/infantum	1	1	Nodule	3.0	Surgical removal for 1	1/1 (100)
No species typing	1	1	Ulcer/Nodule	3.0	Sys Sb^v for 1	1/1 (100)

IL Sb^v: Intra-lesional Pentavalent Antimonials, Sys Sb^v: Systemic Pentavalent Antimonials, LAmB: Liposomal Amphotericin B, Azole: Fluconazole/Itraconazole/ketoconazole.

[a] For any treatment, only patients for which the treatment was known are added up.

[b] According to *Blum* et al.

[c] "Favorable" outcome: cure or improvement after 1st line treatment, when information was available.

of guidelines which are often still based on generally weak evidence from endemic settings only and which do not always clearly highlight the preferred choices, the delay in obtaining species results, the rather good clinical effectiveness of empirical treatments and the perceived convenience of some options (i.e. azoles). As said, there was a trend to better outcome in the group treated according to the species-targeted guidelines and this may convince some clinicians to better adhere to them. However, some factors that could not be recognized during the retrospective file survey may have influenced the outcome. Only robust prospective multicentric studies could answer whether adherence to species-directed guidelines for treatment leads to better outcomes, but such a study would be ethically difficult to set up. In our experience however, the availability of species typing results could help harmonize and optimize the management of CL. Indeed, it is likely that inconsistencies might be minimized, including between experts. Treatments could therefore be simplified, leading to less adverse events and substantial cost sparing with similar efficacy (short course of pentamidine (4 mg/kg/day for 3 days using preferentially the intravenous route [29]), simple wound care or non-prescription of therapies with no clear proof of efficacy). In addition, tailored counselling for species associated with good prognosis or long-term risk (i.e. *L. braziliensis*) can be provided with much more assurance, while uncertainty favors less rational decisions.

5. Conclusion

In conclusion, epidemiology and clinical presentation of leishmaniasis in Belgium were rather similar to previous observations in Europe, with increasing numbers in the last years. Treatment was extremely varied, in particular for CL/MCL, but final outcome was generally good. *Leishmania* species identification is considered helpful for an optimal and standardized management, but whether it really influences therapeutic decisions (and the final outcome) has hardly been investigated. Our study suggests that this "species-directed" approach was not routinely applied in our clinical practice, even if better outcomes were achieved. Pending results of prospective investigations, the present study may serve as a baseline for future comparisons of clinical management and care of leishmaniasis patients.

CRediT authorship contribution statement

Martin Vandeputte: Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Saskia van Henten:** Data curation, Formal analysis, Investigation, Methodology, Software, Writing - original draft. **Johan van Griensven:** Investigation, Writing - review & editing. **Ralph Huits:** Investigation, Writing - review & editing. **Marjan Van Esbroeck:** Data curation, Writing - review & editing. **Gert Van der Auwera:** Data curation, Investigation, Writing - review & editing. **Lieselotte Cnops:** Data curation, Investigation, Methodology, Supervision, Writing - review & editing. **Emmanuel Bottieau:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Writing - review & editing.

Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2020.101885.

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