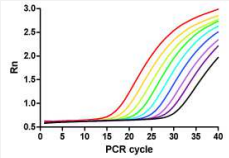
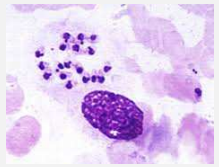
Molecular diagnostics Leishmania

Heeft de moleculaire diagnostiek de zorg mbt Leishmania veranderd?

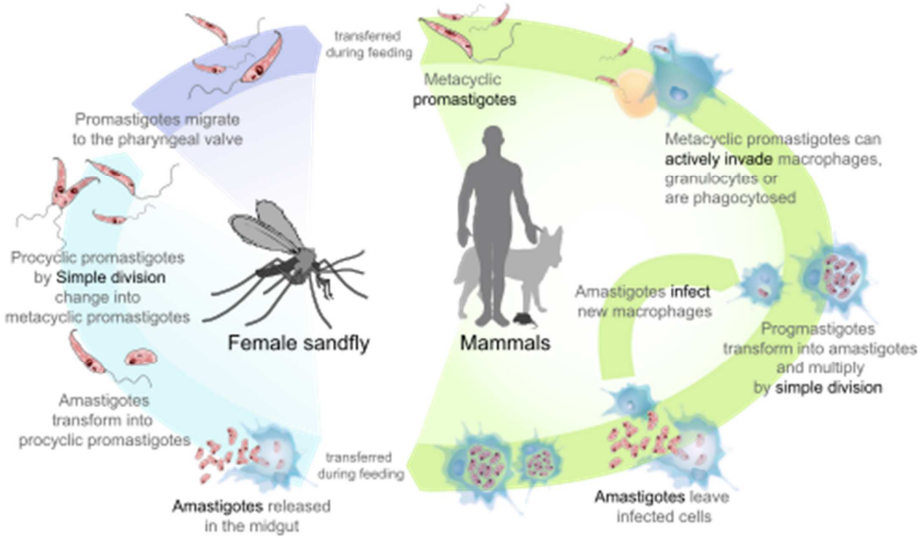




Leishmania

- Order Kinetoplastida;
  - Family Trypanosomatidae
    - Genus Leishmania
      - Subgenus Leishmania and Viannia
- Complex taxonomy
- Morphologically indistinguishable
- Cyclus uniform
- Vector = Sandfly
  - Phlebotomus
  - Lutzimyia

Lifecycle

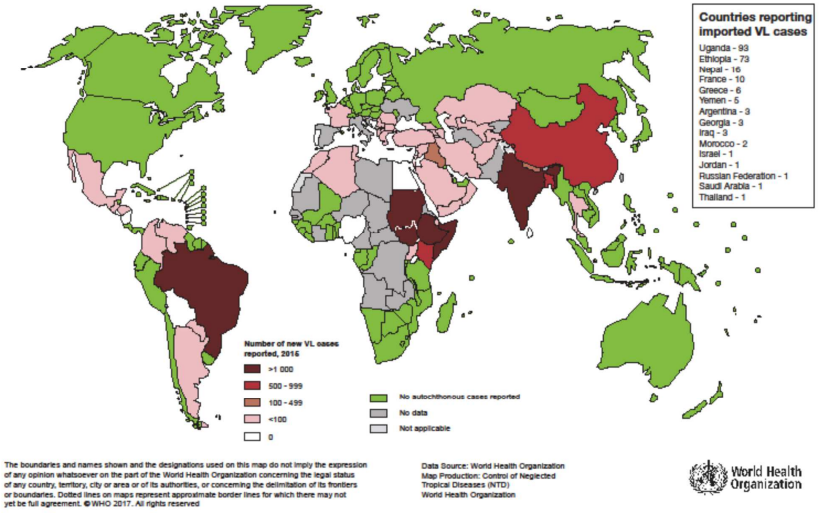


Leishmaniasis is primarily zoonotic with the exception of *Leishmania donovani* and *Leishmania tropica.*

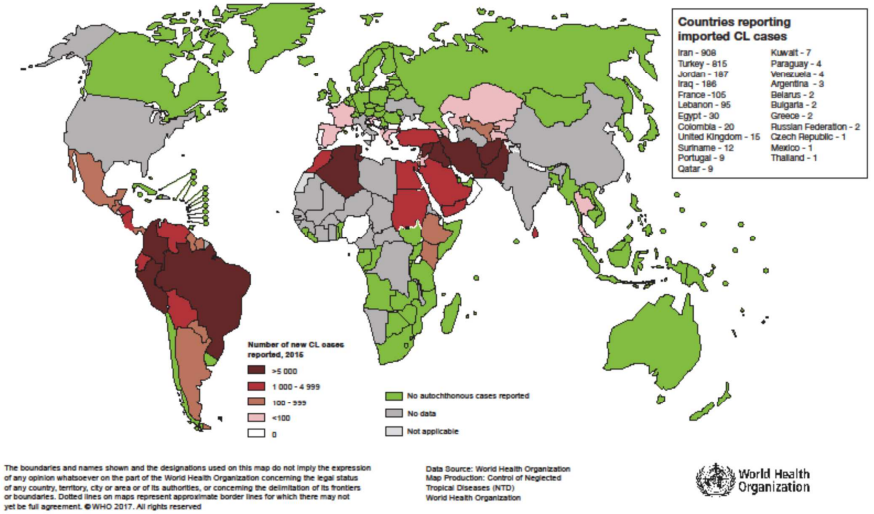
Leishmaniasis – Clinical Classification

- Visceral leishmaniasis
- Cutanuous leishmaniasis
- Muco-cutanuous leishmaniasis
- Parasite, host, geographical location, vector and other factors affect whether the infection becomes symptomatic and whether cutaneous, mucocutaneous or visceral leishmaniasis results.

Status of endemicity of visceral leishmaniasis worldwide, 2015



Status of endemicity of cutaneous leishmaniasis worldwide, 2015



Leishmaniasis – Clinical Classification

- Visceral leishmaniasis
  - *L. donovani* (Asia, Africa) - Kala-azar
    - Non-typical visceral disease (gulf war)
    - HIV co-infection, often reactivation
    - Post kala-azar dermal leishmaniasis (PKDL), develops most commonly after apparent cure from VL.
  - *L. infantum* (Europe, North Africa)
  - *L. chagasi* (South America)

Leishmaniasis – Clinical Classification

- Cutanuous leishmaniasis
  - Old world
    - *Leishmania tropica*
    - *Leishmania major*
    - *Leishmania infantum*
  - New world
    - *Leishmania mexicana*

Leishmaniasis – Clinical Classification

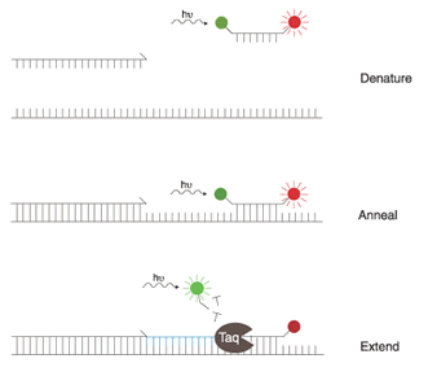
- Muco-cutanuous leishmaniasis
  - *Leishmania braziliensis*
    - Usually months to years after healing CL
    - Mucosal invasion of nose, mouth, throat cavities
    - Necrosis and destruction
    - No spontaneous healing
    - Secondary infection

Detection of Leishmania DNA and Species (complex) identification

- Microscopic diagnosis sometimes very difficult and time-consuming.
- Species (complex) identification has important implications for the clinical management of a patient.

Leishmania SSU real-time PCR

- Target
  - Small Sub Unit rRNA gene
- PCR
  - Product 62 bp
- Detection of all species
- No species differentiation
- Internal inhibition control



Am. J. Trop. Med. Hyg., 65(5), 2001, pp. 583–587

Child, 2 years old, Leukaemia?

Material	Date	Microscopy	PCR
bone marrow	20-04-2000	Poor quality	positive
bone marrow	27-04-2000	*Leishmania*	positive
EDTA blood	27-04-2000	-	positive

Differentiation *L. infantum*

Child, 2 years old, follow up

Sample	Date	Microscopy	PCR
Bone marrow	20-04-2000	Poor quality	Positive
Bone marrow	27-04-2000	*Leishmania*	Positive
EDTA blood	27-04-2000	-	Positive
EDTA blood	03-05-2000	-	Negative
EDTA blood	09-05-2000	-	Negative
EDTA blood	08-08-2000	-	Negative
EDTA blood	06-11-2000	-	Negative

PLOS ONE | PLOS ONE | https://doi.org/10.1371/journal.pone.0185606 September 28, 2017

Real-time PCR in detection and quantitation of *Leishmania donovani* for the diagnosis of Visceral Leishmaniasis patients and the monitoring of their response to treatment

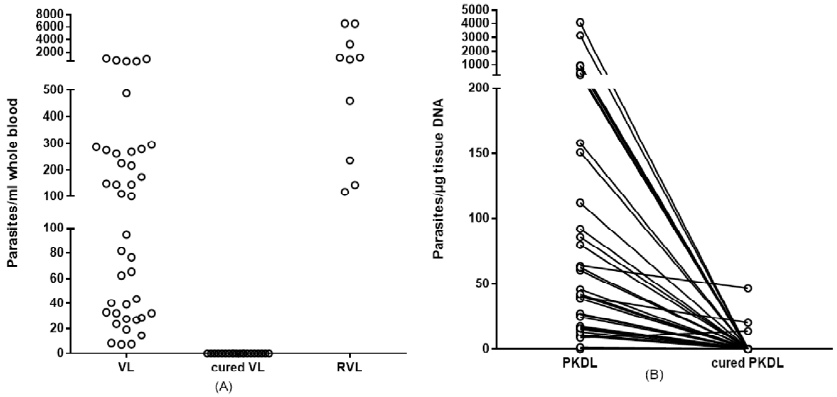
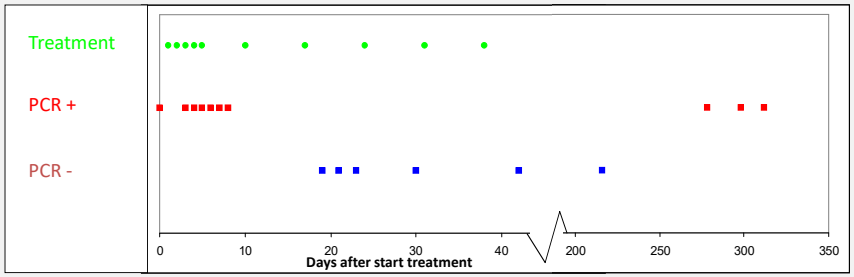


Fig 2. Parasite DNA can be detected in active, but not cured, disease states. In (A), parasite burden in blood samples from VL (n = 40), VL patients deemed to be cured by symptomatic response to treatment (n = 40) and RVL patients (n = 10) were measured. Each point indicates the data obtained from each individual sample. In (B), parasite burden in skin samples from PKDL patients before or after treatment (n = 40) were measured. Each point indicates the data obtained from each individual sample, and before and after data for each patient are linked by the connecting line.

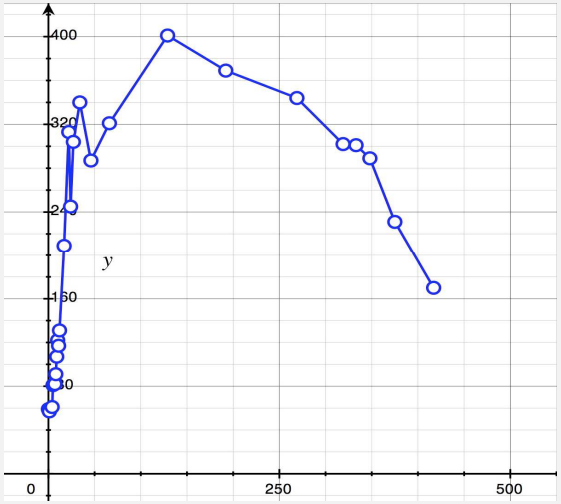
Patient D, 11-07-1968

- vD, 38 yr (F) – Kidney/pancreas TX history
- jan '06: intermittent fever, pancytopenia, abdominal discomfort
- Recent travel history
  - 1995 Gambia
  - 2005 Oregon
  - august 2005 Greece, Peloponessos
- She and her travel companion are frequently bitten by little flies on the beach

Follow-up after treatment using PCR on blood



Trombocyts



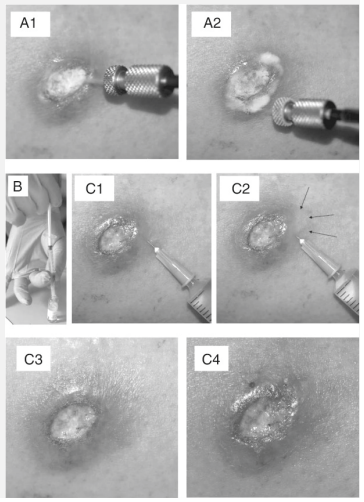
Detection of Leishmania DNA and Species (complex) identification

- Microscopic diagnosis sometimes very difficult and time-consuming.
  - Microscopy 15 minutes followed by PCR
  - Possibility of PCR on EDTA blood and follow-up after treatment
- Species (complex) identification has important implications for the clinical management of a patient.

LeishMan Recommendations for Treatment of Cutaneous and Mucosal Leishmaniasis in Travelers, 2014

Johannes Blum, MD,*†‡ Pierre Buffet, MD,§||¶ Leo Visser, MD,‡# Gundel Harms, MD,** Mark S. Bailey, MD,†† Eric Caumes, MD,‡‡‡ Jan Clerinx, MD,‡§§ Pieter P.A.M. van Thiel, MD,|||| Gloria Morizot, MD,§ Christoph Hatz, MD,*†‡ Thomas P.C. Dorlo,¶¶ and Diana N.J. Lockwood, MD##

- No antileishmanial treatment; simple wound care
- Local treatment
  - Cryotherapy
  - Heat therapy
  - Paromomycin ointment
- Intralesional treatment with pentavalent antimony
- Systemic treatment:
  - Pentavalent antimonials
  - Liposomal amphotericin B
  - Miltefosine



Species based Treatment of CL

Treatment of L. major

a. Up to three lesions, not cosmetically disfiguring, patients not immunosuppressed, option acceptable to patient:

No antileishmanial treatment; simple wound care

b. Up to three lesions with diameters <30 mm—local treatment:

1. "Flash" cryotherapy plus local infiltration with antimonials [**A**][42–46]
2. 15% Paromomycin or 12% methylbenzethonium chloride ointment bid for 10 to 20 days [**A**][47–51]
3. Local heat therapy (50°C for 30 seconds) [**A**][52–55]

c. More than three lesions, diameter >30 mm, delicate location, and/or refractory to local treatment:

1. Miltefosine (50 mg tid × 28 days) [**B**][56–58]
2. Fluconazole (200 mg bid × 6 weeks) [**C**][59–61]
3. Liposomal amphotericin B (18 mg/kg total dose: 3 mg/kg/day, days 1 to 5 and at day 10) [**D**][62]
4. Systemic pentavalent antimonial (Sb 20 mg/kg) and pentoxifylline (3 × 400 mg/20 days) [**A**] or systemic pentavalent antimonial (Sb 20 mg/kg for 10–20 days) [**D**][55,57,63–65]

Treatment of L. tropica, Leishmania infantum/donovani, *and* Leishmania aethiopica

a. Up to three lesions, not cosmetically disfiguring and patients not immunosuppressed, option acceptable to patient:

Simple wound care

b. Up to three lesions with diameter <30 mm—local treatment:

1. Local infiltration with antimonials with or without cryotherapy [**A**] [42,45,46]
2. 15% Paromomycin/12% methylbenzethonium chloride ointment bid for 10 to 20 days [**D**]
3. Local heat therapy (50°C for 30 seconds) [**A**][54,73]

c. More than three lesions, diameter >30 mm, delicate location, and/or refractory to local treatment:

1. Liposomal amphotericin B (18 mg/kg total dose: 3 mg/kg/day, days 1 to 5 and at day 10) [**C**][62,74]
2. Miltefosine (50 mg tid × 28 days) [**D**][75–77]
3. Pentavalent antimonials (Sb 20 mg/kg for 10–20 days) (+/− allopurinol) [**C**][73,78–80]

Species based Treatment of CL

Treatment of L. panamensis

Although many experts consider *L. guyanensis* and *L. panamensis* as a single species complex, we analyzed them separately, since trials were performed on each species. As knowledge of the taxonomy of *Leishmania* increases,

a. Single or few lesion(s), not cosmetically disfiguring, lesions with diameter <30 mm, no lymphatic spread, option acceptable to patient—local treatment considered:

1. 15% Paromomycin/12% methylbenzethonium chloride ointment [**B**][14,16]
2. Local heat therapy [**A**][90]

b. Multiple lesions or large single lesion—systemic treatment:

1. Miltefosine (50 mg tid × 28 days) [**A**][91–94]
2. Pentamidine isethionate (4 mg/kg, three infusions over 5 days) [**A**][35,95]
3. Ketoconazole (600 mg × 28 days) [**B**][39]
4. Pentavalent antimonials (Sb 20 mg/kg for 20 days) [**A**][35,90,96,97]

Treatment of L. braziliensis, Leishmania peruviana

L. braziliensis and *L. peruviana* species are genetically very similar. Data on treatment come from studies of *L. braziliensis* CL.

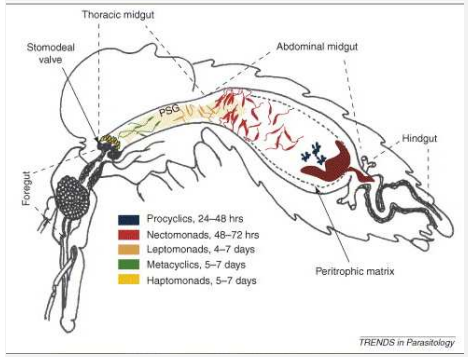
a. Single or few lesion(s), not cosmetically disfiguring, lesion with diameter <30 mm, no lymphatic spread, not from Bolivia—local treatment possible:

1. Local infiltration with antimonials +/− cryotherapy [**B**][17]
2. 15% Paromomycin/12% methylbenzethonium chloride ointment [**B**][18,88]
3. Thermotherapy [**A**][90]

b. All other cases—systemic treatment:

1. Pentavalent antimonials (Sb 20 mg/kg for 20 days) [**A**][37,90,93,94,103–105]
2. Liposomal amphotericin B (18 mg/kg total dose: 3 mg/kg/day, days 1 to 5 and at day 10) [**B**][62,106]
3. Miltefosine (only Bolivia, Brazil) (50 mg tid × 28 days) [**C**][105,107]

Leishmania taxonomy



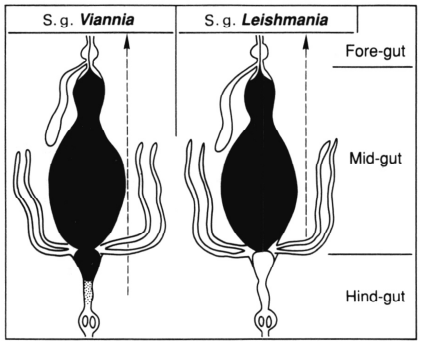


FIG. 1. — Sub-genus *Leishmania* Ross, 1903 and *Viannia* Lainson and Shaw, 1987. Classification based on behaviour in the gut of the sandfly host (according to Lainson and Shaw 1979 and 1987).

Leishmania taxonomy

Leishmania

- *Leishmania donovani species complex*
  - *Leishmania donovani*
- *Leishmania infantum species complex*
  - *Leishmania infantum*
  - *Leishmania chagasi*
- *Leishmania major species complex*
  - *Leishmania major*
  - *Leishmania cf. major*
- *Leishmania tropica species complex*
  - *Leishmania tropica*
- *Leishmania aethiopica species complex*
  - *Leishmania aethiopica*
- *Leishmania mexicana species complex*
  - *Leishmania mexicana*
  - *Leishmania amazonensis*
  - *Leishmania enriettii*
  - *Leishmania venezuelensis*

Viannia

- *Leishmania braziliensis species complex*
  - *Leishmania braziliensis*
  - *Leishmania colombiensis*
  - *Leishmania equatorensis*
  - *Leishmania peruviana*
  - *Leishmania garnhami*
- *Leishmania guyanensis species complex*
  - *Leishmania guyanensis*
  - *Leishmania panamensis*
  - *Leishmania shawi*
- *Leishmania naiffi species complex*
  - *Leishmania naiffi*

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Leishmania taxonomy

Leishmania

- ***Leishmania donovani species complex***
  - ***Leishmania donovani***
- ***Leishmania infantum species complex***
  - ***Leishmania infantum***
  - ***Leishmania chagasi***
- ***Leishmania mexicana species complex***
  - ***Leishmania mexicana***
  - ***Leishmania amazonensis***
  - ***Leishmania enriettii***
  - ***Leishmania venezuelensis***

Leishmania

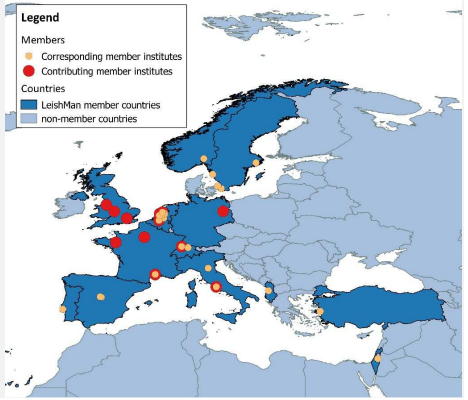
- ***Leishmania donovani species complex***
  - ***Leishmania chagasi***
  - ***Leishmania donovani***
    - ***Leishmania donovani archibaldi***
    - ***Leishmania donovani BHU 1095***
    - ***Leishmania donovani BPK282A1***
    - ***Leishmania donovani donovani***
    - ***Leishmania donovani Ld 2001***
    - ***Leishmania donovani Ld 39***
  - ***Leishmania infantum***
- ***Leishmania mexicana species complex***
  - ***Leishmania amazonensis***
  - ***Leishmania enriettii***
  - ***Leishmania mexicana***
    - ***Leishmania mexicana mexicana***
    - ***Leishmania mexicana MHOM/GT/2001/U1103***
    - ***Leishmania mexicana venezuelensis***
  - ***Leishmania pifanoi***

www.ncbi.nlm.nih.gov

European working group Leishman

- harmonising diagnostic and clinical management of leishmaniasis in Europe
  - to conduct inter-laboratory comparisons and quality controls for diagnosis and parasite collection procedures
  - to establish and validate a consensus on molecular species typing
  - to address taxonomic problems in human-pathogenic species of the *Leishmania* genus
  - to implement permanent exchange between specialists and harmonize treatment recommendations in Europe
  - to collect accurate information on the treatment of cutaneous and mucosal leishmaniasis in Europe

- >70 members
- Three papers on treatment
- Two papers on typing
- Patient database >400 cases,
- Inclusion of VL patients



www.leishman.eu

Surveillance of imported leishmaniasis with regard to diagnostic and therapeutic procedures

JCM
Journals.ASM.org

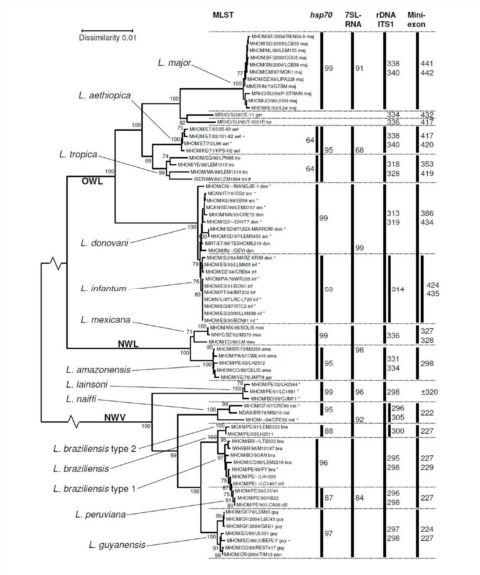
Evaluation of Four Single-Locus Markers for *Leishmania* Species Discrimination by Sequencing

Gert Van der Auwera,[a] Christophe Ravel,[b] Jaco J. Verweij,[c]* Aldert Bart,[d] Gabriele Schönian,[e] Ingrid Felger[f]

Institute of Tropical Medicine, Antwerp, Belgium[a]; University of Montpellier, UMR5290 MIVEGEC, and French Reference Centre on Leishmaniasis, Montpellier, France[b]; Department of Parasitology and Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands[c]; Department of Medical Microbiology, Center for Infection and Immunity Amsterdam, Academic Medical Center, Amsterdam, The Netherlands[d]; Institute of Microbiology and Hygiene, Charité University Medicine, Berlin, Germany[e]; Swiss Tropical and Public Health Institute and University of Basel, Basel, Switzerland[f]

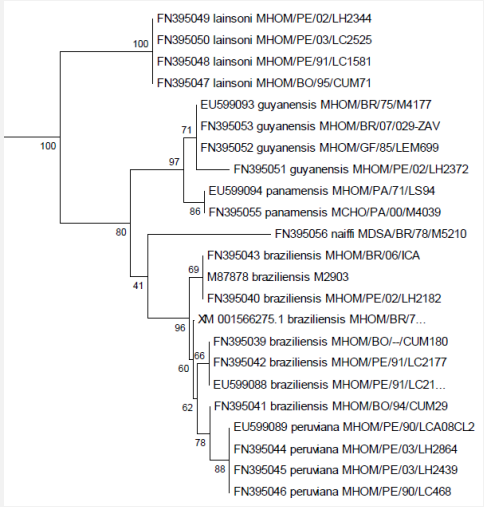
Journal of Clinical Microbiology p. 1098–1104 April 2014 Volume 52 Number 4

Comparison sequence based differentiation of Leishmania species



Journal of Clinical Microbiology p. 1098–1104 April 2014 Volume 52 Number 4

Leishmania differentiation using HSP70 sequencing

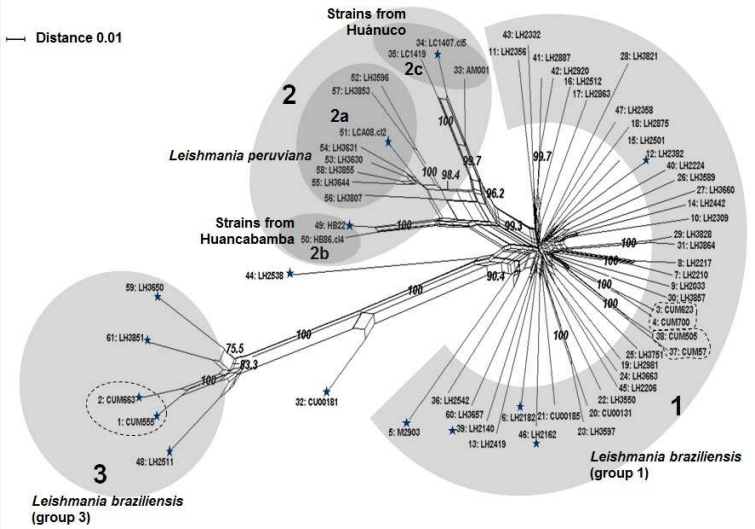


Journal of Clinical Microbiology p. 1098–1104 April 2014 Volume 52 Number 4

Infection, Genetics and Evolution 12 (2012) 1994–2002

Evolution of the *Leishmania braziliensis* species complex from amplified fragment length polymorphisms, and clinical implications

Samwel Odiwuor [a,c,d], Nicolas Veland [b], Ilse Maes [a], Jorge Arévalo [b], Jean-Claude Dujardin [a,d], Gert Van der Auwera [a,*]



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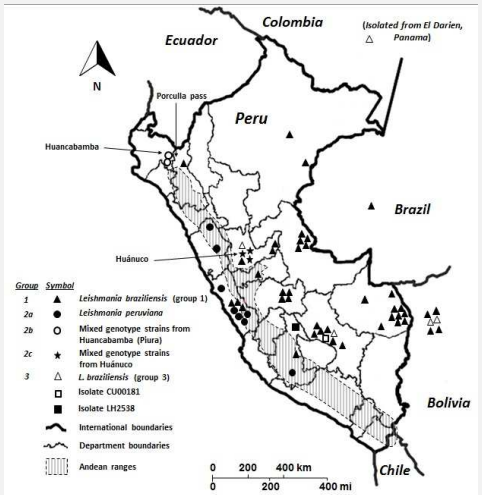

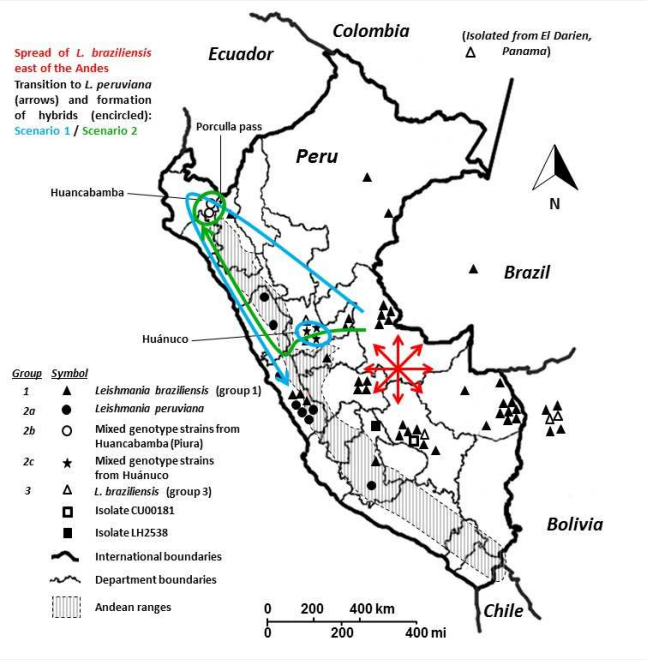

RESEARCH ARTICLE

www.eurosurveillance.org

Comparison of *Leishmania* typing results obtained from 16 European clinical laboratories in 2014

G Van der Auwera [1], A Bart [2], C Chicharro [3], S Cortes [4], L Davidsson [5], T Di Muccio [6], J Dujardin [1 7], I Felger [8 9], MG Paglia [10], F Grimm [11], G Harms [12], CL Jaffe [13], M Manser [14], C Ravel [15], F Robert-Gangneux [16], J Roelfsema [17], S Töz [18], JJ Verweij [19], PL Chiodini [20 21]

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5. The Public Health Agency of Sweden, Stockholm, Sweden
6. Istituto Superiore di Sanità, Rome, Italy
7. Biomedical Sciences, Antwerp University, Antwerp, Belgium
8. Swiss Tropical and Public Health Institute, Basel, Switzerland
9. University of Basel, Basel, Switzerland
10. National Institute for Infectious Diseases (INMI) Lazzaro Spallanzani, Rome, Italy
11. Institute of Parasitology, University of Zürich, Zürich, Switzerland
12. Institute of Tropical Medicine and International Health, Charité-Universitätsmedizin Berlin, Berlin, Germany
13. Hebrew University, Hadassah Medical Centre, Jerusalem, Israel
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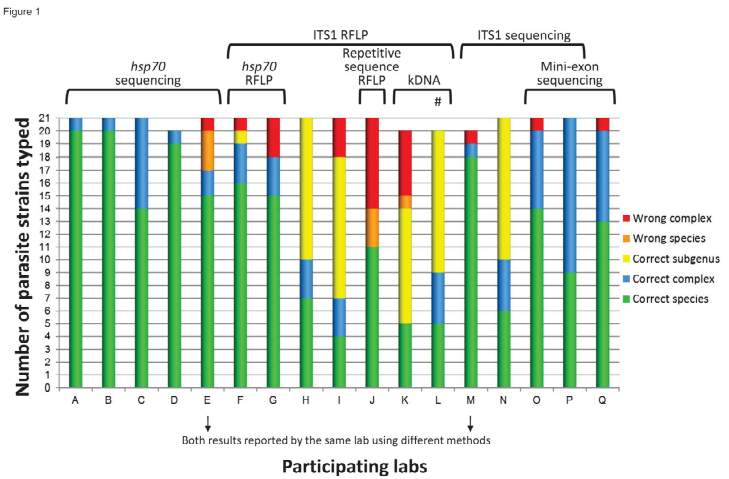
19. St. Elisabeth Hospital, Tilburg, The Netherlands
20. Hospital for Tropical Diseases, London, United Kingdom
21. London School of Hygiene and Tropical Medicine, London, United Kingdom

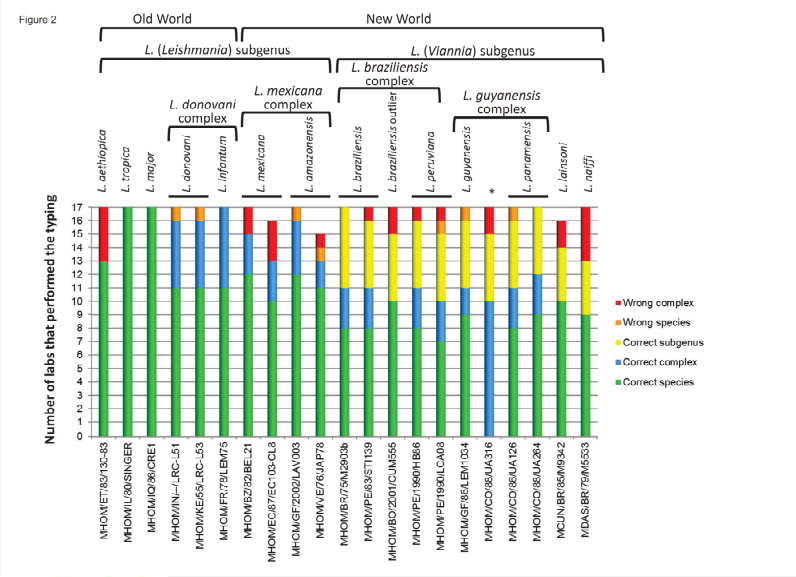
Comparison of *Leishmania* typing results in 16 European clinical laboratories

Reference methods:
multilocus enzyme electrophoresis (MLEE), multilocus sequence analysis (MLSA), amplified fragment length polymorphism analysis (AFLP) , whole genome sequencing



LeishMan Management
ESCMID MANAGING INFECTIONS PROMOTING SCIENCE

Comparison of *Leishmania* typing results in 16 European clinical laboratories



LeishMan Management
ESCMID MANAGING INFECTIONS PROMOTING SCIENCE

Research

Whole genome sequencing of multiple *Leishmania donovani* clinical isolates provides insights into population structure and mechanisms of drug resistance

Tim Downing,[1,10] Hideo Imamura,[2,10] Saskia Decuypere,[2] Taane G. Clark,[3] Graham H. Coombs,[4] James A. Cotton,[1] James D. Hilley,[5] Simonne de Doncker,[2] Ilse Maes,[2] Jeremy C. Mottram,[5] Mike A. Quail,[1] Suman Rijal,[6] Mandy Sanders,[1] Gabriele Schönian,[7] Olivia Stark,[7] Shyam Sundar,[8] Manu Vanaerschot,[2] Christiane Hertz-Fowler,[1,9] Jean-Claude Dujardin,[2,11] and Matthew Berriman[1,11]

Genome Research
www.genome.org

Whole genome sequencing of multiple *Leishmania donovani* clinical isolates provides insights into population structure and mechanisms of drug resistance

Table 1. Coding SNPs showing greatest differentiation between lines resistant and susceptible to SSG treatment

F_{ST}	C[a]	Position	S[b]	R[c]	Type[d]	Gene product	Site	Gene ID
0.54	34	1046281[e]	0.81	0.00	N	Conserved hypothetical protein	H523R[f]	LdBPK_342390
0.44	2	36464	1.00	0.43	N	Phosphatidylinositol 3 kinase	R3452H	LdBPK_020100
0.44	4	337729[g]	1.00	0.43	N	Rhomboid-like serine protease	A113V	LdBPK_040850
0.44	7	383016	1.00	0.43	Syn	Phosphoacetylglucosamine mutase	161V	LdBPK_070930
0.44	12	128965	1.00	0.43	N	Conserved hypothetical protein	Y1230C	LdBPK_120270
0.44	16	690776	1.00	0.43	N	Conserved hypothetical protein	Y1214C	LdBPK_161760
0.44	19	412141	1.00	0.43	Syn	4-coumarate CoA ligase	395V	LdBPK_190970
0.44	21	412303	1.00	0.43	Syn	Hypothetical protein	251P	LdBPK_211240
0.44	24	26882	1.00	0.43	N	Ankyrin/TPR repeat protein	A150V	LdBPK_240130
0.44	25	702972[g]	1.00	0.43	N	Conserved hypothetical protein	A61F	LdBPK_251890
0.44	31	553554[g]	1.00	0.43	Syn	Conserved hypothetical protein[h]	426T	LdBPK_311340
0.44	31	555080	1.00	0.43	N	Conserved hypothetical protein	S268P	LdBPK_311340
0.44	32	357021[g]	1.00	0.43	N	Conserved hypothetical protein	V125L	LdBPK_320990
0.44	35	1773762[g]	1.00	0.43	N	Conserved hypothetical protein	A54T	LdBPK_354470
0.44	36	886126[g]	1.00	0.43	Syn	Conserved hypothetical protein	347T	LdBPK_362330
0.42	33	1316522[e]	0.20	0.86	N	Conserved hypothetical protein	A390V	LdBPK_333140
0.40	34	493541[e]	0.78	0.14	Syn	Amastin-like surface protein	209A	LdBPK_341150

selection in a range of surface- and transport-related genes, including genes associated with drug resistance

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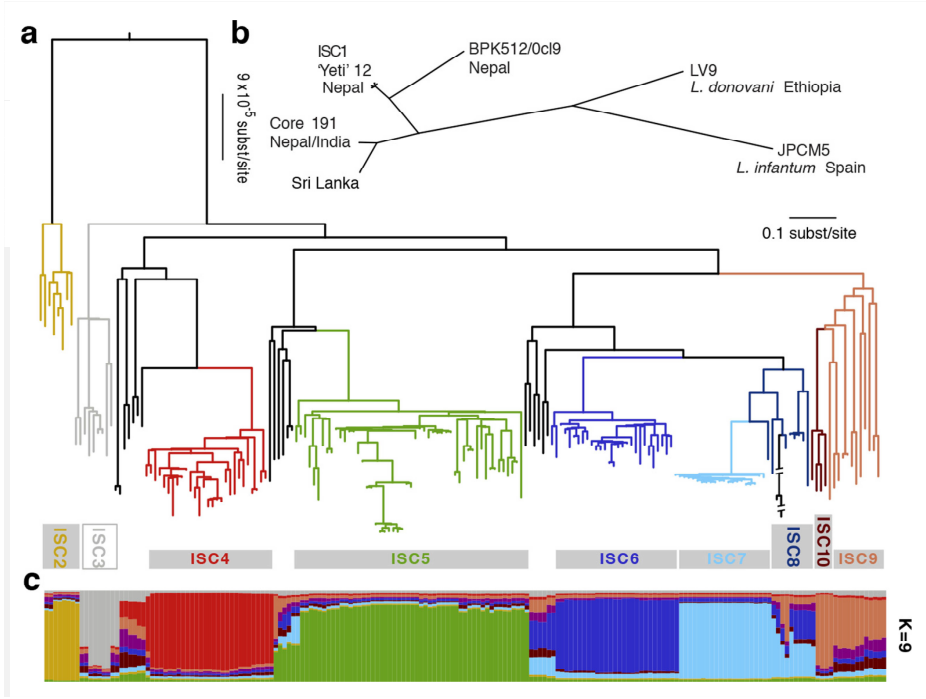
RESEARCH ARTICLE

Evolutionary genomics of epidemic visceral leishmaniasis in the Indian subcontinent

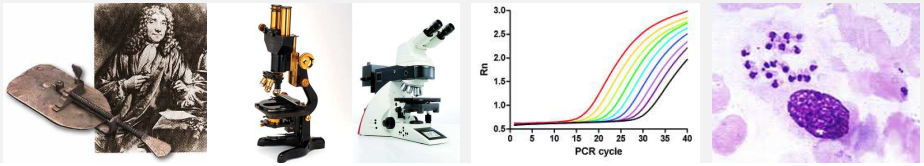
Hideo Imamura[1†], Tim Downing[2,3†‡], Frederik Van den Broeck[1†], Mandy J Sanders[2], Suman Rijal[4], Shyam Sundar[5], An Mannaert[1], Manu Vanaerschot[1§], Maya Berg[1], Géraldine De Muylder[1], Franck Dumetz[1], Bart Cuypers[1], Ilse Maes[1], Malgorzata Domagalska[1], Saskia Decuypere[1,6], Keshav Rai[4¶], Surendra Uranw[4], Narayan Raj Bhattarai[4], Basudha Khanal[4], Vijay Kumar Prajapati[5**], Smriti Sharma[5], Olivia Stark[7], Gabriele Schönian[7], Harry P De Koning[8], Luca Settimo[8,9], Benoit Vanhollebeke[10], Syamal Roy[11], Bart Ostyn[12], Marleen Boelaert[12], Louis Maes[13], Matthew Berriman[2], Jean-Claude Dujardin[1,13*], James A Cotton[2*]



Molecular diagnostics Leishmania

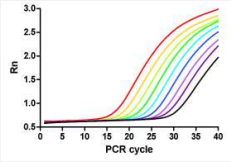
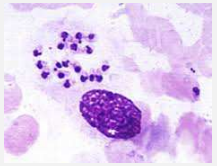
Heeft de moleculaire diagnostiek de zorg mbt Leishmania veranderd?

- Microscopic diagnosis sometimes very difficult and time-consuming.
  - PCR detection is sensitive and specific
  - (Microscopy 15 minutes followed by PCR)
  - Possibility of PCR on EDTA blood and follow-up after treatment
- Species (complex) identification has important implications for the clinical management of a patient.
  - Taxonomy is complex, species definition is not always clear-cut
  - Resolution of identification is target depended
  - No clinical application for WGS yet.



Molecular diagnostics Leishmania

Thanks for listening

Questions?

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