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Leishmaniavirus genetic diversity is not related to leishmaniasis treatment failure

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1 **Title: *Leishmaniavirus* genetic diversity is not related to leishmaniasis treatment failure.**

2 **Running title: LRV1 genetic diversity**

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38 **Abstract**

39 *Objectives*: The outcome of American tegumentary leishmaniasis (ATL) may depend on the presence
40 of the *Leishmania* RNA virus (LRV). This virus may be involved in treatment failure. We aimed to
41 determine whether genetic clusters of LRV1 are involved in this therapeutic outcome.

42 *Methods*: The presence of LRV1 was assessed in 129 *L. guyanensis* isolates from patients treated with
43 pentamidine in French Guiana. Among the 115 (89%) isolates found to carry LRV1, 96 were
44 successfully genotyped. Patient clinical data were linked to the LRV data.

Results: The rate of treatment failure for LRV1-positive isolates was 37% (15/41) versus 40% (2/5) among LRV1-negative isolates (p = 0.88). Concerning LRV1 genotypes, two predominant LRV1 groups emerged, groups A (23% (22/96)) and B (70% (67/96)). The treatment failure rate was 37% (3/8) for group A and 45% (9/20) for group B (p = 0.31).

Conclusion: Neither the presence or genotype of LRV1 in patients with *L. guyanensis* seemed to correlate with pentamidine treatment failure.

Key words: *Leishmania guyanensis*, LRV1, genotype, treatment failure, pentamidine

Introduction

Leishmaniases are infectious diseases caused by *Leishmania* (*Trypanosomatidae*) parasites, which are transmitted through the bites of infected female sandflies. The disease is characterized by a wide range of clinical presentations, from asymptomatic to severe forms, depending on the *Leishmania* species. American tegumentary leishmaniasis (ATL) is characterized by cutaneous lesions and can result in self-healing or spread of the lesions. Localized cutaneous leishmaniasis (LCL) is defined as a skin lesion localized at the point of the vector bite. In some patients, parasites spread throughout the body, causing cutaneous diffuse leishmaniasis (CDL). Mucocutaneous leishmaniasis (MCL) occurs when the parasite spreads from a cutaneous lesion to nasopharyngeal areas of the face, leading to destructive metastatic secondary lesions.

The presence of the *Leishmania* RNA virus (LRV) may be a factor associated with the development of mucosal leishmaniasis (1–4). LRV is a non-enveloped double-stranded RNA virus belonging to the *Totiviridae* family. This virus, initially believed to be exclusively hosted by *Leishmania* parasites, was recently found in another *Trypanosomatidae* of the genus *Blechomonas* (5). LRV has been described in New- and Old-World strains of *Leishmania*, named LRV1 and LRV2, respectively, and has been

69 particularly studied for the *Viannia* subgenus. Six phylogenetic groups of LRV1 can be distinguished in
70 this subgenus, from A to F, with two predominant clusters, A and B. Clusters A to E are comprised of
71 *L. guyanensis* parasites and cluster F includes two isolates from *L. braziliensis* (6).

72 LRV1 appears to play a role in disease progression by directing it towards the severe MCL (1). Indeed,
73 the presence of this virus, carried by *L. guyanensis* parasites, appears to modulate the host immune
74 response in a murine model, leading to a higher susceptibility to the infection, an increase in parasite
75 burden, swelling of the lesions, and metastases. The virus appears to activate host immune
76 receptors, such as Toll-Like Receptor-3 (TLR-3), inducing pro-inflammatory cytokine synthesis and
77 leading to an excessive immune response. The exaggerated immune response directed initially
78 against the parasites simultaneously affects host tissues, leading to their substantial degradation (1).
79 This exacerbation may be amplified by association of the virus with parasitic exosomes (7) but can be
80 prevented by immunization with LRV capsid proteins in mice (8). In humans, LRV1 appears to be a
81 predictive factor of first-line treatment failure and symptomatic relapses (9). However, a recent study
82 has suggested otherwise (10). Here, we aimed to determine whether the presence of LRV1 or one of
83 its genotypes correlates with treatment failure.

84 **Materials and methods**

85 **Patients**

86 The study, conducted in French Guiana, included patients with a diagnosis of tegumentary
87 leishmaniasis due to *L. guyanensis* between February 2012 and May 2016.

88 The diagnosis consisted of a biopsy of the interior of the lesions of the patients, followed by
89 incubation of the biopsy in RPMI-1640 (Gibco®), containing L-glutamine, 20 mM HEPES, and phenol
90 red, supplemented with 20% heat- inactivated fetal calf serum (FCS) (Gibco), 50 IU/mL penicillin
91 (Invitrogen®), 0.05 mg/mL streptomycin (Invitrogen), and nonessential amino acids (Gibco), at 26°C
92 to allow development of the parasite. Part of the culture was used for the routine diagnosis of
93 *Leishmania* species performed by the Cayenne hospital by PCR-RFLP (11) and the other kindly

94 supplied by the Cayenne hospital for LRV1 detection and analysis. Patients diagnosed as positive for
95 *L. guyanensis* but with an unsuccessful culture were not included in the study.

96 In total, 332 cultures were available. The isolates were associated with patient data. Patients without
97 therapeutic outcome data after the first round of treatment and those under 18 years of age were
98 excluded from the study. Finally, 129 patients were included. A sensitivity analysis was carried out to
99 ensure that patient exclusion did not affect the study results (data not shown).

100 **LRV RNA extraction**

101 Upon reaching stationary phase, fresh *Leishmania* cultures were counted under a microscope. Pellets
102 of 1.10^7 parasites were prepared by a 5-min centrifugation at 587 x g and elimination of the
103 supernatant. Cells were preserved at -25°C until use.

104 Total RNA was extracted from *Leishmania* promastigotes using the RNeasy mini kit® according to the
105 manufacturer's recommendations (except for centrifugations of 15 s, which were extended to 30 s
106 because of the characteristics of the centrifuge). The RNA was stored at -80°C until use.

107 **LRV Reverse transcription**

108 RNA reverse transcription was performed using SuperScript™ II Reverse Transcriptase (Invitrogen)
109 with random hexamers (Invitrogen), according to the manufacturer's recommendations.

110 **LRV1 detection by PCR**

111 LRV1 was detected by amplification of a 124-bp fragment by PCR with the LRV1 forward primer,
112 LRV1-F1: 5'- CTGACTGGACGGGGGGTAAT-3' and the LRV1 reverse primer, LRV1-R1: 5'-
113 CAAAACACTCCCTTACGC-3', at a final concentration of 0.2 µM (12). The 25-µL reaction mixture
114 included 1X PCR master mix (BiotechRabbit™), 0.2 µM of each primer, and 2 µL cDNA. A denaturation
115 step at 94°C for 2 min was followed by 40 cycles at 94°C for 30 s, 54°C for 30 s, and 72°C for 1 min.
116 The PCR was completed by a final elongation step at 72°C for 5 min. PCR products were separated on
117 a 2% agarose gel with Midori Green Advance ® to verify the presence of amplification products of the

118 expected size. The reference strain of *L. guyanensis* (MHOM/GF/97/LBC6) was used as a positive
119 control and water as a negative control in each PCR experiment.

120 **LRV genotyping**

121 ***PCR***

122 Positive LRV1 samples were genotyped using the forward primer LRV1s 5'-
123 ATTCGCTAGCTGTYBGGATGGTAGYGTTAC-3' and the reverse primer LRV2as 5'-
124 CATAGCCAAAACGTTCACAWARTGTYGRGTGT-3' (6), amplifying a 779 bp product. These primers
125 target the ORF1 and ORF2 sequences that encompass the sequence amplified by the LRV1-F1/LRV1-
126 R1 primers used for LRV1 detection. The 50-µL reaction mixture included 1X AmpliTaqGold master
127 mix (Applied Biosystem™), 0.2 µM of each primer, and 5 µL cDNA. A denaturation step at 94°C for 5
128 min was followed by 35 cycles at 94°C for 30 s, 62°C for 30 s, and 72°C for 1 min. The PCR was
129 completed by a final elongation step at 72°C for 5 min.

130 Fragments not amplified by the LRV1s/LRV2as primers were amplified using another primer pair
131 proposed by Cantanhêde et. al (13), surrounding the sequence amplified by the LRV1s/LRV2as
132 primers. These primers were LRV1 F orf1 5'-ATGCCTAAGAGTTTGGATTCG-3' and LRV R orf2 5'-
133 AATCAATTTTCCCAGTCATGC-3', amplifying an 850 bp sequence.

134 The 50-µL reaction mixture included 1X AmpliTaqGold master mix (Applied Biosystem™), 0.4 µM of
135 each primer, and 5 µL of cDNA. A denaturation step at 94°C for 2 min was followed by 40 cycles at
136 94°C for 30 s, 56°C for 45 s, and 72°C for 30 s. The PCR was completed by a final elongation step at
137 72°C for 3 min.

138 PCR products were separated on a 2% agarose gel containing Midori Green Advance® to verify the
139 presence of amplification products of the expected size.

140 ***Sequencing***

141 Sample purification and sequencing were performed from 40 µL of PCR product by the sequencing
142 platform of l'Hôpital Cochin (Eurofins France) with the corresponding primers used for fragment
143 amplification. Both strands of each sample were sequenced and the results delivered by the
144 subcontractor, Eurofins.

145 ***Sequence analysis***

146 Sequence complementarity (forward and reverse) obtained for each sample was tested using BioEdit
147 software (version 7.0.5.3) (14) to confirm the sequencing. The consensus sequence resulting from
148 two strictly identical opposed sequences was selected for the genetic analysis. We also included the
149 24 sequences from the study of Tirera et al. (6), 11 of which corresponded to the isolates used in our
150 study. All LRV sequences were aligned with MEGA 7 software (15) using the MUSCLE program. Some
151 alignment corrections were made manually. A phylogenetic tree was constructed using SeaView
152 software (16), based on maximum likelihood phylogenies (PhyML) and the K80 model (default
153 settings).

154 **Clinical data**

155 Clinical data were retrospectively sought for the 129 *Leishmania* samples in the various services of
156 the hospital or health centers spread throughout the territory. Collected data included age, gender,
157 suspected place of infection, number of lesions, lesion size, nodules, papules, satellite papules,
158 lymphangitis, adenopathy, and treatment failure.

159 **Data analysis**

160 R software (version 3.2.0) was used for data analysis. Univariate and multivariate variables were
161 analyzed by logistic regression. Variables included in the statistical analysis were the presence of
162 LRV1, LRV1 genotype, age, gender, suspected place of infection, time between infection and
163 treatment, time between two rounds of pentamidine, number of lesions, lesion size, lymphangitis,
164 adenopathy, nodule, papule, satellite papules, and treatment failure after one or two courses of
165 pentamidine.

166 LRV1 genotypes were separated into six groups, from A to F. Only groups A and B were analyzed. The
167 other groups were excluded due to their small size. Age was divided into two groups: from 18 to 35
168 years and > 35. The suspected locations of infection were grouped into two areas: littoral (Iracoubo,
169 Macouria, Sinnamary, Kourou, Matoury, Cayenne, Rémire-Montjoly) and inland (Régina, Saül, Saint-
170 Elie, Roura, Montsinéry-Tonnegrande, Saint-Georges, Camopi, Trois-Sauts, Saint-Laurent du Maroni,
171 Apatou, Grand-Santi, Papaïchton and Maripasoula). Patients who consulted in French Guiana but
172 were infected in neighboring countries were included in the study. Thus, Suriname and Brazil were
173 also included in the inland group. Lesion number was divided into two groups, the first consisting of
174 patients with one lesion and the second, those with more than one lesion. Lesion size was also
175 divided into two groups, the first consisting of patients with lesions ≤ 2 cm and the second, those > 2
176 cm.

177 Patients included in the study were treated by intramuscular administration of 7 mg/kg pentamidine,
178 divided between two injections given in a single day at different body sites. In the absence of healing
179 of the lesion at least one month after the first treatment (treatment 1), a second dose of
180 pentamidine was administered by the intramuscular route. Treatment failure was considered when
181 patients presented a persistent lesion at least one month after the second course of treatment
182 (treatment 2). Patients showing treatment failure were hospitalized for meglumine antimoniate
183 (Glucantime®) treatment. The influence of LRV on therapeutic failure was determined after the first
184 and second round of treatment.

185 **Ethical approval**

186 Access to these data has been authorized by the Comité de Protection des Personnes (CPP) SUD-EST
187 II, n° ID-RCB: 2017-A00173-50. A declaration was also made to the Commission Nationale de
188 l'Informatique et des Libertés (CNIL), n° peV2056324t.

189 **Results**

190 **LRV and clinical outcomes**

191 The study included 129 *L. guyanensis* isolates, for which the presence of LRV was sought. The results
192 were associated with the available data on the various clinical features of the patients to identify the
193 involvement of this virus in the pathophysiology of the disease (Table 1, see web-only Supplementary
194 Table S1). Thus, among the 115 (89%) *Leishmania* samples harboring LRV1, 63% (73/115) resulted in
195 the patients being unresponsive to the first round of pentamidine versus 57% (8/14) for the LRV-
196 negative patients. In the second round of treatment, 37% of the 41 positive LRV1 isolates (15/41)
197 were associated with treatment failure versus 40% (2/5) for the LRV-negative patients. There was no
198 significant association between treatment failure and the presence of LRV1 ($p = 0.88$).

199 Statistical analysis of the other variables showed that LRV1 did not affect the development of
200 adenopathy, lymphangitis, papules, satellite papules, nodules, or the size or number of lesions.
201 Neither age nor gender influenced the development of LRV1.

202 Geographical analysis showed a much stronger presence of LRV1 inlands than at the coastline (OR =
203 9.8, $p = 0.0008$).

204 **LRV genotyping**

205 LRV1 amplification by PCR using the various primers, as part of the sequencing, is shown in Figure 1.
206 Among the 115 LRV1 isolates, 96 were successfully sequenced and used to construct a phylogenic
207 tree. Thirteen other sequences, from the study of Tirera et al., were also included (6). The phylogenic
208 tree (see web-only Supplementary Figure S1) highlighted five groups, from A to E. The groups A and B
209 were predominant, accounting for 22 (23%) and 67 (70%) of the LRV1 isolates, respectively. Groups D
210 and E accounted for 1 (1%) and 6 (6%) LRV1 isolates. No LRV1 isolates used in this study belonged to
211 groups C or F.

212 **LRV genotypes related to clinical outcome**

213 Among the 96 genotyped LRV isolates, 89 were distributed within clusters A and B. In group A, 59%
214 (13/22) of the LRV isolates were associated with the unresponsiveness of patients to the first round
215 of pentamidine and 63% (42/67) in group B. In the second round of treatment, 37% (3/8) of the LRV

216 isolates of group A were associated with treatment failure versus 45% (9/20) in group B. None of the
217 LRV1 groups seemed to be associated with treatment failure (p = 0.31) or to the development of
218 adenopathy, lymphangitis, papules, satellite papules, nodules, large lesions, or the number of lesions.
219 The LRV1 groups did not appear to be associated with age, gender, or geographic area (Table 2, see
220 web-only Supplementary Table S1).

221 **Discussion**

222 The presence of LRV1 has been shown to be a risk factor for therapeutic failure (9,17). In the present
223 study, we found no correlation between the presence of LRV1 or its genotypes in *L. guyanensis*
224 parasites and treatment failure, either after the first or second course of treatment of pentamidine.
225 The main difference, and limitation, of this study relative to the others was that this study was
226 retrospective and included patients with non-standardized clinical monitoring. Our results contrast
227 with those of Bourreau et al. and Adaui et al. (9,17), but are in accordance with those of Christen et
228 al. (10). A prominent difference between our study, that of Bourreau et al. (9), and that of Christen et
229 al. (10) was the diagnostic methodology used. Bourreau et al. performed LRV1 detection directly on
230 biopsies, whereas we detected LRV1 from cultures. Christen et al. used both methodologies, biopsies
231 or cultures, depending on the case (personal communication). However, according to Bourreau et al.,
232 LRV1 prevalence was higher in parasite cultures (87%) than in biopsies (58%). Such a higher
233 prevalence of LRV1 in cultures was observed in our study and that of Christen et al., 89% and 85%,
234 respectively. Indeed, low amounts of LRV1 in a biopsy may render the virus undetectable by
235 molecular biology, whereas it may be detected in culture. This may lead to bias, especially since,
236 according to Ives et al., the magnitude of the immune response induced by LRV1 should depend on
237 its amount (1). This could explain the absence of a correlation between the presence of LRV1 and
238 treatment failure in the present study. Nevertheless, Adaui et al. found a correlation between the
239 presence of LRV1 in cultivated *L. braziliensis* isolates and treatment failure (17). However, Bourreau
240 et al. reported that treatment failure did not correlate with the LRV1 load per parasite (9) and
241 another study reported that the LRV1 burden (genome equivalent) did not correlate with the state of

242 the lesion (active lesion, lesion in the process of scarring, or scar), as the amount of LRV1 was highly
243 variable, regardless of the state of the lesion (18). Another difference observed between these
244 various studies was that Adaui et al. and Bourreau et al. included relapsing patients, which was not
245 the case in our study or that of Christen et al. (9,10,17). Pereira et al. also reported that LRV1 was
246 detected in patients with disease reactivation (19). Thus, LRV1 may be only responsible for relapses.
247 Future studies to investigate the association of LRV1 genotypes with disease should therefore only be
248 carried out on samples from relapsing patients.

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259

260 **Conflict of interest**

261 The authors declare that they have no competing interests.

262

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Authors' contributions

The conception and design of the study: GP, PC and MG. Acquisition of data: all the authors. Analysis and interpretation of data: SB, GP, MG. Drafting the article: GP, PC, SB, MG. Revising it critically for important intellectual content: all authors. Final approval of the version to be submitted: all authors.

References

1. Ives A, Ronet C, Prevel F, Ruzzante G, Fuertes-Marraco S, Schutz F, et al. Leishmania RNA Virus Controls the Severity of Mucocutaneous Leishmaniasis. Science. 2011 Feb 11;331(6018):775–8.

2. Cantanhêde LM, da Silva Júnior CF, Ito MM, Felipin KP, Nicolete R, Salcedo JMV, et al. Further Evidence of an Association between the Presence of Leishmania RNA Virus 1 and the Mucosal Manifestations in Tegumentary Leishmaniasis Patients. Bates PA, editor. PLOS Neglected Tropical Diseases. 2015 Sep 15;9(9):e0004079.

3. Ito MM, Catanhêde LM, Katsuragawa TH, da Silva Junior CF, Camargo LMA, Mattos R de G, et al. Correlation between presence of Leishmania RNA virus 1 and clinical characteristics of nasal mucosal leishmaniosis. Brazilian Journal of Otorhinolaryngology. 2015 Sep;81(5):533–40.

4. de Carvalho RVH, Lima-Junior DS, da Silva MVG, Dilucca M, Rodrigues TS, Horta CV, et al. Leishmania RNA virus exacerbates Leishmaniasis by subverting innate immunity via TLR3-mediated NLRP3 inflammasome inhibition. Nat Commun. 2019 Dec;10(1):5273.

290 5. Grybchuk D, Akopyants NS, Kostygov AY, Konovalovas A, Lye L-F, Dobson DE, et al. Viral
291 discovery and diversity in trypanosomatid protozoa with a focus on relatives of the human
292 parasite *Leishmania*. Proceedings of the National Academy of Sciences. 2018 Jan
293 16;115(3):E506–15.

294 6. Tirera S, Ginouves M, Donato D, Caballero IS, Bouchier C, Lavergne A, et al. Unraveling the
295 genetic diversity and phylogeny of Leishmania RNA virus 1 strains of infected Leishmania
296 isolates circulating in French Guiana. Tibayrenc M, editor. PLOS Neglected Tropical Diseases.
297 2017 Jul 17;11(7):e0005764.

298 7. Atayde VD, da Silva Lira Filho A, Chaparro V, Zimmermann A, Martel C, Jaramillo M, et al.
299 Exploitation of the Leishmania exosomal pathway by Leishmania RNA virus 1. Nature
300 Microbiology [Internet]. 2019 Jan 28 [cited 2019 Feb 4]; Available from:
301 http://www.nature.com/articles/s41564-018-0352-y

302 8. Castiglioni P, Hartley M-A, Rossi M, Prevel F, Desponds C, Utzschneider DT, et al. Exacerbated
303 leishmaniasis caused by a viral endosymbiont can be prevented by immunization with its viral
304 capsid. PLoS neglected tropical diseases. 2017;11(1):e0005240.

305 9. Bourreau E, Ginouves M, Prévot G, Hartley M-A, Gangneux J-P, Robert-Gangneux F, et al.
306 Presence of *Leishmania* RNA Virus 1 in *Leishmania guyanensis* Increases the Risk of First-Line
307 Treatment Failure and Symptomatic Relapse. Journal of Infectious Diseases. 2016 Jan
308 1;213(1):105–11.

309 10. Christen J-R, Bourreau E, Demar M, Lightburn E, Couppié P, Ginouvès M, et al. Use of the
310 intramuscular route to administer pentamidine isethionate in Leishmania guyanensis cutaneous
311 leishmaniasis increases the risk of treatment failure. Travel Medicine and Infectious Disease
312 [Internet]. 2018 Mar [cited 2018 Apr 12]; Available from:
313 http://linkinghub.elsevier.com/retrieve/pii/S1477893918300401

314 11. Simon S, Veron V, Carme B. Leishmania spp. identification by polymerase chain reaction–
315 restriction fragment length polymorphism analysis and its applications in French Guiana.
316 Diagnostic Microbiology and Infectious Disease. 2010 Feb;66(2):175–80.

317 12. Ginouvès M, Simon S, Bourreau E, Lacoste V, Ronet C, Couppié P, et al. Prevalence and
318 Distribution of Leishmania RNA Virus 1 in Leishmania Parasites from French Guiana. The
319 American Journal of Tropical Medicine and Hygiene. 2016 Jan 6;94(1):102–6.

320 13. Cantanhêde LM, Fernandes FG, Ferreira GEM, Porrozzi R, Ferreira R de GM, Cupolillo E. New
321 insights into the genetic diversity of Leishmania RNA Virus 1 and its species-specific relationship
322 with Leishmania parasites. Clos J, editor. PLOS ONE. 2018 Jun 18;13(6):e0198727.

323 14. Hall T. Bioedit: A user-friendly biological sequence alignment editor and analysis program for
324 windows 95/98/NT. Nucleic acids symp ser. 1999;(41):95–8.

325 15. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0
326 for Bigger Datasets. Mol Biol Evol. 2016;33(7):1870–4.

327 16. Galtier N, Gouy M, Gautier C. SEAVIEW and PHYLO_WIN: two graphic tools for sequence
328 alignment and molecular phylogeny. Comput Appl Biosci. 1996 Dec;12(6):543–8.

329 17. Adaui V, Lye L-F, Akopyants NS, Zimic M, Llanos-Cuentas A, Garcia L, et al. Association of the
330 Endobiont Double-Stranded RNA Virus LRV1 With Treatment Failure for Human Leishmaniasis

Caused by *Leishmania braziliensis* in Peru and Bolivia. Journal of Infectious Diseases. 2015 Jun 8;213(1):112–21.

18. Ogg MM, Carrion Jr R, BOTELHO ACDC, Mayrink W, Correa-Oliveira R, Patterson JL. quantification of leishmaniavirus RNA in clinical samples and its possible role in pathogenesis. The American journal of tropical medicine and hygiene. 2003;69(3):309–313.

19. Pereira L de OR, Maretti-Mira AC, Rodrigues KM, Lima RB, Oliveira-Neto MP de, Cupolillo E, et al. Severity of tegumentary leishmaniasis is not exclusively associated with Leishmania RNA virus 1 infection in Brazil. Memórias do Instituto Oswaldo Cruz. 2013 Aug;108(5):665–7.

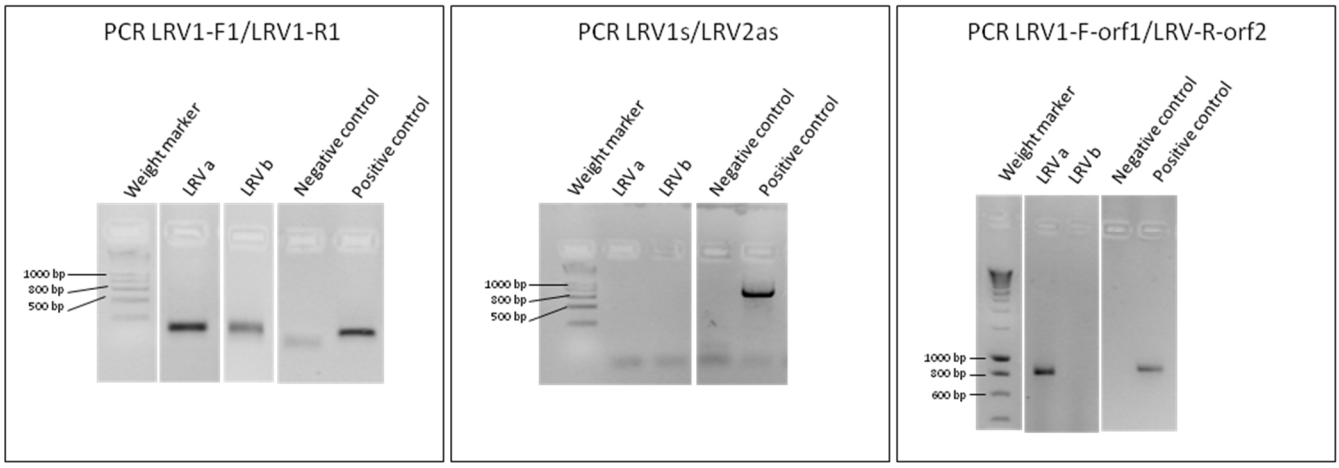

Figure 1 : LRV PCR with the different primers : LRV1-F1/LRV1-R1 [12] used for the LRV detection, LRV1s/LRV2as [6] and LRV1-F-orf1/LRV-R-orf2 [13] used for the LRV genotyping. LRVa corresponded to the non-amplified sample using LRV1s/LRV2as primers, but amplified using the LRV1-F-orf1/LRV-R-orf2 primers. LRVb corresponded to the non-amplified sample using LRV1s/LRV2as primers or LRV1-F-orf1/LRV-R-orf2 primers.

Table 1 : Statistical analysis of variables tested with LRV

				Univariate analysis	
		LRV-	LRV+	OR	p-value
Age	18-35 years hold	5	53	0,65	0,46
	> 35 years hold	9	62		
Gender	female	4	12	3,04	0,10
	male	8	73		
Time between two rounds of pentamidine	1 month	1	20	0,28	0,29
	> 1 month	3	17		
Time between infection and treatment	≤ 50 days	4	44	0,56	0,40
	> 50 days	6	37		
Treatment 1	cure	6	42	1,30	0,64
	failure	8	73		
Treatment 2	cure	3	26	0,87	0,88
	failure	2	15		
Suspected place of infection	Littoral	6	8	9,87	0,0008
	Inland	6	79		
Lesion size	≤ 2 cm	1	41	0,16	0,12
	> 2 cm	3	20		
Number of lesions	1	8	64	1,02	0,97
	>1	6	49		
Lymphangitis	absent	7	49	1,17	0,80
	present	5	41		
Adenopathy	absent	8	66	0,70	0,58
	present	4	23		
Papule	absent	11	69	6782796,24	0,99
	present	0	6		
Satellite papules	absent	10	70	0,86	0,89
	present	1	6		
Nodule	absent	9	59	0,86	0,84
	present	3	17		

Table 2 : Statistical analysis of variables tested with LRV genotypes

		LRV group		Univariate analysis	
		A	B	OR	p-value
Age	18-35 years hold	12	29	1,58	0,35
	> 35 years hold	10	38		
Gender	female	4	6	2,43	0,21
	male	12	46		
Time between two rounds of pentamidine	1 month	5	11	1,07	0,93
	> 1 month	1	7		
Time between infection and treatment	≤ 50 days	6	29	0,58	0,37
	> 50 days	8	22		
Treatment 1	cure	9	25	1,21	0,70
	failure	13	42		
Treatment 2	cure	5	11	3,33	0,31
	failure	3	9		
Suspected place of infection	Littoral	2	4	1,47	0,67
	Inland	18	47		
Lesion size	≤ 2 cm	7	23	1,12	0,88
	> 2 cm	3	11		
Number of lesions	1	12	39	0,81	0,67
	>1	10	26		
Lymphangitis	absent	10	30	1,07	0,90
	present	8	27		
Adenopathy	absent	11	44	0,42	0,13
	present	7	12		
Papule	absent	14	47	0,82	0,87
	present	1	3		
Satellite papules	absent	15	48	ND	0,99
	present	0	2		
Nodule	absent	10	41	0,44	0,21
	present	5	9		

ND : Not determined