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1 Full title: Translocating *Mycobacterium ulcerans*: an experimental model.

2 Short title: Buruli ulcer, a rat model.

3

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20 **ABSTRACT**

21 *Mycobacterium ulcerans* is a non-tuberculous environmental mycobacterium
22 responsible for extensive cutaneous and subcutaneous ulcers in mammals, named
23 Buruli ulcer in patients. *M. ulcerans* has been seldom detected in the feces of
24 mammals but not in patients, nevertheless the detection and isolation of *M. ulcerans*
25 in animal feces does not feet with the current epidemiological schemes for the
26 disease. Here using an experimental model in which rats were fed with 10^9 colony-
27 forming units of *M. ulceran*s, we detected *M. ulcerans* in feces of challenged rats for
28 two weeks and along their digestive tract for 10 days. *M. ulcerans* was further
29 detected in the lymphatic system including cervical and axillary lymph nodes and the
30 spleen, but not in any other tissue including the healthy and breached skin, 10 days
31 post-challenge. These observations indicate that in some herbivorous mammals, *M.*
32 *ulcerans* contamination by the digestive route may precede translocation and limited
33 infection of the lymphatic tissues without systemic infection. These herbivorous
34 mammals may be sources of *M. ulcerans* for exposed populations but are unlikely
35 reservoirs for the pathogen.

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36 **INTRODUCTION**

37 *Mycobacterium ulcerans* is an environmental slowly growing, non-tuberculous
38 mycobacterium responsible for progressively extending cutaneous and
39 subcutaneous ulcer named Buruli ulcer [1]. Buruli ulcer is a World Health
40 Organization-notifiable neglected infection which has been notified by 34 countries
41 over the last ten years [2]. Accordingly, Buruli ulcer is a tropical infection mainly
42 affecting rural populations in South America, West Africa, Australia, South-China and
43 Japan [3]. Although a genetic trait has recently been described among a 7-individual
44 family in whom two individuals suffered Buruli ulcer and carried a specific deletion on
45 chromosome 8 [4], in addition to previously reported deletion in the NRAMP-1 gene
46 [5], nevertheless Buruli ulcer is not a contagious infection but is rather resulting from
47 contacts with *M. ulcerans*-contaminated environments [2]. Accordingly, it's been
48 reported that *M. ulcerans* is being cultivated from aquatic Hemiptera [6]. indeed *M.*
49 *ulcerans* DNA has also been detected in some animals including *Thryonomys*
50 *swinderianus* (here designed agoutis, the word commonly used in West Africa) [7-8]
51 rabbits and rats, which have all in common to be rodent mammals [9]. Moreover, two
52 isolates of *M. ulcerans* have been reported from possum feces collected in
53 Melbourne, region, Australia although characterization and repository reference were
54 not provided [10]. These observations led to suggest that possums and
55 *Pseudocheirus peregrinus* may play a role in the epidemiology of Buruli ulcer in
56 endemic Australian regions [11].

57 Furthermore, we recently reported the isolation of *M. ulcerans* from *T.*
58 *swinderianus* feces collected in the vicinity of the Kossou Dam, Côte d'Ivoire,
59 although we were unable to sub-culture this isolate [8]. These observations
60 suggested that *T. swinderianus*, an herbivorous mammal rodent may contaminate its

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61 digestive tract by the oral route after eating *M. ulcerans*-contaminated food; possibly
62 acting as a secondary source of infection for populations as it is catched as brush
63 meat and eaten after unprotected, manual evisceration [7] Likewise, it has been
64 suggested that the small terrestrial mammals *Mastomys natalensis* can play a
65 potential role in the natural history of *M. ulcerans* [12]. Also, we recently observed
66 the PCR-based detection of the ketoreductase B gene (KR-b) and the IS*2404* and
67 IS*2606* insertion sequences in one tenth of spleen specimens collected from *T.*
68 *swinderianus* in the area of Yamoussoukro area, Côte d'Ivoire , and in spleen of
69 common ringtail possums in some areas of Victoria endemic for *M. ulcerans* disease
70 [7-10].

71 Altogether, these observations pointed to the medical interest in
72 understanding the mode of contamination and pathology of *M. ulcerans* in *T.*
73 *swinderianus*. We therefore developed an experimental model of oral route
74 contamination in rat, asking four questions relative to the survival and excretion of
75 the pathogen in the digestive tract, its translocation, its systemic dissemination and
76 its capacity to infect previous aseptic skin lesion.

77

78 **MATERIALS AND METHODS**

79 **Ethics Statements.** The experimental protocol, registered by the "Ministère de
80 l'Enseignement Supérieur et de la Recherche" under reference number
81 2018081011226001, was approved by the Institutional Animal Care and Use
82 Committee of Aix-Marseille University "C2EA- 14", France. All animal handling was
83 carried out in compliance with the rules of Décret N° 2013–118, Février 7, 2013,
84 France. The experimental procedures on rats were carried out in accordance with
85 European law and in agreement with Animal: Reporting In Vivo Experiments

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86 (ARRIVE Guidelines http://www.nc3rs.org.uk). We used Long-Evans rats (Charles
87 River Laboratories, L'Arbresle, Lyon, France). Animals were housed in protected
88 environmental area, in individual transparent cages (one rat per cage) in individually
89 ventilated Allentown technologies caging systems (Allentown, Pennsylvania, USA)
90 with free access to standard diet including dehydrated rodent feed pellets and sterile
91 water until the experiment. Efforts have been made to minimize the number of
92 animals, and to limit their stress the environment has been enriched with litter and
93 cardboard tunnels. The rats were observed daily for any signs of distress or adverse
94 events. To minimize animals suffering all invasive procedures have been performed
95 under full general anesthesia. The animals were sacrificed by injection of lethal dose
96 of Pentobarbital preceded by full general anesthesia. All experiments were
97 performed in a biosafety level 3 laboratory of the University Hospital Institute (IHU),
98 Marseille, France.

99 ***M. ulcerans* inoculum.** *M. ulcerans* strain CU 001, a clinical isolate from Ghana [13]
100 was cultured on Middlebrook 7H10 supplemented by OADC during a 6-week
101 incubation at 30°C. Colonies were suspended on sterile phosphate buffered saline
102 (PBS) tube and the bacterial was vigorously vortexed for 10 min using 3-mm sterile
103 glass beads (Sigma-Aldrich, Saint-Quentin- Fallavier, France) and passed three
104 times through a 29 Gauges in order to eliminate bacterial aggregates. The
105 mycobacterial suspension was then calibrated at optic density of 5 McFarland
106 (equivalent to 10^9 colony-forming units (CFU)/mL.
107

108 **Rat infection protocol.** Animal experimentations were performed on 16 rats (8
109 males and 8 females) aged 8 weeks (Charles River Laboratories) weighing between
110 220 g and 250 g. These animals obtained with complete health reports, were found

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111 to be healthy and free of infection and were housed under conditions free of specific
112 pathogens. Each rat was placed into an individual plastic cage with free access to
113 water and food. In order to induce subcutaneous lesions in the shoulders, rats were
114 full general anaesthetized with intraperitoneal injection of a mixture of Ketamine (80
115 mg/kg) and Xylazine (10 mg/kg). One gram of sterile glass powder mixed with 300
116 µL of sterile PBS were injected under the skin of the right shoulder and 300 µL of
117 PBS under the skin of the left shoulder as a negative control. Digestive inoculation
118 was performed after the shoulder skin lesions resolved (i.e., 7 days after skin lesions
119 were made). Briefly, the rats were manually restrained and sterile, single-use 1-mL
120 syringes were used to administer the *M. ulcerans* suspensions directly into the rats'
121 mouth, respecting the swallowing cycle in order to ensure that rats swallowed the
122 entire administered suspension: 300 µL of sterile PBS were administered to 4 rats (2
123 males and 2 females) forming the negative control group and 300 µL of
124 mycobacterial suspension at 10^9 CFU/mL were administered to 12 challenged rats (6
125 females and 6 males).
126
127 **Animal follow-up and sample collection.** Rat behavior was observed daily until the
128 day of euthanasia. In the post-infectious period, animals were observed carefully
129 daily for any abnormal behavior including swelling/bleeding of the injection site,
130 ruffled coat, hunched posture, signs of pain or distress. Feces were collected directly
131 from the exit of the rectum on the first day of challenge and then every two days until
132 20 days after challenge. Ten days after challenge, rats of group n°1 of 6 infected rats
133 (3 males and 3 females) and two controls (1 male and 1 female) were sacrificed by
134 intraperitoneal injection of lethal dose of Pentobarbital (100-150 mg/kg) preceded by
135 full general anesthesia as previously described. Organs were carefully collected.

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136 Another group n°2 of 6 infected rats (3 males and 3 females) and one male and one
137 female control rats were followed in the same way until 60 days after infection, then
138 sacrificed by intraperitoneal injection of a mixture intraperitoneal injection of lethal
139 dose of Pentobarbital (100-150 mg/kg) preceded by full general anesthesia as
140 previously described above and euthanized to collect a second set of organs.
141 **PCR detection of *M. ulcerans* DNA.** All collected organs were stored in Eppendorf
142 tubes at 4°C for one day. Then, one piece of each organ was transferred to another
143 1.5 mL-Eppendorf tube containing 500 µL of sterile PBS. The organs were vigorously
144 crushed using single used sterile piston and 200 µL of organ juice were put into a 1.5
145 mL-Eppendorf tube containing a mixture of 200 µL G2 lysis buffer, 20 µL proteinase
146 K and a small quantity of glass powder. Tube underwent 3 cycles of FastPrep 24™-5
147 (MP Biomedicals, Strasbourg, France) before being heated at 56°C for 2 hours.
148 Then, 200 µL of supernatant were used to extract DNA using the EZ1 apparatus
149 according to the manufacturer's recommendations (Qiagen, GmbH, Germany).
150 Extracted DNA was stored at 4°C. Detection of *M. ulcerans* DNA was performed by
151 using real-time PCR (RT-PCR) and a CFX thermal cycler (BIO-Rad, Marnes-la-
152 Coquette, France) using specific primers targeting the ketoreductase B gene (KR-b)
153 and the IS*2404* and IS*2606* insertion sequences, as previously described [14]. The
154 negative controls of our RT-PCR reactions were formed from the same reaction mix
155 as our samples switching only the 5 uL of DNA by 5 uL of ultrapure™ DNase/RNase-
156 Free Distilled Water (Invitrogen France), thus one negative control is placed after
157 every 5 samples on a Light cycler 480 multiwell plates 96-well plate (Roche).
158
159 ***M. ulcerans* culture.** One piece of spleen collected from every rat, was crushed in
160 500 µL of sterile PBS using sterile single-used piston and 2 X 150 µL of spleen juice

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161 were inoculated on two Middlebrook 7H10 agar plates incubated at 30°C for 2
162 months. In addition, the feces collected at 3, 14- and 20-days post-infection were
163 incubated for 10 min at room temperature in 2 mL NAOH (1M) and centrifuged at
164 3,500 g for 10 min. The pellet was resuspended in 1 mL of 5% oxalic acid and
165 incubated for 10 min at room temperature before centrifugation at 3,500 g for 10 min.
166 The pellet was then resuspended into 2 mL home-made Trans MUI decontamination-
167 preculture medium incubated for 5 days at 30°C. The mixture was then vortexed 30
168 times and 200 µL were plated onto Trans MUg, a homemade Middlebrook-based
169 medium supplemented with with 10% oleic acid, bovine albumin, dextrose and
170 catalase enrichment (OADC, Becton Dickinson), oxytetracycline (40 µg/mL),
171 polymyxin E (80 µg/mL) and voriconazole (50 µg/mL) for 3 months at 30°C. Trans
172 MUg medium was previously tested and shown to respect the viability of *M. ulcerans*
173 (no published data).
174
175 **RESULTS**
176 **Rat clinical follow-up.** During a 60-day follow-up post-infection, all rats in the
177 control group and all rats in the challenged group remained apparently healthy and
178 showed no pathological clinical sign, no pain and no weight loss. In addition, the
179 subcutaneous lesions induced by the glass powder do not show any pathological
180 signs during the duration of the experimentation.
181 **RT-PCR test results.** In all RT-PCR assays, the negative controls remained
182 negative. At 10 days post-challenge, the RT-PCR detection of *M. ulcerans* DNA
183 remained negative on the internal organs and feces collected in control group rats
184 whereas it was positive in some *M. ulcerans*-infected rats: RT-PCR tests revealed a
185 simultaneous detection of IS*2606* insertion sequence and the KR-B gene in cervical

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186 lymph nodes, axillary lymph nodes, spleen and the digestive tract in males and
187 female (Table 1). Also, *M. ulcerans* DNA was detected in the feces of infected rats
188 up to 15- and 17-days post-infection, in males and females, respectively. At 60 days
189 post-challenge, the RT-PCR detection of *M. ulcerans* DNA remained negative on the
190 internal organs and in feces collected in control and challenged groups (Table 1).
191 **Culture results.** No colony of *M. ulcerans* was isolated from the feces and the
192 spleen samples collected in 4 negative-control group rats after three-month
193 incubation. The same observations were reported after three-months of cultivation of
194 feces and spleens of 12 *M. ulcerans*-infected rats
195

196 DISCUSSION

197 We have previously reported the molecular detection of *M. ulcerans* DNA in the
198 digestive tract and the spleens of wild agoutis caught in Côte d'Ivoire: and its culture
199 in one case [7-8] In the present work, we developed an experimental model of
200 gastrointestinal infection in rats to confirm that our field observations were the result
201 of probable gastrointestinal contamination of wild agoutis. We chose the rat model as
202 the laboratory mammal closest to agouti, sharing its general morphology (in terms of
203 size and weight), a herbivorous rodent diet and a body temperature of 37°C, which
204 also makes it a relevant model of human infection [15].
205 The rat model showed that these rodents can be infected by *M. ulcerans*
206 through the digestive tract, allowing us to answer our first question. Indeed, the
207 negative control rats and the experimental negative controls that we introduced at all
208 stages of our experiments, remained negative in both the RT-PCR and the culture-
209 based experiments carried out on the feces and internal organs RT-PCR assays of

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210 uninfected rats. This fact allowed us to interpret the positive results we observed, as
211 authentic and not a mere result of laboratory contamination.
212 Our experimental results thus confirm that some wild rodents are digestively
213 infectable with *M. ulcerans,* as it has been previously observed for wild agoutis in
214 Côte d'Ivoire [7-8]. and for possums in Australia [10]. Accordingly, our results
215 reporting the *M. ulcerans* DNA detected in feces and in internal organs, suggests
216 that some wild rodents may participate in the natural cycle of the pathogen. This
217 observation indicates that direct contact with the feces of these wild rodents, when
218 they are prepared as bushmeat for example, constitutes a circumstance of
219 contamination of populations by live *M. ulcerans*, and a source of Buruli ulcer.
220 In a second step, *M. ulcerans* was detected by RT-PCR but not cultured, in
221 the spleen and some lymph nodes of challenged rats but not from negative control
222 rats. This observation indicates that *M. ulcerans* has probably the ability to penetrate
223 the digestive mucosa through mechanisms whose determination was not part of the
224 objectives of this experimental work, and to be kept by the lymphatic system in which
225 it is destroyed. Accordingly, this observation suggests that at some stage of this
226 translocation process, mycolactones are no longer synthesized, or secreted or
227 inactivated. This experimental result corroborates the fact that *M. ulcerans* has never
228 been detected in a tissue or organ at a distance from its inoculation point, which also
229 suggests that the cytotoxic activity of mycolactones is local and not systemic, without
230 clarifying the role of mycobacterial inhibition and mycolactone inhibition themselves.
231 Interestingly, it is possible to determine mycolactones in Buruli ulcer lesions, but not
232 in patients' blood [16]. It should be noted that translocation property is shared with
233 mycobacteria of the *Mycobacterium tuberculosis* complex, in which experimental
234 translocation has been shown for *Mycobacterium canettii* [17] and *M. tuberculosis*

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235 [18], there are no experimental data or clinical observations to our knowledge for
236 mycobacteria of the *Mycobacterium leprae* complex.
237 After translocation, the fate of *M. ulcerans* differs considerably from that of
238 mycobacteria of the *M. tuberculosis* complex. The latter spread into the lung and
239 other highly vascularized organs [18], while *M. ulcerans* did not spread into any
240 organs in the rat model.
241 Altogether, the observations here reported indicate that in some herbivorous
242 mammals, *M. ulcerans* contamination by the digestive route may precede
243 translocation and limited infection of the lymphatic tissues without systemic infection.
244 These herbivorous mammals may participate as sources of *M. ulcerans* for exposed
245 populations, but do not participate as reservoirs for the pathogen.

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255 **REFERENCES**

256

257 1. MacCallum P, Tolhurst JC, Buckle G, Sissons HA. A new mycobacterial
258 infection in man. J Pathol Bacteriol. 1948; 60: 93–102.
259 2. Zingue D, Bouam A, Tian RBD, Drancourt M. Buruli Ulcer, a Prototype for
260 Ecosystem-Related Infection, Caused by Mycobacterium ulcerans. Clin
261 Microbiol Rev. 2017; 31: e00045-17.
262 3. Merritt RW, Walker ED, Small PL, Wallace JR, Johnson PD, Benbow ME, et
263 al. Ecology and transmission of Buruli ulcer disease: a systematic
264 review. PLoS neglected tropical diseases. 2010; 4: e911.
265 4. Vincent QB, Belkadi A, Fayard C, Marion E, Adeye A, Ardant MF, et
266 al. Microdeletion on chromosome 8p23. 1 in a familial form of severe Buruli
267 ulcer. PLoS neglected tropical diseases. 2018 ; 12 : e0006429.
268 5. Stienstra Y, van der Graaf WT, te Meerman GJ, The TH, de Leij LF, van der
269 Werf TS. Susceptibility to development of Mycobacterium ulcerans disease:
270 review of possible risk factors. Tropical Medicine & International Health.
271 2001 ; 6: 554-562.
272 6. Portaels F, Meyers WM, Ablordey A, Castro AG, Chemlal K, de Rijk P. et al.
273 First cultivation and characterization of Mycobacterium ulcerans from the
274 environment. PLoS Negl Trop Dis. 2008; 26: e178.
275 7. Hammoudi N, Dizoe AS, Regoui S, Davoust B, Drancourt M, Bouam
276 A. Disseminated Mycobacterium ulcerans Infection in Wild Grasscutters
277 (Thryonomys swinderianus), Côte d'Ivoire. The American journal of tropical
278 medicine and hygiene. 2019; 101: 491-493.

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.04.977447; this version posted March 4, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

279 8. Zingue D, Panda A, Drancourt M. A protocol for culturing environmental
280 strains of the Buruli ulcer agent, Mycobacterium ulcerans. Sci Rep. 2018; 8:
281 6778.
282 9. Narh CA, Mosi L, Quaye C, Dassi C, Konan DO, Tay SC. et al. Source
283 tracking Mycobacterium ulcerans infections in the Ashanti region,
284 Ghana. PLoS neglected tropical diseases. 2015; 9 : e0003437.
285 10. O'Brien CR, Handasyde KA, Hibble J, Lavender CJ, Legione AR, McCowan
286 C,et al. Clinical, microbiological and pathological findings of Mycobacterium
287 ulcerans infection in three Australian Possum species. PLoS neglected
288 tropical diseases. 2014 ; 8: 1.
289 11. Fyfe JA, Lavender CJ, Handasyde KA, Legione AR, O'Brien CR, Stinear TP,et
290 al. A major role for mammals in the ecology of Mycobacterium ulcerans. PLoS
291 neglected tropical diseases. 2010; 4 :8.
292 12. Dassi C, Mosi L, Akpatou B, Narh AC, Quaye C, Konan OD, Bonfoh
293 B. Detection of Mycobacterium ulcerans in Mastomys natalensis and potential
294 transmission in Buruli ulcer endemic areas in Côte d'Ivoire. Mycobact Dis.
295 2015; 5: 2161-1068.
296 13. Drancourt M, Jarlier V, Raoult D. The environmental pathogen Mycobacterium
297 ulcerans grows in amphibian cells at low temperatures. Appl Environ
298 Microbiol. 2002; 68: 6403-4.
299 14. Fyfe JA, Lavender CJ, Johnson PD, Globan M, Sievers A, Azuolas J, Stinear
300 TP Development and application of two multiplex real-time PCR assays for
301 the detection of Mycobacterium ulcerans in clinical and environmental
302 samples. Appl. Environ. Microbiol. 2007; 73: 4733-4740.

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303 15. Gordon CJ. Thermal biology of the laboratory rat. *Physiology & behavior*.
304 1990; 47: 963-991.
305 16. Wadagni A, Frimpong M, Phanzu DM, Ablordey A, Kacou E, Gbedevi M, et
306 al. Simple, rapid Mycobacterium ulcerans disease diagnosis from clinical
307 samples by fluorescence of mycolactone on thin layer chromatography. PLoS
308 neglected tropical diseases. 2015; 9: 11.
309 17. Bouzid F, Brégeon F, Lepidi H, Donoghue HD, Minnikin DE, Drancourt M.
310 Ready experimental translocation of Mycobacterium canettii yields pulmonary
311 tuberculosis. Infection and immunity. 2017; 85: e00507-17.
312 18. Fellag M, Loukil A, Saad J, Lepidi H, Bouzid F, Brégeon F, Drancourt
313 M.Translocation of Mycobacterium tuberculosis after experimental
314 ingestion. PloS one. 2019; 14 : 12

Table 1. Detection of *M. ulcerans* in the organs and feces collected in rats challenged with the pathogen by the oral route. Red squares indicate the absence of detection, green squares indicate a positive detection. “A-B-C-D” denote negative control, non-challenged rats; “E-P” denote challenged rats. The numbers indicate the time (days) when M. ulcerans DNA was detected in the feces.

		Male rats					Female rats			
		A	E	F	G		B	H	I	J
Rats sacrificed at 10 days postinfection	Left lesion									
	Right lesion									
	Cervical ganglion									
	Axillary ganglion									
	Heart									
	Lung									
	Spleen									
	Kidney									
	Liver									
	Stomach									
	Small intestine									
	Large intestine									
	Secum									
	Peyer plate									
	Mesenteric nodes									
	Feces		**1---9**	**1---10**	**1---10**			**1---10**	**1---10**	**1---10**

		Male rats				Female rats			
		C	K	L	M	D	N	O	P
Rats sacrificed at 60 days postinfection	Left lesion								
	Right lesion								
	Cervical ganglion								
	Axillary ganglion								
	Heart								
	Lung								
	Spleen								
	Kidney								
	Liver								
	Stomach								
	Small intestine								
	Large intestine								
	Secum								
	Peyer plate								
	Mesenteric nodes								
	Feces		**1---15**	**1---14**	**1---15**		**1---15**	**1---17**	**1---14**

320

321