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Terrestrial Small Mammals as Reservoirs of *Mycobacterium ulcerans* in Benin[▽]

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***Mycobacterium ulcerans*, the causative agent of Buruli ulcer (BU), is considered an environmental pathogen. Different mycobacteria were detected in 68 (12%) out of 565 small mammals collected in areas in Benin where BU is endemic. Although *M. ulcerans* was not found, we suggest that more research on *M. ulcerans* in African (small) mammals is needed.**

Mycobacterium ulcerans is the causative agent of Buruli ulcer (BU), a serious skin disease (7, 29). Epidemiological evidence strongly associates BU with aquatic ecosystems (29). *M. ulcerans* DNA has been identified in water, fish, aquatic insects, detritus, leeches, crustaceans, mollusks, and mosquitoes (13, 18, 20, 25, 36). However, the difficulty in culturing the bacillus from environmental specimens and the low bacillary concentration shown by PCR (28) strongly suggest that *M. ulcerans* does not multiply in these specimens. Recent findings in Australia show high concentrations of *M. ulcerans* DNA in possum feces in sites where BU is endemic (C. O'Brien et al., presented at the WHO Annual Meeting on Buruli Ulcer, Geneva, Switzerland, 2008; C. O'Brien et al., presented at the WHO Annual Meeting on Buruli Ulcer, Geneva, Switzerland, 2009; J. Fyfe et al., presented at the WHO Annual Meeting on Buruli Ulcer, Geneva, Switzerland, 2010). *M. ulcerans* DNA also has been found in mosquitoes trapped in the same sites of endemicity where the possum feces were collected (18) and in feces of the black rat *Rattus rattus* (Linnaeus, 1758) (O'Brien et al., presented at the WHO Annual Meeting on Buruli Ulcer, Geneva, Switzerland, 2009; Fyfe et al., presented at the WHO Annual Meeting on Buruli Ulcer, Geneva, Switzerland, 2010). Similarly, in West Africa, mammals present in watery environments, such as rodents and insectivores (17), could be a reservoir of *M. ulcerans*. African rodents and insectivores (shrews) can carry pathogenic mycobacteria (9) and are sensitive to experimental infection with *M. ulcerans* (1, 6, 14, 24, 34). Moreover, emergence of BU has been associated with environmental disturbances (29), which could also alter the transmission of rodent-borne diseases (26). To date, only one study has attempted to systematically culture *M. ulcerans* from rodents in an area of Africa where BU is endemic (Uganda) (31), but since the development of PCR assays, no such study has been carried out. In this study we hypothesize that small terrestrial mammals are part of the reservoir of *M. ulcerans* in which the bacteria can multiply and from which the environment can be contaminated.

By using Sherman live traps and box traps (9), 326 rodents and 222 shrews were caught around bodies of water and in the houses of three villages with high BU endemicity and three villages with low BU endemicity (Table 1) in the dry (January and February) and wet (October and November) seasons of 2006. Animal species identifications (Table 2) were based on external and/or cranio-dental analysis and were confirmed by molecular analysis. Cytochrome *b* gene sequences were compared to those presented by several researchers (8, 21, 23, 27, 32, 35). From each animal, a piece of liver, the spleen, a lung, the mesenteric lymph nodes, and external lesions, if present, were kept in semisolid transport medium (12) at −20°C until further analysis. The organs of each animal were pooled for analysis by culture and PCR or analyzed individually when the animal presented external or internal lesions. Culture and identification of mycobacteria were performed as described earlier (9), but with inoculation at 30 to 32°C (22) and additional use of charcoal medium (30). DNA was extracted using the modified Boom method (2, 10) and amplified in a nested PCR specific for all mycobacteria (9) and specific for *M. ulcerans* (33). From 49 (8.7%) animals, nontuberculous mycobacteria were cultured, but no *M. ulcerans* was isolated. Most of the mycobacterial isolates in this study can cause disease in humans (Table 3). Twenty-six animals (4.6%) were positive for mycobacteria by PCR, but no *M. ulcerans* was detected. Com-

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TABLE 1. Location of the study villages

CDTUB[a]	Village	No. of BU cases in 2005-2006	Level of BU endemicity	Geographical coordinate	
				Latitude	Longitude
Lalo	Tandji	25	High	6.94122098	1.97169973
	Adjassagon	2	Low	7.00175107	1.95172347
Zagnanado	Houedja	6	High	7.13986237	2.44355033
	Agonvè	2	Low	7.25431818	2.45778188
Allada	Sedje Houégoudo	11	High	6.74590738	2.37206443
	Ahozonnoude	0	Low	6.8136599	2.37536363

[a] CDTUB, Centres de Dépistage et Traitement d'Ulcère de Buruli.

bining culture and PCR, a total of 68 animals (12.0%) were carriers of mycobacteria. In 14 (15.5%) out of 90 fecal samples collected from a subset of the trapped animals, mycobacteria were detected by PCR, but *M. ulcerans* was not detected. Whether rodents and shrews can, indeed, transmit mycobacteria, e.g., by excretion in their feces, should be investigated further experimentally.

Although a slightly higher presence of mycobacteria was found in the animals trapped in the villages with high BU endemicity (13.8%) than in villages with low BU endemicity (9.8%), the difference was not statistically significant ($P = 0.162$). Eddyani et al. (11) did find more mycobacteria in amoebae in areas of high BU endemicity than in areas of low BU endemicity.

Similar to findings of a previous study carried out in Tanzania (9), the presence of mycobacteria in shrews (21.2%) was significantly higher than in rodents (6.1%) ($P < 0.001$). For shrews, a significantly higher presence of mycobacteria was found in the wet season (33.3%) than in the dry season (10.3%) ($P < 0.001$). On the other hand, for rodents we found that in the dry season relatively more mycobacteria were present (8.5%) than in the wet season (2.7%) ($P = 0.025$). These findings could be due to a difference in behavior or feeding habits between shrews and rodents. Shrews mainly forage on invertebrates from the ground surface and among leaf litter (4, 5). Several mycobacteria have already been found in several invertebrates (15, 16, 18, 20, 28). It is possible that the seasonal distribution of mycobacteria in shrews observed in this study is a consequence of a seasonal distribution of mycobacteria in the invertebrates on which the shrews forage although no information is available on seasonality of mycobacteria in invertebrates. In other studies on environmental mycobacteria, more mycobacteria were found in the environment (soil and water) in the dry season than in the wet season (3), which could be a possible explanation for the seasonality of mycobacteria in rodents.

TABLE 2. Number of animals collected and number of animals positive for mycobacteria per animal species

Animal species	No. of animals collected	No. of animals positive for mycobacteria
Rodents		
Rattus rattus (Linnaeus, 1758)	78	4
Lemniscomys striatus (Linnaeus, 1758)	66	5
Mastomys natalensis (Smith, 1834)	64	3
Praomys misonnei Van der Straeten & Dieterlen, 1987	52	5
Praomys cf. *derooi* Vanderstraeten & Verheyen, 1978	38	1
Praomys sp. n. 1	7	0
Uranomys ruddi Dollman, 1909	7	0
Mus (*Nannomys*) spp.	6	1
Dasymys bentleyae (Thomas, 1892)	3	0
Hybomys cf. *trivirgatus*	1	0
Hylomyscus sp.	1	0
Mastomys erythroleucus (Temminck, 1853)	1	1
Mus sp.	1	0
Taterillus gracilis (Thomas, 1892)	1	0
Thryonomys swinderianus (Temminck, 1827)	14	1
Xerus erythropus Desmarest, 1817	3	0
Insectivores		
Crocidura cf. *foxi* Dollman, 1915	146	31
Crocidura olivieri (Lesson, 1827)	56	13
Crocidura spp.	20	3

The fact that *M. ulcerans* was not found in the animals collected in the present study could be due to several factors. The size and type of the traps favor certain species of rodents and shrews. Some animal species are too large to enter the traps or too small to trigger them. Additionally, several animal species were caught in low numbers only. The prevalence of BU in humans varies between 0 and 5.61% in villages in the district of Lalo (Benin) (19). In order to have 95% probability of trapping at least one positive individual, assuming a prevalence of between 5 and 10%, we would need to test between 30 and 80 animals per species in a certain area, which is more than the numbers we have trapped for most species.

The fact that we did not find *M. ulcerans* DNA in the feces of *R. rattus* trapped in Benin although it has been detected in the same species in Australia could be due to a lower sensitivity of our methods (gel-based PCR in the present study versus real-time PCR in the study of C. O'Brien et al. (presented at the WHO Annual Meeting on Buruli Ulcer, Geneva, Switzerland, 2009). However, it is also possible that in Australia *R. rattus* obtained *M. ulcerans* only from eating contaminated possum feces while a similar source of *M. ulcerans* is absent in Benin.

M. ulcerans disease in wild and domestic animals has never been described in the literature from any of the West and Central African countries, probably because of the lack of attention to diseases in wild (and domestic) animals in this region. Taking all the above into consideration, we do

TABLE 3. Mycobacteria isolated from small mammals in areas of high and low BU endemicity assigned to risk groups[a]

Risk group and isolated mycobacterium[b]	Small mammal (*n*)[c]	Field code	Location (BU endemicity level)	Trapping site	Season	Comment[d]
Risk group 2						
Mycobacterium scrofulaceum	*Crocidura* cf. *foxi* (3)	BN899, BN902, BN934	Zagnanado (high)	Near water body	Wet	
	Crocidura olivieri (2)	BN906, BN961	Zagnanado (high)	Near water body	Wet	
	Crocidura cf. *foxi* (2)	BN968, BN974	Zagnanado (low)	Near water body	Wet	
	Crocidura cf. *foxi* (1)	BN986	Allada (high)	Near water body	Wet	
	Crocidura olivieri (1)	BN997	Allada (high)	Near water body	Wet	
	Crocidura olivieri (1)	BN998	Allada (high)	House	Wet	
	Crocidura olivieri (1)	BN1062	Allada (low)	Near water body	Wet	Feces PCR+
Mycobacterium simiae	*Crocidura* cf. *foxi* (1)	BN321	Lalo (high)	Near water body	Dry	
	Lemniscomys striatus (1)	BN1007	Lalo (high)	Near water body	Dry	
	Crocidura olivieri (1)	BN825	Lalo (low)	Near water body	Wet	
	Crocidura cf. *foxi* (2)	BN912, BN959	Zagnanado (high)	Near water body	Wet	
	Crocidura sp. (1)	BN947	Zagnanado (high)	Near water body	Wet	
	Crocidura cf. *foxi* (1)	BN341	Zagnanado (high)	Near water body	Dry	
	Mastomys erythroleucus (1)	BN410	Zagnanado (high)	Near water body	Dry	Spleen, ML
	Mus (Nannomys) sp. (1)	BN980	Zagnanado (low)	Near water body	Wet	
	Crocidura cf. *foxi* (1)	BN987	Allada (high)	Near water body	Wet	
	Crocidura olivieri (1)	BN1017	Allada (high)	House	Wet	
Mycobacterium avium complex	*Crocidura* cf. *foxi* (1)	BN1020	Allada (high)	Near water body	Wet	
	Praomys misonnei (1)	BN308	Lalo (high)	Near water body	Dry	Lung
	Crocidura sp. (1)	BN875	Lalo (high)	House	Dry	
	Praomys misonnei (1)	BN346	Zagnanado (high)	Near water body	Dry	Wound on back
Mycobacterium intracellulare	*Crocidura olivieri* (1)	BN300	Lalo (high)	House	Dry	
	Crocidura cf. *foxi* (1)	BN969	Zagnanado (low)	House	Dry	
Mycobacterium asiaticum (-like)	*Crocidura* sp. (1)	BN1061	Allada (low)	House	Wet	
	Praomys misonnei (1)	BN308	Lalo (high)	Near water body	Dry	ML
Mycobacterium shimoidei-like	*Mastomys natalensis* (1)	BN474	Zagnanado (low)	House	Dry	Tail
Risk group 1						
Mycobacterium interjectum	*Crocidura olivieri* (1)	BN900	Zagnanado (high)	Near water body	Wet	
	Crocidura cf. *foxi* (1)	BN1002	Allada (high)	Near water body	Wet	
	Mus (Nannomys) sp. (1)	BN980	Zagnanado (low)	Near water body	Wet	
Mycobacterium lentiflavum	*Crocidura* cf. *foxi* (1)	BN337	Zagnanado (high)	Near water body	Dry	Spleen
Mycobacterium triplex	*Crocidura* cf. *foxi* (1)	BN478	Zagnanado (low)	Near water body	Dry	
Not assigned to a risk group						
Mycobacterium paraffinicum (-like)	*Crocidura* cf. *foxi* (1)	BN456	Zagnanado (low)	Near water body	Dry	Spleen, lung
	Mastomys natalensis (1)	BN517	Zagnanado (low)	Near water body	Dry	Lung
Mycobacterium saskatchewanense	*Crocidura* cf. *foxi* (1)	BN206	Lalo (high)	Near water body	Dry	
Mycobacterium sherrisii	*Crocidura* cf. *foxi* (1)	BN967	Zagnanado (low)	Near water body	Wet	
	Crocidura cf. *foxi* (2)	BN1015, BN1027	Allada (high)	Near water body	Wet	Feces PCR+
	Mastomys natalensis (1)	BN958	Zagnanado (high)	House	Wet	
Mycobacterium colombiense	*Crocidura olivieri* (1)	BN1001	Allada (high)	Near water body	Wet	
	Crocidura cf. *foxi* (2)	BN1002, BN1030	Allada (high)	Near water body	Wet	Feces PCR+
Mycobacterium angelicum	*Crocidura olivieri* (1)	BN1043	Allada (low)	Near water body	Wet	
Mycobacterium barombii	*Crocidura* cf. *foxi* (1)	BN990	Allada (high)	Near water body	Wet	
Mycobacterium spp.	*Crocidura cf. foxi* (2)	BN970, BN982	Zagnanado (low)	Near water body	Wet	
	Crocidura olivieri (1)	BN291	Lalo (high)	House	Dry	

[a] Leão et al. (22).
[b] Risk group 2 contains pathogens that pose a moderate individual risk and of which disease with average severity exists in the community. Risk group 1 contains pathogens that pose a low risk of infection for both the human individual and the community. Diseases are never or rarely described in normal adults (22).
[c] *n*, number of animals.
[d] The body site from which the mycobacterium is isolated (ML, mesenteric lymph nodes) is mentioned. If no body site is mentioned, the mycobacterium was isolated from the pooled organs. Feces PCR+, the feces sample was also positive by PCR.

not reject our initial hypothesis that rodents or shrews are part of the reservoir; instead, we broaden it to other mammals.

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