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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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