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Potential Role for Fish in Transmission of *Mycobacterium ulcerans* Disease (Buruli Ulcer): an Environmental Study

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This study reports a potential role that fish may play in the transmission of *Mycobacterium ulcerans* disease (Buruli ulcer). Fish found positive for *M. ulcerans* DNA all appear to feed on insects or plankton and are believed to concentrate *M. ulcerans* from this usual food source. These observations provide additional data supporting our previous hypothesis on sources of *M. ulcerans* and modes of transmission.

Mycobacterium ulcerans is an environmental organism that causes Buruli ulcer (BU), a necrotizing disease of skin and bone that prevails in West and Central Africa. Although BU is closely associated with tropical wetlands, the epidemiology of the disease remains poorly understood. However, the source of *M. ulcerans* in nature is becoming better defined from epidemiological data and environmental molecular biological findings. Beginning in 1996, *M. ulcerans* DNA has been detected in multiple studies of environmental specimens by PCR using accepted *M. ulcerans*-specific primers targeting the repetitive sequence IS*2404* (11, 12). *M. ulcerans* was first detected in aquatic insects collected from the environment in Benin by Portaels et al. (9). In another study, also in areas of Benin where BU is endemic, it was shown that small fish were positive for *M. ulcerans* by PCR (10). Observations in areas of Ghana where BU is endemic yielded similar results (5).

Marsollier et al. (7) demonstrated that *M. ulcerans* may multiply in the salivary glands of aquatic insects following experimental infection and that the bites of such insects transmitted *M. ulcerans* to laboratory mice. Members of the Hemiptera are aggressive predators of snails and fish, making them likely reservoirs of *M. ulcerans* as the studies mentioned above suggest (1, 7, 10).

Portaels et al. (10) formulated a hypothesis for a source and mode of transmission of *M. ulcerans* to animals and humans. This hypothesis proposed that environmental mycobacteria in the bottoms of swamps may be mechanically concentrated by small water-filtering organisms (e.g., microphagous fish, snails, mosquito larvae, small crustaceans, and protozoa); these organisms may then be ingested by aggressive predators such as aquatic insects. Some fish feed on insects and insect larvae and may in turn concentrate *M. ulcerans* from these sources. This hypothesis led to this more extensive study on fish in areas of Ghana and Benin where BU is endemic.

In October 2001, 40 fish were collected from four bodies of water in the Ga district of Ghana, where BU is endemic. The four sampling sites were in two villages: Otuaple and Ayikai Dobro. These fish belong to the families Cichlidae (*Oreochromis niloticus*, *Tilapia guineensis*, *Hemichromis bimaculatus*, and *Hemichromis fasciatus*) and Poeciliidae (an *Aplocheilichthys* sp.) (Table 1). In March 2002, 85 fish were collected from swamps around four villages of southern Benin where BU is endemic: Lalo, Houedja, Monzougoudo, and Tevedji. These fish belong to the families Cichlidae (*T. guineensis*, *Sarotherodon galilaeus galilaeus*, *H. bimaculatus*, and *H. fasciatus*), Aplocheilidae (*Epiplatys bifasciatus*), and Citharinidae (*Neolebias ansorgii* and *Neolebias unifasciatus*) (Table 1).

Large fish (5 to 15 cm long) (which included all members of the Cichlidae from Ghana and the *Sarotherodon* sp. from Benin) were dissected before analysis; only the gills and intestines were analyzed. The gills and intestines of *S. galilaeus galilaeus* fish were analyzed separately, while these organs were combined before analysis of the fish from Ghana. The rationale for analyzing the gills of the Cichlidae separately was that the Cichlidae are microphagous fish that have gill apparatuses with very fine processes capable of trapping particles as small as bacteria (6). No dissection was carried out on small fish (1 to 3 cm long). All specimens were tested for *M. ulcerans* DNA by IS*2404* nested PCR (12).

As shown in Table 1, the Citharinidae (the *Neolebias* spp.) all tested negative for *M. ulcerans*. Among the cichlids, one *H. bimaculatus* fish tested positive for *M. ulcerans*. Five (9.8%) of 51 *S. galilaeus galilaeus* fish were positive for *M. ulcerans* DNA: in one fish both the intestines and gills were positive, in two fish only the gills were positive, and in two fish only the intestines were positive (data not shown). One of eight *Epiplatys* fish tested positive. Of the *Aplocheilichthys* fish, six (75.0%) of eight were positive for *M. ulcerans* DNA. The positivity rate of the *Aplocheilichthys* fish was significantly higher than that of any other species ($P = 0.04$). There was no significant difference between the findings in Ghana and Benin or between the sampling sites in Ghana and Benin (data not shown). None of the collected fish showed signs of disease. Statistical analysis was done with Epi-Info 6.0 software.

The higher positivity rate of the Poeciliidae could be related to the fact that they feed exclusively on insects and insect

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TABLE 1. IS*2404* PCR results for fish collected in Ghana and Benin

Fish feeding type	Family	Species	No. of PCR-positive fish/total no. (%)		
			Ghana	Benin	Total (Ghana + Benin)
Planktivorous	Cichlidae	*Oreochromis niloticus*	0/11		0/11
		Tilapia guineensis	0/12	0/5	0/17
Zooplanktivorous[a]		*Sarotherodon galilaeus galilaeus*		5/51 (9.8)	5/51 (9.8)
		Hemichromis fasciatus	0/2	0/1	0/3
		Hemichromis bimaculatus	1/7 (14.3)	0/3	1/10 (10.0)
Subtotal			1/32 (3.1)	5/60 (8.3)	6/92 (6.5)
Omnivorous	Citharinidae	*Neolebias ansorgii*		0/10	0/10
Carnivorous		*Neolebias unifasciatus*		0/7	0/7
Subtotal				0/17	0/17
Insectivorous	Aplocheilidae	*Epiplatys bifasciatus*		1/8 (12.5)	1/8 (12.5)
Insectivorous	Poeciliidae	*Aplocheilichthys* sp.	6/8 (75.0)		6/8 (75.0)
Total			7/40 (17.5)	6/85 (7.1)	13/125

[a] The species that fed on zooplankton were juveniles.

larvae (3). This suggests that these fish can serve as passive reservoirs of *M. ulcerans* by eating insects of species that are known to be PCR positive for *M. ulcerans*, making them potential transmitters of BU. This is also true for the *Epiplatys* sp. Some planktivorous fish (cichlids) also tested positive for *M. ulcerans*. Plankton such as *Daphnia* spp. and protozoa may act as filters for *M. ulcerans* in water or serve as hosts that support active multiplication of *M. ulcerans* (10). Indeed, Portaels et al. found that the water flea *Daphnia* can take up *M. ulcerans* in experimental aquaria artificially seeded with *M. ulcerans* (10). Other mycobacteria multiply in protozoa; *M. avium*, for example, can replicate in amoebae (2, 13), and *M. avium*, *M. intracellulare*, and *M. scrofulaceum* multiply in *Tetrahymena pyriformis* (14). Drancourt et al. (4) have cultured *M. ulcerans* in association with amphibian cell lines, which suggests that amphibians may also be reservoirs of *M. ulcerans*. Of particular interest is the observation that the mycobacteria were present intracellularly.

The earlier hypothesis of Portaels et al. (10) that *M. ulcerans* could be concentrated in the gills of microphagous fish can now be revised in light of our present results. Microphagous fish may concentrate *M. ulcerans* in the intestines as well as in the gills: 3 of the 51 *Sarotherodon* sp. fish tested were positive for *M. ulcerans* in both organs. Fish species found positive for *M. ulcerans* (the Poeciliidae, Aplocheilidae, and Cichlidae) all feed on insects or plankton, and they seem to concentrate *M. ulcerans* in their gills and possibly support their replication in vivo (7, 10). Further research on the exact localization of the *M. ulcerans* DNA in fish may help us to better understand their role in the transmission of BU. The positivity of fish for *M. ulcerans* suggests that animals that prey on fish, for example, fish eagles and other birds, are candidates for the dissemination of *M. ulcerans*. This could help explain the typical sporadic distribution of BU in most areas of endemicity.

In a recent study, Portaels et al. (F. Portaels, K. Chemlal, A. Ablordey, M. Debacker, A. Guédénon, P. A. Fonteyne, C. R. Johnson, R. Kotlowsky, A. Martin, W. M. Meyers, C. Uwizeye, C. Zinsou, and P. Elsen, unpublished data) found that the positivity rate for *M. ulcerans* in insects from some swamps in Benin where BU is endemic declined from 2000 to 2002, and that the decline correlated directly with a lower incidence of BU in patients from the same area over the same time period. Fish collected from the same areas, however, did not show reductions in their positivity rates. This observation supports the hypothesis that fish may be passive reservoirs of *M. ulcerans* but are not usually responsible for direct transmission and possibly explains why the PCR positivity rate of fish is not as closely correlated with the incidence of BU as the positivity of insects.

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