

Entomological investigations carried out from 2002 to 2010 into the involvement of water bugs (Heteroptera - Hemiptera) in transmission of Mycobacterium ulcerans to humans in C te d'Ivoire (West Africa)

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Ulcer is a disease caused by a mycobacterium present in the environment: *Mycobacterium ulcerans*. This communicable disease occurs essentially in wet tropical regions, and in particular in west Africa where it is endemic. It is the third most common mycobacterial disease affecting humans after leprosy and tuberculosis, although it is more prevalent than either leprosy or tuberculosis in some rural areas of several countries (Benin, Côte d'Ivoire and Ghana). This has led WHO to act, and in 1998 to declare Buruli ulcer an « emerging disease » and to recognize it as a neglected tropical disease. Its development is a source of concern in Côte d'Ivoire, the country most affected in the world, with an aggregate number of 30 000 cases and more than 2000 cases detected each year. It particularly affects children living in isolated rural areas around bodies of stagnant or slowly flowing water. In order to control the disease, it is essential fully to understand its epidemiology. In this connection, there are several hypotheses on the mode of transmission of *M. ulcerans* to humans. Since 1999, the involvement of water bugs belonging to the order of the hemiptera has been invoked by Portaels. In 2002, this hypothesis was confirmed by Marsollier et al. for water bugs of the genus *Naucoris* taken from the region of Daloa in Côte d'Ivoire, where the disease is endemic. In 2008, Portaels also found *M. ulcerans* in samples taken from the environment (Gerridae) in Ghana. In 2007, studies began in Côte d'Ivoire into the specific diversity, biology, ecology, ethology and role of aquatic heteroptera in the transmission of *M. ulcerans* to humans. Samples of aquatic heteroptera were collected each month from different aquatic environments in endemic areas of Côte d'Ivoire. The insects were identified by family, genus and occasionally species. Their distribution, population dynamics and ecological distribution in the water points investigated were correlated with human activities. Monospecific batches of water bugs were regularly composed in order to identify the molecular signatures of *M. ulcerans* using PCR at the bacteriology laboratory of the Institut Pasteur in Côte d'Ivoire and at the bacteriology laboratory of the Groupe d'Etudes des Interactions Hôtes-Pathogènes (Host-Pathogen Study Group) at the University Teaching hospital in Angers, France. Eighteen (18) species belonging to 8 families were identified. After the aquatic insects collected had been identified, 283 monospecific batches were composed and sent to the Institut Pasteur in Côte d'Ivoire (IPCI) for PCR. Twenty four (24) of the 283 batches i.e. 8,5% containing the following, 14 *Diplonychus* sp, 2 *Naucoris* sp, 3 *Micronecta* sp, 2 *Ranatra fusca*, 2 *Anisops* sp and 1 *Laccotrephes ater*, respectively belonging to the families Belostomatidae, Naucoridae, Corixidae, Ranatridae and Nepidae tested positive under PCR. Thirty five (35) samples of saliva were collected from specimens of the genus *Diplonychus*. Six of the samples (i.e. 17%) tested positive under PCR. Out of 109 other monospecific batches sent to the laboratory in Angers, France, 33 (i.e. 30%) tested positive under PCR. They comprised 11 batches of *Diplonychus* sp (Belostomatidae), 8 batches of *Micronecta* sp (Corixidae), 2 batches of *Laccocoris* sp (Naucoridae), 4 batches of *Ranatra fusca* (Ranatridae), 3 batches of *Anisops* sp, 1 lot de *Anisops sardea* et 1 lot de *Enithares* sp (Notonectidae), 2 batches of *Plea pullula* (Pleidae) and 1 batch of de *Laccotrephes* sp (Nepidae). Clearly, not only is *Diplonychus* sp the genus most commonly found, it is also that most affected by *M. ulcerans*. This justifies the decision to breed this genus in the laboratory since 2008, in order to improve our understanding of its biology and ethology and to standardize physical and chemical parameters so as to determine the best conditions for breeding the insect which would provide an animal model for experimental infections. We have now bred six successive generations in the laboratory. To conclude, although some aquatic heteroptera that host *M. ulcerans* are strictly phytophagous, (e.g. the Corixidae), the great majority of water bugs are carnivorous predators that are hosts and vectors of *M. ulcerans*. The absence of a reliable key for determining the family, genus and species in central and west Africa has led us to draw up an iconographic catalogue to determine the taxonomy of these insects.

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