



Survey of *Legionella* spp. in Mud Spring Recreation Area

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Legionella genera are parasites of FLA, and intracellular bacterial replication within the FLA plays a major role in the transmission of disease. At least 13 FLA species—including Acanthamoeba spp., Naegleria spp., and Hartmannella spp.—support intracellular bacterial replication. In the study, Legionellae were detected with microbial culture or by direct DNA extraction and analysis from concentrated water samples or cultured free-living amoebae, combined with molecular methods that allow the taxonomic identification of these pathogens. The water samples were taken from a mud spring recreation area located in a mud-rock-formation area in southern Taiwan. Legionella were detected in 15 of the 34 samples (44.1%). Four of the 34 samples analyzed by Legionella culture were positive for Legionella, five of 34 were positive for Legionella when analyzed by direct DNA extraction and analysis, and 11 of 34 were positive for amoebae-resistant Legionella when analyzed by FLA culture. Ten samples were shown to be positive for Legionella by one analysis method and five samples were shown to be positive by two analysis methods. However, Legionella was detected in no sample by all three analysis methods. This suggests that the three analysis methods should be used together to detect Legionella in aquatic environments. In this study, *L. pneumophila* serotype 6 coexisted with *A. polyphaga*, and two uncultured Legionella spp. coexisted with either *H. vermiformis* or *N. australiensis*. Of the unnamed Legionella genotypes detected in six FLA culture samples, three were closely related to *L. waltersii* and the other three were closely related to *L. pneumophila* serotype 6. *Legionella pneumophila* serotype 6, *L. drancourtii*, and *L. waltersii* are noted endosymbionts of FLA and are categorized as pathogenic bacteria. This is significant for human health because these Legionella exist within FLA and thus come into contact with typically immunocompromised people.