



## 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.