

PA-129 **CULTIVATION OF TWO IS2404 POSITIVE
MYCOBACTERIUM SPP. FROM THE ENVIRONMENT OF
ASANTE AKIM DISTRICT OF GHANA**

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Background Buruli ulcer (BU) is one of the neglected tropical diseases. *Mycobacterium ulcerans* is the aetiologic agent of Buruli ulcer. Many extensive studies have failed to isolate *M. ulcerans* in pure culture from the environment, even in highly endemic areas of BU. We investigated the role of macro-invertebrates as possible hosts or vectors for *M. ulcerans* by attempting to cultivate *M. ulcerans* from these organisms.

Methods The study was conducted in 5 villages in the Asante Akim District of Ghana for 10 months. Primary detection of *M. ulcerans* was done by real-time PCR targeting insertion sequence IS2404 coupled with the detection of IS2606 and Ketone reductase genes for increased sensitivity and specificity. Primary cultures were done using routine bacteriological media for culturing mycobacteria, L-J and special enrichment liquid broth, BACTEC®.

Results The overall rate of detection of IS2404 in the general macro-invertebrate population was 12.8%. Cluster of CT-values was observed around a mean value of 35.88 and range values of 28.35–38.61. Statistically, there were no significant differences between the various CT-values obtained, $p > 0.05$. The difference in Δ CT values (IS2606-IS2404) for homogenate sample obtained from Naucoridae which was positive for the three targets on *M. ulcerans* genome was estimated to be 1.77. The present study reports the cultivation of two IS2404 positive *Mycobacteria spp.* from two aquatic macro-invertebrates of the families Belostomidae and Notonectidae both of the order Hemiptera. The isolate from Belostomidae was identified as either *M. ulcerans* or *M. marinum* with 98% identities that from Notonectidae was 98% identical to *M. neoaurum*. The organisms are yet to be passaged through mice footpad and fully characterised.

Conclusions For the first time *M. neoaurum* species was reported to have harboured IS2404 element. Aquatic Hemiptera are highly suspected to be vectors or hosts for *M. ulcerans* and they may transmit the pathogen to humans through biting.