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In-vivo culturing of *Setaria digitata* life stages (*micro filariae*-L3 larvae) in *Culex quinquefasciatus* mosquitoes

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Setaria species (Filarioidea, Nematoda) are commonly found in the abdominal cavities of ungulates and the disease is mild in its normal host cattle. However, when it infects hosts such as sheep, goats and horses, the infectious larvae of *S. digitata* migrate erratically into the central nervous system causing a serious and often fatal neuropathological disorder commonly known as epizootic cerebrospinal setariosis or lumbar paralysis. This has an impact on livelihood of livestock farmers. In addition, *S. digitata* has been used as a model organism for human filarial parasites due to its ready availability and also its close resemblance to human filarial parasites in many respects including morphology, histology antigenicity and response to drugs. Therefore, it was necessary to develop an *in-vivo* culturing method to study the biology of life stages (L1, L2, and L3) of this socioeconomically important parasite. In this study, attempts were made to establish *in-vivo* culturing of *Setaria digitata* life stages using its intermediate host, the mosquito *Culex quinquefasciatus*.

A *Culex quinquefasciatus* colony was established using wild caught mosquitoes. They were fed with chicken blood and the F1 generation was obtained from eggs they have laid. In this study, instead of microfilaria infested blood feeding, microfilariae isolated from dissected adult worms were microinjected in to 3 day old female mosquitoes using a pulled borosilicate capillary injector under a dissection microscope. PBS (0.5 µl) containing 20 microfilariae were injected in to the thorax of the mosquito following anesthetization by exposing to chloroform for around 45-60 secs. The development of larval stages (L1 to L3) from microfilariae within the mosquito was monitored by dissecting them at predetermined intervals and visualizing under a light microscope. Within 24 hrs, 20% of the microfilariae transformed in to L1. Transformation of microfilariae in to 100% of L1, L2, L3 occurs in 3, 6, 10 days respectively. The outcome of this work demonstrates the vector competence of *Culex quinquefasciatus* for *S. digitata* and also enables us to study the functions of the genes, in the transformation of microfilariae to larval stages of *S. digitata* in mosquitoes using gene knock-down techniques such as siRNA mediated RNA interference.