ABSTRACT FORM

INVESTIGATION OF LATENCY ASSOCIATED WITH *KOI HERPESVIRUS* INFECTION IN CARP (*CYPRINUS CARPIO*)

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Koi Herpesvirus (KHV) or Cyprinid Herpesvirus-3 causes severe disease and mass mortalities in populations of common carp (*Cyprinus carpio carpio*) and ornamental koi (*Cyprinus carpio koi*). The first major outbreaks of KHV disease were seen in Israel in the late 1990s. Since then the disease has spread rapidly around the world and has had a major economic impact with devastating losses reported in both intensive and extensive carp culture facilities. The disease affects fish of all sizes and has a permissive water temperature range of 18- 28^{o}C with an optimum range of 22-25^{o}C. The scientific literature contains numerous studies of the clinical disease and diagnosis of KHV infection but very few studies have investigated latent infection associated with KHV.

Laboratory studies and field investigations have provided evidence to suggest that KHV has the ability to establish a latent infection in carp. *In vivo* infection experiments suggest that long term survivors of high morality KHV outbreaks may remain latently infected with the virus for long periods. Both viral reactivation and detection of viral DNA have been reported in otherwise healthy survivors long after the initial outbreak. Assaying for KHV DNA alone is not a reliable method of confirming the presence of a latent infection for two main reasons. Firstly at this stage KHV DNA is present in low quantities and is therefore difficult to detect. This increases the chances of obtaining false negatives. Secondly detection of viral DNA alone cannot distinguish between latency and an abortive infection. To identify latent infection we need to be able to detect active viral gene transcription. During herpesvirus latency, gene transcription is largely confined to non-protein encoding genes which act as gene regulators e.g. microRNAs (miRNAs). MiRNAs are a class of non-coding RNA transcripts typically 17-25nt in length. They play important roles in gene regulation through targeted cleavage or translational-repression of specific messenger RNA (mRNA). In recent years several research groups have shown that miRNAs are highly expressed during latency in other herpesviruses and that they play a large role in the maintenance of latency and reactivation of lytic infections. They may also play a role in attenuating the innate and adaptive immune responses of the host.

Using computational analysis we have identified regions of the KHV genome that could theoretically give rise to miRNA precursors (pre-miRNAs). We designed microarrays with probes to detect mature miRNAs derived from 5' and 3' arms of 2825 predicted pre-miRNAs. These microarrays were hybridized with fluorescently labelled RNA from in-vitro infections. Probes targetting 64 mature miRNAs from predicted pre-miRNAs showed signal strengths of at least double or dramatically above background levels and their associated control probes. This may represent the first identification of miRNAs in a virus of a lower vertebrate organism. Due to their strong association with latency in other herpesviruses, KHV encoded miRNAs have the potential to be used as biomarkers for use in diagnosis of latent KHV infections within populations of otherwise healthy fish.