In vitro isolation and molecular identification of reptarenavirus in Malaysia

ABSTRACT

Boid inclusion body disease (BIBD) is a viral disease of boids caused by reptarenavirus. In this study, tissue from naturally infected boid snakes were homogenized and propagated in African Monkey kidney (Vero) and rat embryonic fibroblast (REF) cells. Virus replication was determined by the presence of cytopathic effect, while viral morphology was observed using transmission electron microscopy. Viral RNA was amplified using RT-PCR with primers specific for the L-segment of reptarenavirus; similarly, quantification of viral replication was done using qPCR at 24–144 h postinfection. Viral cytopathology was characterized by cell rounding and detachment in both Vero and REF cells. The viral morphology showed round-to-pleomorphic particles ranging from 105 to 150 nm which had sand-like granules. Sanger sequencing identified four closely associated reptarenavirus species from 15 (37.5 %) of the total samples tested, and these were named as follows: reptarenavirus UPM-MY 01, 02, 03, and 04. These isolates were phylogenetically closely related to the University Helsinki virus (UHV), Boa Arenavirus NL (ROUTV; BAV), and unidentified reptarenavirus L20 (URAV-L20). Comparison of deduced amino acid sequences further confirmed identities to L-protein of UHV, L-polymerase of BAV and RNA-dependent RNA polymerase of URAV-L20. Viral replication in Vero cells increased steadily from 24 to 72 h and peaked at 144 h. This is the first study in South East Asia to isolate and characterize reptarenavirus in boid snakes with BIBD.

Keyword: Boid inclusion body disease; Reptarenavirus; Cell culture; Viral isolation; Sanger sequencing; Electron microscopy