

MOLECULAR AND PHENOTYPIC ANALYSIS OF HIGHLY PATHOGENIC H5N1 AVIAN INFLUENZA VIRUSES FROM LAO PDR, 2006-2007.

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Highly pathogenic influenza A (H5N1) viruses re-emerged in Lao PDR in 2006 and continued to circulate in 2007 affecting 255 villages in five different provinces over a three month period. In this study, we analyzed 21 influenza H5N1 viruses isolated from chickens and ducks: 4 viruses isolated in 2006 and 17 in 2007. Antigenic analysis with a panel of anti-H5 monoclonal antibodies showed altered antigenic properties of three 2007 isolates. Sequence analysis of the hemagglutinin (HA) revealed that all isolates contained the HA cleavage-site sequence PLRERRRKRK identical to H5N1 strains found in Thailand and Southern China. Phylogenetic analysis of the HA genes shows similarity between 2006 and 2007 influenza strains which cluster to the recently described clade 2.3.4. No mutations were detected in NA protein that were previously reported to confer resistance to NA inhibitors. However, analysis of antiviral susceptibility in an enzyme inhibition assay revealed that 2 of 21 isolates possessed a decreased susceptibility to oseltamivir carboxylate with IC₅₀ values 20-100 fold higher than for other isolates. In addition, a separate isolate had an S31N amino-acid substitution in the transmembrane region of M2 protein that has been described in amantadine-resistant variants. Our results suggest that H5N1 influenza viruses became established among avian species in Lao PDR in 2007 and continued to evolve. Interestingly, this evolution included mutations in the NA and M2 which resulted in decreased sensitivity to antivirals. Therefore, continued surveillance to monitor the evolution of avian influenza viruses is required.