Molecular Epidemiology and Isolation of Very Virulent Marek's Disease Virus from India

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Marek's disease (MD) suspected samples were collected from poultry farms in Tamilnadu, Karnataka and Maharashtra. ICP4 gene based PCR is specific for serotype 1 MD virus and will not amplify the serotype 3 MD virus (HVT vaccine virus). Using this ICP4 PCR, 22 out of 173 samples were positive indicating that these isolates belonged to MD virus serotype 1. Of the PCR products from 22 isolates, 6 were sequenced. All the 6 samples had nucleotide sequence homologies ranging from 95.2% to 98.4% with the vvMDV serotype 1 reference strain, Md5. To further confirm the very virulent nature of these isolates, a 132-bp repeat PCR was performed. This PCR is used to differentiate very virulent serotype 1 MDV from the attenuated serotype 1 MDV such as CVI988, which is being used as a vaccine in other countries, based on the presence of multiple bands on gels. All the field isolates showed the presence of only 2 repeats (approximately 300 bp product) indicating the very virulent nature of these isolates. Eight field isolates positive for the 132-bp repeat sequences were sequenced. All the 8 samples had nucleotide sequence homologies ranging from 92.3% to 99.7% with the vvMDV serotype 1 reference strain, RB1B. The present study confirms the presence of vvMD viral genome in poultry flocks of India. The MDV field isolates were initially passaged in duck embryo fibroblast (DEF) cells then passaged in chicken embryo fibroblast (CEF) cells. Cytopathic effect (CPE) such as small plaques for virulent MDV and big plaques for HVT infected CEF cells was observed. As it took long time to produce CPE in cell culture (7 days), an attempt was made to know the effect of apoptosis induced by the MDV field isolate and HVT vaccine in CEF cells by using flow cytometry. After 72 hours post infection, the apoptosis induced for the uninfected control cells was 19.2% whereas for the MDV infected field virus, it was 40.2% and for the vaccine virus HVT, it was 27.7% per cent. It could be seen that the field virus induced higher rate of apoptosis than the vaccine strain that possibly could contribute to the higher virulence associated with such very virulent strains.