Standardization of Molecular Based Approach in Diagnosis of Recent Outbreaks of Infectious Laryngotracheitis (ILT) in Layers by Polymerase Chain Reaction in Namakkal, Tamilnadu

B Puvarajan, K Sukumar, J Johnson Rajeswar, TJ Harikrishnan, and GA Balasubramaniam

Department of Veterinary Public Health and Epidemiology, Veterinary College and Research Institute, Orathanadu - 614625 Thanjavur

During 2011-2012, a study was conducted on explosive outbreak of infectious laryngotracheitis (ILT) in layers flocks of Hyline and Babcock with a number ranging from 1,20,000 to 3,00,000 layers housed in cage system to standardize the polymerase chain reaction targeting a relatively conserved region of the thymidine kinase gene for the rapid detection of infectious laryngotracheitis virus in Thaligai village, Mettukattanpalayam village and Anna nagar of Namakkal District, Tamilnadu. The clinical findings of the disease were gasping, coughing, gurgling, marked dyspnea and expectoration of vigorously in a blood stained mucus obstructing the trachea or larynx. Some layers showed existence of dried blood around the nostrils and lower beaks, closed eye or eyes, lacrimation and the egg production was decreased by 30%. The morbidity rate was high, mortality rate reached 8 %. The disease was diagnosed by isolation of the causative agent from the dead and sick birds trachea suspension onto the chorioallantoic membrane (CAM) of 10 day old embryonated chicken eggs by fourth passage and identified by Agar Gel Immuno Diffusion test using reference hyperimmune serum. Twenty CAM samples were subjected to Polymerase chain reaction analysis for Tk gene of 647 bp and simultaneously from the affected trachea of birds suspected for ILT. DNA was extracted from tracheal and infected CAM of ILTV samples by using Bio-Basic® Genomic DNA Extraction kit and thymidine kinase gene was amplified by using PCR system 9600 Thermocycler. Twenty CAM and tracheal samples were positively amplified by polymerase chain reaction. A procedure was developed for rapid detection of infectious laryngotracheitis virus by polymerase chain reaction of the conserved region of viral thymidine kinase gene containing DNA fragments. This suggests a polymerase chain reaction procedure for early diagnosis and less time consuming test for the detection of field strains of infectious laryngotracheitis virus in layers.