A Cost-Effective, Sensitive, and Specific Assay for the Detection of Avian Rotavirus and Picornavirus and Development of Predictive Models of Virus Prevalence via a Retrospective Analysis of Avian Enteric Disease in the United States

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Avian rotavirus and picornavirus are among the leading causes of gastrointestinal illnesses in poultry species around the world, and therefore, entail major economic loss for the global livestock industry. Conventional diagnostic methods, due to high costs and equipment requirements, have been unable to curb this loss. Hence, the goal was to utilize highly applicable molecular techniques, namely, Reverse-Transcription Polymerase Chain Reaction, to develop a sensitive, specific, and cost-effective diagnostic assay for Avian rotavirus and picornavirus.RT-PCR primers were designed from all published sequences of the viruses. Conserved regions were manually searched for primer sites based on predefined primer design criteria that emphasized high genome binding capability and safeguarding against mispriming. Combined with the primers, positive and negative control samples underwent standard RT-PCR thermal cycles.Analysis of these initial RT-PCR products provided evidence of erroneous amplification; to correct this, an extensive full factorial experiment in which key PCR variables were altered against one another was conducted. The RT-PCR protocol that yielded the best results (89% sensitive/86% specific) was validated by field testing with poultry farms in the Midwest (~65% decrease in time for diagnostic results and ~72% decrease in overall costs) and was used to screen 980 samples of enteric disease cases from 20 US states. Phylogenetic analyses and examination of data isolated by geographic region, time of disease onset, and poult age offered valuable insight into many of the viruses' most important characteristics; this could be key to the development of new treatment strategies, and, in conjunction with the assay, could stimulate major advancement of the livestock industry.

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