Cairo University
Faculty of Veterinary medicine
Department of Virology

Development of Recombinase Polymerase Amplification (RPA) assays for detecting *Avian influenza viruses*

Thesis presented by

By

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For the degree of Philosophy of Doctor in Veterinary Medical Science
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Supervision sheet

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Abstract

Avian influenza virus can lead to severe economic losses in the poultry industry.The most common types of AIV in Egypt are highly pathogenic AI H5N1 and low pathogenic AI H9N2. AI H5N1 caused economic damage and significant threat to public health and AI H9N2 cause severe economic losses in poultry industry especially when co-infection took place with other pathogens .Therefore, the rapid detection of AI H5,H9 viruses is very important in order to control the disease. In this study, Reverse Transcription Recombinase Polymerase Amplification assay (RT-RPA) assay for the detection of AI subtype H5, H9 was developed. An *in-vitro* transcribed RNA standard for AI H5, H9 and standard virus titration by EID_{50} for AI H9N2 was developed and used to determine the assay sensitivity. The AI H5 RT-RPA assay was able to detect one RNA molecule/ul and AI H9 RT-RPA can detect one EID_{50} or one RNA molecule/ul within 8 minutes than H5 real-time RT-PCR that detect one copy of RNA and H9 real-time RT-PCR detect one log 10 EID_{50} in 90 minutes. AI H5 and H9 RT-RPA assay did not detect nucleic acid extracted from AI H5 and H9 negative samples or from other pathogen producing respiratory manifestation in poultry. The clinical performance of the AI H5 and H9 RT-RPA assay was tested in 30 samples; the sensitivity of AI H5 and H9 RT-RPA and real-time RT-PCR were 100%.AI H5 RT-RPA was able to detect multiple mutant strains of AI H5N1 field samples and other strain as AI H5N2 and AI H5N8 in the presence of up to fourteen mutations. In conclusion, AI H5 and H9 RT-RPA were faster, simple, sensitive, specific and portable than real-time RT-PCR.

Key words: Avian influenza, AI H5N1, AI H9N2, RT-RPA, real-time RT-PCR, diagnosis.

Dedication

Dedicated to my family

….. Father,

….. Mother

…….. My Brother

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