

Detection of avian influenza virus and Newcastle Disease virus by duplex One Step RT PCR

Research Article

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Abstract: Newcastle disease Virus (NDV), a member of the *Paramyxoviridae* family, and Influenza virus, from the *Orthomyxoviridae* family, are two main avian pathogens that cause serious economic problems in poultry farming. NDV strains are classified into three major pathotypes: velogenic, mesogenic, and lentogenic. Avian influenza viruses (AIV) are also divided into: low pathogenic (LPAI) and highly pathogenic (HPAI) strains. Both viruses are enveloped, single stranded, negative-sense RNA viruses which give similar symptoms ranging from sub-clinical infections to severe disease, including loss in egg production, acute respiratory syndrome, and high mortality, depending on their level of pathogenicity. This similarity hinders diagnosis when based solely on clinical and post mortem examination. Most of the currently available molecular detection methods are also pathogen-specific, so that more than one RT-PCR is then required to confirm or exclude the presence of both pathogens. To overcome this disadvantage, we have applied a One Step Duplex RT-PCR method to distinguish between those two pathogens. The main objective of the project was to develop a universal, fast, and inexpensive method which could be used in any veterinary laboratory.

Keywords: Newcastle disease virus • Influenza virus • Differentiation • One Step RT-PCR • Duplex

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1. Introduction

Avian influenza virus (AIV) is a member of the *Orthomyxoviridae* family. It has a spherical, enveloped capsid composed of a matrix protein. The virus contains linear, segmented, single-stranded RNA of negative polarity, covered by nucleoprotein. Eight segments encode twelve proteins. On the surface of the virion, there are ion channels and two main glycoproteins, hemagglutinin and neuraminidase, which are responsible for the diversity of this virus. Highly pathogenic avian influenza (HPAI) strain cause severe symptoms in the respiratory tract, as well as in the digestive and nervous systems which lead to a high mortality rate of up to 100%. This group includes, in particular, some H5 and H7 influenza strains. Other strains cause milder symptoms: apathy, decrease in laying capacity, and mild respiratory tract disorders, and are known as LPAI (low pathogenic avian influenza)

[1,2]. The differences in virulence level have a molecular basis, namely changes in the cleavage site of HA0. In HPAI strains, in the cleavage site, there is a region rich with basic amino acids. This motif can be cleaved by the majority of enzymes present in various cells of the host organism [3].

Newcastle disease virus (NDV) belongs to *Mononegavirales* order, *Paramyxoviridae* family, and *Avulavirus* genus. The viral particle is composed of a matrix protein, surface glycoproteins (hemagglutinin-neuraminidase and a fusion protein), and has a lipid double layer on its surface. The genome of NDV is linear, non-segmented, single-stranded RNA encoding 8 proteins [4]. NDV strains are classified into one of the three major pathotypes: velogenic, mesogenic, and lentogenic [5], according to pathogenicity *in vivo*. Similarly to influenza virus, NDV pathogenicity has a molecular background. The product of F gene of highly pathogenic strains

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