OMICS Group Conferences
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International Conference on
Oceanography & Natural Disasters
August 21-23, 2013 Holiday Inn Orlando International Airport, Orlando, FL, USA

Development of 'flock-side' diagnostic assays and surveillance methodologies for the detection of *avian influenza virus* (AIV) in South Africa

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The South African commercial agriculture sector is periodically affected by viral diseases that threaten food security, injure rural economies, and potentially disrupt trade relations. Between 2004 and 2012, three outbreaks of avian influenza virus (AIV) were reported to occur within the South African ostrich industry. The persistence of AIV within the ostrich population and the inability completely to eradicate the virus poses a risk of an outbreak of a highly pathogenic avian influenza (HPAI) to the commercial poultry industry, thus significantly threatening the country's food security. The absence of suitable disease surveillance and monitoring technologies, coupled with inadequate diagnostic facilities at the 'flock-side' are the major obstacles in controlling AIV outbreaks in South Africa. In this proof of concept study and in partnership with Axxin, we developed a 'flock-side' diagnostic assay for the molecular detection of avian influenza. The assay development encompasses three components that include virus isolation and concentration followed by isothermal amplification of avian influenza subtypes in South Africa and finally, an adequate detection method to detect the amplified products. Genomic RNA was extracted from avian influenza viral stocks (high and low pathogenic strains) using the QIAamp RNA extraction kit and LAMP (Loop mediated isothermal amplification) assays, specific for the amplification of the matrix (M) gene as well as the hemagluttinin (HA) genes of South African H5 and H7 AIV isolates were carried out using the Axxin Lamp device. We further compared the specificity and sensitivity of the LAMP assay to published conventional RT-PCR as well as real-time RT-PCR assays. The LAMP assays were optimized at 65°C for 30min and were able to detect both high and low pathogenic AIV strains with high sensitivity and specificity. The detection limit of the assay was determined to be 100-fold higher than conventional RT-PCR assays and comparable to real-time RT-PCR assays. These preliminary results indicate that the simple and versatile diagnostic platform is sensitive enough for the on-site detection of avian influenza infections. Multiplexing the developed assays will enable accurate identification of the virus subtype circulating during an outbreak and therefore facilitate the establishment of appropriate contingency plans to control the spread of the virus and ultimately assist in food security and economic prosperity in South Africa.

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