(Abstract)

ISOLATION, CHARACTERIZATION AND EVALUATION OF REAL TIME RT-PCR PROCEDURES FOR DIAGNOSIS OF CLINICAL SAMPLES OF FOOT AND MOUTH DISEASE VIRUS IN NEPAL

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Foot and Mouth Disease (FMD) is severe, highly contagious disease of cloven - hoofed animals that affects cattle, buffaloes, sheep, goats, pigs and various wildlife species. Different countries have a different FMD status which requires separate approach for defining the diagnostic and control strategy. A variety of new diagnostic tests and procedures have been developed to improve FMD laboratory diagnosis. A study on evaluation of different diagnostic techniques of FMD was conducted during November 2010 to August 2011 at FMD and TAD’s Laboratory and Central Veterinary Laboratory, Kathmandu. During the research period samples from different parts of the Nepal were collected and were used for the different diagnostic techniques like cell culture isolation, ELISA and real time PCR. The aim of this study is to evaluate the contemporary diagnostic tools and the ability to detect FMD virus or viral genome in field samples and cell culture fluids using an ELISA, TaqMan real-time RT-PCR.
The objective of this study was to characterize FMD type O, A, C and ASIA-1 virus isolated from different districts of Nepal where the outbreak had occurred between 2010 and 2011. FMD type O and type Asia-1 virus were detected in this study. Viruses were adapted to BHK-21 cells or were directly used for typing by sandwich ELISA and the virus sample were used for extraction of viral RNA for RT-PCR reaction with the universal primer for all type of FMDV. Thus the probe/primer set are designed from highly conserved regions of FMD viral genome - IRES (internal ribosomal entry site) ensuring detection of all seven serotypes of FMD virus.

Key words: Foot and Mouth Disease (FMD), Cell culture isolation, Immunological assay and Nucleic acid Recognition assay.