Name: Omnia Hamdy Muhammad Refaei
Date of Birth: 27/1/1988
Place of birth: Cairo Egypt
Title of M.V.Sc thesis: Role of ticks in the epidemiology of lumpy skin disease
Supervisors:
Prof.Dr. Adel A. Fayed
Prof.Dr. Amr A. El-Sayed
Prof.Dr. Ausama A. Yousif

Abstract

Lumpy skin disease (LSD) is an economically important arthropods porn viral disease of cattle. Lumpy skin disease virus (LSDV) is a *Capripoxvirus* that belongs to subfamily *Chordopoxvirinae* of family *Poxviridae*. LSDV is the etiological agent of LSD. *Capripoxvirus* members are a group of genetically and antigenically similar viruses. LSD is endemic in most countries of Africa and the Middle East, and is an exotic threat to European countries. Live attenuated SPPV is used as a vaccine in endemic LSD control. Cattle vaccinated with a LSD vaccine containing SPPV seed can develop LSD due to induction of partial protection in vaccinated animals, or as a result of vaccine seed contamination with non-highly-attenuated LSDV. LSDV field control efforts and vaccine production require differentiation between LSDV and SPPV. PCR assays that differentiated LSDV from other capripoxviruses were described; however, the techniques used were either dependent on size differentiation of PCR products, the use of restriction enzymes, or melt-curve analysis. In this study, a simple LSDV-specific PCR assay was developed to reliably differentiate between both viruses. Primers were designed based on whole genome sequence analysis to amplify LSDV DNA spanning parts of an envelope protein gene and an adjacent core virion protein gene. The assay design allowed amplification of a long (1452 bp) LSDV PCR product to facilitate sequence comparisons and generation of purified DNA templates for determination of the test detection sensitivity. In addition, an alternative reverse primer allowed the amplification of a short (502 bp) PCR product that can be used for rapid single-step diagnosis and in-process vaccine seed identification. The test was found to have high specificity and sensitivity either by conventional or Real-Time formats. The LSDV-specific PCR developed provides a rapid, simple, and reliable tool for diagnosis and vaccine quality control that can be incorporated in LSD control programs. Recent experimental evidence show that ticks play a role in LSDV transmission, and that LSDV can be transmitted transovarial and transstadial in ixodid ticks. In addition to experimental evidence, several aspects of tick involvement in virus epidemiology during natural outbreaks need to be investigated. Among which, is the presence of LSDV in ticks infesting diseased animals and contacts during natural infection, whether all ticks on infected animals become infected with LSDV, and whether there is evidence to support viral replication in ticks during natural infection. Our study was aimed at detecting LSDV DNA in the viscera of two species of ixodid (hard) ticks collected from the skin of naturally infected unvaccinated cattle during the 2014 LSDV outbreak in Egypt. Ticks and animals were tested using the diagnostic LSDV-specific PCR technique. Tested animals were PCR-positive. Twenty eight *Boophilus* and *Rhipicephalus* ticks were tested. Eighteen ticks were PCR-positive (64.3%). One of two ticks collected separately from an apparently healthy contact animal during the same outbreak was positive to LSDV DNA. The duration ticks spent on the animals was not related to the detection of LSDV DNA in their viscera. Semi-quantitative analysis of LSDV-specific PCR products of DNA extracts from tick viscera suggests active virus replication in ticks. This work supports experimental evidence of hard tick involvement in LSD epidemiology, and the importance of tick control in LSD control programs.
Keywords: *Capripoxvirus*; Differentiation; Lumpy skin disease virus; PCR; Vaccine quality control; Ixodid ticks; Natural outbreak; Replication