Journal of Applied Biological Sciences 10 (2): 01-03, 2016
ISSN: 1307-1130, E-ISSN: 2146-0108, www.nobel.gen.tr

The Molecular Detection of Lumpy Skin Disease Virus from Infected Cattle in Turkey

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Received: February 11, 2016
Accepted: March 28, 2016

Abstract

LSD is endemic in Africa but through the last years disease begins to spread to the other countries. In Turkey, the disease has been detected for the first time in 2013 in East Mediterranean Province and then continued to spread and caused enormous economic losses through the other regions of country. In this study, we examined blood (n=1), skin nodules (n=2) from three cows suspected of LSD and conjunctival swab samples (n=1) from cow with conjunctivitis and corneal opasity (suspected of MCF) that were administered to our laboratory from four different dairy cattle herds between 2014 to 2015. By performing PCR and using specific primers for viral attachment protein and as a result, we have demonstrated that all the mentioned animals are positive. According to the our data, LSD could been seen in animals with different clinical conditions located in different regions of our country, and immediate transportation of LSDV suspicious samples to the efficient laboratories and correct diagnosis of the disease is an important factor for prevention of spreading, control (like quarantine/vaccination) of the disease and avoiding serious economical losses.

Keywords: Cattle, Conjuctivitis, Lumpy skin disease, PCR

INTRODUCTION

Lumpy skin disease virus (LSDV), a member of Capripoxvirus genus and Poxviridae family, is a DNA virus which is antigenically related to Sheeppox and Goatpoxviruses. Virus transmission occurs mainly via insect vectors however other routes like secretion and excretion sometimes play role in the spreading of infection among the cattle [1]. The most characteristic symptoms of disease are the enlargement of lymph nodes and nodule-like lesions on the skin of infected animals. Besides that, fever, salivation, lacrimation, conjunctivitis and corneal opacity are common symptoms. Due to the mentioned symptoms the LSD's differential diagnosis must be performed from other diseases like Malignant Catarhal Fever (MCF). Morbidity rate of the virus is high but the mortality is generally rare. However due to the milk yield drop, abortion, infertility, weight loss and decreased skin quality, this infection causes important economical losses[2].

LSD is endemic in Africa but the last years disease begins to spread to the other countries. In Turkey, the disease has been detected for the first time in 2013 in East Mediterranean Province and then continued to spread and caused enormous economic losses through the other regions of country. The disease would mainly be transferred to infection-free areas by transport of infected animals and vectors. The risk of that depends on the virus prevalence in the country of origin and the number of animals illegally moved in [3,4].

By considering all the facts about this disease, we have analysed the required materials from blood sample, skin nodules, conjunctival swab from animals showing conjunctivitis and corneal opacity and tested by PCR using specific primers for viral attachment protein.

MATERIAL and METHOD

PCR and Sequence Analysis

We have examined blood sample (n=1), skin nodules (n=2) from three cows suspected of LSD and conjunctival swab sample (n=1) from cow with conjunctivitis and corneal opasity (suspected of MCF) that were administered to our laboratory from four different dairy cattle herds between 2014 to 2015 (Figure 1). DNA extraction from the samples were carried out according to Sambrook and others [5,6]. The detection of LSD DNA was performed by PCR using specific primers coding region of viral attachment protein as described by OIE [3] with some minor modifications. For the amplification, the following set of primers were used: 5'- TCC GAG CTC TTT TCT TAC TAT-3' and 5'-TAT GGT ACC TAA ATT ATA TAC GTA AAT AAC-3' generating a product of 192 bp [3]. In addition, all samples were tested for BoHV-1, BoHV-2, BoHV-4 and MCF. Briefly, 3µl of DNA was subjected to thermocycling in a 30µl reaction mixture containing 2.5U Taq DNA Polymerase (Fermentas, Lithuania), 3.5mM dNTP mix (25 mM Fermentas, Lithuania), 10 pmol of each primers, 1.5 mM MgCl2, 10X PCR reaction buffer. Thermal cycling conditions were, first denaturation at 95°C for 5 min followed by 95°C for 45 sec, 35 cycles of 50°C for 50 sec, 72°C for 1 min. In addition, reaction tubes were kept further 10 min at 72°C for final extension. PCR products were visualized in transilluminator after electrophoresis in 1% agarose gel containing ethidium bromide. The amplified products of Turkish field strains (n=2) were then gel purified using a commercial kit (GeneMark, Taiwan). Sequence analysis of the PCR products were determined using CEQ8000 Genetic Analysis system (Beckman Coulter, USA). Phylogenetic analysis based on partial viral attachment protein gene sequences of LSD's was performed using the Neighbour-Joining method by MEGA6 software [8].

RESULTS

All samples (n=4) were found positive for LSD, and negative for other tested viruses (BoHV-1, BoHV-2, BoHV-4 and MCF) (Figure 2). Then, two samples that were PCR positive for LSD, confirmed with sequencing analysis and phylogenetic trees were presented in Figure 3. It should be noted that only animal with conjunctivitis/corneal opasity was LSD positive, this is an important finding for sampling of suspected animals.

DISCUSSION and CONCLUSION

LSD is endemic in many African and Asian countries, and it is rapidly spreading throughout the Middle East, including Turkey. The outbreaks that have occurred in Turkey most likely originated by the introduction of LSD from neighbor countries like Syria, thus suggesting that political unrest may facilitate the disease spreading [7]. In Turkey, there is a large number of cattle movements from provinces that have Lumpy skin disease.The introduction of infected animals is the most important way to introduce LSDV into a country, in particular by spreading to long-distances. The spread of LSD is usually limited to a short distance when infected animals are not moved to non-affected areas. The active movement of flying vectors can be a route for LSD introduction into a naive country from a short distance, e.g. from infected areas close to the borders.

By considering all the facts about this disease, immediate transportation of LSDV suspicious samples to the efficient laboratories and correct diagnosis of the disease is an important factor for prevention of spreading, control (like quarantine/vaccination) of the disease and avoiding serious economical losses. Homologous vaccines are more effective than sheep pox strain vaccines. The safety of the vaccines should be improved and the development of vaccines for differentiating between infected and vaccinated animals is recommended [7]. Active surveillance, rapid detection and prompt culling of infected herds are effective measures for LSD control. The role of vectors for LSD transmission should be further investigated in both controlled environments and the field. The cooperation of the EU with neighbouring countries should be encouraged to prevent transboundary disease spread.

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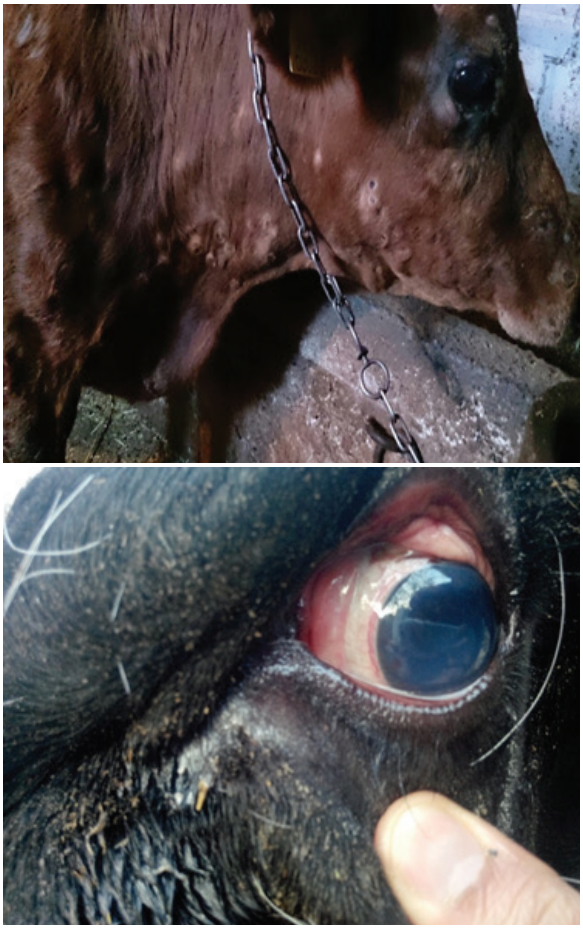

Figure 1. Images taken from clinically infected animals.

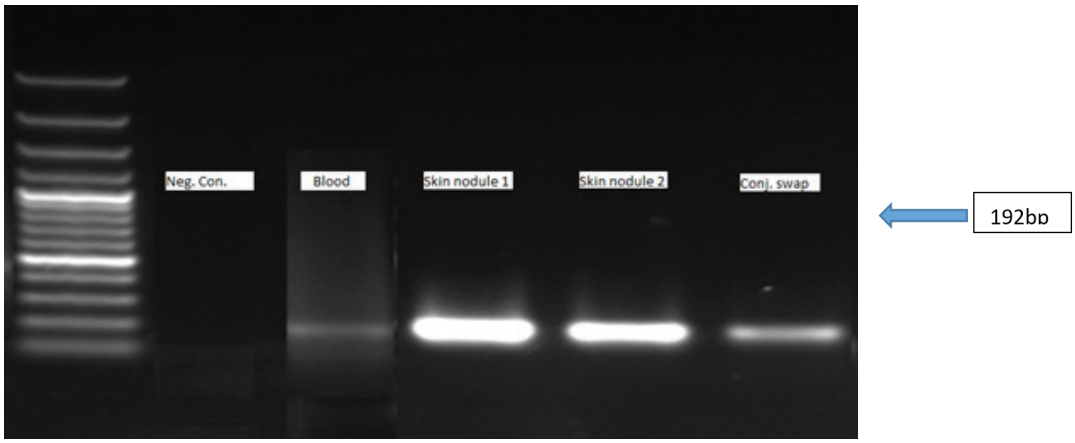

Figure 2. Agarose gel image of PCR products of the samples

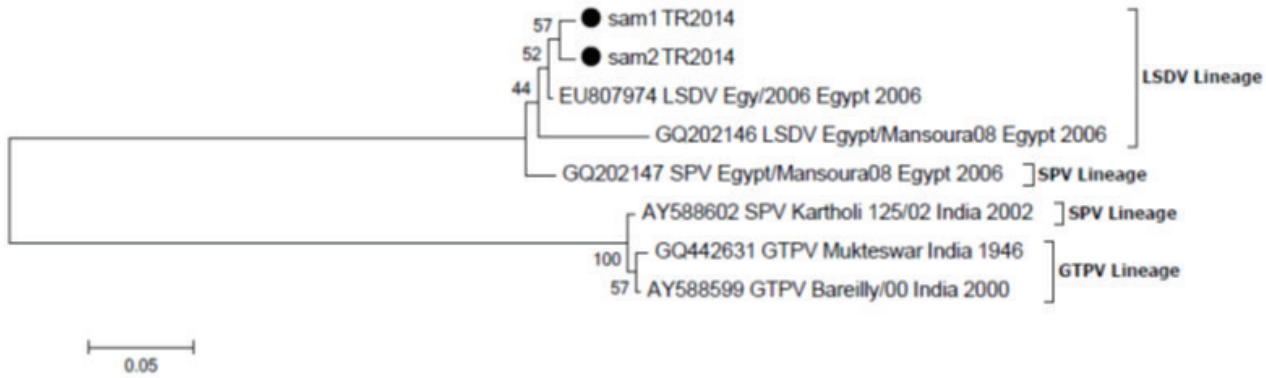

Figure 3. Phylogenetic analysis based on partial viral attachment protein gene.