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"Molecular diagnosis of LSD virus in cattle"

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Summary

LSD is a serious infectious skin disease which considered one of the most economically important viral diseases that infect cattle. The disease is endemic in Egypt and further isolation and characterization of the LSD virus is required to provide more reliable methods for detection and control of outbreak of the virus so, the present study show trials for molecular detection and isolation of the virus.

The applied experiments revealed that: -

1- Trails for detection of LSDV in skin biopsy samples (nodules and scabs), blood samples, serum samples from cattle, sheep and goat and rectal swab sample by using real-time PCR and all the samples were positive with the lowest Ct values for skin biopsy samples.

2- The detection of LSDV in three skin nodular samples of clinically suspected cattle by three blind passages on MDBK cell cultured. The isolated virus induced the characteristic CPE which appeared in the form of cell rounding, cell aggregation and cells coalesce together forming clusters and the CPE increased gradually till 70-90% of the sheet become completely detached.

3- The detection of LSDV in three skin nodular samples of clinically suspected cattle by three blind passages on CAM of SPF-ECE. The isolated virus induced the characteristic lesion on CAM which appeared in the form of congestion and clotting of blood in CAM Blood vessels, presence of pock lesion in the form of small, scattered, numerous white foci and turbidity of the membrane.

4- The passages on MDBK cell culture and on CAM were subjected to real-time PCR to detect the difference in Ct values between the original samples and

their passage. The passages showed higher Ct values than their original samples.

5- Serum samples from cattle, sheep and goat were examined by ELISA for detection of antibodies against Capripoxviruses and only one serum sample from cattle (showed signs of LSD two months before collecting the sample) was positive. Serum samples were collected after one month from the previous three negative cattle to be tested again by ELISA to detect the presence of antibodies. This time the three serum samples give positive result with ELISA test.

6- Molecular identification of LSDV in (13) Nodular samples and (2) scab samples using conventional PCR for the amplification of fusion protein coding gene. 11 nodular samples and only one scab sample were positive showing specific PCR product at the correct expected size of fusion protein coding gene (410 bp). Four samples were applied to re-PCR to increase the concentration of the PCR product and the sharpness of bands. Three of them gave sharp clear bands (410 bp) while the fourth one which was considered negative gave a faint band at the same size (410 bp).

7- Further molecular identification of LSDV in (13) Nodular samples, (2) scab samples and sheeppox vaccine using conventional PCR for the amplification of RBO30 gene. Sheeppox vaccine was used as reference to approve the specificity of RBO30 primers. The two scab samples and (7) nodular samples were positive while (6) nodular samples and the sheeppox vaccine were negative.

8- Sequencing of purified PCR products obtained from four nodular samples.

9- Alignment of the four LSDV strains in this study with each other which revealed 100% nucleotide identity between strains in this study. Then the strains in our study were aligned with other published LSDV, SPV and GPV strains available in gene bank database and they show high percent of nucleotide identity with LSDV strains ranged from 100-99%.

10- Construction of phylogenetic tree to calculate, examine the evolutionary relationships of the sequence and show the relationship between LSDV strains of the study and LSDV, SPV and GPV strains available in gene bank database. The phylogenetic analysis show that our strains are closely related to LSDV group than to GPV and SPV.