Recent isolation and identification of a lumpy skin disease virus from Qaluobia Province, Egypt 2016

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A B S T R A C T

Cases of skin nodules eruptions suspected for lumpy skin disease virus (LSDV) are becoming more frequent in Qaluobia Province, Egypt in cattle populations during summer and autumn of 2016. In this study, LSDV associated with the outbreak was isolated from skin nodules of unvaccinated cattle on chorioallantoic membrane (CAM) of specific pathogen free embryonated chicken eggs (SPF-ECEs) and Madin derby bovine kidney (MDBK) cell line. The isolate induced pock lesion on CAM and rounding, aggregation and detachment of MDBK cells. Identification of LSDV isolate was confirmed by neutralization test and polymerase chain reaction (PCR) targeting LSDV fusion protein encoding gene. In conclusion, LSDV is still circulated in Egypt and further molecular and biological characterization is required.

Key words: LSDV; cattle; chick embryos; Neutralization, PCR; Egypt.

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1. INTRODUCTION

Lumpy skin disease virus (LSDV) is a Capripoxvirus within the subfamily Chordopoxvirinae, family Poxviridae (Babiuk et al. 2008). There is only one serotype of LSD virus, and it is very closely related serologically to the virus of sheep and goat pox (SGP) (Burdin, 1959). Natural infection has not been reported in any other ruminant species with the exception of a report of five cases in water buffalo (Bubalus bubalis) in Egypt (Ali et al., 1990).

The genome of LSDV is 151 kbp long with a central coding region bounded by identical 2.4 kbp-inverted terminal repeats and coded for 156 putative genes (Tulman et al, 2001). The capripox virus genomes are highly conserved; more than 95% homology among LSDV, SPPV and GTPV can be ascertained (Tulman et al., 2002).

In Egypt, LSDV was first isolated and identified from cattle during two outbreaks in Suez and Ismailia governorates on 1989 (House et al., 1990). In early 2006, a LSD outbreak has invaded cattle in different localities of Egypt, exerting severe economic losses to livestock industry (El-Kholy et al., 2008).

LSDV is usually associated with high morbidity and low mortality causes economic losses because of decreased weight gain, permanent damage to hides, decreased milk production and infertility (Woods, 1988).

In infected cattle, LSDV exists in nodules, crusts of skin lesions, blood, saliva, nasal discharge, semen and milk (Babiuk et al. 2008).

Transmission of LSDV is likely to be through mechanical transmission of the virus by a variety of blood- feeding vectors especially by Aedes aegypti mosquitoes (Chihota et al., 2001).

In the majority of animals the superficial lymph nodes are enlarged. Skin nodules, the characteristic feature of the disease, appear before or during the second rise in body temperature, four to ten days after the initial febrile response (Coetzer and Tuppurainen 2004).

The tentative diagnosis of LSD is usually based on characteristic clinical signs, and the clinical diagnosis is confirmed by using conventional PCR (Tuppurainen et al., 2005). The key objective of this work is a trial for isolation and identification of LSDV in skin nodule samples from clinically infected cattle at Qaluobia province during the year 2016.

2. MATERIALS AND METHODS

2.1. Clinical history and sample collection

Between May and August 2016, cattle from different farms in Qaluobia Province, Egypt, were reported to have developed skin nodules. Most of the cattle had severe symptoms where nodules and scabs scattered all over their body parts including vulva, teat, and forelimbs. The cattle were unvaccinated against LSDV. Skin nodules were aseptically collected either on phosphate buffer saline or frozen until they were used for later study.

2.2. Virus isolation in ECE and MDBK cell line

Three Skin nodules homogenate was prepared in phosphate buffered saline (PBS, PH 7.4) with 100 U/ml penicillin and 100 mg/ml streptomycin. The homogenate was lysed by freezing and thawing three times, and the supernatant was purified by centrifugation at 6000 xg for 5 min at 4°C and filtration through a 0.45 mm pore-size cellulose acetate filter. SPF-ECEs after 9 days of incubation were inoculated with 0.2ml of the supernatant, which was dropped onto through chorioallantoic membrane (CAM) (House et al 1990). The sample was grown at least three times in CAM to increase the titer of the virus. The eggs were daily examined till 5–7 days post-inoculation. The embryos that died within the first 24 h were classified as nonspecific deaths. Signs on CAM were recorded and subjected to LSDV identification.

While suspected prepared sample were inoculated on MDBK cell line according to (OIE, 2010) and examined daily for the presence of cytopathic effect (CPE).

2.3. Neutralization technique

It was carried out on MDBK cell for all examined nodular samples line according to house et al (1990) where LSDV infection was confirmed using the LSDV reference antisera (supplied by Department of Pox Virus Vaccine Research and Production, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt). That prevents CPE produced on MDBK cells by inoculated virus.

2.4. DNA extraction and PCR

DNA was extracted from 4 nodular samples by using the DNeasy Tissue Kit (Qiagen, USA) according to the manufacturer's instructions. The primers used were: LSD 5' - ATGGACAGAGCTTTATCA-3'(Forward), LSD 5' - TCATAGTGTTTGTACTTCG -3` (Reverse). The reaction conditions were: 94 ◦C for 2min, 35 cycles of 94 ◦C for 20 s, 52 ◦C for 30 s and 72 ◦C for 50 s and a final extension step of 72 ◦C for 5 min. The primers were specific for LSDV fusion protein-encoding. The expected size of the PCR product was 410 bp according to El-Bagoury et al, (2009).

3. RESULTS

3.1. Virus isolation

Isolation of LSDV on CAM of ECE revealed congestion and clotting of blood in CAM Blood vessels, presence of pock lesion in the form of small, scattered, numerous white foci **(Fig. 1).** While characteristic CPE of the virus were rounding, aggregation and detachment of MDBK cells (Fig. 2).

3.2. Neutralization assay

The characteristic CPE produced by inoculated samples were inhibited by the specific antisera of LSDV indicate the isolate was LSDV.

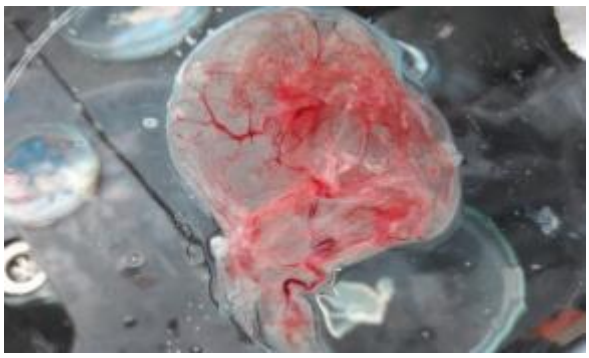

Fig.1. Pock lesion of LSDV on CAM numerous, small, scattered white foci and congestion

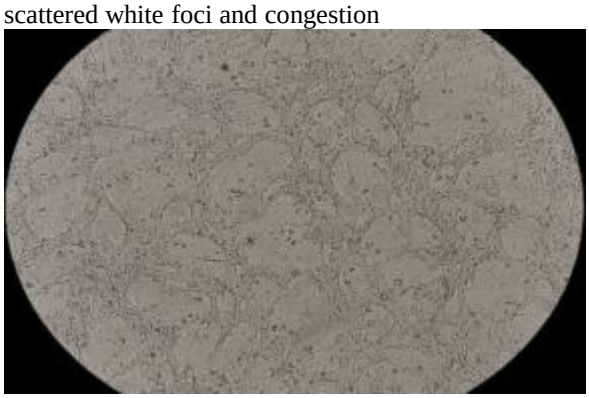

Fig. 2. CPE of LSDV isolate on MDBK cell line in the form rounding, aggregation of cells and cells detachment (Magnificent power: 10 x)

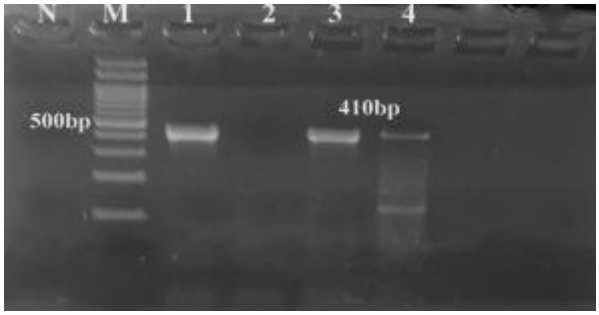

Fig. 3. All the nodular samples were positive except one sample, Lane N: negative control. Lane m: High molecular weight nucleic acid marker (100bp). Lanes 1, 3, and 4: Positive nodular samples. Lane 2: Negative nodular sample

3.3. PCR

The primer targeted the LSDV envelope protein-like gene was succeeded to amplify the specific products (410bp) from the extracted DNA products of original skin sample, infected CAM and embryo liver using PCR (fig.3).

4. DISCUSSION

According to the findings of this study, the LSDV was isolated from a recent outbreak in Qaluobia Province, Egypt in cattle populations during 2016. The isolate was able to replicate in CAMs and chick embryos of SPF-ECE like other Egyptian isolate (House et al., 1990; Tamam 2006; El-Kenawy and El-Tholoth, 2010; El-Nahas et al, 2011). Although most Egyptian LSDV strains were successfully induce lesion on CAM that varied from thickening and congestion (El-Kenawy and El-Tholoth, 2011) to clearly visible Pock lesion (House et al., 1990) that appeared like numerous, small, scattered white foci (El-Nahas et al,2011).

House et al., (1990) demonstrated that neutralization has high accuracy to be used for the diagnosis and Sero-surveillance analysis of LSD in the target population. The neutralization was able to detect and identify LSDV antigen in infected CAM and MDBK cells that infected by chick embryo homogenate emphasis the responsibility of the virus on the signs observed on CAM and chick embryo.

LSD, goat pox and sheep pox viruses are serologically identical, and so their specific identification relies exclusively on the use of molecular tools (Le Goff et al 2009). Although recent molecular studies suggest that the Capripoxvirus genus including SPPV, GTPV and LSDV are very similar in terms of antigenic characteristics, these viruses are phylogenetically distinct and can be differentiated by accurate molecular techniques (Bhanuprakash et al., 2006)

The PCR using primer targeted the LSDV fusion protein-like gene was sensitive to detect LSDV strain in its original skin samples (El-Bagoury et al., 2009).

Conclusion: LSDV isolate was circulated in Egypt and further molecular sequencing for more specific sufficient target were needed and cross neutralization using monoclonal antibodies between our strain and the used neethling type vaccine recommended.

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