j.Egypt.vet.med.Assoc 76, no 4. 497- 502 (2016)

# IDENTIFICATION OF LUMPY SKIN DISEASE FROM NATURALLY INFECTED CATTLE AT EL FAYOUM, EGYPT (2015)

By

**El-Sayed, M.M. El-Sayed; H.M. El-Saeid; Samah. Iman. Bastaweesy;**

**Samah.E.M. El-Saman and anwar, A.Z.**

Department of Medicine and Infectious Diseases,

Faculty of Veterinary medicine, Cairo University, Egypt

## ABSTRACT

Lumpy skin disease virus (LSDV) was isolated from skin biopsies collected from clinically diseased cattle at El-Fayoum governorate. The virus isolated on the chorioallantoic membrane of Embryonated chicken egg (ECE) and propagated on cell culture of MDBK cells followed by conventional PCR assay.

**Key words:**

Lumpy skin disease virus, Embryonated chicken egg and PCR)

## INTRODUCTION

Lumpy skin disease is considered one of the most serious diseases of cattle caused by lumpy skin disease virus (LSDV) type (Neethling strain), which together with sheep poxvirus (ShPV), and goat poxvirus (GPV) related to *capripoxvirus* genus within *chordopoxvirus* subfamily of the *poxviridae* **(Woods 1988 and Fenner, 1996).** Lumpy skin disease is potential for rapid spread and ability to cause severe economic losses. Capripoxvirus are antigenically indistinguishable from each other and able to induce heterologous cross protection and the complete nucleotide sequences of the capripoxvirus genomes are 97% similar **(Davies, 1991 and Tulman *et al.*, 2002).** The investigation about the LSD virus is aided by the isolation of LSDV on the chorioallantoic membrane of embryonated chicken egg also isolated on the cell culture (MDBK) cells.

## MATERIAL AND METHODS

### Tissue specimens:

Twenty skin nodules collected from clinically infected cattle showing signs of lumpy skin disease. The skin nodules were preserved at -80˚c for isolation purpose.

**Cultures for virus isolation:**

Nine to ten - day-old SPF- embryonated chicken eggs (ECE) were inoculated via chorioallantoic membrane (CAM) route. The cell culture used for isolation is Madin Darby Bovine kidney (MDBK) cells propagated with Eagle's minimum essential medium (EMEM) supplemented with 2%fetal bovine serum (FBS).

**Reference Virus:**

Lumpy skin disease virus (LSDV) Neethling strain (lumpyvax).

**Molecular Identification of Virus Isolates.**

**Extraction of Viral DNA:**

Viral DNA was extracted from skin biopsy by Using DNeasy Blood and Tissue Kit (Qiagen, Germany) and stored at-20°C until used in PCR.

**Polymerase Chain Reaction (PCR) Assay:** It was performed according to the procedures of **Ireland and Binepal, 1998.**The PCR primers were designed for the gene for viral attachment protein were used. PCR reaction was applied in a total volume of 50 µl containing: 1 X PCR buffer (20 mM Tris HCl pH 8.4 and 50 mM KCl); 1.5 mM MgCl2; 0.2 mM deoxynucleosides triphosphates mixture (dATP, dCTP, dGTP and dTTP); 20 pmol of each primer; 2.5 units (U) Thermus aquaticus Taq polymerase of 0.1µg of extracted viral DNA and nuclease-free sterile double distilled water up to 50.0 µl. Then the resulting mixture was subjected to precise thermal profile in a programmable thermocycler as follows: One cycle of: 94 °C for 2 min; 40 cycles of: 94°C for 50 seconds, 50° C for 50 sec and 72° C for 1 min; followed by one final cycle of 72° C for 10 min.

**Analysis of PCR Amplification Products (Amplicons):**

The resulting PCR amplicons (10-15 µl) were analyzed by 2% agarose gel Electrophoresis.

**Virus isolation on CAM:** SPF ten-day-old Embryonated chicken egg (ECE) was inoculated with the processed skin samples via chorioallantoic membrane (CAM) method. 0.2 ml from the prepared samples was inoculated via chorioallantoic membrane (CAM) of 9-11-day old, according to **Van Rooyen *et al.* (1969)**. The inoculated egg incubated at 37˚c for 5-7 days.

**Virus isolation on MDBK cell line:**

The prepared tissue samples were inoculated for three passages into cell culture according to the method described by **(Carn and Kitching, 1995)**.

## RESULTS

The chorioallantoic membrane's inoculated with field samples revealed pock lesions scattered all over the membrane around the site of inoculation. The size and shape of pock lesions differ from each other. The Characteristic pock lesions were observed after 1st passage and became clear after the 3rd passage as shown in Fig. (1, 2). A clear cytopathic effect (CPE) appeared on the inoculated MDBK cells with the field samples after third passage as cell rounding, cell aggregation, coalesce together forming clusters that scattered all over the monolayer within 72 hr PI and gradually increased till 70-80 % of sheet was completely detached as shown in Fig. (3, 4). The molecular diagnosis: The DNA extract were amplified by the specific primers giving DNA fragment of 192bp as the expected amplicon size of LSDV. Shown in Fig. (5).

**Fig. (1):** Pock lesions scattered around the blood vessels of CAM in the first passage with field samples.

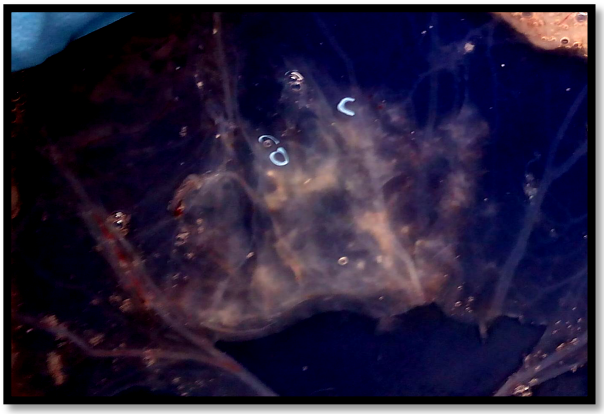

**Fig. (2):** pock lesions on CAM at the 3rd passage.

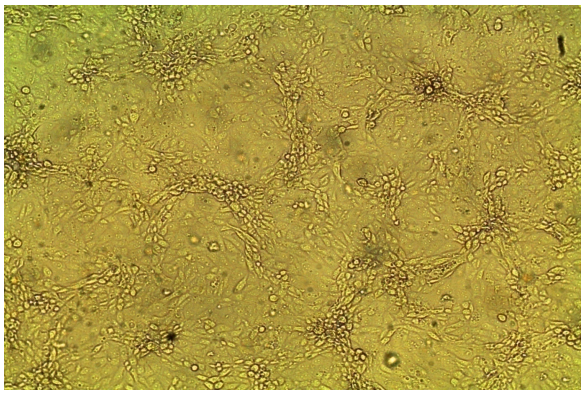

**Fig. (3):** MDBK cells showing cell clustering after inoculation with field samples at 3rd passage 4 days PI.

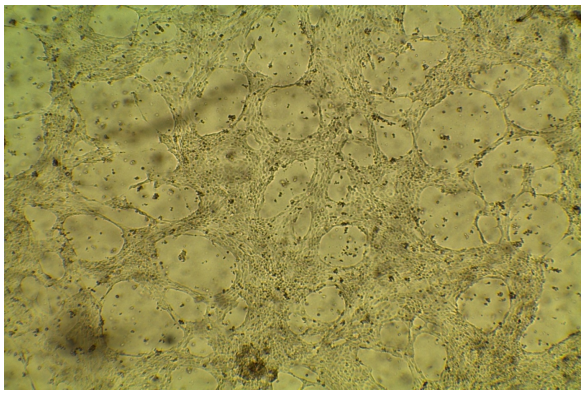

**Fig. (4):** MDBK cells showing detachment follow inoculation of field samples 8days PI at 3rd passage

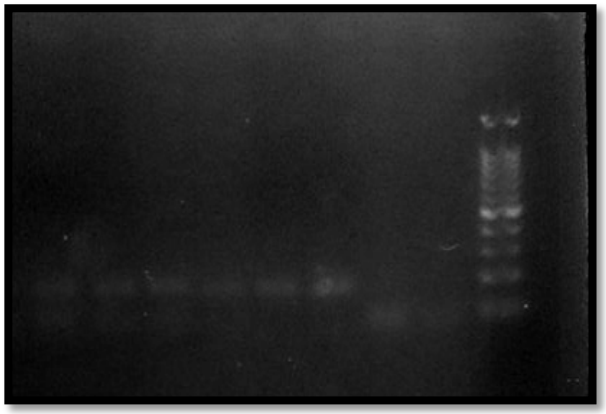

**Fig. (5):** PCR assay for detection of Capri pox virus using Ireland and Binepal primers giving amplicon size of 192bp.

**Lane1:** DNA ladder

**Lane2:** -ve control

**Lane3:** negative sample

**Lane4:** positive control

**Lane5:** positive sample

**Lane6:** positive sample

**Lane7:** positive sample

**Lane8:** positive sample

## DISCUSSION

The present study deals with identification of Lumpy skin disease virus. Skin samples collected from clinically diseased cattle and isolated on cell culture (MDBK) cells and on fertile chicken egg with further identification followed by molecular assay as PCR. Lumpy skin disease virus was isolated from samples collected from naturally infected cattle by inoculation on CAM of SPF-ECE. Characteristic pock lesions were observed after the 1st passage and became clear after the 3rd passage. **(House *et al.*, 1990)** who reported that lumpy skin disease virus can be grown on the chorioallantoic membrane (CAM) of embryonated chicken eggs. Maximum yield of LSDV was obtained in the CAM of 7- to 9-day-old embryos inoculated at 33.5°C and 35°C for 5 Characteristic pock lesions were observed after the 1st passage and become clear after the 3rd passage. The trial of isolation on the cell culture as MDBK cells showed a clear typical CPE characterized by cell rounding, cell aggregation, coalesce together to form clusters scattered all over the monolayer within 72 hr. post inoculation and gradually increased till 70-80 % of sheet was completely detached. These results were justified by PCR assay which is rapid and sensitive method. This result is agreed with **Sajid *et al.* (2013)** who reported that PCR give more positive results than cell culture technique.

## CONCLUSION

Lumpy skin disease is a growing problem in cattle production causing great economic losses. During isolation on ECE found the virus titer increase with every passage which is cleared on the membrane and justified when compared with high titer neethling strain.

After isolation of the field samples, attenuated LSD isolate and neethling strain on all cell culture types found that the virus shows a clear CPE on MDBK cells faster than all included cells.

## REFERENCES

**Carn, V.M. and Kitching, R.P. (1995):** The clinical response of cattle following infection with lumpy skin disease (Neethling) virus. Arch. Virol., 140:503-513.

**Davies, F. G. (1991):** Lumpy skin disease, an African capripox virus disease of cattle. Br. Vet. J., 147:489 - 503.

**Fenner, F. (1996):** Poxviruses. In B.N. Fields, D.M. Knipe and P.M. Howley (ed.), Fields virology. Lippincott-Raven, Philadelphia, pp: 2673-2702.

**House, J.A.; Wilson, T.M.; El Nakashly, S.; Karim, I.A.; Ismail, I.; El - Danaf, N.; Moussa, A.M. and Ayoub, N.N. (1990):** The isolation of lumpy skin disease virus and bovine herpesvirus-4 from cattle in Egypt. J. Vet. Diagn. Invest., 2 (2):111-115.

**Ireland, D.C. and Binepal, Y.S. (1998):** Improved detection of capripoxvirus in biopsy samples by PCR. J. Virol., 74:1-7.

**Sajid, A.; Chaudhary, Z.I.; Maqbol, A.; Anjum, A.A.; Sadique, U.; Hassan, Z.U.; Rafiullah and Shahid, M. (2013):** comparative sensitivity of PCR and cell culture technique for the identification of goatpox virus. J. animal and plant sciences, 23(1 Suppl.): 31-34.

**Tulman, E. R.; Afonso, C.L.; Lu, Z.; Zsak, L.; Sur, J.H.; Sandy baev, N. T.; Kerembekova, U. Z.; Zaitsev, V. L.; Kutish, G. F. and Rock, D. L. (2002):**Genomes of sheep pox and goat pox viruses. J. Virol., 76 (12): 6054 - 6061.

**Van Rooyen, P.J.; Munz, E.K. and Weiss, K.E. (1969):** The optimal conditions for the multiplication of Neethling-type lumpy skin disease virus in embryonated eggs. Onderstepoort J. Vet. Res., 36:165-174.

**Woods, J.A. (1988):** Lumpy skin disease-A Review. Tropical Animal Health and Production, 20:11-17.