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Comparative propagation and titration of lumpy skin disease virus on different cell cultures types

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ABSTRACT

Lumpy skin disease virus is a member of the genus Capripoxvirus within the Poxviridae family that infects cattle and causes considerable economic losses. Providing a suitable cell culture for virus propagation is essential goal to be used for virus isolation and vaccine production. The present work deals with a novel cell culture, the ovine lamb heart (OLH), to investigate its benefit for LSDV propagation in comparison with the use of African green monkey kidney cells and Madin-Darby bovine kidney cell cultures. Ten serial passages of The Lumpy skin disease virus in each cell culture revealed a similar cytopathic effect recorded in the peak virus titer (6.0, 5.5, and 5.0 Log_{10} $TCID_{50}$/ml in OLH, Vero, and MDBK cell cultures, respectively) by the 6th day post-cell infection at the time of harvest of the highest titer by studying the virus growth curve in each cell culture. The virus neutralization test (VNT) and direct fluorescent antibody technique (FAT) using specific anti-LSDV sera confirmed the presence of all used cell cultures. Therefore, it was concluded that OLH cell culture is suitable for the propagation of LSDV, and more research is needed to evaluate its use for vaccine preparation.

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INTRODUCTION

Lumpy Skin Disease (LSD) is the pox disease of cattle and is of high economic importance in the cattle industry in which milk and meat production are deceased **(Babiuk *et al.*, 2008; OIE, 2017)**. The first case of LSD was observed in Zambia in 1929 (**RAS, 1931)** and rapidly spreading in cattle crossing across African countries.LSD is affected different animal species, especially cattle. It is a member of the Poxviridae family with the Neethling strain. insect vectors are the main route of Transmission of the disease. Viremia, nodules on the skin, sit-fast formation, and reduction in milk and meat production are the main characteristics of this disease. It was spread through the Middle East into Southeast, Europe, Russia, and Western Asia during the past five years **(AL- Sabawy *et.al.*, 2020).**The lumpy skin disease virus genome length is 151 kbp in length (**Lojkic *et al.*, 2018).**

Cell culture technique is the process in which human, animal, or insect cells are grown in a favorable artificial environment. There are two types of cell cultures: the first is established cell lines and the second is established cell strains. Animal cell culture became a common laboratory technique In the mid1900s and is now become one of the major tools used in the field of research sciences because of its economic value (**Anju Verma *et al.*, 2020).**

Cytopathic effect (CPE) of Lumpy Skin Disease Virus on Vero cells does not produce until the 4th blind passage, but upon adaptation, it produces high viral titers in the cultured cells. The growth curve analysis of Vero cell-adapted LSDV initiates synthesizing its genome at 24 hours post-infection and reaches its peak level at 96 HPI. The evidence of progeny virus particles was observed at 36–48 hours (h) with a peak titer at 120 h. The cytopathic effect (CPE) of the Lumpy Skin Disease Virus on Madin-Darby bovine kidney (MDBK) cells is characterized by the formation of foci (multifocal areas of hyperplastic cells) **(Naveen Kumar *et al.*, 2021).**

Faetal ovine heart (FOH-SA) was established by serial passage of fetal ovine heart cells and found that this cell line is permissive to peste des petits ruminants (PPR), lumpy skin disease (LSD), *sheep pox*, RVF, and camelpox viruses (**Khalid *et al.*, 2022)**. This work aims to determine the most suitable cell culture for the propagation of LSDV aiming to obtain the maximum titer in order to increase the yield of LSDV vaccine production economically.

MATERIALS AND METHODS

1. Lumpy skin disease virus (LSDV)

Live attenuated Lumpy Skin Disease Virus with titer 4.5 Log_{10} $TCID_{50}$/ml was supplied by the Department of Pox Vaccines Research (DPVR); Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt.

2. Positive anti-LSD serum

Positive LSD antiserum unconjugated and conjugated with FITC were supplied by DPVR, VSVRI, and used to confirm the incidence of LSDV in infected cell culture.

3. Cell cultures

3.1. Cell lines

3.1.1. Madin-Darby bovine kidney (MDBK) cells **(Yatinder Singh *et al.*, 2001; Khandelwal *et al.*, 2017); Mashaly *et al.*, 2020**) were available at VSVRI and grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with antibiotics and 10% fetal calf serum.

3.1.2. African green monkey kidney (Vero) cell line established by **Yasamura and Kawatika (1967); Mashaly *et al.*, (2020)** was supplied by VSVRI.

3.2- Primary cell culture

Primary ovine fetal heart cell culture (OLH) **(Khalid *et al.*, 2022)** was prepared from freshly slaughtered lamb hearts and aseptically incised and used to prepare primary cell cultures. The heart muscles were thinly minced washed several times with Hank's balanced salt solution and trypsinized with 0.25% trypsin under stirring for half an hour. Then the supernatant was sieved through double sterile gauze and centrifuged at 1000 RPM for 5 minutes. The obtained cell pellet was cultured into Roux flasks containing complete growth minimum essential medium (MEM supplied by (Gibco), 10 % fetal bovine serum; Gentamycin 50µg/ml with adjusted pH 7.2-7.4 with 4.5% sodium bicarbonate followed by incubation at 37°C.

4. cell culture media, serum, chemicals, and Equipments

4.1. MEM supplemented with 10% heat-inactivated FBS, filter sterilized

4.2. Trypsin-EDTA in DPBS without calcium or magnesium, filter sterilized

4.3. DPBS without calcium or magnesium, filter-sterilized

4.4 . $25cm^2$, $50cm^2$and $75cm^2$ tissue culture flasks with vented caps, 15mL conical tubes, Serological pipets, 96-well plate, sterile biological Cabinet, humidified 37°C incubator with 5% CO2, Centrifuge, and inverted microscope.

5. Adaptation of LSDV to African green monkey (Vero), Madin Darby Bovine Kidney (MDBK), and Ovine Lamb Heart (OLH) cell cultures

In order to adapt LSDV to Vero, MDBK, and OLH cell cultures, 500 µl inoculum of the virus was used to infect the cells for 1 h followed by the addition of maintenance medium and observation of CPE during sequential passage(s) **(Kumar *et al.*, 2021).** Ten sequential passages in each cell culture were made. The growth curve and kinetics of synthesis of the LSDV genome of the P10 virus were studied in the three cell cultures as described by (**Coves-Datson *et al.*, (2020)**. After complete CPE was observed, the cells were freeze-thawed twice and used to reinfect fresh cells. The virus titer of each passage was determined in the corresponding cell culture by $TCID_{50}$ according to **(Reed and Muench (1938), and OIE, (2018).**

6. Growth curve of LSDV in tested cell cultures

Vero, MDBK, and OLH cell monolayers were prepared in tissue culture tubes where each cell culture was infected with its propagated LSDV at its 10^{th} passage. Infected tissue culture tubes were picked up every day for hours up to 6 days post-cell infection and kept at -80°C till subjected to virus titration.

7. Confirmation of the incidence of LSDV in cell cultures

7.1. Virus neutralization test

Samples of each virus passage in each of the used cell cultures were subjected to a virus neutralization test to confirm the incidence of LSDV using specific LSDV immune serum according to (**OIE, 2018), EU Reference Laboratory for Capri pox Viruses, 2022).**

7.2. Direct FAT

Direct fluorescent antibody technique was carried out on the tenth LSDV passage in each type of Vero, MDBK, and OLH cell culture using specific anti-LSDV serum conjugated with fluorescein isothiocyanate according to (**Ehizibolo *et al.*, 2013).**

RESULTS

Table 1: LSDV passages in Vero cell line

Passage number	Onset of CPE (Days post-infection)	Time of harvesting (Days post-infection)	Virus titer (Log_{10} $TCID_{50}$/ml)
1	3	7	2.0
2	3	7	2.5
3	3	7	3.0
4	3	6	3.5
5	3	6	4.0
6	3	6	4.0
7	3	6	4.5
8	3	6	4.5
9	2	6	5.0
10	2	6	5.5

Table 2: LSDV passages in MDBK cell line

Passage number	Onset of CPE (Days post infection)	Time of harvesting (Days post infection)	Virus titer (Log_{10} $TCID_{50}$/ml)
1	3	7	2.0
2	3	7	2.0
3	3	7	2.5
4	3	7	3.0
5	3	6	3.5
6	3	6	4.0
7	2	6	4.0
8	2	6	4.0
9	2	6	5.0
10	2	6	5.0

Table 3: LSDV passages in primary OLH cell culture.

Passage number	Onset of CPE (Days post infection)	Time of harvesting (Days post infection)	Virus titer (Log_{10} $TCID_{50}$/ml)
1	Hidden	10	Hidden
2	Hidden	10	Hidden
3	4	8	2.5
4	4	8	3,0
5	4	8	3.0
6	4	7	3.5
7	3	7	4.0
8	3	7	4.5
9	3	6	5.5
10	3	6	6.0

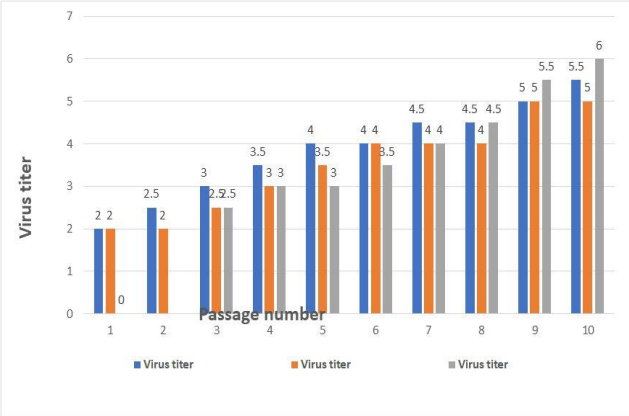

Fig. 1: LSDV titer (Log_{10} $TCID_{50}$/ml) in VERO, MDBK and OLH

Table 4: Growth curve of LSDV in different cell cultures using the 10th passage

Infected cell culture	LSDV titer (Log_{10} $TCID_{50}$/ml)					
	Days Post Infection					
	1st	2nd	3rd	4th	5th	6th
Vero	2.0	2.8	4.0	4.5	5.0	5.5
MDBK	2.0	2.6	3.5	4.0	4.5	5.0
OLH	2.0	2.9	4.1	4.6	5.2	6.0

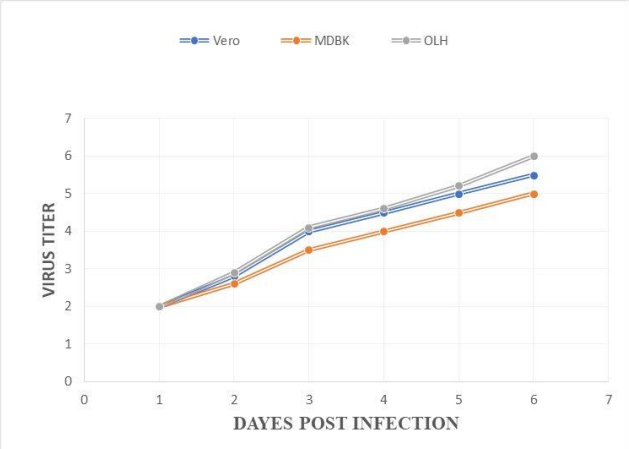

Fig. 2: Growth curve of LSDV in VERO, MDBK and OLH

Cytopathic effect (CPE) of LSD virus characterized by cell rounding, foci formation (cell clustering), and degenerative changes was observed in Vero cells (Fig.3) .

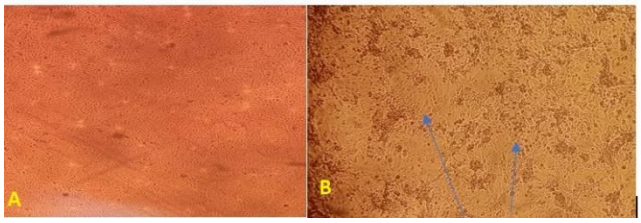

Fig. 3: Vero cell line. A:Normal cell. B: CPE of LSDV (72HPI).

Typical poxvirus cytopathic effect (CPE) was obseved,which characterized by cell rounding and becoming refractile then developing irregular shapes with long, thin projections (Fig. 4). LSDV replicated well on MDBK cells with approximately 2.5 log_{10} growth rate by day 2 post-infection. This indicates that MDBK cells are able to support the growth of LSDV to a high titer.LSDV quantitation is currently based on the determination of $TCID_{50}$ in primary cells).

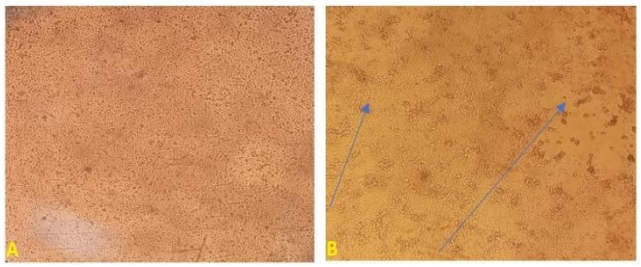

Fig. 4: MDBK cell line: A: Normal cell. B: CPE of LSDV (96HPI).

Similar description of LSD-CPE in Vero and MDBK cell cultures was recorded by with best harvesting time 72hr PI to obtain a maximum titer for subsequent vaccine production**.**

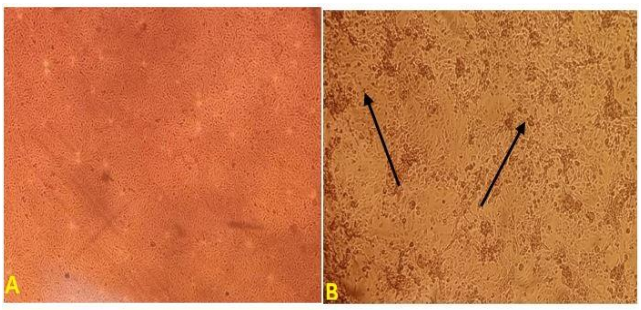

Fig. 5: OLH cell culture A: Normal cell. B: CPE of LSDV.

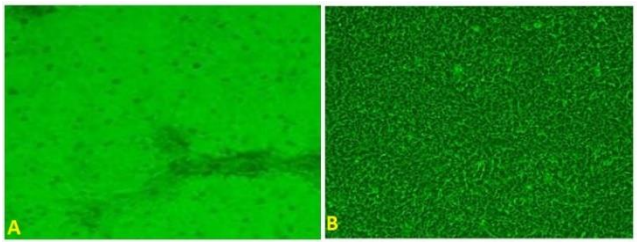

Fig 6: Direct FAT on OLH cell culture: A. Negative B. Positive

DISCUSSION

Capripox viruses grow slowly in mammalian cell culture of animal origin and require several passages to propagate these viruses and CPE of LSDV cannot be detected before day 4 post-inoculation up to 14 days **(Tuppurainen *et al.*, 2017).** The results of the current study came to be confirmed with this fact where detectable CPE of LSDV was able to be obvious by the 3rd days post infection of Vero and MDBK cell up to the 9th passage in Vero and 7th passage in MDBK cells then began to appear by the 2nd day in both cultures (Tables 1 and 2).

Vero cell culture was able to produce higher virus titer (5.5 Log_{10} $TCID_{50}$/ml) than that obtained by MDBK cell (5.0 Log_{10} $TCID_{50}$/ml) by the 10th passage. This finding agrees with (**Mashaly *et al.*, 2020)** who concluded that Vero cell was considered the best susceptible cell for the propagation of LSDV. Similar characterized CPE of LSD virus in Vero and **MDBK was described by (Omyma, 2008; OIE, 2017; Zhivodeorova, *et al.*, 2017).** CPE of LSDV on Vero cells was similar to those observed previously in MDBK cells (**Fay *et al.*, 2020**) characterized by cell clustering, cell rounding, and degeneration. Vero cell-adapted LSDV grew similar to the virus titer obtained in primary cells (**Babiuk *et al.*, 2007)** and MDBK cells **(Fay *et al.*, 2020)**, suggesting their suitability for LSDV propagation.

The tabulated results in Table (3) showed that the CPE of LSD was hidden for 2 successive passages in OLH and began to be obvious on the 3rd passage at the 4th day up to the 6th passage then it was detectable by the 3rd day post cell infection up to the 10th passage yielding virus titer 6.0 Log_{10} $TCID_{50}$/ml (Table 3) higher than that obtained by Vero and MDBK. CPE in OLH cells could be attributed to the fact that it could not be for several passages but can be detected after its adaptation before day 4 post-inoculation up to 14 days **(Tuppurainen *et al.*, 2017).** On the other side, although all used cell cultures yielded their harvests by the 6th day post infection, OLH yielded the highest virus titer (6.0

Log_{10} $TCID_{50}$/ml) followed by Vero (5.5 Log_{10} $TCID_{50}$/ml) and lastly MDBK (5.0 Log_{10} $TCID_{50}$/ml).

It was noted that the propagation of Pox viruses on embryonic heart (EH) cells showed no significant difference in viral replication among different passages from passage 5th to the 20th for Lumpy Skin Disease Virus. But there was a clear difference between the titer obtained on embryonic heart cells and the titer obtained on other cells. The titers obtained on EH reach 7.0 (Log_{10} $TCID_{50}$/ml), however on other cells, the titer does not reach to7.0 (Log_{10} $TCID_{50}$/ml). Lamb heart cells have the characteristics of cell lines such as storing in liquid nitrogen at -180, repeatable batches and the advantages of primary cells such as producing a high number of viral progenies for LSD, camel pox, and Ecthyma (**Rhazi *et al.*, 2021)**.

It was noted that propagation of pox viruses in Lamb heart cells give titer higher than that of primary lamb testis in which Lamb hear cells have specific type-1 membrane glycoprotein receptors for pox viruses is a hypothesis that should be confirmed with specific markings. The use of Lamb hear cells for the cultivation and propagation of poxviruses guarantees viral homogeneity and stability **(Halima *et al.*, 2020 Khalid *et al.*, 2022).** The observed CPE induced by LSDV in primary OLH was nearly similar to that described in Vero cell lines and MDBK and (Fig. 8) agreed with what reported previously by (**Babiuk *et al.*, 2007 and OIE, 2017)** who concluded that LSDV isolated on Primary cells of ovine, caprine and bovine origin.

Regarding the following up of LSD virus titer in Vero, MDBK and OHL cell cultures, Table (4) and Fig (2) showed a gradual daily increase in the virus titer revealing that the optimum time of virus harvesting is the 6th day post infection of Vero, MDBK and OLH cell cultures yielding virus titer 5.5, 5.0 and 6.0 Log_{10} $TCID_{50}$/ml respectively. Although there are no data that discuss the growth curve of LSD virus in cell cultures, the present results suggest that there is a difference in the virus growth in Vero, MDBK and OLH revealing faster growth rate in OLH than in Vero than in MDBK. These results agree with **(Salnikov *et al.*, 2018; Fay *et al.*, 2020; Munyanduki *et al.*, 2020; Petra *et al.*, 2020**) who mentioned MDBK cells have been commonly used for the in-vitro propagation of LSDV at high titers.

Similar conclusion was stated by **Khalid *et al.*, (2022)** but it disagrees with **Mashaly *et al.*, (2020)** who obtained the highest LSDV titer by the 4th day (96 hours post cell infection) the thing which could be attributed to virus passage number (more adapted) or the used multiplicity of infection (MOI.).The faster growth rate correlated with absence of immune cells and decrease of immune regulation pathways in the cell lines used while the destructive action of LSDV on cell culture and the detachment of infected cells from the surface of the prescription leading to decrease the titer, This result is in general agreement with **OIE (2017).**

Virus neutralization test and direct FAT (Fig. 6) confirmed the incidence of LSDV in infected Vero, MDBK, and primary OLH cell cultures. VNT is considered to be the most specific serological method for diagnostics of LSD **(EFSA, 2015; OIE, 2016; EFSA, 2017 and Milena Samojlovic *et al.*, 2019).** Application of FAT to determine the incidence of LSDV in cell cultures (Fig. 6) agreed with what was reported previously by **Gari *et al.*,** (**2008); Gamil *et al.*, (2019); Chuanwen Jiang *et al.*, (2022)** who concluded that fluorescence assay can conveniently and sensitively detect the LSDV.

CONCLUSION

It was concluded that the ovine lamb heart (OLH) cell culture represents a suitable and best one for propagation of LSDV and more researches are in needed to evaluate its use for vaccine preparation

Conflict of interest

The authors declare that no prospective conflicts of interest exist.

REFERENCES

AL-SABAWY, H.B., AL-HAMDANY, E.K., AL-SULTAN, A.A., and RDAM, S.A., 2020. High light on lumpy skin disease in Iraq and the Middle East. Journal of Applied Veterinary Science Volume 5 Issue, Page 94-103. HTTPS://DX.DOI.ORG/10.21608/JAVS.2020.8560

ANJU VERMA, MEGHA VERMA, and ANCHAL SINGH, 2020. Animal tissue culture principles and applications, Animal Biotechnology,269-293.https://doi.org/10.1016/B978-0-12-811710-1.00012-4

BABIUK, S., PARKYN, G., COPPS, J., LARENCE, JE., and SABARA, M.I., 2007. Evaluation of an ovine testis cell line (OA3.Ts) for propagation of capripoxvirus isolates and development of an immunostataing technique for viral plaque visualization. J Vet Diagn Invest, 19: 486–491.https://doi.org/10.1177/104063870701900505

CHUANWEN,J., DAGANG, T., YUANCHENG., HAO,Y., BINGRONG, X., YINGYU, C., CHANGMIN, H., HUANCHUN, C., SHENGSONG, X., ANDAIZHEN, G., 2022. Sensitive and Specific Detection of Lumpy Skin Disease Virus in Cattle by CRISPR-Cas12a

Fluorescent Assay Coupled with Recombinase P. Amplification Genes,13:734. https://doi.org/10.3390/genes13050734

COVES DATSON, E.M., KING, S.R., LEGENDRE, M., GUPTA, A., CHAN, S.M., GITLIN E., KULKARNI V. V., GARCIA P. J., SMEE F. D., LIPKA E., EVANS E. S., TARBET B. E., ONO A., and MARKOVITZ D. M., 2020. A molecularly engineered antiviral banana lectin inhibits fusion and is efficacious against influenza virus infection in vivo. Proc Natl Acad Sci USA, 117: 2122–2132. https://doi.org/10.1073/pnas.1915152117

EFSA (EUROPEAN FOOD SAFETY AUTHORITY) 2015. Scientific opinion on lumpy skin disease: EFSA Panel on Animal Health and Welfare. EFSA Journal, 13 (1): 3986, 67 pp.https://doi.org/10.2903/j.efsa.2015.3986

EFSA (EUROPEAN FOOD SAFETY AUTHORITY) 2017. Scientific report on lumpy skin disease: I. Data collection and analysis. EFSA Journal, 15 (4): 4773, 54 pp. https://doi.org/10.2903/j.efsa.2017.4773

EHIZIBOLO, D., NWOSUH, C., EHIZIBOLO, E., and KIA, G., 2013. Comparison of the fluorescent antibody test and direct microscopic examination for rabies diagnosis at the National Veterinary Research Institute, Vom, Nigeria. African Journal of Biomedical Research,12 (1):73-76 https://hdl.handle.net/1807/54163

EUROPEAN UNION REFERENCE LABORATORY FOR CAPRIPOX VIRUSES, 2022.The sixth annual combined workshop for Capripox virus and PPinfections.https://www.eurlcapripox.

FAY, C., COOK, C.G., WIJESIRIWARDANA, N., TORE, G., and OMTET, L., 2020. Madin-Darby bovine kidney (MDBK) cells are a suitable cell line for the propagation and study of the bovine poxvirus lumpy skin disease virus. J Virol Methods 285:https://DOI.10.1016/j.jviromet.2020.113943

GAMIL, S., GAMIL, Z., AYMAN, H., MAHMOUD, A., MOSTAFA A., KHALED A., MANAL, H.K., HALA, A., and AHMED, Z., 2019. Detection of lumpy skin disease virus in cattle using real-time polymerase chain reaction and serological diagnostic assays in different governorates in Egypt in 2017. Vet World, 12 (7): 1093-1100. https://doi:10.14202/vetworld.2019.1093-1100

GARI, G., BITEAU-COROLLER, F., LEGOFF, C., CAUFOUR, P., and ROGER, F., 2008. Evaluation of indirect fluorescent antibody test (IFAT) for the diagnosis and screening of lumpy skin disease using Bayesian method. Vet. Microbiol., 129: 269–280. https://doi.org/10.1016/j.vetmic.2007.12.005

HALIMA, R., IKRAM, T., KARIMA, M., OUMAIMA, B., NAJETE, S., MERYEM, A., KHALID, O., T., MATTHIAS, L., and MEHDI, E., 2020. Development of diploid embryonic sheep heart cells and sensitivity study of three poxviruses: Lumpy skin disease, Camel pox viruses, and Ecthyma; FST. https://orcid.org/0000-0002-7561-2429

HOUSE, J., WILSON, T., NAKASHLY, S., KARIM, I., and ISMAIL, I., 1990. The isolation of lumpy skin disease virus and bovine herpesvirus-4 from cattle in Egypt. J Vet Diagn Invest, 2: 111–115. https://doi.org/10.1177/104063879000200205

KHALID,M., SULEIMAN, M., ALJULIDAN, G., HUSSEIN, and HABIB,N., 2022. Establishment of a novel fetal ovine heart cell line by spontaneous cell fusion bioRxiv Cell Biology.https://doi.org/10.1101/2022.07.14.500071

KHANDELWAL, N., CHANDER, Y.,RAWAT,K., RIYESh,T., and NISHANTH, C., 2017. Emetine inhibits replication of RNA and DNA viruses without generating drug resistant V. variants. Antiviral Res, 144:196.https://doi.org/10.1016/j.antiviral.2017.06.006

KUMAR, N., CHANDER, Y., KUMAR, R., KHANDELWAL, N., RIYESH, T., and CHAUDHARY, K., 2021. Isolation and characterization of lumpy skin disease virus from cattel in India PLoS ONE 16 (1) : 0241022. https://doi.org/10.1371/journal.pone.0241022

LOJKIC, I., SIMIC, I., KRESIC, N., and BEDEKOVIC, T., 2018. Complete Genome Sequence of a Lumpy Skin Disease Virus Strain Isolated from the Skin of a vaccinated Animal. GenomeAnnounce, 6. 10.1128/genomeA.00482-18. https://doi.org/10.1128/genomea.00482-18

MILENA, S., VLADIMIR, P., GURJANOV, V., and DIANA ,L., 2019. Detection of antibodies against Lumpy skin disease virus by Virus neutralization test and ELISA methods; Acta Veterinaria, 69 (1): 47-60. https://doi.org/10.2478/acve-2019-0003

MASHALY, M., AYMAN, D., MOMTAZ, S., and HUSSEIN, H., 2020. Comparative growth kinetic of Egyptian LSDV isolate in Vero and MDBK cell line. J. of Virol. Sci., Vol. 7: 66- 76, ISSN: 2636-2937.

MUNYANDUKI, H., DOUGLASS, N., OFFERMAN, K.,CARULEI, O., and WILLIAMSON, A., 2020. Influence of the lumpy skin disease virus (LSDV) superoxide dismutase homologue on host transcriptional activity, apoptosis and histopathology. J Gen Virol, 101: 645–650. https://doi.org/10.1099/jgv.0.001423

NAVEEN,K.,YOGESH,C., RAM,K.,NITIN,K.,THACHAMVALLY,R., KHUSHBOO,C., KARUPPUSAMY, S., SANJIT, K., ANAND, K., MADHURENDU, K., GUPTA,Y., SANJAY, B., and BHUPENDRA, N., 2021. Isolation and characterization of lumpy skin disease virus from,cattleIndia.: 10.1371/journal.pone.0241022. https://doi.org/10.1371/journal.pone.0241022

OIE 2016. Terrestrial Manual: Lumpy skin disease. Chapter 2.4.13

OIE 2017. Lumpy skin Disease OIE Terrestrial Mannual. Chapter 2.4.13.

OIE., 2018. Sheep pox and goat pox. In: Terrestrial Animal Health Code. World Organization for Animal Health, Paris, France

OMYMA, M. 2008. Recent Isolation and identification of lumpy skin disease virus from cattle in Egypt. Egyptian Journal of Comparative Pathology and Clinical Pathology,7 (3): 234-237

PETRA, C., FAY, C., COOK, N., WIJESIRIWARDANA, G., LOIC, C., ALIX, C.,

BARBARA, S., GRAHAM, F., ISMAR, R., and PHILIPPA, M., 2020. Madin-Darby bovine kidney (MDBK) cells are a suitable cell line for the propagation and study of the bovine poxvirus lumpy skin disease virus. Journal of Virological Methods,Vol.285: 113943.https://doi.org/10.1016/j.jviromet.2020.113943

RAS, M. 1931. Pseudo-Urticaria of Cattle. Government of Northern Rhodesia: Department of Animal Health Emerging and Re-emerging Infectious Diseases of Livestock pp 309–326 https://doi.org/10.1007/978-3-319-47426-7_14

REED, J., and MUENCH, H., 1938. A simple method for estimating fifty percent (50%)endpoints**.** Amer.j.Hyg..,27:493497.https://doi.org/10.1093/oxfordjournals.aje.a118408

RHAZI, H., SAFINI, N., MIKOU, K., ALHYANE, M. and LENK, M., 2021. Comparative Sensitivity Study of Primary Cells, Vero, OA3. Ts and ESH-L Cell Lines to Lumpy Skin Disease, Sheep pox, and Goat pox Viruses Detection and Growth. J Virol Methods, 293: 114164, https://doi.org/10.1016/j.jviromet.2021.114164

SALNIKOV, N., USADOV, T., KOLCOV, A., ZHIVODEROV, S., and MORGUNOV, Y., 2018. Identification and characterization of lumpy skin disease virus isolated from cattle in the Republic of North Ossetia-Alania in 2015.Transbound Emerg Dis, 65: 916–920. https://doi.org/10.1111/tbed.12818

TUPPURAINEN, M., VENTER, H., SHISLER, L., GARI, G., MEKONNEN, A., JULEFF, N., LYONS, A., DE CLERCQ, K., UPTON, C., BOWDEN, R., BABIUK, S., and BABIUK, A., 2017. Capripoxvirus Diseases:Current status and opportunities for control. Transboundary and Emerging Diseases, 729-745. HTTPS://DOI.ORG/10.1111/TBED.12444

YASAMURA and Kawatika 1967. Studies on virus in tissue culture cells. World Health Organization, PP 390-398. https://doi.org/10.1002/9780471729259.mca04es11

YATINDER, S., ONGADA, A., and CHEPKWONY, C., 2001. Alternative cell lines for the propagation of lumpy skin disease virus. Journal of Veterinary Research , 68:151-153.

ZHIVODEOROVA, V., ANISIMOVA, I., KUSHNIR, D., USADOV, R., YURKOV, G., SALNIKOV,I., and LUNITSIN ,V., 2017.Permissivity of various cell cultures to lumpy skin disease,virusbiologyAgricultural,52(6)1265-1272. https://doi:10.15389/agrobiology.2017.6.1265eng

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