Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Molecular detection and phylogenetic analysis of recent isolate of lumpy skin disease virus from clinical cases in Menofia governorate between 2018 to 2020 based on GPCR gene

Adel E. Abdelsalam[1,2], Ayman S. El-Habbaa[1], Eman M. Abo Hatab[2], Saad S.A. Sharawi[1]

2 Department of Virology, Faculty of Veterinary Medicine, Benha University, Moshtohor, Tukh, Egypt
1 Department of Virology, Animal Health Research Institute, Agricultural Research Center, Giza, Egypt

ARTICLE INFO

Keywords

LSDV

PCR

Phylogenetic analysis

Egypt

Received 24/04/2023
Accepted 07/05/2023
Available On-Line 01/07/2023

ABSTRACT

Lumpy skin disease (LSD) is a viral illness of cattle population that causes highly significant losses in economy. In recent times, the illness has spread throughout various countries of Middle East, Egypt is among of them. The purpose of present research was to investigate the genetic make-up of LSD virus during outbreaks of Menofia governorate from period between 2018 to 2020 and its association to wild field and vaccination strains and isolates in database. Nodular skin tissues (n = 30), whole blood (n=50) and milk (n=10) samples were taken from apparently diseased cows for LSDV molecular detection based on GPCR gene. By PCR testing, 60 of the 90 samples tested were positive for LSDV. The amplified products of 7 positive samples found in the nodular tissue (n= 3), buffy coat (n= 3) and milk (n= 1) of cattle from Menofia governorate were subjected to sequencing and thus the evolutionary tree was built. Furthermore, the sequence alignment of the Menofia governorate LSDV strains revealed remarkable similarity ranging from 99% to 100% in between and with the LSDV genome of database strains and 95% to 96 % with vaccinal strains. To summarize GPCR gene sequencing and phylogenetic investigations showed only minor genetic differences among LSDV wild strains from various areas, as well as a close link between virulent field strains and homologous vaccines.

1. INTRODUCTION

Lumpy skin disease is a cow infectious eruptive illness caused by the lumpy skin disease virus (LSDV), which included in Capripoxvirus and is a member of the Poxviridae family (Sprygin et al., 2016). Both the dairy and beef industries are heavily impacted by the disease as it causes sharp decrease in milk production, sterility which may be temporary or permanent, abortion, damage to skin and loss of body physical condition (Lu et al., 2021).

LSD was firstly discovered in Zambia in 1929, since then, the disease has spread to the bulk of African nations and the Middle East, including Egypt, and it has now expanded to include the European and Western Asian regions (OIE, 2018).

LSD was discovered in Egypt in May 1989 in the Suez and Ismailia regions through African imported cattle. Then, within five to six months, it quickly spread to 22 Egyptian provinces (Ali et al., 1990; House et al., 1990). Several outbreaks of the disease resurfaced in various Egyptian provinces in 2006, 2011, 2014, and 2017 (Sharawi and Abdelrahim, 2011; El-Nahas et al., 2011; El-Tholoth and El-Kenawy, 2016; Helmy et al., 2017).

Biting insects or flies are the primary vectors of LSDV (Issimov et al., 2020). LSD can be present in two forms; acute or subacute, with severity changing according to the host breed and virus strain (Abutarbush and Tuppurainen, 2018).

Controlling of LSD necessitates quick and reliable diagnostic tools for making a definitive diagnosis. The most often used procedures for diagnosing LSD are viral isolation (VI), electron microscopy, and serological approaches such as (ELISA and VNT) and PCR (Selim et al., 2019). **T**he PCR technique is the most effective way of differentiating parapox and Capripox viruses (Reisberg et al., 2013).

The genomic analysis indicated LSDV is genetically closed to sheep and goatpox viruses with at least 96% genomic homogeneity (Babiuk et al., 2008). To detect and characterize LSDV and remaining other viruses of genus Capripox, the majority of specific and sensitive molecular approaches that target GPCR, P32, and RPO30 genes are used (Le Goff et al., 2009) used GPCR to perform genetic characterization and phylogenetic study of LSDV, which then has been used in numerous studies in recent years.

The current research performed to look into genetic characteristics of LSDV detected in Egypt's Menofia province using the GPCR gene, as well as the genetic link between the detected LSDV and remaining LSDV and vaccinal strains.

* Correspondence to: dradelelsaid132@gmail.com

2. MATERIAL AND METHODS

2.1.approval Ethics

All experimental methods were carried out in the Virology Research Laboratory, Virology Section, Faculty of Veterinary Medicine, Benha University, Egypt, and were approved by the Benha University Animal Ethical Committees. with ethical approval number (BUFVTM 09-01-2023) and at the Animal Health and Research Institute (AHRI) in Giza, Egypt, in accordance with its requirements for virus identification, biosafety, and good microbiological methods.

2.2. *Sampling and samples preparation*

Thirty skin nodule samples were collected surgically under aseptic condition and round local anesthesia was performed. Collected samples transported to the laboratory in viral transportation media, 50 whole blood samples (5 ml) from each animal were collected in EDTA vacutainers by jugular venipuncture and 10 milk samples were collected in clean sterile falcon tubes. In addition, clinical manifestations of suspected examined animals were documented. All collected samples were prepared according to (Burleson et al., 1997) and kept at − 20 ◦C till examination using PCR. Furthermore, a commercial SERVAC LSDV Neethling Vaccine which currently used in Egypt was also collected to compare its genetic characteristics with LSDVs strains from the clinical samples.

2.3. Viral DNA extraction

Following the manufacturer's instructions, of Thermo Scientific GeneJET Genomic DNA Purification Kit, Pub.No. MAN0012663.*2.4* DNA of collected samples was extracted**.**

2.4. PCR amplification

The GPCR gene was amplified using specific primer according to Mashaly et al. (2020) as follow LSDV Forward (5′-AGT ACA GTT AGT AGC GCA ACC-3′) and LSDV Reverse (5′-GGG TGA ACT ACA GCT AGG TAT C-3′) to amplify 554 bp fragment of GPCR gene. PCR and cycling procedures are performed according to Amin et al. (2021) following AmpliTaq Gold™ 360 Master Mix kit manufacture instructions and analysis to PCR product using 1.5 agrose gel electrophoresis.

2.5. Sequencing

In accordance with the manufacturer's recommendations. The resulting fragments were purified by QIA quick genomic purification kit and sequenced in one way using a BigDye® Terminator v3.1 Cycle Sequencing Kit on a 3500 Genetic Analyzer in the United States.2.6.

2.6. Phylogenetic investigation.

Using NCBI BLAST, the nucleotide sequences of the GPCR genes were initially compared and matched with those of reference virulent and vaccine CaPV strains in GenBank. According to (Hall, 1999) BioEdit Version 7.0 software ClustalW Multiple alignment algorithm was used to perform a comparative alignment of the GPCR gene sequences and Sequence identities and divergences were estimated. MEGA 7 software was used to create a phylogenetic tree using the GPCR gene nucleotide sequences according to Saitou and Nei (1987).

3. RESULTS

3.1. Polymerase chain reaction.

Out of 90 suspected cases, 60 cases gave positive bands with specific GPCR at expected product size "554 bp" (Figure 1). Furthermore, while LSDV was found in all 30 nodule samples, it was only found in 26 of 50 blood samples and 4 of 10 milk samples.

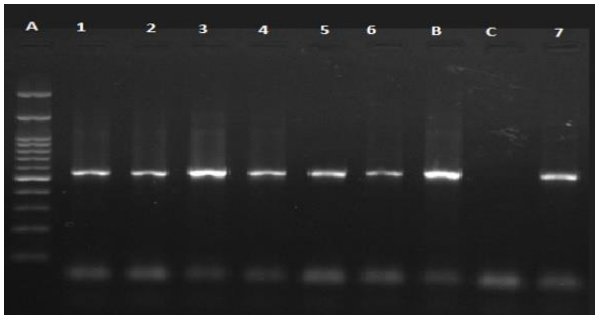

Fig (1). Gel electrophoresis of G protein-coupled chemokine receptor gene-based polymerase chain reaction. Lane A:100bp DNA ladder (Marker). Lane B: control positive. Lane C: control negative. Lanes 1-7: positive study field strains showing expected band at size 554 bp.

3.2. Sequencing

Seven positive samples were sequenced and submitted under Accession numbers (OQ679861, OQ679862, OQ679863, OQ679864, OQ679865, OQ679866 and OQ679867) in GenBank.

3.3. Phylogenetic analysis

Seven Menofia LSDV strains are closely related to each other as well as virulent variants from various places with identity percentage ranged from 99% to 100% and 95% to 96% with LSDV Neethling vaccine (figure-2)

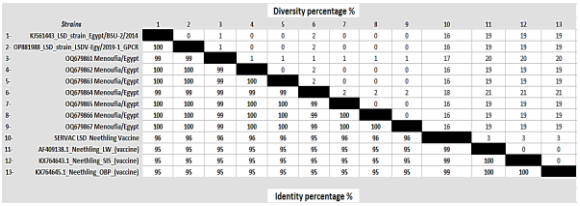

Fig (2). Identity and diversity percentage between field and vaccinal strains.

The phylogenetic tree constructed from the nucleotide sequences of the GPCR gene (Figure-3) revealed that five out of seven Menofia LSDV strains of current study were segregated with each other in same group with previously studied Egyptian strains and isolates but remaining two strains, OQ679861 and OQ679864 located in separated clade also we noted that current study SERVAC LSDV Neethling Vaccine, Egypt strain located with other reference vaccinal strains in a separate clade. Multiple sequence alignments of the GPCR gene demonstrated that seven field LSDVs strains of the Menofia governorate differ from Lumpy Skin virus of live attenuated Neethling vaccine just in a 12-nucleotide deletion (Figure-4). Comparative sequencing analysis of GPCR show that field LSDV strains differ only in two amino acids (AAs) substitution from the LSDV Neethling vaccine. (S/N50 and M/I101) and four AAs deletion at positions 3-6 (S3, T4, I5, and L6) was observed in Neethling vaccine sequenced in this study and vaccine obtained from GenBank but not in LSDV field strain (figure-5)

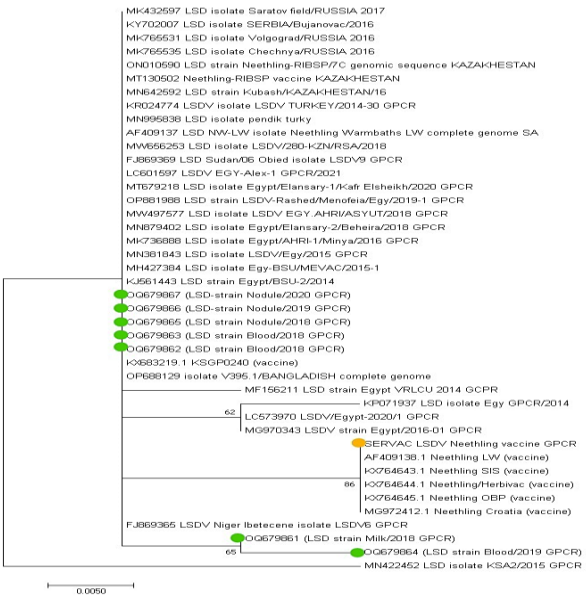

Fig (3). Phylogenetic analysis of the G protein-coupled chemokine receptor gene. The tree was generated using MEGA 7 program by the neighbor-joining analysis. Bootstrap confidence values were calculated on 1000 replicates according to the maximum likelihood approach. Sequences obtained in this study are labeled green for field strain and yellow for vaccinal strain.

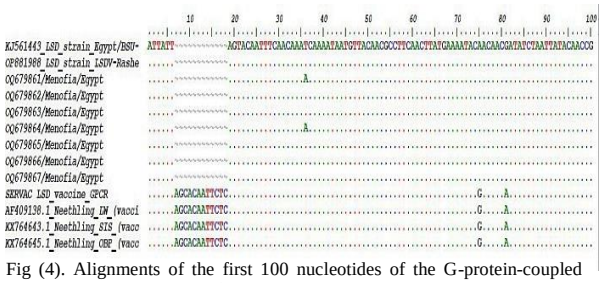

Fig (4). Alignments of the first 100 nucleotides of the G-protein-coupled chemokine receptor gene of lumpy skin disease virus (LSDV) strains and those of other recovered from GeneBank; deletion of 12 nucleotides from position 7 to 18 are evident in the sequences of the study new LSDV strains when compared with those of vaccinal strain.

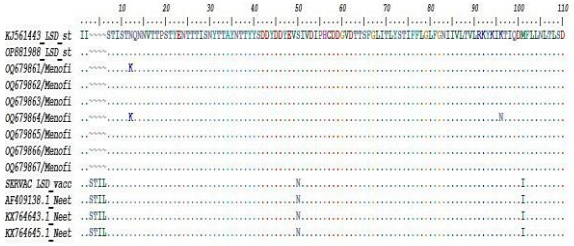

Fig (5). Differences in amino acid motifs between LSDV field and vaccine strains in G protein-coupled receptors gene.

4- DISCUSSION

In Egypt, LSD diseases is now considered endemic. Although the intensive immunization campaigns carried out by Egyptian veterinary agencies, LSDV is still prominent and widespread across the country., and it frequently reappeared during the 2018-2020 summer season causing severe economic losses, as a result, the current study presents a molecular characterization of Menofia outbreak LSDV as well as a its relation with other field and vaccination strains using GPCR gene sequence analysis. The current return of the disease could be attributed to livestock movement across districts and the widespread of open livestock markets in many provinces (Sudhaka et al., 2020). LSD typically appeared during the summer season, which is characterized by wet hot climate. This weather condition is favorable for insect vectors reproduction that transmit the disease (Tuppurainen et al., 2011). The polymerase chain reaction has high specificity and sensitivity and recommended by Ochwo et al. (2019) for detecting the virus from many samples types as nodular tissue and buffy coat. The PCR analysis detect LSDV in all examined 30 skin nodules, whereas 26 out of 50 buffy coats were positive for LSDV. This finding gets in agreement with prior research (Ochwo et al., 2019), who reported that the skin nodules tested positive for LSDV more than buffy coat samples utilized for molecular testing. This may be due to collection of blood from cattle after viremic stage of the disease that circulated for a short time 4-11 day before skin nodular eruption This finding is corresponds with prior research (Zeynalova et al., 2016), which showed that skin nodules are superior samples for LSDV PCR detection than blood samples. Out of 10 milk samples only 4 samples tested positive for LSDV and this result agree with (Sharawi and Abd El-Rahim, 2011) who demonstrated that PCR found LSDV nucleic acid in 50% of whole milk samples and 60% of pelleted sedimented samples.

The 554bp amplified product of the GPCR gene of seven positive Menofia LSDV field strains were sequenced alongside with local vaccinal strains and compared to other wild and vaccinal strains published in GenBank. Comparative sequencing analysis found that all seven LSDV strains are highly similar with identities percentage ranging about 99% to 100% in between, confirming the spread of the same strain. Menofia LSDV strains were discovered to be more closely related to the LSDV Neethling vaccine strain with 95 to 96 % identity while the only difference between them is a two amino acids substitution and a four amino acids deletion at locations 3-6 this result agreed with previous study (Rouby et al., 2019) who proved that LSDV isolate dissimilar only in 4 amino acids substitutions and 4 amino acids deletion at positions 30-33. All our study LSDV strains of Menofia governorate were 99 to 100 % identity with wild type LSDV strains from the African, Asian and European countries. This result shows the LSDV genome's conserved character, despite the virus's propagation in multiple geographical regions (Selim et al., 2019). Phylogenetically, Menofia LSDV wild strains were found in the same group with other LSDV strains in GenBank and vaccinal strains, comprising SERVAC LSDV Neethling Vaccine from Egypt, revealing negligible genetic change across distinct LSDV isolates from diverse locales and confirming the excellent stability of LSDVs, as previously described by Kara et al. (2013). Menofia LSDV strains are more closely linked to Neethling vaccinal strain of Egypt. The close genetic relationship between wild LSDV strains and the LSDV Neethling vaccine strain has previously been described by Kara et al. (2013) and recent studies have advised the use of homologous vaccines for the management of LSDV infections, in conjunction with adequate immunization coverage and appropriate control methods (Tuppurainen and Galon, 2016).

5. CONCLUSION

Based on field observations, sequencing, and phylogenetic analyses of LSDV current study establishes the prevalence of virus between cattle in Egypt. The results revealed genetic similarities among LSDVs currently circulated in Egypt and those from African countries. Furthermore, GPCR gene based phylogenetic analyses revealed a close relation among the Egyptian wild and Neethling vaccine strains. We recommended that performing further genetic analysis using full-genome sequencing to field and

vaccination strains to decide that there is variation between them or no.

6. REFERENCES

1. Abutarbush, S.M. and Tuppurainen, E.S.M., 2018. Serological and clinical evaluation of the Yugoslavian RM65 sheep pox strain vaccine uses in cattle against lumpy skin disease. Transboundary and emerging diseases, 65(6), 1657–1663.
2. Ali, A.A., Esmat, M., Attia, H., Selim, A., Abdel-Hamid, Y.M., 1990. Clinical and pathological studies on lumpy skin disease in Egypt. The Veterinary record, 127(22), 549–550.
3. Amin, D.M., Shehab, G., Emran, R., Hassanien, R.T., Alagmy, G.N., Hagag, N.M., Abd-El-Moniem, M.I.I., Habashi, A.R., Ibraheem, E.M., Shahein, M.A., 2021. Diagnosis of naturally occurring lumpy skin disease virus infection in cattle using virological, molecular, and immunohistopathological assays. Veterinary world, 14(8), 2230–2237.
4. Babiuk, S., Bowden, T.R., Boyle, D.B., Wallace, D.B., Kitching, R.P., 2008. Capripoxviruses: an emerging worldwide threat to sheep, goats and cattle. Transboundary and emerging diseases, 55(7), 263–272.
5. Burleson, F.G., Chambers, T.M., Wiedbrauk, D.L., 1997. Virology: a laboratory manual. Academic Press, Harcourt Brace Jovanovich, San Diego.
6. El-Nahas, E.N., El-Habla, E.S., El-bagoury, G.F., Radwan, M.E.I., 2011. Isolation and Identification of Lumpy Skin Disease Virus from Naturally Infected Buffaloes at Kalyobiya, Egypt. Global Veterinaria 7(3): 234–237.
7. El-Tholoth, M. and El-Kenawy, A.A., 2016. G-Protein-Coupled Chemokine Receptor Gene in Lumpy Skin Disease Virus Isolates from Cattle and Water Buffalo (Bubalus bubalis) in Egypt. Transboundary and emerging diseases, 63(6), e288–e295.
8. Hall, T.A. (1999) BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41, 95-98.
9. Helmy, N.M., Ahmed, S.A., Mohamed, Z.Y., 2017. Molecular, clinico-pathological and sero-diagnosis of LSDV in cattle at Sharkia and Fayoum Governorates. J. Virol. Sci.1:1-11
10. House, J.A., Wilson, T.M., El Nakashly, S., Karim, I.A., Ismail, I., El Danaf, N., Moussa, A.M., Ayoub, N.N., 1990. The isolation of lumpy skin disease virus and bovine herpesvirus-4 from cattle in Egypt. Journal of veterinary diagnostic investigation: official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc, 2(2), 111–115.
11. Issimov, A., Kutumbetov, L., Orynbayev, M.B., Khairullin, B., Myrzakhmetova, B., Sultankulova, K. and White, P.J., 2020. Mechanical Transmission of Lumpy Skin Disease Virus by Stomoxys Spp (Stomoxys Calsitrans, Stomoxys Sitiens, Stomoxys Indica), Diptera: Muscidae. Animals: an open access journal from MDPI, 10(3), 477.
12. Kara, P.D., Afonso, C.L., Wallace, D.B., Kutish, G.F., Abolnik, C., Lu, Z., Vreede, F.T., Taljaard, L.C., Zsak, A., Viljoen, G.J., et al., 2003. Comparative sequence analysis of the South African vaccine strain and two virulent field isolates of Lumpy skin disease virus. Archives of virology, 148(7), 1335–1356.
13. Le Goff, C., Lamien, C. E., Fakhfakh, E., Chadeyras, A., Aba-Adulugba, E., Libeau, G., Tuppurainen, E., Wallace, D. B., Adam, T., Silber, R., et al., 2009. Capripoxvirus G-protein-coupled chemokine receptor: a host-range gene suitable for virus animal origin discrimination. The Journal of general virology, 90(Pt 8), 1967–1977.
14. Lu, G., Xie, J., Luo, J., Shao, R., Jia, K., Li, S., 2021. Lumpy skin disease outbreaks in China, since 3 August 2019. Transboundary and emerging diseases, 68(2), 216–219.
15. Mashaly, M.M., El-Deeb, A.H., Shahein, M.A., Hussein, H.A., 2020. Molecular Characterization and cytopathogenecity of lumpy skin disease virus in Egypt. Faculty of Veterinary Medicine, Cairo University, Egypt.
16. Ochwo, S., VanderWaal, K., Munsey, A., Nkamwesiga, J., Ndekezi, C., Auma, E., Mwiine, F.N., 2019. Seroprevalence and risk factors for lumpy skin disease virus seropositivity in cattle in Uganda. BMC veterinary research, 15(1), 236.
17. OIE. 2018. Lumpy skin disease. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Office International des Epizooties, World Organization for Animal Health, Paris, pp. 1159–1171.
18. Reisberg, K., Selim, A.M., Gaede, W., 2013. Simultaneous detection of Chlamydia spp., Coxiella burnetii, and Neospora caninum in abortion material of ruminants by multiplex real-time polymerase chain reaction. Journal of veterinary diagnostic investigation: official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc, 25(5), 614–619.
19. Rouby, S.R., Bazid, A.H., Wasfy, M., El-Sayed, M., 2019. Capripoxviruses: Exploring the genetic relatedness between field and vaccine strains from Egypt. Veterinary world, 12(12), 1924–1930.
20. Saitou, N. and Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution, 4(4), 406–425.
21. Selim, A., Abdelrahman, A., Thiéry, R. and Sidi-Boumedine, K., 2019. Molecular typing of Coxiella burnetii from sheep in Egypt. Comparative immunology, microbiology and infectious diseases, 67, 101353.
22. Sharawi, S.S. and Abd El-Rahim, I.H., 2011. The utility of polymerase chain reaction for diagnosis of lumpy skin disease in cattle and water buffaloes in Egypt. Revue scientifique et technique (International Office of Epizootics), 30(3), 821–830.
23. Sprygin, A., Artyuchova, E., Babin, Y., Prutnikov, P., Kostrova, E., Byadovskaya, O., Kononov, A., 2018. Epidemiological characterization of lumpy skin disease outbreaks in Russia in 2016. Transboundary and emerging diseases, 65(6), 1514–1521.
24. Sudhakar, S.B., Mishra, N., Kalaiyarasu, S., Jhade, S.K., Hemadri, D., Sood, R., Bal, G.C., Nayak, M.K., Pradhan, S.K., Singh, V.P., 2020. Lumpy skin disease (LSD) outbreaks in cattle in Odisha state, India in August 2019: Epidemiological features and molecular studies. Transboundary and emerging diseases, 67(6), 2408–2422.
25. Tuppurainen, E. and Galon, N., 2016. Technical Item II Lumpy Skin Disease: Current Situation in Europe and Neighbouring Regions and Necessary Control Measures to Halt the Spread in South-East Europe. Technical Report. OIE Regional Commission, Europe.
26. Tuppurainen, E.S., Stoltsz, W.H., Troskie, M., Wallace, D.B., Oura, C.A., Mellor, P.S., Coetzer, J.A., Venter, E.H., 2011. A potential role for ixodid (hard) tick vectors in the transmission of lumpy skin disease virus in cattle. Transboundary and emerging diseases, 58(2), 93–104.
27. Zeynalova, S., Asadov, K., Guliyev, F., Vatani, M., Aliyev, V., 2016. Epizootology and Molecular Diagnosis of Lumpy Skin Disease among Livestock in Azerbaijan. Frontiers in microbiology, 7, 1022.