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A Phylogenetic Study Of Marek's Disease Virus Glycoprotein L In Iraqi Layers' Farms

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ABSTRACT

Marek's virus disease (MDV) is an important avian disease of tumorigenesis nature, immunosuppression outcome, and significant economic impacts. The disease is controlled by mass vaccination supported with good management practices that restrict tumor formation. However, these practices failed in controlling virus spread which continues to evolve and cause field infections, implying a possible negative role for vaccine in driving virus mutations and virulence. In this study, 160 clinical samples were collected from 50 layers' flock from ten different Iraqi provinces, were examined by real-time PCR against the polyprotein (pp38) gene of MDV using specific primers. The results revealed that 28 samples originated from 12 farms of four provinces were positive. Next, eight positive samples were selected according to their high yield viral genome which was represented by high Cycle threshold (Ct) ratio, amplified by glycoprotein L (gL) specific primers through conventional PCR. The PCR products were sent to commercial sequence company (Macrogen/ South Korea) and Upon results feedback, sequence alignment and phylogenetic relatedness study of viral glycoprotein L was carried out. The results found multiple Single Nucleotide Polymorphisms (SNP) among the local strains compared with reference one, where six out of eight samples carry a gL genome that matched 98-100% of circulating viruses previously described from the region. Also, the results found that two sequences showed a highly variable sequence with 93% and 85% identity match with other described viruses sequence of gL. This study represents the first attempt to investigate the gL of MDV in Iraqi poultry farms.

Keywords: *Glycoprotein L, Marek's disease virus, Phylogenetic analysis, real-time PCR, Sequence variation.*

1. INTRODUCTION

Poultry production is regarded as one of the fast-growing economies all around the world that provides high-quality protein for billions of people (Food and Agriculture Organization, 2013), however, intensive increase in poultry farms has been accompanied by many challenges, especially avian pathogens like Marek's virus Disease (Dima and Girma, 2023, Zeghdoudi et al., 2023) Marek's virus disease is an infectious and highly contagious disease of chicken that is caused by an alpha Herpesvirus known as Gallid herpesvirus-2, characterized by infiltration of infected T-cells into many visceral organs including the liver, spleen, kidney, heart, lung, Proventriculus, as well as nervous system including the brain and peripheral nerves (World Organization for Animal Health, 2023).

Marek's disease virus carries a large linear double-strand DNA genome that weighs about 180kb and is characterized by the presence of 2 main segments, the unique long (UL), and the unique short (US). Each one of these segments is flanked with two specific sequences known as the Terminal Repeat sequence (TR) and the Internal Repeat sequence (IR). This genome encodes at least 100 ORFs that are involved in different aspects of virus pathogenesis and replication (Liao et al., 2021) including several enveloped proteins known as gB, gC, gD, gE, gH, gI, gK, gM and gL (Davison and Nair, 2004).

UL1 is the viral gene that encodes for Glycoprotein L. Glycoprotein L is found in a heterodimer form with glycoprotein H, both are involved in the mechanism of virion entry, in which gL is folded with gH, a process essential to activate gH properly to bind host cell receptor and the ectoderm domain of gH is engulfing the gL in a sandwich structure (Gonzalez-Del Pino and Heldwein, 2022).

Glycoprotein L plays an essential role in MDV entry into host cells, as it mediates the processes of attachment, fusion, penetration, assembly, and cell–to-cell entry, as well as it contributes in MDV specific cytotoxic immune responses (Hassanin et al., 2013, Abd-Ellatieff et al., 2018). Also, it was suggested that mutated UL1 plays an associated role with increased viral virulence (Shamblin et al., 2004).

Several studies suggested that mutations within the UL1 region may interrupt virus virulence and aid in overcoming vaccine coverage (Santin et al., 2006, Tavlarides-Hontz et al., 2009, Shaikh et al., 2013), although it was very difficult to correlate these mutations with developing viral virulence (Dunn et al., 2019).

In Iraq, the economic importance of Marek's Virus Disease brings special attention to this disease as compared with other important avian pathogens like Avian influenza (Khamas, 2008, Allawe and Hidayat, 2022), Newcastle disease virus (AL-Zuhariy, 2017), Infectious Laryngiotrachitis virus (Allawe, 2016) and the related immunosuppressive Infectious bursal disease (Jumaa et al., 2020). Accordingly, many studies have been conducted to investigate the clinical form of MDV and to describe some of its genetic properties (Zahid, 2008, Wajid et al., 2013, Najem et al., 2024, Anas, 2023).

The increased rate of incidence of MDV in Iraqi layers farm and the shortage of related molecular studies have emerged the need for extensive analysis of the virus field strains, especially its virulence genes, so this study was designed. This study helps to analyze and better understand the phylogeny of the glycoprotein L, as one of the suggested virulence genes, and it can be a part of improving the related control strategies and vaccine usage.

2. MATERIALS AND METHODS

2.1. Ethical approval

Ethical approval was granted through the local committee of animal care and use at the College of Veterinary Medicine within the University of Baghdad (Number 1240 on 2024/06/27).

2.2. Samples collection

In this study, a total of 160 tissue samples were collected from Marek's disease virus suspected flocks in 50 farms from ten Iraqi governorates. The collected samples include liver, spleen, kidney, and feather follicles from acute cases. These samples were delivered, crushed, and prepared for viral nucleic acid extraction at the Virology laboratory in the Central Veterinary Laboratories/ Iraqi veterinary department.

2.3.DNA extraction

Viral DNA was extracted using a QIAamp DNA Kit from Qiagen according to manufacturer instructions. The extraction process yields 200 µl of viral DNA that is stored at -70°C until the test.

2.4. Real-time PCR for detection of Marek's virus

In this study, a Taqman Real-time PCR uses a specific primers-probe set as in (Table 1) to detect a region of the pp38 gene of MDV. Promega reaction mix buffer was used and prepared by mixing the following components: 2X buffer mix, 0.4 µl of enzyme mix, 2.05 µl DEPC, 1µl of each primer, 0.5µl of the fluorescent Taqman probe, and 0.05 µl of Rox passive reference stain. the mix constitutes 15µl of reaction buffer, and was completed to the final volume 20 µl by adding 5µl of the suspected sample.

Table 1 The primer-probe set used in this study for the detection of the pp38 gene by real-time PCR

Oligo Name	Sequence 5'-3'	Reference
MDV PP38F	GAG CTA ACC GGA GAG GGA GA	(Baigent *et al.*, 2016)
MDV PP38R	CGC ATA CCG ACT TTC GTC AA	
MDVPP38 Pr	FAM-CTC CCA CTG TGA CAG CC-BHQ1	

The investigation tests were carried out using the Applied Biosystem Fast 7500 thermocycler under the following conditions as recommended by the reaction mix manufacturer: initial denaturation and hot start activation at 95°C for 2 minutes followed by 40 cycles of 95°C for denaturation and 60°C for annealing and extension step that elapsed for 3, and 30 seconds respectively. The fluorescence signal was read during the extension step using the FAM filter, and the results analysis was carried out according to instrument settings.

2.5. Conventional PCR and electrophoresis for Marek's virus glycoprotein L

The next step involved amplification of the glycoprotein L gene from positive samples utilizing specific primers recommended by (Hassanin et al., 2013) to amplify a region of 576 bp (Table 2). eight positive samples with high yields Ct value. and from different Iraqi governorates were selected for the detection and analysis of glycoprotein L.

Table 2 Primers used for amplifying gL for this experiment

Oligo	Oligo sequence	Product size
gL/FP	5'-ATG AAA ATT TAT AGA GTA CTC GTG-3'	576 bp
gL/RP	5'-GGC ATT GGC TCG TCG GCT -3'	

The amplification process used GoTaq®G2 Green Master Mix/ Promega for the test. The master mix was ready to use at 12.5µl/sample with 1ul of both upstream and downstream primer at 10 pmol/µl, and 5.5 µl of nuclease-free water/ sample after that 5µl of DNA template was added for each test to complete the reaction volume to 25µl. The thermal condition was optimized before the test and the following conditions were set: 95°C for 2 minutes as the initial denaturation step, followed by 40 cycles of 95°C for 30 seconds for denaturation, 55°C for 30 seconds for annealing, and 72°C for 1 minute for extension step. It ended with a final elongation step of 72°C for 10 minutes. The whole amplification protocol was applied through the ProFlex PCR System from Applied Biosystems™.

The product was visualized on 1.5% agarose run under 70 volts for 90 minutes. The samples were stained with ethidium bromide and a promega ladder was used as a marker for these products.

2.6. Genetic sequencing of Glycoprotein L

Positive PCR products were packed and sent to Macrogen, Inc. / South Korea for gene sequence analysis, feedback results were analyzed through MEGA11 software, and a phylogenetic tree was created from these data through Bio Edit Sequence Alignment Editor software. The phylogenetic tree was created by alignment of the sequence data with a reference strain of a very virulent Gallid alphaherpesvirus 2 published by (Tulman et al., 2000) under the accession Number (NC_002229.3).

3. RESULTS

3.1. Marek's disease virus detection by real-time PCR

The real-time PCR results (Figure 1A, and 1B) represent the amplification plot of Marek's disease positive samples which target the pp83 gene by TaqMan probe-based real-time PCR, some of the samples yield a high Cycle threshold (Ct) value between 26-31, while other samples yield a Ct value of 33-35.

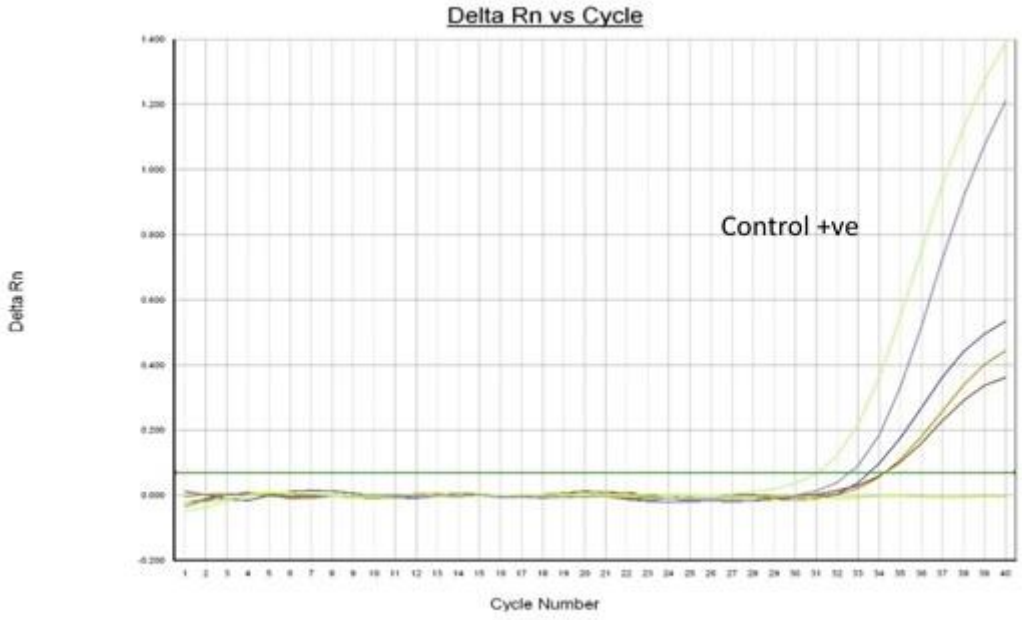

Figure 1A represent the amplification plot of Marek's virus positive samples with Taqman real-time PCR tested in Applied Biosystem 7500 fast real-time PCR with FAM as a reporter molecule in comparison with Positive control. The samples showed a moderate to low Ct value, although within the range of positive results

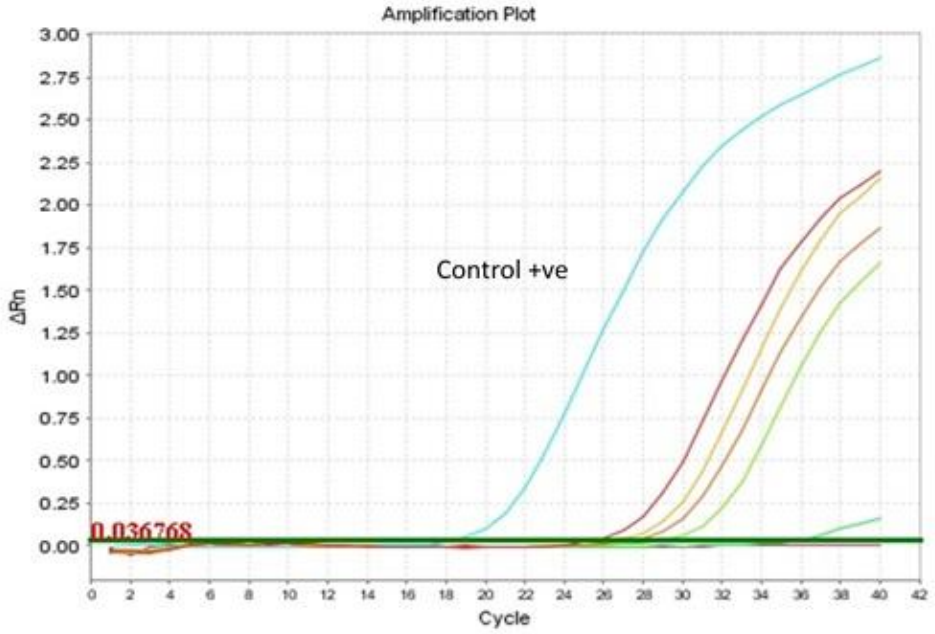

Figure 1B the amplification plot of Marek's virus-positive samples with Taqman real-time PCR tested in Applied Biosystem 7500 fast real-time PCR with FAM as a reporter molecule in comparison with Positive control. The samples showed a high Ct value between 26-31, these samples represent a good candidate for genes analysis step

3.2. Conventional PCR results for gL

The PCR amplification process that target the gL specific gene, yields the expected PCR product of about 576 bp (Figure 2) when the product was separated by electrophoresis in 1.5% agarose stained with ethidium bromide dye and separated with 70volts for 90 minutes. At analysis of sequence feedback, the sequence chains were trimmed from the terminus leaving about 490 bp that is located between the nucleotide bases 19270-19750 when compared with the reference sequence.

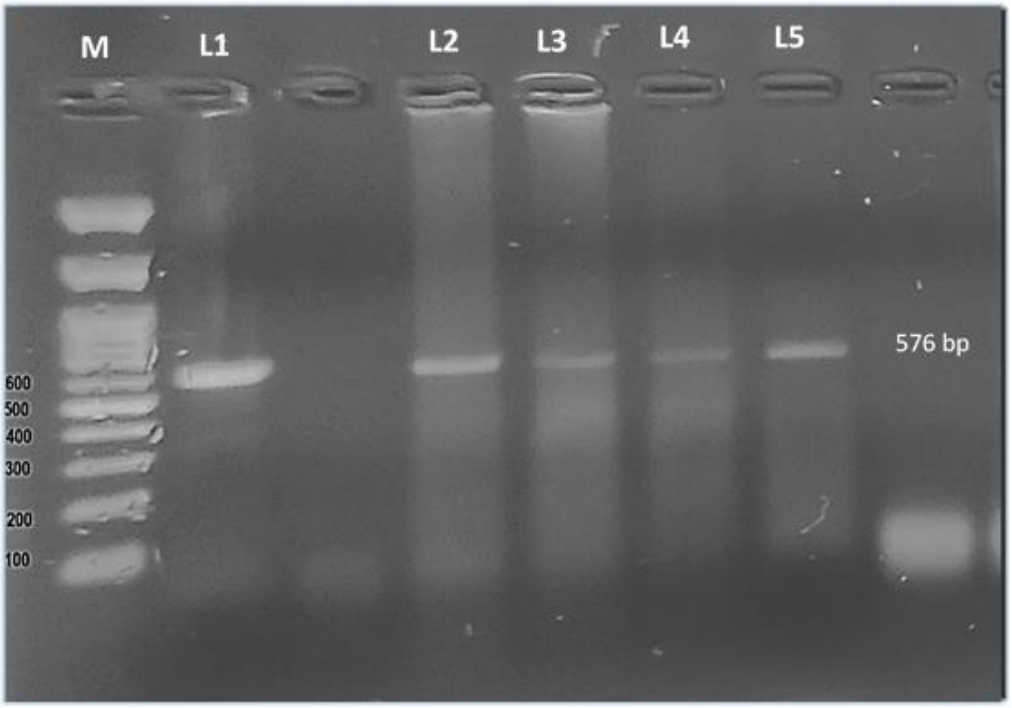

Figure 2 Gel electrophoresis with Amplification bands of gL with 576bp weight in comparison with 100 bp Marker, agarose at 1.5%, bands stained with ethidium bromide, electrophoresis at 70 volts for 1.5 hours. The ladder is of 100bp band from promega.

3.3. Sequence analysis of glycoprotein L and Phylogenetic relations

The deep analysis of sequence results (Figures 3A, and 3B) showed that out of those 8 sequences analyzed through this experiment, 6 of them (L208, L211, L227, L5, L1, and L2) showed high similarity with the reference gene sequence with only a few Single Nucleotide Polymorphisms (SNP), while Samples L3 and L4 showed the most variable changes among the tested sequences with multiple variations compared with the reference sequence (Figure 3 A, and3B).

The sample sequence L2 showed a complete matching with the reference sequence, while sample L1 showed only replacing AG to GC at position 19756-19757.

Sample L208, L211, and L227 showed an SNP at site 596 where G is replaced by A, Sample L211 also showed two SNPs at position 19662 and 19682 which also share this SNP with sample

Sample L208 showed four SNPs at positions 19596, 19682,19691, and 19755 in which all of them replaced G with A.

Sample L227 also showed two SNPs at 19596, and 19691 where G is converted into A, while another SNP showed replacing A into T at position 19394. Additionally, GAG was converted into AGC at position 19754-19756.

Sample L5 showed an exchange of G to A at position 19691, while AG was replaced with GC at 19756-19757.

Sample L211 showed replacing of G into A at positions 19376,19569,19662,19682, and 19691.

The phylogenetic tree appears in (Figure 4) showed that the sequenced samples were divided into three clusters, two of the are closely related as they comprise six samples (L1, L2, L227, L5, L208, and L211) that showed high homology of 98-100% with previously described strains from Egypt, Tunisia, and Japan as well as with the standard strain. The third cluster involved the samples (L3 and L4) that express much more variation 93% and 85% from the previously described strains as well as from the reference strain.

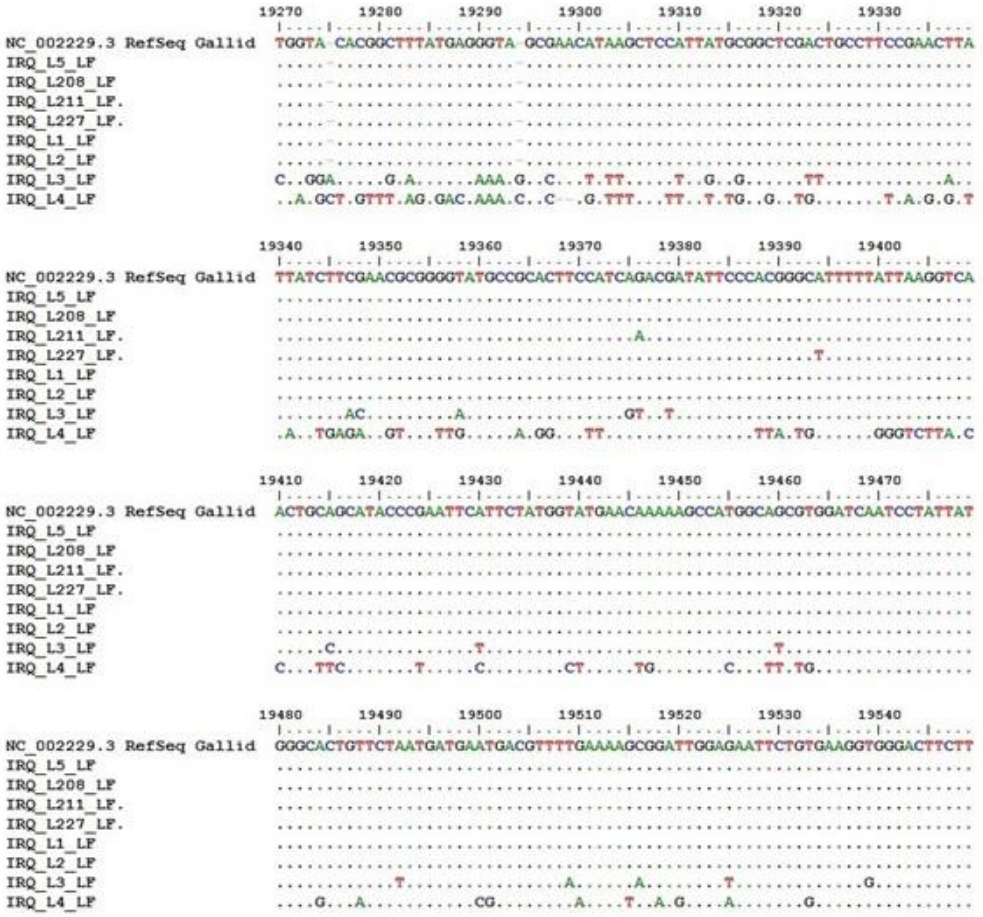

Figure 3A the first part of the alignment scheme in which the genetic analysis of the eight genes for gL, illustrates the highly matching sequences of the first six samples compared with variation in samples L3 and L4 that showed multiple variations. All eight genes are aligned with the standard control gene NC-002229.3. Alignment process was done with MEGA 11 software.

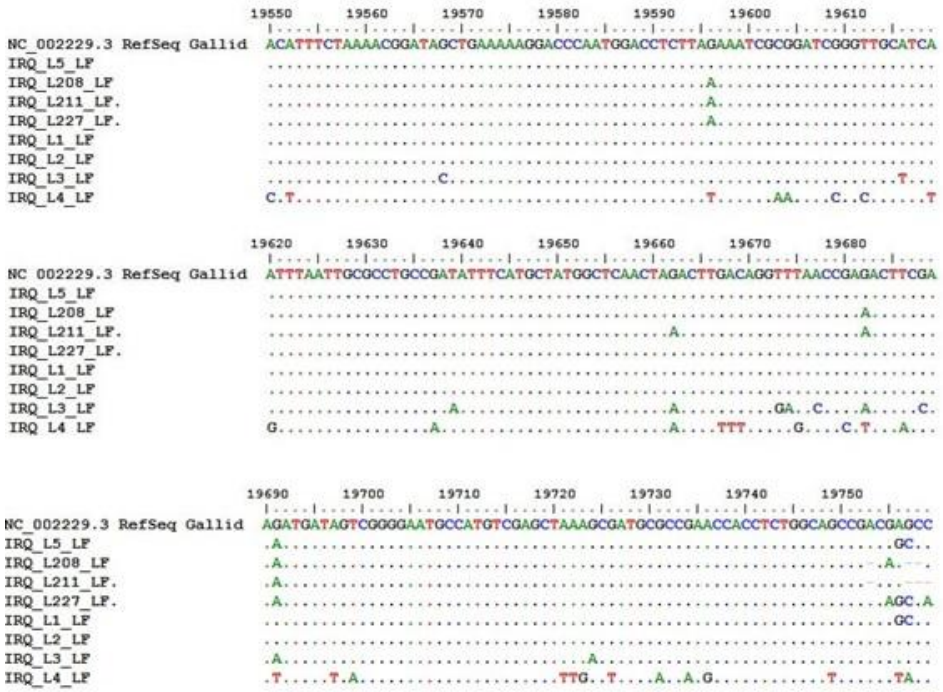

Figure 3B the second part of the alignment scheme illustrates how the samples L3, and L4 showed less variation than the first segment of the gene, it also illustrated clearly how the other samples express a shared SNP between them. Alignment process was done with MEGA 11 software.

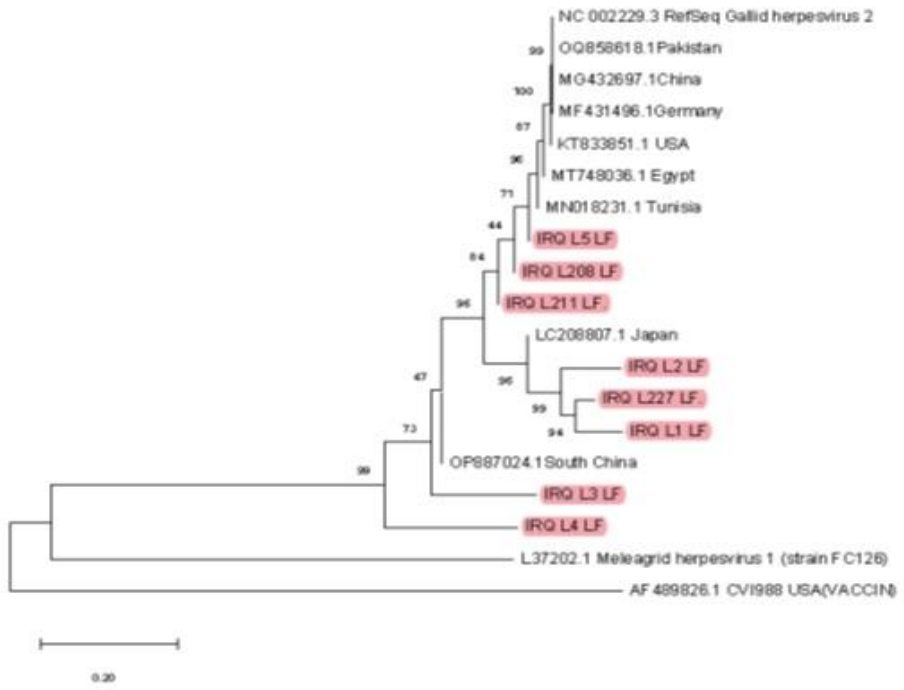

Figure 4 the phylogenetic analysis of gL for the Iraqi isolates and their relation with other strains analyzed from different countries, the samples separated into three clusters, two of them are related as they present the six samples with high matching rate with the egyptian, Tunisian, and Japanese strain. The remaining two samples got a separate location as they have higher variation from the standard as well as from the other local strains. Data was analyzed by Bio Edit Sequence Alignment Editor software.

4. DISCUSSION

Marek's disease is considered one of the important avian diseases with significant economic importance correlated with its immunosuppression and oncogenic nature (Boodhoo et al., 2016).

The disease is controlled by vaccination either inovo or at hatching where the vaccine approved an efficacy in preventing tumor formation. Over time, with continuous vaccine administration, higher virulent MDV strains emerged with a suggested possibility of vaccine-positive selection (Gimeno, 2008, Reddy et al., 2017, Murata et al., 2020). Upon performing molecular studies on emerging virulent strains, there were many mutations in different genes that were involved in virus virulence like the oncogene Meq, PP38, UL1, and UL44 (Lachheb et al., 2020).

This study describes the genetic sequence analysis of glycoprotein L and its genetic phylogeny as one of the important virion surface epitopes that is responsible for virion attachment and fusion with host cells as well as cell-to-cell spread (Wu et al., 2001, Abd-Ellatieff et al., 2018). This study was accomplished in two stages: initial detection of MDV, and Genetic analysis of gL.

The first stage of this study investigated the positive Marek's disease virus samples which were detected among the total study collected samples through the Taqman real-time PCR technique, and was selected according to its high sensitivity, specificity, and efficacy as advised by (Cao et al., 2013, Bulbula et al., 2022) especially that this study selected the primers that target pp38 gene with capability to detect only virulent MDV-1 strains and not vaccine strains as was designed and recommended by (Baigent *et al.*, 2016). A total of 12 flocks were detected positive out of the total 50 flocks tested.

The next stage of this study was the selection of eight positive samples based on criteria of geographical and high-concentration gene copies according to their Ct value to ensure a good quality and quantity of gL gene product using gene-specific primers. The PCR product was sent to a commercial sequence company, and sequence feedback was analyzed.

The Results analysis revealed that, SNP mutations were seen in all sequenced genes at different frequencies that suggest different alleles for these mutated genes which agreed with (Kim et al., 2023). Some of the described SNPs were shared among different gene sequences like at position 19691 where A Substitutes G in four sample genes (208, 2011, 227, and L5) as well as at position 19596 were also an A Substitutes G in three samples (208, 211, and 227) which may reflect some dominant or fixed mutation at these sites especially with the scientific fact that, Herpesvirus are highly stable viruses with low mutation rates that may reach $1.6x10^{-5}$ according to (Trimpert et al., 2017).

The results of this study also agree with the findings of (Hassanin et al., 2013, Lachheb et al., 2020) that the sequences of local gL genes did not record the deletion of 12 nt reported previously by (Tavlarides-Hontz et al., 2009, Shaikh et al., 2013) which were suggested as the indicator of virulence strains by (Shamblin et al., 2004), while this study, on the other hand, differs from (Lachheb et al., 2020) in that the gL sequence from local strains showed variable degree of mutations between them as well as with reference strain. The lack of previous studies in Iraq on this gene sequence prevents the understanding of the developing history of these mutations and if they are significant to the Iraqi strains or are just under more development under the influence of vaccine usage in this country (Yehia et al., 2021).

The sequence alignment of the eight taxa through the Basic Local Alignment Search Tool (BLAST) as well as with MEGA11 software for phylogenetic analysis showed that the sequences under investigation follow two genetic forms: the first one shows the high identity sequences with strains previously isolated from Egypt, Tunisia, and Japan (Gifu strains) with the matching of 98-100%, where L1 showed 100% with Tunisian and Gifu 3 strains and more than 99% with other strains including some reference strains like CVI988, RB1B, GA, and MD5. This identity also was found with L2, L208, while the identity of 98-100% was shown with samples L211, L227, and L5. These results, with the phylogenic analysis, represent the high homology of this gene gL as compared with other strains circulating within the region in Egypt and Tunisia. However, these results did not give full regional overview of the gene mutation due to the lack of more information about circulating strains in other countries of the Middle East region where Iraq is a part of (Hassanin et al., 2013, Abd-Ellatieff et al., 2018, Lachheb et al., 2020).

On the other hand, special attention should be given to the second genetic form that is represented by samples L3 and L4, where L3 showed a low homology of about 93% with Gifu, Tunisian, and Egyptian strains, in comparison sample L4 showed a higher variation with an identity of 85% only with Gifu and Tunisian strains and 84.81% with Egyptian strains, and these results need more follow-up and investigation process to understand if this variation represent a selective mutation within the field Mark's virus as described previously by (Tavlarides-Hontz et al., 2009).

The limitation of this study is that mutations found cannot be used to directly describe virus virulence as this needs to perform in vivo pathogenicity study to investigate the role of these mutations in changing virus virulence and survival upon challenges as agreed with (Shaikh et al., 2013, Kim et al., 2023). Nevertheless, the study results can be indirectly connected to disease virulence as these mutated virus strains were collected from clinically infected and vaccinated layer flocks which can suggest that, these mutations have a neutral role on the virus virulence if not improve it in synergism with other viral genes mutations (Conradie et al., 2020, Bertzbach et al., 2020) and it send critical alert to select a higher matching vaccine according to the available molecular characteristics of the recent circulating field strains (Ahmed, 2020).

As a future study, it is highly suggested to increase the quantity of test samples and vary their sources like broilers and backyards as this will support to understand mutation patterns and will be very good additive information for research, especially that backyards are not vaccinated against MDV which will release them from vaccine pressure.

In conclusion, this study illustrates the molecular analysis and phylogenetic description of MDV gL from local Iraqi strains. Upon alignment, the results revealed multiple SNP among the eight studied gL gene from the local strains in comparison with the standard strain. the results indicated that six sequences showed a high homology with strains previously analyzed from Egypt, Tunisia, and Japan with sequence matching of 98-100%, while the other two sequences showed higher variation of 93% and 86% respectively. These variations appeared more clearly when analyzed to build on the phylogenic tree, in which the six highly homologues sequence strains were separated into two groups. The other highly variable strains occupy another branch of the tree which prove the mutation process within the local strains and that the clinically virulent circulating strains incorporated these mutant gL genes. Continuous follow up and analysis of more samples and multiple virulent genes beside gL are important to more understanding of mutation pattern and pathological feature of the circulating virus which will reflect on enhancing and developing control strategies of MDV.

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6. CONFLICT OF INTEREST

There is no conflict of interest with this article.

REFERENCES

[1] ABD-ELLATIEFF, H. A., ABOU RAWASH, A. A., ELLAKANY, H. F., GODA, W. M., SUZUKI, T. & YANAI, T. 2018. Molecular characterization and phylogenetic analysis of a virulent Marek's disease virus field strain in broiler chickens in Japan. *Avian Pathology,* 47**,** 47-57.

[2] AHMED, A. I. 2020. Molecular characterization of infectious bursal disease virus isolated from naturally infected broiler chickens in Erbil, Iraq. *The Iraqi Journal of Veterinary Medicine,* 44**,** 21-27.

[3] AL-ZUHARIY, M. T. 2017. Isolation and identification of the Newcastle disease virus from field outbreaks in broiler and layer flocks in Iraq: Mushtaq TB AL-Zuhariy1; Sahar Hamdi Abdulmaged1; Raed HS Rabee2 and Amer AA AL-Baldawi3. *The Iraqi Journal of Veterinary Medicine,* 41**,** 23-27.

[4] ALLAWE, A. B. 2016. Molecular characterization of field isolates of Avian Infectious Laryngeotracheitis Virus from different farms in Iraq. *The Iraqi Journal of Veterinary Medicine,* 40**,** 31-35.

[5] ALLAWE, A. B. & HIDAYAT, K. A. 2022. Identification and isolation of low pathogenic avian influenza (LPAI) virus from chicken farms in Iraq. *International Journal of Health Sciences***,** 12666-12679.

[6] ANAS, M. 2023. Detection of pathotype of Marek's Disease in layer chicken in Iraq. *University of Thi-Qar Journal of agricultural research,* 12**,** 25-34.

[7] BERTZBACH, L. D., CONRADIE, A. M., YOU, Y. & KAUFER, B. B. 2020. Latest insights into Marek's disease virus pathogenesis and tumorigenesis. *Cancers,* 12**,** 647.

[8] BOODHOO, N., GURUNG, A., SHARIF, S. & BEHBOUDI, S. 2016. Marek's disease in chickens: a review with focus on immunology. *Veterinary research,* 47**,** 1-19.

[9] BULBULA, A., BORENA, B., TADESSE, B., ALIY, A. & NEGESSU, D. 2022. Isolation and molecular detection of Marek's disease virus from outbreak cases in chicken in South Western Ethiopia. *Veterinary Medicine: Research and Reports***,** 265-275.

[10] CAO, W., MAYS, J., DUNN, J., FULTON, R., SILVA, R. & FADLY, A. 2013. Use of polymerase chain reaction in detection of Marek's disease and reticuloendotheliosis viruses in formalin-fixed, paraffin-embedded tumorous tissues. *Avian Diseases,* 57**,** 785-789.

[11] CONRADIE, A. M., BERTZBACH, L. D., TRIMPERT, J., PATRIA, J. N., MURATA, S., PARCELLS, M. S. & KAUFER, B. B. 2020. Distinct polymorphisms in a single herpesvirus gene are capable of enhancing virulence and mediating vaccinal resistance. *PLoS pathogens,* 16**,** e1009104.

[12] DAVISON, F. & NAIR, V. 2004. *Marek's disease: an evolving problem*, Elsevier.

[13] DIMA, F. & GIRMA, A. 2023. Review on marek's disease and its economic importance. *J Bacteriol Infect Dis 2021; 4 (4): 1-2. 2 J Bacteriol Infect Dis 2021 Volume 4 Issue,* 4**,** 3.

[14] DUNN, J. R., BLACK PYRKOSZ, A., STEEP, A. & CHENG, H. H. 2019. Identification of Marek's disease

virus genes associated with virulence of US strains. *Journal of General Virology,* 100**,** 1132-1139.

[15] Food and Agriculture Organization. (2013) Poultry Development Review. Access online https://www.fao.org/4/i3531e/i3531e.pdf.

[16] GIMENO, I. M. 2008. Marek's disease vaccines: a solution for today but a worry for tomorrow? *Vaccine,* 26**,** C31-C41.

[17] GONZALEZ-DEL PINO, G. L. & HELDWEIN, E. E. 2022. Well Put Together—A Guide to Accessorizing with the Herpesvirus gH/gL Complexes. *Viruses,* 14**,** 296.

[18] HASSANIN, O., ABDALLAH, F. & EL-ARABY, I. E. 2013. Molecular characterization and phylogenetic analysis of Marek's disease virus from clinical cases of Marek's disease in Egypt. *Avian diseases,* 57**,** 555-561.

[19] JUMAA, R. S., ALLAWI, A. B. & JABBAR, R. N. 2020. Genetic analysis of field isolates of infectious bursal disease virus in Iraqi farms.*The Iraqi Journal of Veterinary Medicine,* 44**(1):**18-28

[20] KHAMAS, E. J. 2008. Avian influenza (H9N2) outbreak in Iraq. *The Iraqi Journal of Veterinary Medicine,* 32**,** 223-230.

[21] KIM, T., HEARN, C. J., MAYS, J., VELEZ-IRIZARRY, D., REDDY, S. M., SPATZ, S. J., CHENG, H. H. & DUNN, J. R. 2023. Phenotypic Characterization of Recombinant Marek's Disease Virus in Live Birds Validates Polymorphisms Associated with Virulence. *Viruses,* 15**,** 2263.

[22] LACHHEB, J., MASTOUR, H., NSIRI, J., KABOUDI, K., CHOURA, I., AMMOUNA, F., AMARA, A. & GHRAM, A. 2020. Newly detected mutations in the Meq oncogene and molecular pathotyping of very virulent Marek's disease herpesvirus in Tunisia. *Archives of virology,* 165**,** 2589-2597.

[23] LIAO, Y., LUPIANI, B. & REDDY, S. M. 2021. Latest insights into unique open reading frames encoded by Unique Long (UL) and Short (US) regions of Marek's Disease Virus. *Viruses,* 13**,** 974.

[24] MURATA, S., MACHIDA, Y., ISEZAKI, M., MAEKAWA, N., OKAGAWA, T., KONNAI, S. & OHASHI, K. 2020. Genetic characterization of a Marek's disease virus strain isolated in Japan. *Virology journal,* 17**,** 1-13.

[25] NAJEM, H. A., KHALEEFAH, I. A. & ALMAYAHI, W. M. 2024. Genotypic Characterization of Marek's Disease Virus from Clinically Suspected Layer in Basrah, South of Iraq: https://doi. org/10.12982/VIS. 2024.025. *Veterinary Integrative Sciences,* 22**,** 349-361.

[26] REDDY, S. M., IZUMIYA, Y. & LUPIANI, B. 2017. Marek's disease vaccines: Current status, and strategies for improvement and development of vector vaccines. *Veterinary microbiology,* 206**,** 113-120.

[27] SANTIN, E. R., SHAMBLIN, C. E., PRIGGE, J. T., ARUMUGASWAMI, V., DIENGLEWICZ, R. L. & PARCELLS, M. S. 2006. Examination of the effect of a naturally occurring mutation in glycoprotein L on Marek's disease virus pathogenesis. *Avian diseases,* 50**,** 96-103.

[28] SHAIKH, S. A., KATNENI, U. K., DONG, H., GADDAMANUGU, S., TAVLARIDES-HONTZ, P., JAROSINSKI, K. W., OSTERRIEDER, N. & PARCELLS, M. S. 2013. A Deletion in the Glycoprotein L (gL) Gene of US Marek's Disease Virus (MDV) Field Strains Is Insufficient to Confer Increased Pathogenicity to the Bacterial Artificial Chromosome (BAC)–Based Strain, RB-1B. *Avian Diseases,* 57**,** 509-518.

[29] SHAMBLIN, C. E., GREENE, N., ARUMUGASWAMI, V., DIENGLEWICZ, R. L. & PARCELLS, M. S. 2004. Comparative analysis of Marek's disease virus (MDV) glycoprotein-, lytic antigen pp38-and transformation antigen Meq-encoding genes: association of meq mutations with MDVs of high virulence. *Veterinary microbiology,* 102**,** 147-167.

[30] TAVLARIDES-HONTZ, P., KUMAR, P. M., AMORTEGUI, J. R., OSTERRIEDER, N. & PARCELLS, M. S. 2009. A deletion within glycoprotein L of Marek's disease virus (MDV) field isolates correlates with a decrease in bivalent MDV vaccine efficacy in contact-exposed chickens. *Avian diseases,* 53**,** 287-296.

[31] TRIMPERT, J., GROENKE, N., JENCKEL, M., HE, S., KUNEC, D., SZPARA, M. L., SPATZ, S. J., OSTERRIEDER, N. & MCMAHON, D. P. 2017. A phylogenomic analysis of Marek's disease virus reveals independent paths to virulence in Eurasia and North America. *Evolutionary Applications,* 10**,** 1091-1101.

[32] TULMAN, E., AFONSO, C., LU, Z., ZSAK, L., ROCK, D. & KUTISH, G. 2000. The genome of a very virulent Marek's disease virus. *Journal of virology,* 74**,** 7980-7988.

[33] WAJID, S. J., KATZ, M. E., RENZ, K. G. & WALKDEN-BROWN, S. W. 2013. Prevalence of Marek's disease virus in different chicken populations in Iraq and indicative virulence based on sequence variation in the EcoRI-Q (meq) gene. *Avian diseases,* 57**,** 562-568.

[34] World Organization for Animal Health (2023). *Marek's Disease in OIE Terrestrial Manual, Paris*. Available from: http://www.oie.int/fileadmin/Home/eng/Healthstandards/tahm/2.03.13MAREK DIS.pdf

[35] WU, P., REED, W. & LEE, L. 2001. Glycoproteins H and L of Marek's disease virus form a hetero-oligomer essential for translocation and cell surface expression. *Archives of virology,* 146**,** 983-992.

[36] YEHIA, N., EL-SAYED, H. S., OMAR, S. E., ERFAN, A. & AMER, F. 2021. Genetic evolution of Marek's disease virus in vaccinated poultry farms. *Veterinary World,* 14**,** 1342.

[37] ZAHID, A. 2008. Field evaluation of Marek's disease infection in vaccinated parent flock. *Al-Qadisiyah Journal of Veterinary Medicine Sciences,* 7.

[38] ZEGHDOUDI, M., LATIFA, M., SELOUA, M., RIMA, S., MARDJA, T. & LEILA, A. 2023. Updating of epidemiological and pathological features of Marek's disease in laying hens and broilers. *Veterinární medicína,* 68**,** 443.