MOLECULAR DETECTION OF PATHOTYPE OF MAREK'S DISEASE VIRUS IN LAYERS CHICKEN IN IRAQ

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ABSTRACT

Marek's disease virus (MDV) infects chickens and is among the most common tumor in animals. The first MDV vaccinations that prevented cancer and reduced losses in the chicken industry were live-attenuated vaccines. Despite the fact that the current gold standard vaccination effectively guards against the disease, because of the Meq gene the virus is continuously mutating to become more virulent. the two vaccine generations previously given protection was able to be overcome by emerging field strains. In the present study collecting the tissue samples from infected, A standardized PCR was used. 132-bp tandem repeat region targeted for serotype-1 MD viruses. in Further, the oncogenes' characterization. PCR and nucleotide sequencing were used to detect Meq gen and viral interleukin 8 (vIL-8) gen. Meq gene sequence analysis for different clinical cases from Iraq revealed 100 % homology with Indian strain, Japan strain, Turkish strain, Germany strain (very virulent). and with (very very virulent) Iranian strains showed 100% identity with the Iraq strain. With the use of more GenBank sequences, a phylogenetic analysis of oncogenes was carried out. Finally, based on nucleotide and phylogenetic analyses of the viruses, we get to the conclusion that MDV strains found in the current outbreaks in Iraq could be classified as virulent or very very virulent pathotypes.

Keywords: virulent, Meq gene, VIL 8

مجلة العلوم الزراعية العراقية- 2025 :56 (4):1316-1324 قدوري وحميد

التحري الجزيئي لفايروس الماريك في الدجاج البياض في العراق

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المستخلص

يصيب فيروس مرض ماريك (MDV) الدجاج وهو من بين أكثر أنواع الأورام شيوعًا بين الحيوانات. كانت أول لقاحات MDV التي حالت دون الإصابة بالأورام وقللت من الخسائر في صناعة الدواجن لقاحات حية مضعفة. على الرغم من فعالية التطعيم المعياري الذهبي الحالي، بسبب وجود Meq جين الفيروس يتحور باستمرار ليصبح أكثر ضراوة. سابقا تمكّن جيلا اللقاح من توفير حماية لهما والتغلب على سلالات الحقول الناشئة. في هذه الدراسة تم جمع عينات الأنسجة، واستخدام PCR موحد. منطقة تكرار ترادفيه 132–bp تستهدف فيروسات النمط المصلي 1 MD. علاوة على ذلك، توصيف الجينات المسرطنة. تم استخدام تسلسل النيكلوتيدات للكشف عن Meq gen و interleukin 8 (vIL–8) gen. أظهر تحليل تسلسل الجينات Meq لحالات سريرية مختلفة من العراق تماثلًا بنسبة 100 ٪ مع السلالة الهندية، والسلالة اليابانية، والسلالة التركية، والسلالة الألمانية (شديدة الضراوة). ومع سلالات إيرانية (شديدة الضراوة) أظهرت نسبة التشابه 100٪ مع سلالة العراق. مع استخدام المزيد من متواليات في البنك الجيني، تم إجراء تحليل النشوء والتطور للجينات المسرطنة. أخيرًا، استنادًا إلى تحليلات النيوكليوتيدات والتطور الوراثي للفيروسات، توصلنا إلى استنتاج مفاده أن سلالات MDV الموجودة في العراق يمكن تصنيفها على أنها أنواع خبيثة أو شديدة الضراوة.

الكلمات المفتاحة: عالي الضراوة، الميك جين، انترلوكين 8.

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INTRODUCTION

Marek's disease virus (MDV) is an alpha herpes and it has a fatal lymphoma mortality rate of up to 100% in chickens, Clinical signs of the lymphoproliferative disease include immunosuppression, torticollis, ataxia, and paralysis; however, it is a highly cell-associated, lymphotropic -herpesvirus that inflicts neoplastic disease and paralysis in chickens (29). The disease has been managed by vaccination with attenuated viruses, and this case offers the first proof that an antiviral vaccine may control cancer (6). Marek's disease has a complex etiology that includes lymphoid cell cytolytic and latent infection. as well as CD4+ T cell oncogenic transformation in susceptible chickens (6). Meleagrid alphaherpesvirus 1, Gallid alphaherpesvirus 2 (serotype1), and Gallid alphaherpesvirus 3 (serotype2) are members of the genus Mardivirus, previously described by three different serotypes (turkey herpesvirus [HVT], serotype 3, unless otherwise called MDV, the virus serotype 1 acts as the family's prototype virus (31). Also, Gallid herpesvirus type 1 (GaHV-1) is a member of the genus Iltovirus, the causative agent of infectious laryngotracheitis (ILT), and acute respiratory infection of layers chickens (4). Classical Marek's disease: involves paralysis of a limb or limbs, an infection of the neck's regulating nerves may cause torticollis and vagal involvement will cause the crop to enlarge; breathing problems and crop enlargement are possible side effects (20). Along with peripheral nerve lesions, lymphomatous infiltrations, and tumors are commonly seen in the skin, skeletal muscle, and visceral organs; the proventriculus, ovary, spleen, liver, kidneys, lungs, heart, and adrenals are among the organs that are frequently harmed (20). Acute Marek's disease early starts of despair, paralysis, and mortality (four to eight weeks prior to tumor growth) (11,36). Various degrees of edema brought on by inflammation in the brain stem, cerebellum, and cerebrum are among the post-mortem lesions. Non-specific: There are other signs of diarrhea, anorexia, paleness, and weight loss (11). There are still incidences of Marek's disease (MD), and MDV strains showed persistent virulence evolution. that causes immunological failure (32). MD outbreaks have become more frequent recently in various provinces and based on strain virulence and host susceptibility (32). A disease that causes immunosuppression is an important health problem in chickens and generates economic losses in birds due to susceptibility to other diseases (17). Due to the connection between the field's selection of highly contagious strains and the development of vaccines, the virulence of MDV strains tends to rise over time (30). Attention has been drawn to gene mutation as a major cause for the increased oncogenicity and pathogenicity (14). Continuous field strain evolution, probably as a result of strong selection pressure imposed on the widespread use of such insufficient vaccinations, is the cause of vaccine failure (2,9). In Iraq used HVT+ CVI988 vaccine for Marek's disease (24,33). The severity of the disease in Iraq and the high mortality rate in Iraq is evidence of the present of a very virulent virus and as a result of genetic mutations (24,34,35). The pathology an MDV isolate induces in non-vaccinated and vaccinated chickens, as well as its ability to survive the effects of vaccination, are used to pathotype the isolate (30). The United States Department of Agriculture developed this pathotyping method known as the ADOL assay (Avian Diseases and Oncology Laboratory assay); the ADOL test evaluates an isolate's ability to cause lymphoproliferative lesions in chickens that were given vaccinations. The isolates can be classified as mild MDV, virulent, very virulent MDV, and very virulent plus MDV strains based on the results of the ADOL assay as table 1 (21).

Table 1. lists different MDV reference pathotypes.

pathotype	Reference strains
Virulent(V)	JIM/102W, 596A, 617A.
Very virulent (VV)	RBIB,549A,653A,643P,595.
Very virulent plus (VV+)	584A,610A,645,648A,776, 660A,651.

Early cytolytic phase, latent infection, late cytolytic phase, and productive infection of the feather-follicle epithelium have all been identified as stages in the pathogen of MD (7). Clinical signs, postmortem changes, histological changes, and PCR were used for the diagnosis of MDV (23). PCR was used to

aids in a rapid, accurate detection and identification of poultry disease (5,18). It's used for the detection of MDV-1 and differentiation of field strains and vaccine strains (8,37). The aim of this study was to identify the presence of MDV co-infection in an infected flock in field conditions, and to determine new strains that had emerged.

MATERIALS AND METHODS

Filed sample collection

A total of 150-layer chickens were taken from different farms in Iraq involving. 50 chickens from 5 farms in Baghdad,30 chickens from 3 farms in Al-Kut, 21 chickens from 3 farms in Al Anbar,28 chickens from 5 farms in AL Nagefe,23 chickens from 3 farms in Karbala provinces) the feather follicle, spleen, kidney, lung, Gizzard, sciatic nerve, and proventriculus these samples were removed from each chicken in a sterile dissection. stored in labeled containers and transported immediately with ice to the laboratory and stored at -30c until processing (3,38)**.** collected from postmortem dead birds for virological and molecular analyses in sterile sample containers. some of these organs were kept in 10% formal saline for histopathological analysis (22).

DNA extraction

The extraction of DNA was done from tissues by Bosphore Viral DNA Extraction Spin Kit, a kit constructed with the silica membrane column separation method. The kit involves separating and purifying the nucleic acids from each component of cells. to extract them from biological samples. Thus, the several stages required for DNA isolation include disruption/lysis of the samples, inhibition of nuclease activity, binding of the DNA to the silica membrane, removal of contaminants, and recovery of the nucleic acid. Nanodrop was used to detect the DNA's absorbance at 260 nm and 280 nm wavelengths. The resulting granules were suspended in DNase-free water and then subjected to PCR.

PCR standardization

targeting the region of 132 bp repeated

Initial denaturation at 94°C for 4 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min, elongation at 72°C for 1 min, and final elongation for 10 min are the most suitable conditions for PCR of the 132 bp repeat region. at 72ºC and hold for 5 min at 4ºC.sequences of primer used for PCR targeting 132 bp region are: the first primer is forward composed of 22 bp 5' ATG CGA TGA AAG TGC TAT GGA G 3 and the second primer is reverse composed of 22bp 5' ATC CCT ATG AGA AAG CGC TTG a 3'.

Oncogene PCR standardization (Meq and vIL8)

The optimum conditions for PCR of Meq and vIL-8 genes: Initial denaturation at 94ºC for 4 min, 35 cycles of denaturation at 94ºC for 1 min, annealing at 59.4ºC for 1 min, elongation at 72ºC for 1 min, final elongation at 72ºC for 10 min and hold at 4ºC for 5 min. And PCR amplification of oncogenes by using specific primer (27). Meq gen: the first primer is forward composed from 22bp 5' GGC ACG GTA CAG GTG TAA AGA G 3, revers primer composed from 22bp 5' GCA TAG ACG ATG TGC TGC TGA G 3'. VIL8 gen: the first primer is forward composed from 22bp 5' GAG ACC CAA TAA CAG GGA AAT C 3' and revers primer composed from 22bp 5' TAG ACC GTA TCC CTG CTC CAT C 3'.

Oncogene sequence analysis

Meq, and vIL-8 genes' PCR results have been sent to Macrogen, South Korea, for sequencing. Using the MEGA 6 program to analysis of oncogene, the obtained nucleotide sequences of the vIL-8 and Meq genes of the MDV were analyzed and compared with other reference sequences from the GenBank database. vIL-8 and Meq genes' phylogenetic analysis. Using a Neighbor-joining approach with 1000 bootstrap replications, phylogenetic analysis was performed using MEGA 6 software (15).

RESULTS AND DISCUSSION

Clinical examination and necropsy

Poultry aged 120 to 240 days were provided the samples. The necropsy of the 20 flocks of chickens under examination indicated widespread or localized lymphomas in the liver, spleen, gonads, enlargement of the feather follicles and visceral tumor, these findings agree with (25,26,39). These chickens are paralyzed, enlargement in the sciatic nerve, a lack of cross-striations and edematous these results agree with (10,28).

Virus identification : By using PCR to amplify the samples, their amplicons undergo agarose gel electrophoresis analysis. For every isolate, and second step Amplification of 132 bp tandem repeats region was done using a positive MD DNA sample by PCR to detect and distinguish between vaccine strains and serotype-1 field MDV strains (7,41). The samples that will be identified as positive for 132 bp repeat region, all the tissue samples were found to be PCR amplificons containing a 446 bp product which is serotype 1-specific positive, as in Figure 1.

The third step involved using a DNA sample that had tested positive for a 132 bp repeat region to further amplify oncogenes. Standardization of PCR targeting oncogenes (Meq and vIL-8) These genes are known to be present in MDV-1, strains and are associated with oncogenicity (19,40,42). The presence of viral vIL-8 in all tissue samples was negative, while the presence of the Meq gene was positive. The purified PCR product of positive samples of Meq genes from Iraq was sent to sequencing, as in Figure 2.

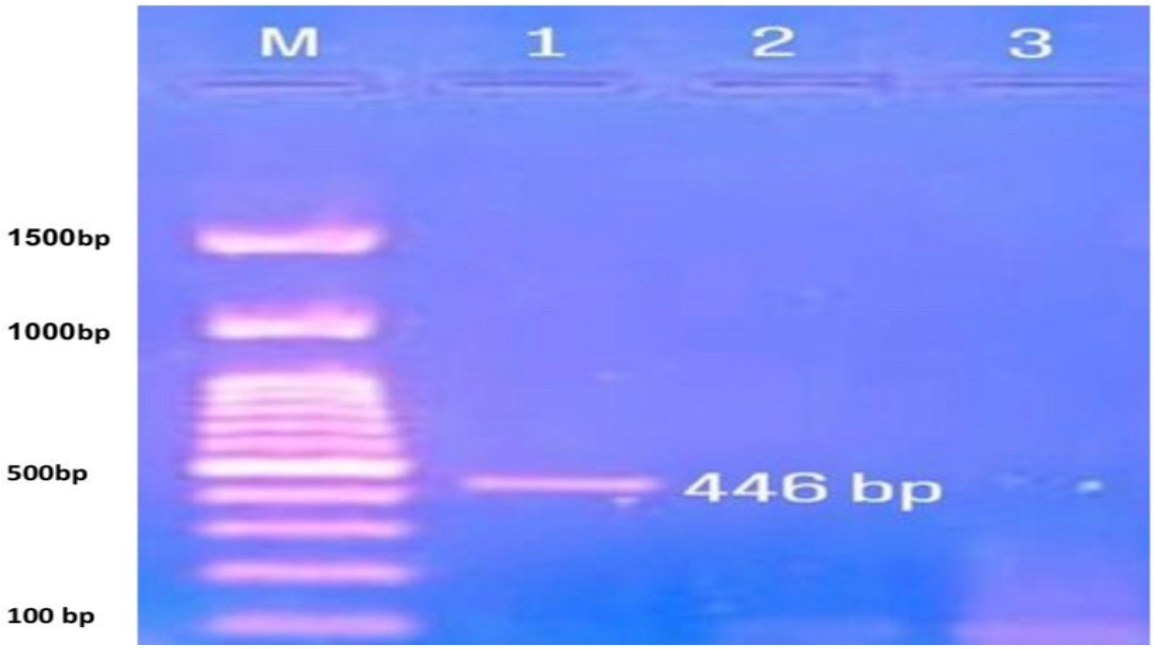

Figure 1. Amplification results stained with ethidium bromide. Agar gel electrophoresis of PCR products. M= 100bp DNA ladder, Lanes 1= positive sample with amplicon size of 446bp of repeat region.

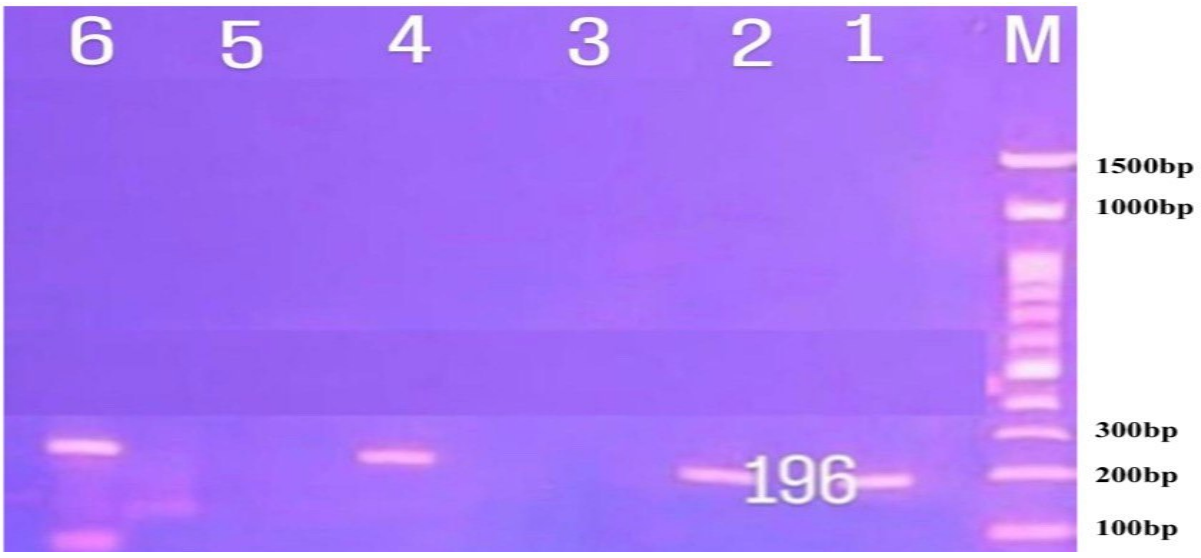

Figure 2. Amplification results stained with ethidium bromide. Agar gel electrophoresis of PCR products. M= 100bp DNA ladder, Lane 1,2,4,6= positive sample with amplicon size of 196bp of Meq gen.

Gene Sequencing of Meq genes

The Meq gene's PCR products have been extracted from an agarose gel and then sent to be sequenced by Genomics Macrogen Company in South Kore. the accession numbers for the Iraqi field sequences that were submitted to GenBank case 1 (OP524124), case 2 (0P524125), case 3 (OP524126), case 4 (OP524127),

The representative nucleotide sequences were compared with field MDV sequences using MEGA 6.0 software. the results showed 77 to 100 % homology with very virulent Indian strain KM067122, MK584541 (13). very virulent Japan strain LC385873(23). very virulent Turkish strain MN956505(1). very virulent German strain MT797631(12) and very virulent to very virulent plus with Iranian strain MW846300 (16). as in Table 2.

Table 2. The accession numbers for the sequences and their strain identification that were submitted to GenBank.

NO.	Isolate	Origin	Year	Pathotype	Percent Ident.	Accession number
1	Gallid herpesvirus 2 strain KeralaMty-F1 Meq protein (Meq) gene, partial cds.	India	2018	V virulent	Case1 100 % Case2 100 % Case4 96.6%	KM067122
2	Gallid alphaherpesvirus 2 MD239 Meq gene for Meq oncoprotein, complete cds.	Japan	2012	V virulent	Case1 100% Case2 100% Case 4 99 %	LC385873
3	Gallid alphaherpesvirus 2 isolate KeralaMty-2F Meq protein (Meq) gene, partial cds.	India	2019	V virulent	Case1 100% Case2 100% Case 98.4%	MK584541
4	Gallid alphaherpesvirus 2 strain MDV/Tur/2019 MEQ oncoprotein (Meq) gene, partial cds.	Turkey	2021	V virulent	Case1 100% Case2 100% Case 3 100% Case4 96.6%	MN956505
5	Gallid alphaherpesvirus 2 clone RB1B-N_Meq, complete sequence.	Germany	2020	V virulent	Case2 77%	MT797631
6	Gallid alphaherpesvirus 2 isolate UT-PCR9380 Meq gene, partial cds.	Iran	2021	Vv virulent	Case1 100% Case2 100% Case 3 100% Case 4 95%	MW846300

Phylogenetics analysis

The result of Meq gene sequences and a result of the selected sequences were compiled and compared with the different MDV nucleotide sequences database around the global in the GenBank NCBI. All reported sequences were aligned with isolated MDV sequences in this study. The tree was constructed using the neighbor-joining method in MEGA 6.0 Figure 3.

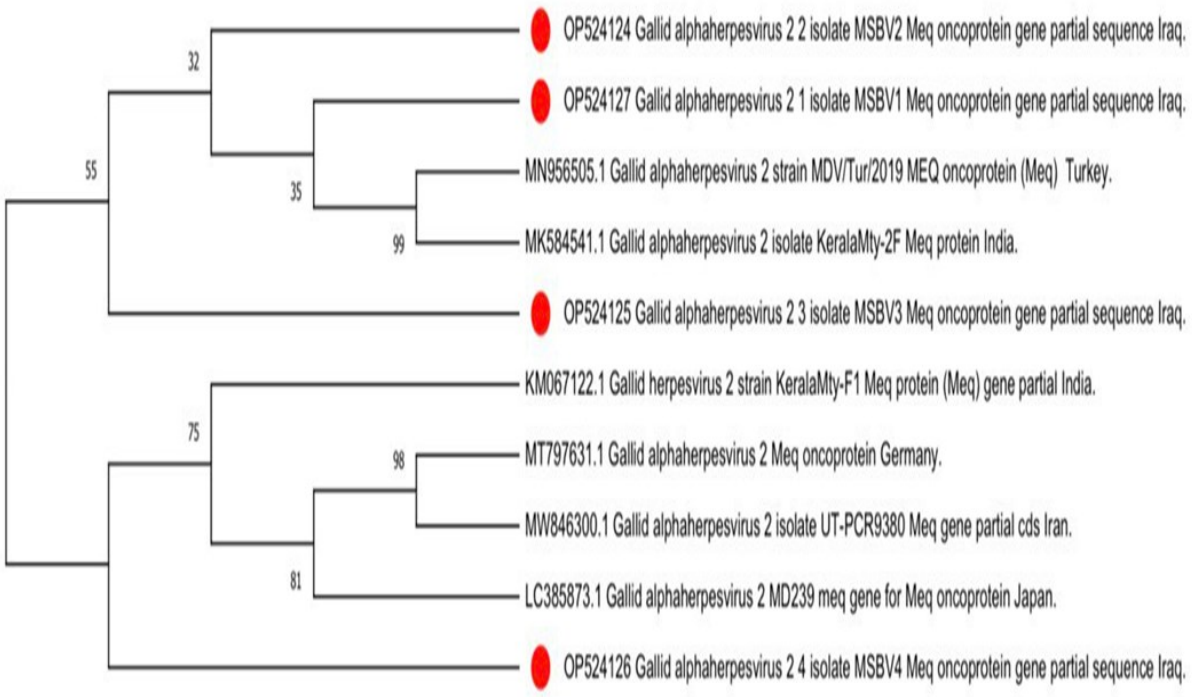

Figure 3. Phylogenetic analysis of Meq gene of and reference MDVs based on nucleotide sequences.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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