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Detection of pathotype of Marek's Disease in layer chicken in Iraq

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Abstract:

Marek's Disease is a viral disease that affects poultry, particularly chickens. It is caused by the Marek's Disease Virus (MDV), which belongs to the herpesvirus family. The disease is characterized by the formation of tumors in various organs, including the nerves, skin, and internal organs such as the liver, spleen, and kidneys. A layer poultry farm of one day old, vaccinated with Marek's disease virus vaccine. The flock was infected with a virulent Marek's disease virus with a mortality rate of up to 60%. Histopathological examination of affected tissues is an important tool for the diagnosis of the disease. The histopathological finding of tissue sections from chickens with MDV shows extensive proliferation of lymphocytes of various sizes. The confirmation was done by PCR and standardized targeting a 132 bp tandem repeat region specific for serotype-1 MD viruses Meq gene was carried out by PCR and nucleotide sequencing, The sequence analysis of Meq gene of different clinical cases from Iraq homology with very virulent strain and Phylogenetic analysis of oncogenes was carried out with other available sequences in the GenBank we conclude that MDV strains obtained in the present outbreaks in Iraq could be designated as very virulent pathotypes based on nucleotide and phylogenetic analysis of the viruses.

Key word : meg gene , very virulence, MDV,Iraq.

I. INTRODUCTION

Marek's disease, or neurolymphomatosis, is a lymphoproliferative disease that damages the peripheral nervous system. It is also associated with the development of visceral lymphomas in visceral organs like the liver, kidneys, and ovaries, as well as a rare infiltration of mononuclear cells in the brain and the eye. the etiological factor A DNA-containing virus with a hexagonal nucleocapsid and a shell of 150 to 160 nm in diameter(1).The bird that once suffered from the disease remains the virus for the duration of its life and constantly releases it into the environment. The virus that causes Marek's disease commonly replicates in cell nuclei rather than the cytoplasm or extracellular space(2). 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, 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(3).Acute Marek's disease early starts of despair, paralysis, and mortality (four to eight weeks before tumor growth). Various

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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(4). MD outbreak occurred from 32 to 47 weeks and All chickens received in hatchery repeated vaccination against Marek's disease (Rispens CVI 988 strain + HVT)(5). Within a few weeks of infection, MDV establishes malignant lymphomas in chickens, despite intensive vaccination (6). Marek's disease (MD), a lymphoproliferative disease in chickens induced by MDV, is one example where the virulence of a given strain can be defined as its ability to cause disease. (e.g., nerve lesions, visceral tumor), MDV over the past 40 years, has undergone three major shifts in virulence, the most recent two occurring after the widespread introduction of new and more effective MD vaccines. (7). In the field, acute forms of MD, including visceral lymphomas, cause the majority of economic losses; however, virus-induced immunosuppression. Clinical signs of MD include weight loss, paralysis, and depression while, in internal organ bursal/thymic atrophy, neurologic disorders, and rapid onset of T cell lymphomas that can infiltrate lymphoid tissues, visceral organs, and peripheral nerves(8). MD virus infects through the respiratory route, infecting lymphocytes and macrophages in the lung. It then moves to lymphoid organs, causing acute cytolytic infection. After replication in B lymphocytes, the virus infects CD4+ T lymphocytes, causing the formation of lymphomas. Blood lymphocytes transport the pathogen to the skin, where it replicates in feather follicles before spreading to the environment(9). MD can appear in several forms, with lymphoproliferative diseases being the most common forms these indicate the development of mononuclear inflammatory nerve lesions and/or visceral lymphomas., mononuclear inflammatory cells, or lymphomatous infiltration in the eye and skin(10).The stages of MD pathogenesis have been defined as early cytolytic phase, latent infection, late cytolytic phase, and fully productive infection of the feather-follicle epithelium(10).

II. MATERIAL AND METHOD

Postmortem examination and sample collection

Fifteen (150) tissue samples were collected from vaccinated layer flocks from (Abo_Gurabe, AL Kute, Al Anbar, AL Nagefe, and Karbala provinces) These flocks suffered from (paralysis syndrome, weight less, paleness, and blindness) the morbidity and mortality reach to 40-70%. The moribund bird was subjected to necropsy and tissue samples were collected including a feather follicle, a sciatic nerve, a spleen, kidney, lung, Gizzard, and proventriculus. these samples were stored in labeled containers and transported immediately with ice to the laboratory and stored at -30c until processing.

Histopathological examination

Histological Sections Study Preparation was down in the Histological unit/ College of Veterinary Medicine. The histological sections have been prepared as: The samples were fixed using 10% Neutral Buffered Formalin for 24 to 48 hours. After washing and dehydration, the remaining formalin was removed and placed in a 90% graded ethanol solution. The clearing was performed three times to remove the ethanol solution. Infiltration was completed twice at 56-58°C. Embedding was done in 56-58°C liquid paraffin-filled containers, and the samples kept at room temperature

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until solidified. The samples were then frozen and sectioned 5 micrometers thick using a rotary microtome. The samples were then placed in a water bath at 50-55°C for staining.

PCR standardization

targeting the region of 132 bp repeated

The most suitable conditions for PCR targeting the 132 bp repeat region are initial denaturation at 94°C for 4 minutes, 35 cycles of denaturation, annealing, elongation at 72°C, and final elongation for 10 minutes. The primer sequences are forward and reverse, containing 22bp 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 a specific primer . 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 reverse primer composed from 22bp 5' TAG ACC GTA TCC CTG CTC CAT C 3'.

III. RESULT

The case history

352 samples were collected from suspected infected birds, those samples were included (liver, feather follicle, spleen, kidney, lung, proventriculus, gizzard, sciatic nerve, and ovary) it collected from (20) poultry farms in distributed provinces (Baghdad, Al Kute, Al Anbar, Al Najaf, Karbala). The sample collected from chickens suffering from unilateral paralysis, weight less, paleness, blindness in one eye, were stored in labeled containers and transported immediately with ice to the laboratory and stored at -20c until further processing. Some of those samples were kept in formalin for histopathological examination.

The clinical signs and gross lesion

The chicken suffered from paralysis in the wing and leg, Feather loss around the neck, with enlargement of feather follicles. In the majority of MD cases, the spleen, proventriculus, lung, sciatic nerve, mesentery, and crop. All organs showed enlargement with diffuse tumor nodules of various sizes (figure 1). Organs from like crops, a sciatic nerve, and proventriculus collected from the dead bird in 10% formalin saline were subjected to histopathological examination, The main histopathological feature of suspected affected birds was characterized by focal and diffuse proliferation of pleomorphic lymphocytes in various tissue with evidence of structural distraction. (Figure 2).

PCR targeting 132 bp repeat region

Standardization of PCR targeting 132 bp repeat region was done using a positive MD DNA sample, the tissue samples were found to be positive for serotype-1-specific MD yielding a 416 bp product in PCR amplification (Figure 3).

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PCR targeting oncogene

The DNA samples, which were positive for 132 bp repeat region, were further used for amplification of oncogenes. Standardization of PCR targeting oncogenes (Meq and vIL-8) was done. the tissue samples were found to be negative for the presence of VIL 8 and positive for the presence Meq gene. the amplification of Meq gene 1020 bp, these 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 (OP524128), case 2 (0P524129), case 3 (OP524130), and case 4 (OP524131). (figure 4)

Nucleotide sequence analysis of oncogenes (Meq gene) and Phylogenetic analysis

The obtained nucleotide sequences of Meq were verified by NCBI-BLAST and sequences showed homology with Meq of different serotype-1-specific MDV strains. The accession numbers for oncogenes from Iraqi regions OP524128, 0P524129, OP524130, and OP524131were MN817546, MW380121, KU229907, LC589272, MT797629 respectively. All reported sequences were aligned with isolated MDV sequences in this study. The tree was constructed using the neighbor-joining method in MEGA 11 (Figure 5).

Figure 1. A. Paralysis of wing and leg. B. enlarged feather follicles due to lymphoid infiltration. c. Enlarged and diffuse tumor nodules in the proventriculus. D. Multiple lymphomas in mesenteric tissue.

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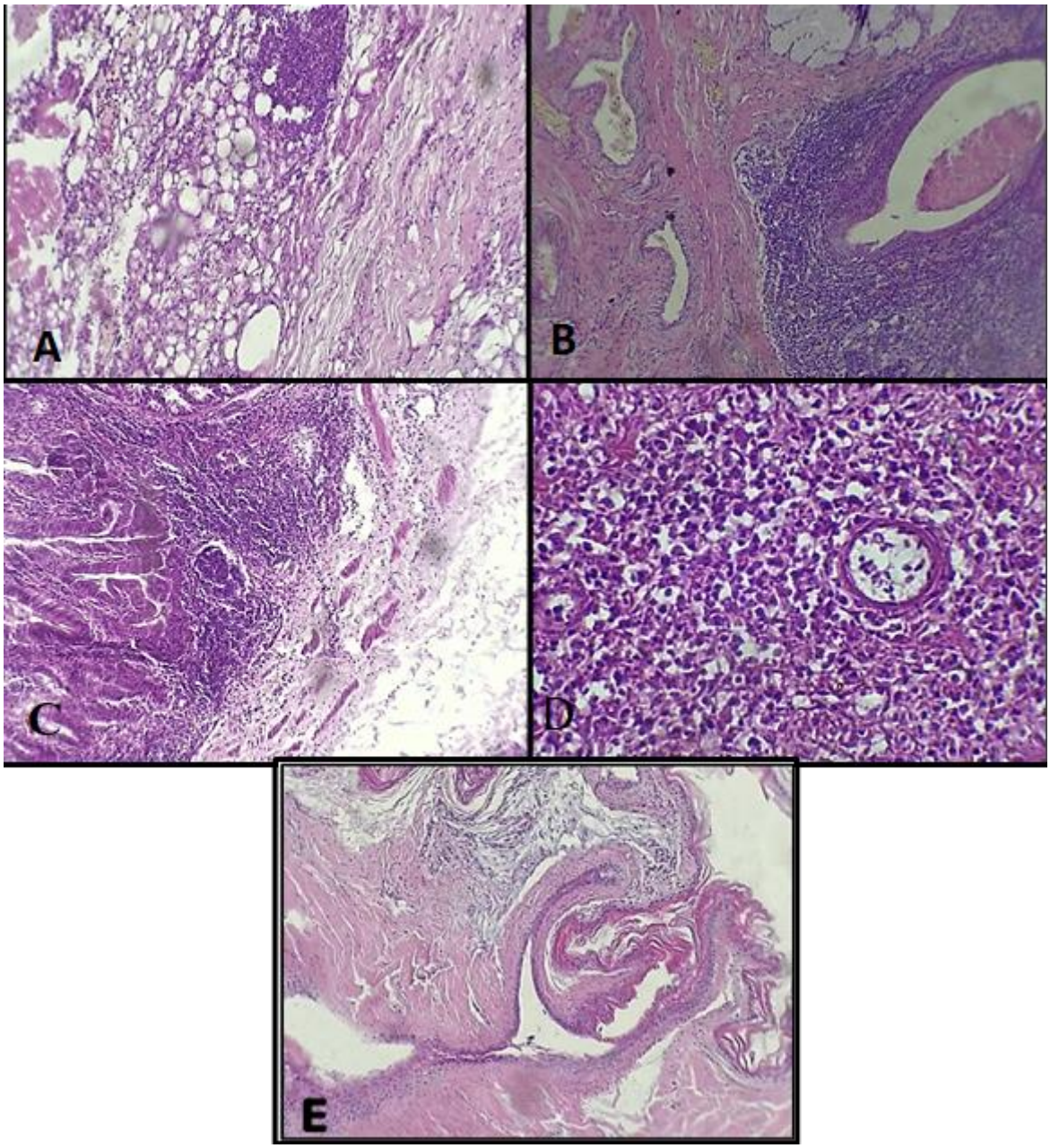

Figure.٢ A. Nerve show focal proliferation of pleomorphic lymphoid cell may be seen in perineural tissue with evidence of intraneuritic inflammatory edema. **B.** Marked thickness of crop mucosa coated due to extensive of infiltration of pleomorphic lymphocyte accompanied with fibrines thickness of mucus membrane and great Dilation of adjacent lymphatic vessels. **C.** proventricular shows Diffuse and marked lymphoid cell proliferation (variable size lymphocyte) in both mucosa and L.P with evidence of atrophy of gut association lymphoid tissue together with submucosal inflammatory edema. **E.** the skin focal epidermal hyperplasia and hyperkeratosis with mild lymphocytic infiltration mainly in upper dermis accompanied with fragmentation of dermal collagen fiber.

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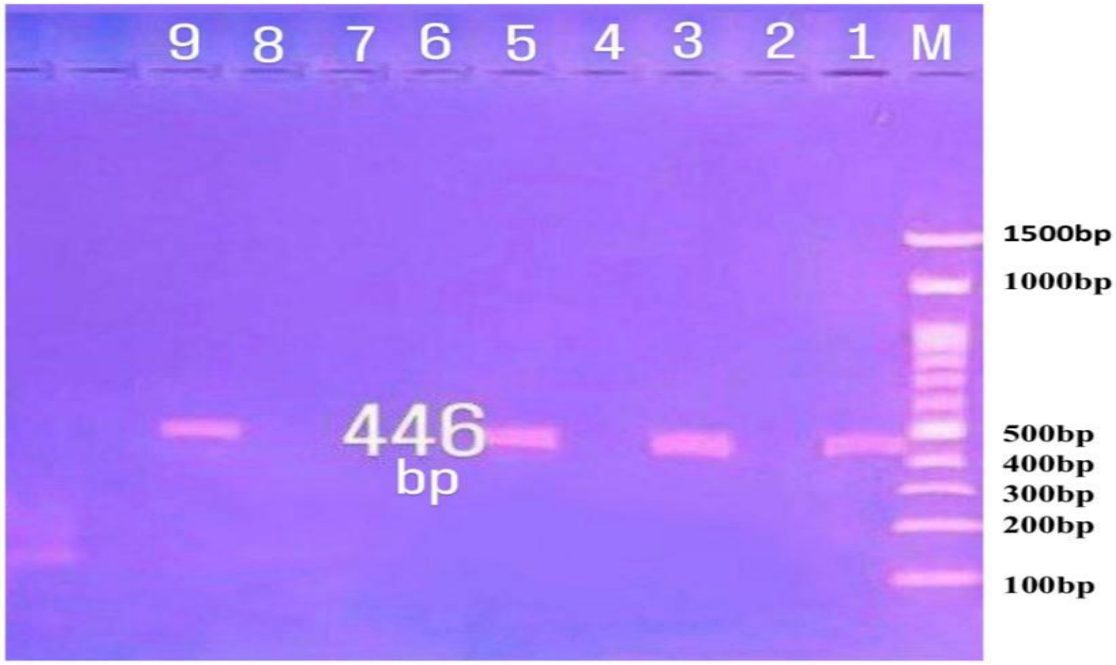

Figure 3. Amplification results stained with ethidium bromide. Agar gel electrophoresis of PCR products. M= 100bp DNA ladder, Lanes 1,3,5,9= positive sample with amplicon size of 446bp of repeat region.

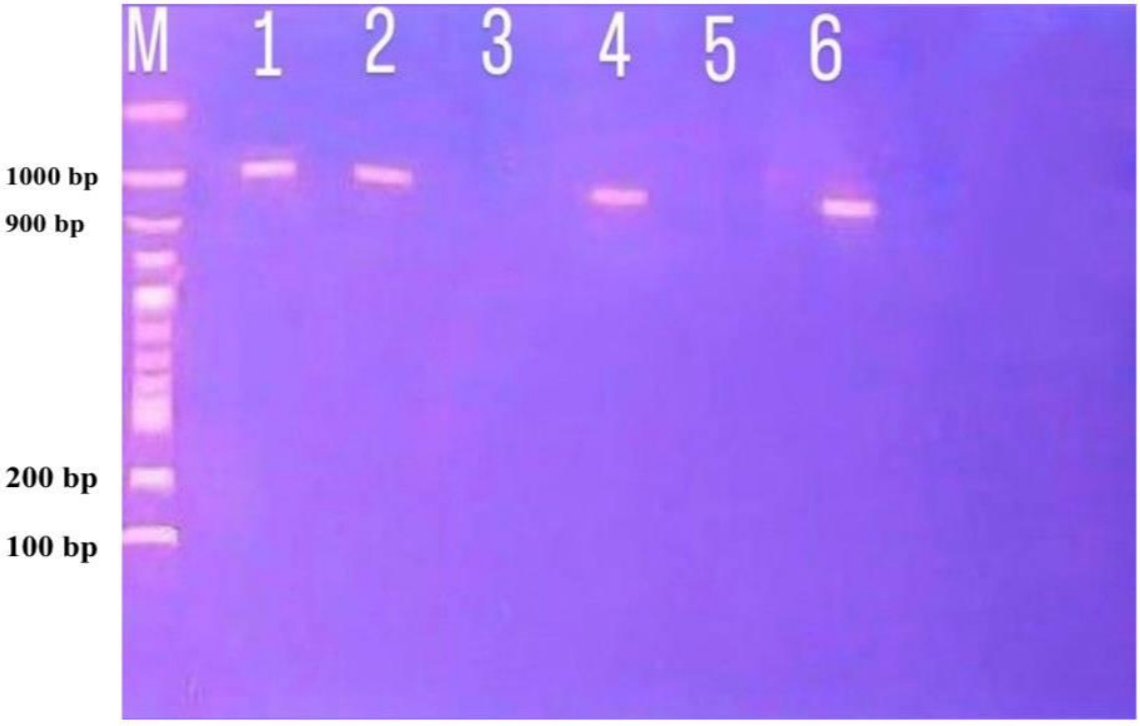

Figure 4. Amplification results stained with ethidium bromide. Agar gel electrophoresis of PCR products. M= 100bp DNA ladder, Lane 1,2,4,6= positive sample with amplicon size of 1020bp of Meq gen.

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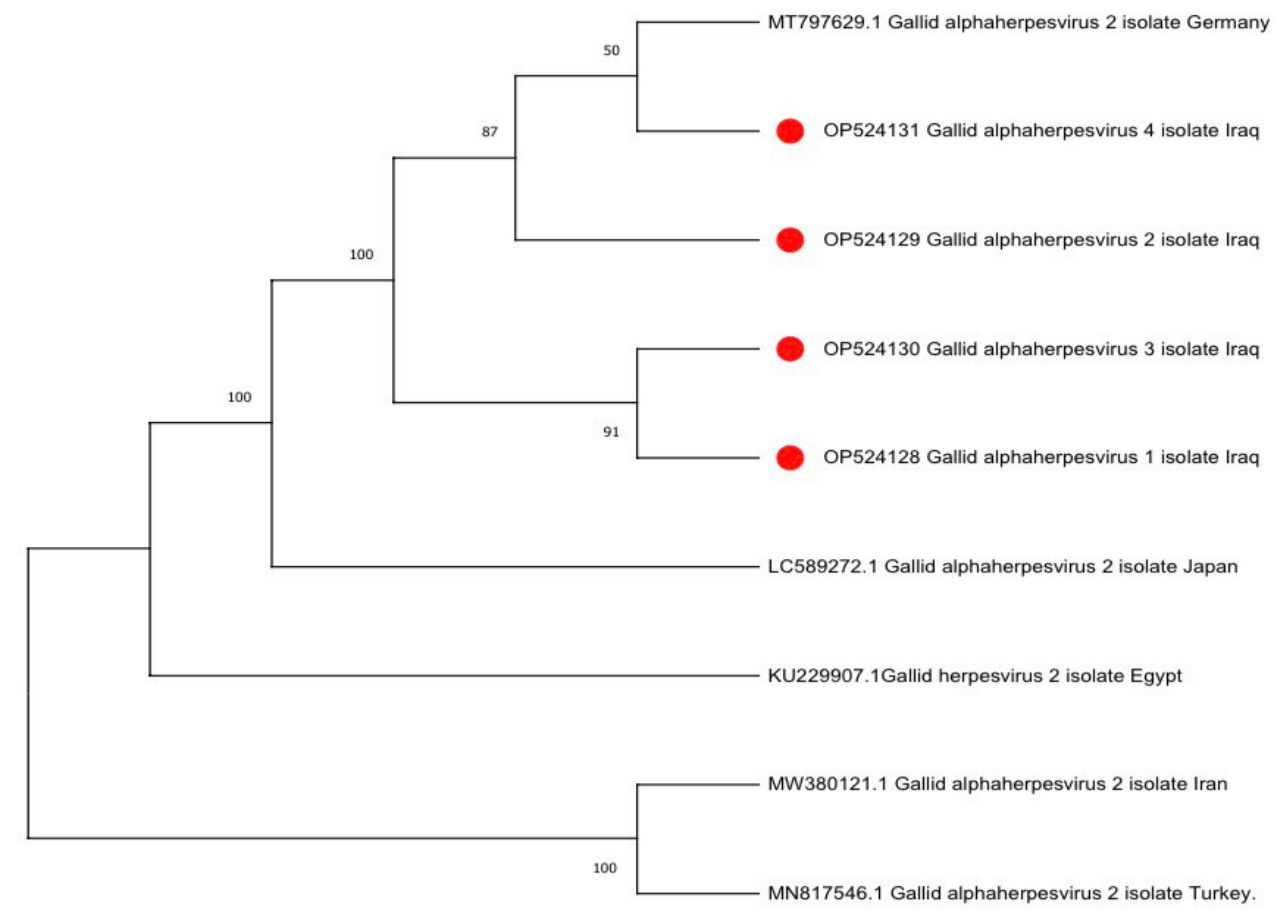

phylogenetic analysis of Meq gene nucleotide sequence of Iraqi isolates with other reference sequences

IV. DISCUSSION

Marek's disease is one of the most significant chicken diseases and is responsible for major economic losses to the worldwide poultry field(11). MDV was diagnosed using typical diagnostic procedures including clinical signs, postmortem change, histological changes, and polymerase chain reaction(12). 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 (13). Clinical surveillance showed leg and wing paralysis, weight loss, depression, all of which are similar with those reported by(14). Peri peripheral nerve dysfunction involves progressive paresis and spastic paralysis of one or more extremities. Involvement of the vagus nerve can cause crop dilation, gasping, and incoordination,A characteristic clinical presentation is a bird with one leg stretched forward and the other back due to unilateral paralysis, Chickens with MD lymphomas may appear normal but have extensive neoplastic involvement when euthanized. Nonspecific signs include weight loss, paleness, anorexia, and diarrhea, the Death often results

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from starvation and dehydration due to the inability to access food or water, or from flock mates' trampling(15). Leukotic tumors involving feather follicles (skin leukosis) (16). The tumor mass's histological findings revealed a homogeneous proliferation of lymphoblast, small to medium lymphocytes grouped in a disorganized fashion. big pleomorphic nuclei with big and visible nucleoli were characteristics of tumor cells(5).

in the spleen Marek's disease is characterized by periarteriolar lymphoid sheath expansion by pleomorphic lymphoid cells in the spleen. There may only be a very small number of foci for this lesion. Pleomorphic lymphoid cells' growth of the periarteriolar lymphoid sheath. Grossly splenic enlargement(17). In the lungs, diffuse lymphoblastic infiltration was seen, particularly in the parabronchial, second-order bronchial, and ectobronchial plate, Blood was present in the capillaries, which had edema and dilated blood vessel walls(2). microscopic lesions of Marek's disease in peripheral nerves are characterized by marked cellular infiltration, numerous proliferating lymphoblastic cells, and edema(16). In skin Keratoacanthoma crater-like depression with raised edges, keratin accumulation, and loss of epithelium(18). In the crop, the mucosa and submucosa showed moderate infiltration of lymphoid cells, with frequent mitotic activity. Cellular infiltrate proliferation was observed in the deepest glands. The crop mucosa showed signs of catarrhal with bacterial aggregates, and the same infiltrate also affected the muscular stomach's mucosal membrane with minor extension. These findings support the lymphoproliferative process(5). PCR was used to aid in the rapid, accurate detection and identification of poultry disease (12). after the detection of MDV in tissue sample the study uses PCR targeting the 132 bp repeat region for specific detection of serotype-1 MDV strains, distinguishing pathogenic strains from vaccine strains in field samples(11). 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). 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(20). 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(21). The severity of the disease in Iraq and the high mortality rate in Iraq is evidence of the presence of a very virulent virus and as a result of genetic mutations(22).

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