Molecular characterization of field isolates of Avian Infectious Laryngeotracheitis Virus from different farms in Iraq

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Summary

This study was conducted to detect virulent isolates of avian infectious laryngeotracheitis virus in Iraq by Real Time-Polymerase Chain Reaction with the amplification of glycoprotein G gene which is responsible for virulence of the virus. Seventy samples (larynx and trachea) were collected from different farms in Iraq to investigate presence of avian infectious laryngeotracheitis virus (detection of virulent isolates from other vaccine strains). Five samples out of seventy samples were virulent isolates (positive result) by using Real Time-Polymerase Chain Reaction utilizing flurescein amidite labeled probe specific for detection of isolates that have G gene (by amplification of G gene) for the first time in Iraq. These virulent isolates were negative by using Real Time-Polymerase Chain Reaction utilizing Quasar-labeled probe specific for the detection of attenuated isolates that lack G gene and targeted a region within glycoprotein J downstream from the sequence of glycoprotein G.

Keywords: Avian infectious laryngeotracheitis virus, Polymerase Chain Reaction Glycoprotein G.

Introduction

Avian Infectious laryngotracheitis (ILT) virus is an alpha herpes virus that causes acute respiratory disease in chickens (1). The main clinical signs of ILT are manifested by nasal secretion, conjunctivitis, gasping, coughing, and expectoration of bloody mucus (2). Live attenuated ILT virus vaccines were used widely in some countries, but ILT infection did not stop, therefore the used vaccine was unable to protect chickens against infection with ILT virus (3-6). ILT virus encodes glycoprotein G (gG), a glycoprotein that is conserved in most members of the subfamily Alphaherpes virinae (7 and 8). The role of gG in vitro has been investigated in a number of different alphaherpes viruses. Many researches demonstrated that gG is the virulence factor in ILT virus and that deletion of gG from the genome of alphaherpes virus lead to attenuation of the virus in its natural host (9).

In Iraq, ILT was isolated and detected by molecular technique (10), in order to detect the virulence of field isolates and detection of virulent isolates from other vaccine strains, this study was conducted.

Materials and Methods

Seventy samples (Infected larynx and trachea) were collected from different farms in Iraq. Ten samples were collected from Jezera farm At Al- Siwara city (brown table egg layer 28 weeks), 20 samples were collected from Abbas Kareem Co. at Diala city (broiler breeder 31 weeks), 20 samples were collected from Al-safaa Co.at Diwania city (brown table egg layer 28 weeks), 51 samples were collected from Wasit Co. at Wasit City (broiler breeders 32 weeks), and 5 samples were collected from Karbala farms at Karbala city (brown table egg layer 30 weeks). DNA extraction kit (Intron-Korea) was used for extraction of DNA from infected samples with ILT virus.

Primers: Specific for detection of virulent isolates (G positive isolates). Forward primer; CAGCTCGAAGTCTGAAGAGACA. Reverse primer AGCGAGCATACTAGGGAAACGGT FAM-labeled probe FAM-TGAGCGGCTTCAG TAACATAGGATCGA-BHQ-1. Primers specific for detection of attenuated isolates (G negative isolates). Forward primer CAGCTCGAAGTC TGAAGAGACA. Reverse primer GCTGCACG CCAACTCCTATG. Quasar-labeled probe Quasar 670-TGTGCGGGTGAAACCGTAAATT ACG- BHQ-2.

FAM=flurescein amidite
Quasar=Cyanine (CY5) replacement
BHQ=Black Hole Quencher

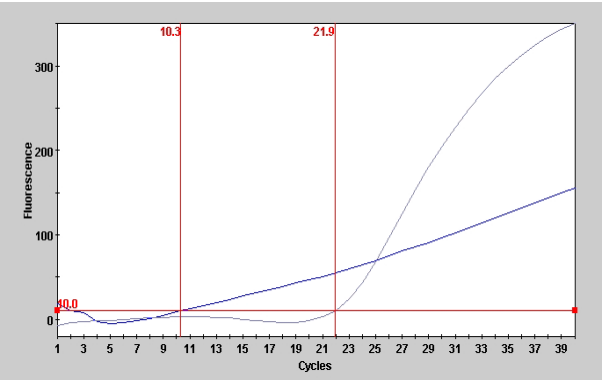
All primers were manufactured by (Alpha DNA, Montreal, Quebec) and were imported

by URUK Center. Larynx and trachea were ground for extraction of DNA. DNA was extracted from prepared samples by DNA extraction kit.

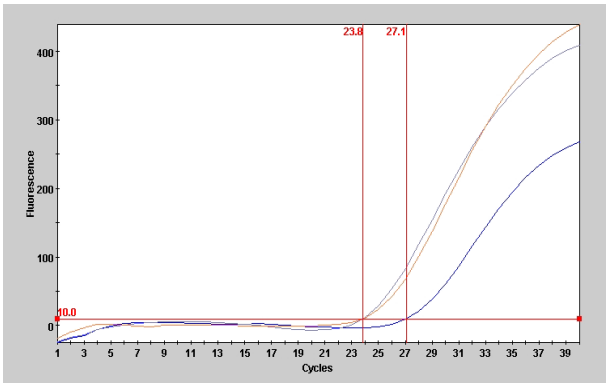
Amplification of extracted DNA: RT- PCR was used to differentiate virulent ILT isolates from attenuated ILT isolates by using two sets of primers, the first primer pair was used to detect region of glycoprotein G which mainly present in virulent isolates with a specific FAM –labeled probe while the second primer pair was used to detect attenuated ILT isolates (isolates that lack glycoprotein G) targeted region of J sequence downstream of glycoprotein G with a specific Quasar –labeled probe.

Results and Discussion

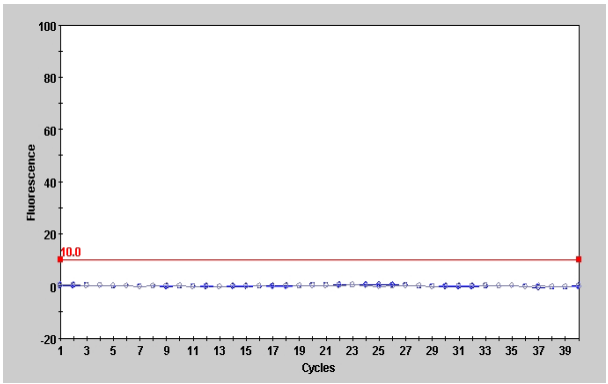
Results of Real Time-polymerase chain reaction for detection of virulent isolates: The result of amplification of extracted DNA revealed that five samples out of seventy samples were virulent isolates and showed positive result by RT-PCR with the amplification of G gene as seen in (Fig. 1 and 2). Results of Real Time-polymerase chain reaction for detection of attenuated isolates: The five samples showed negative result by using real - time polymerase chain reaction utilizing Quasar-labeled probe specific for the detection of attenuated isolates that lack G gene and targeted a region within glycoprotein J downstream from the sequence of glycoprotein G as seen in (Fig. 3 and 4).



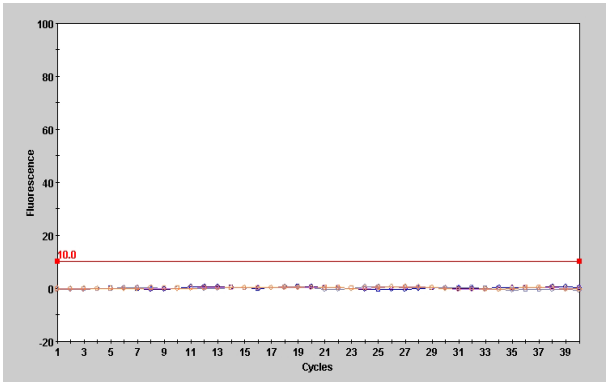
Figure, 1: RT- PCR amplification plot of G gene of ILT virus of two laryngeal and tracheal samples indicate positive result with FAM labeled probe: ILTV RT-PCR wild type for larynx and trachea of brown table egg layers (28 weeks old) from Jezera farm At Al- Siwara. ILTV RT-PCR wild type for larynx and trachea of brown table egg layers (28 weeks old) from Jezera farm At Al- Siwara.



Figure, 2: RT- PCR amplification plot of G gene of ILT virus of three laryngeal and tracheal samples indicate positive result with FAM labeled probe:
Abbas Kareem Co.of broiler breeders 31 weeks, Diala.
Al-safaa Co.brown table egg layers 28 weeks, Diwania.
Wasit Co.broiler breeders 32 weeks at Wasit City.



Figure, 3: RT- PCR amplification plot of glycoprotein J for detection of attenuated isolates that lack G gene of ILT virus of two laryngeal and tracheal samples indicate negative result with Quasar labeled probe: ILTV RT-PCR wild type for larynx and trachea of brown table egg layers (28 weeks old) from Jezera farm At Al- Siwara. ILTV RT-PCR wild type for larynx and trachea of brown table egg layers (28 weeks old) from Jezera farm At Al- Siwara.



Figure, 4: RT- PCR amplification plot of glycoprotein J for detection of attenuated isolates that lack G gene of ILT virus of three laryngeal and tracheal samples indicate negative result with Quasar labeled probe.
Abbas Kareem Co.of broiler breeders 31 weeks, Diala.
Al-safaa Co.brown table egg layers 28 weeks, Diwania.
Wasit Co.broiler breeders 32 weeks at Wasit City.

Live attenuated ILT virus vaccine was used in the world but participated in the emergence of virulent strains (5 and 6). Differentiation between virulent ILT isolates and vaccine strains was responsible for controlling of ILT viral infection (11). Live attenuated ILT virus vaccine was used in the world but participated in the emergence of virulent strains (5 and 6). Differentiation between virulent ILT isolates and vaccine strains was responsible for controlling of ILT viral infection (11). The combination of polymerase chain reaction technique with a technique of restriction fragment length polymorphism (RFLP) analysis of one and many viral genes and regions of genome have enabled the characterization of different strains within a region or a country (4). Many reports revealed that some field strains were closely related to and likely to be derived from vaccine strains; others were true 'wild types' (12). Many genes were tested by different researchers and included glycoprotein G (gG), TK (thymidine kinase), glycoprotein E (gE) and unique long (UL47) (13). There were 36 restriction enzymes used by (14), while others have used as few as four.

Researchs of gG deletion mutants of other alphaherpesviruses in vivo, such as Herpes simplex virus 1 (HSV-1), have shown only a slight attenuation of virulence (15-17). In these researchs, the role of gG in attenuation was again obscured by the unknown effect upon the expression of adjacent genes.

The relevance of gG in vivo in viral pathogenicity has not been extensively investigated. The role of gG in infectious laryngeotracheitis virus was investigated by characterizing gG-deficient mutants of ILT virus in vivo and in vitro. The in vivo studies revealed that gG-deficient ILT virus was significantly attenuated compared with wild type ILT virus. Birds inoculated with the initial gG-deficient mutant, (DgG) ILT virus, had less severe clinical signs and better weight gain than birds inoculated with wild ILT virus. The extensive investigation into DgG ILT virus pathogenicity also investigated attenuation with respect to clinical signs, mortality rate and weight gain (4).

In this study, five samples out of seventy were detected as virulent isolates (positive result) by RT-PCR technique with amplification of glycoprotein G which responsible for virulence of isolates in agreement with (4) which explains that the glycoprotein G responsible for virulence of most ILT strains and strains that lack glycoprotein G was attenuated strains. RT-PCR technique with amplification of glycoprotein G was useful for detection of virulent isolated in agreement with (18) by using FAM labeled probe for detection of virulent isolates. The advantages of using RT-PCR technique in compare with other conventional virus isolation or conventional PCR followed by electrophoresis was rapid method and can be performed in less than two hours in agreement with (19 and 20) who has been described this technique for rapid detection of ILT virus. The five positive samples showed negative result in agreement with (17) by using Quasar labeled probe for detection of attenuated isolates, this negative result indicate that the five positive samples were virulent ILT viruses.

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التوصيف الجزيئي للعزلات الحقلية لفايروس التهاب الحنجرة والرغامي المعدي الطيري من حقول مختلفة في العراق

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الخلاصة

أجريت هذه الدراسة لتحديد العزلات الضارية لفايروس التهاب الحنجرة والرغامي المعدي الطيري في العراق باستعمال تقنية تفاعل سلسلة انزيم البلمرة بتضخيم الجين الخاص بكلايكوبرروتين ج المسؤول عن ضراوة الفايروس. جُمِعَت سبعون عينة (حنجرة ورغامي) من حقول مختلفة في العراق للتحري عن وجود فايروس التهاب الحنجرة والرغامي المعدي الطيري وتحديد العزلات الضارية من المضعفة. خمس عينات من أصل سبعين عينة وحُدِّدت كعزلات ضارية (اعطت نتيجة موجبة) باستخدام تقنية تفاعل سلسلة انزيم البلمرة باستعمال مجس معلم بصبغة الفلورسين أميديت خاص بتحديد العزلات التي تحوي الجين ج (بتضخيم الجين ج) ولأول مرة في العراق. أظهرت العزلات الضارية نتيجة سالبة عند استعمال تقنية تفاعل سلسلة انزيم البلمرة باستعمال مجس معلم بصبغة الكوازار الخاصة بتحديد او تشخيص العزلات المضعفة الفاقدة للجين الخاص بكلايكوبرروتين ج مستهدفة منطقة ضمن الكلايكوبروتين جي في اتجاه التسلسل الخاص نفسه بكلايكوبرروتين ج.

الكلمات المفتاحية: التهاب الحنجرة والرغامي المعدي الفايروسي، فايروس، تقنية تفاعل سلسلة انزيم البلمرة، كلايكوبروتين ج.