

INVESTIGATION OF INFECTIOUS LARYNGEOTRACHEITIS VIRUS IN IRAQI CHICKEN FARMS

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ABSTRACT

Twenty samples of Laryngeal and Tracheal tissues from laying chickens of two flocks with suspicious infectious laryngeotracheitis were tested for the detection of the causative virus by using virus isolation in chicken embryo fibroblast (CEF) cell culture and chicken embryos by chorioallantoic membrane inoculation and conventional polymerase chain reaction (PCR). The virus was isolated from three samples (larynx and trachea) out of ten samples at Al-Sawara city on (CEF) cell culture and produce pock lesions on chorioallantoic membrane of infected chicken embryos in which the virus was isolated.

Positive PCR results were detected in the three clinical samples, isolated virus in CEF cell culture and isolated virus in chicken embryos (inoculated on chorioallantoic membrane).

INTRODUCTION

Avian infectious laryngotracheitis (ILT) is a viral respiratory disease which is included within List B of the Office International des Epizooties (OIE). Avian infectious laryngotracheitis caused by: Gallid Herpesvirus I also known as: GHV-1 — Infectious Laryngotracheitis Virus (1,2). Infectious laryngotracheitis virus is a member of the family Herpesviridae, subfamily Alpha herpesvirinae, genus Varicellovirus. The species is named *Gallid Herpesvirus-1 (GHV-1)* (3). *Gallid Herpes virus* causes respiratory disease in chickens and pheasants (2). Severity of disease varies from mild to peracute, with mortality in peracute outbreaks exceeding 50 % (3). GHV-1 can remain latent in carriers after infection as with other herpesviruses and then will be shed intermittently, relapsing with stress (4). While the distribution of ILT is world-wide (5), chicken flocks which are endemically infected with ILT virus (ILTV) often occur only in some regions of countries or even in particular (multiple-age) production sites, regardless of whether these are industrial or backyard flocks. However, outbreaks of disease continue to occur periodically whenever ILTV strains can move from persistently infected flocks into non-vaccinated chickens. Unpredictability regarding ILT disease cases is increased by the irregular or emergency-only vaccination for ILT in some countries (2). The currently available ILT vaccines are manufactured with modified live strains, which can also establish latent infections and cause disease when allowed to spread from bird to bird

(4). In Iraq many viral causes of respiratory infection in chickens were isolated and studied like infectious bronchitis virus(6), but infectious laryngotracheitis virus still, has not been studied also we have no information about presence of this virus in Iraqi chickens farms, therefore in this study, we investigated the presence of Avian infectious laryngotracheitis in Iraq and the virus was demonstrated and diagnosed by using several traditional methods and new molecular techniques.

MATERIALS AND METHODS

1-Collection of samples: Ten samples of Larynx and Trachea were collected from different poultry farms at Al-Sawara city and ten samples (Larynx and Trachea) were collected from AL-Haditha Company.

2-Preparation of samples: Larynx and Trachea were grounded with mortar and pestle and antibiotics (10000 I.U. Penicillin and 10 mg of Streptomycin/ml) were added according to (7).

3-Chicken embryo fibroblast cell culture (CEF) was prepared according to (8) at Virology lab. at College of Veterinary Medicine/University of Baghdad. Prepared samples were inoculated into CEF cell culture. Control cell cultures were treated with 0.5ml/Flask of phosphate buffer saline. Cell culture was examined daily for presence of CPE. Crystal violet stain, 1% solution prepared in 10% formalin used for staining and fixation of cell culture.

4- Chicken embryos (ten days old) were used for virus isolation for detection of pock lesions. Prepared samples were inoculated into chicken embryos by chorioallantoic membrane (CAM) inoculation and incubated at 37.8 °C and candled daily for detection of pock lesions until the five days of incubation according to (7).

5-Extraction of DNA: Intron biotechnology DNA kit extraction was used for extraction of DNA from prepared trachea and larynx samples, infected CEF cell culture and infected chorioallantoic membrane (CAM).

6-Conventional Polymerase chain reaction: Conventional Polymerase chain reaction was conducted at URUK Laboratory. PCR kit produced by Intron biotechnology (Korean company) was used for amplification of extracted DNA. PCR was conducted for extracted DNA of prepared trachea and larynx of two chicken flocks and isolated virus in CEF cell culture and infected CAM.

7- Gel electrophoresis for separation of amplified cDNA: 2% of agarose (USA Biologica) was used and the method of gel electrophoresis was conducted according to (9) method, then 1.5 µl of ethidium bromide (10mg/ml) solution was added and poured in gel chamber until the agarose solidify, finally the amplified DNA was loaded into the agarose wells. 10µl of loaded amplified DNA was added to each lane of gel Agarose and

10µl of marker (1000 bp) was added to first lane of electrophoresis. The amplified DNA separated by electrophoresis at 100 volts for 60 minutes.

RESULTS

1-Clinical Observations

Samples (Larynx and Trachea) were collected from chickens at Al-Sawara farms suffering from respiratory infection. The observed clinical signs in those sick chickens were depression, difficulty in respiration ,congestion of eye and gasping as seen in figure(1).



Figure 1: chicken suffered from depression and gasping

2-Post mortem examination:

Post mortem examination has been showed congestion of trachea with purulent exudate as seen in figure 2 and 3:



Figure 2



Figure 3

Figure 2 and 3: congested trachea with purulent exudates

3-Results of virus isolation into tissue culture:

Chicken embryo fibroblast cell culture inoculated with prepared samples from infected chickens at Al-Sawara farms showed cellular changes after one passage after 1 days Post inoculation. Cytopathic effects completed after 5 days of inoculation. However the fourth and fifth passage showed complete cytopathic effect (CPE) after 3 days Post inoculation, characterized by swollen cells which lead eventually to cell rounding and degeneration of floating cells in the cell culture medium and foci of degenerated cells (Fig.4) but no cytopathic effects were seen in control CEF cell culture (Fig.5). Further passages of the harvested virus lead to CPE became more obvious as noticed in (Figure 6) but no cytopathic effects were noticed in control CEF cell culture as seen in (Fig.7):

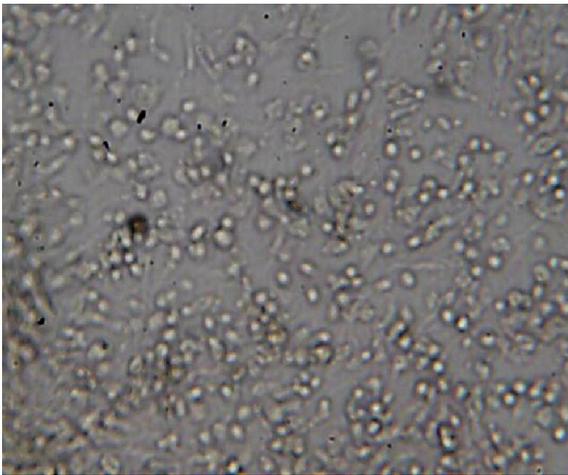


Figure 4: Chicken Embryo Fibroblast Cell Cultures after 5 Days of Virus Inoculation with Isolated Virus . Explain Focal Rounding of Cells (2 passage).100X.

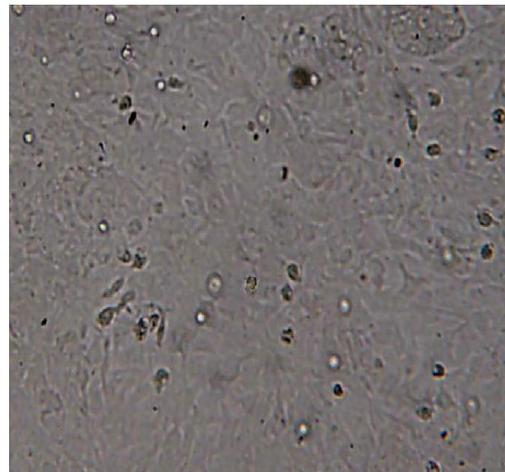


Figure 5: normal chicken embryo fibroblast Cell culture

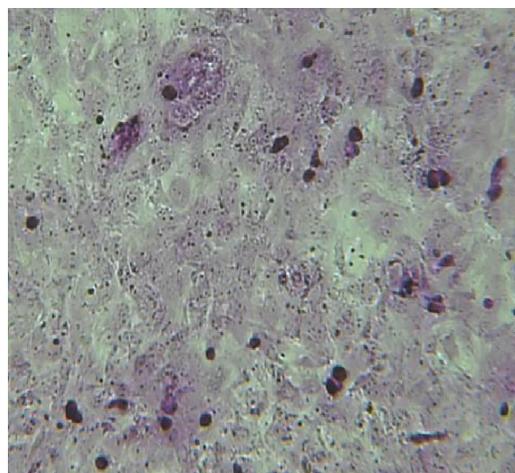
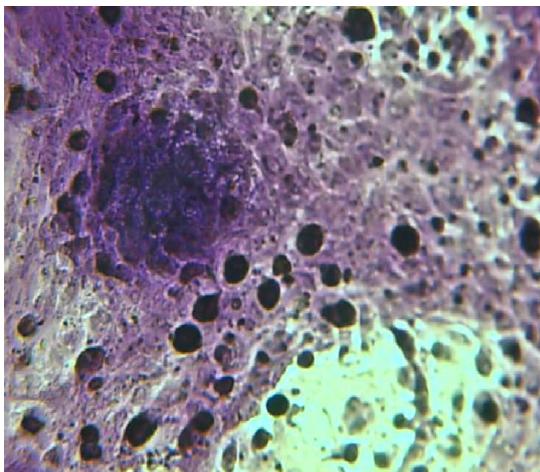


Figure 6: chicken embryo fibroblast Cell Cultures after 5 Days of Virus Inoculation with Isolated Virus . Explain Focal rounding of Cells with syncytia (5 passage).100X.

Figure 7: normal chicken embryo fibroblast Cell culture

4-Results of virus isolation into chicken embryos

Results of virus isolation into chicken embryos by chorioallantoic membrane inoculation revealed general odema and development of lesions (pock lesions) on chorioallantoic membrane as noticed in figure 8 but lesions were not observed on control chorioallantoic membrane as noticed in figure 9:

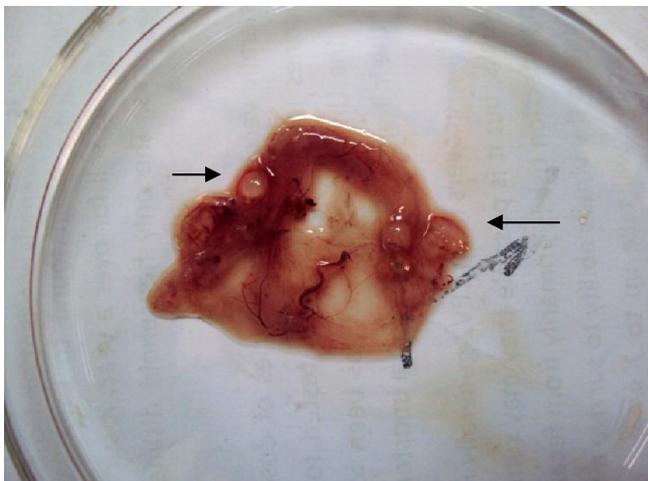


Figure 8: congestion and thickening of chorioallantoic of chorioallantoic with pock lesions of infected chicken embryo

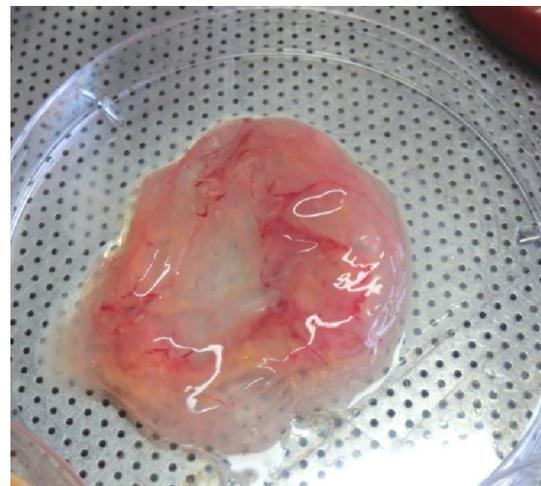


Figure 9: normal membrane of control chicken embryo

5- Detection of ILT by conventional-PCR:

The result of PCR amplification performed on the extracted DNA was confirmed by electrophoresis.

The result of the successful binding of the specific primer representing part of M gene(Matrix gene) with extracted DNA appeared as single band under UV light illuminator using ethidium bromide as specific DNA stain ,only the band with expected size 477 bp was observed in Figures 10,11.

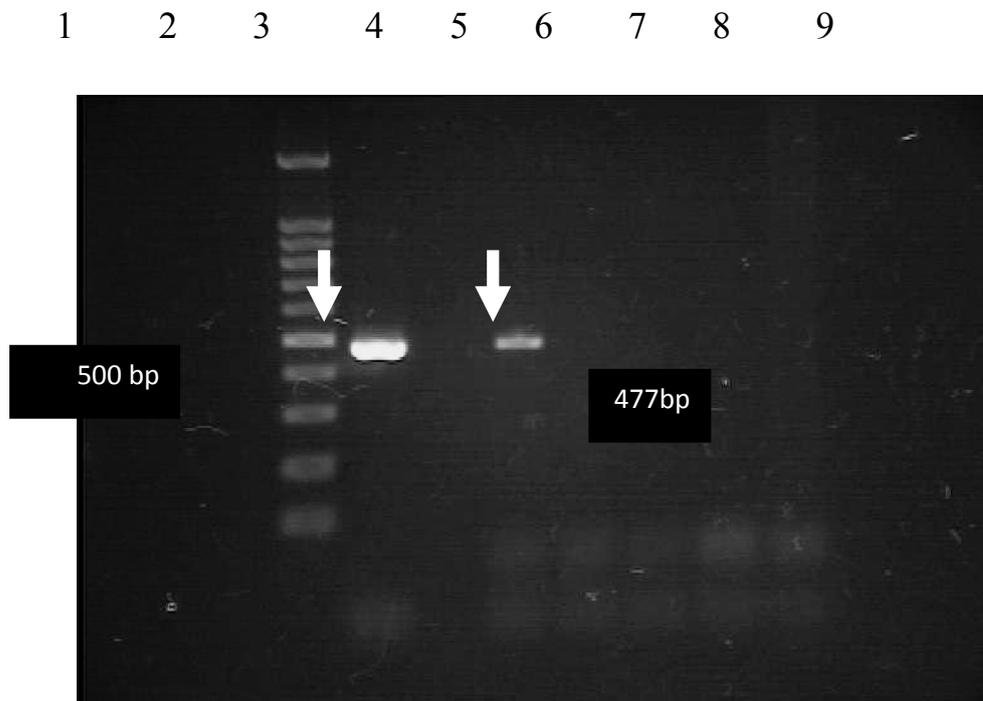


Figure 10: Trachea samples (from AL-Sawara farms) PCR Amplification Product of M gene 477 bp. Lane 1: marker (100-1000) bp; Lane 2: positive control ,Lane 3: negative control, Lane 4: infected trachea,Lane:5-9 negative results.

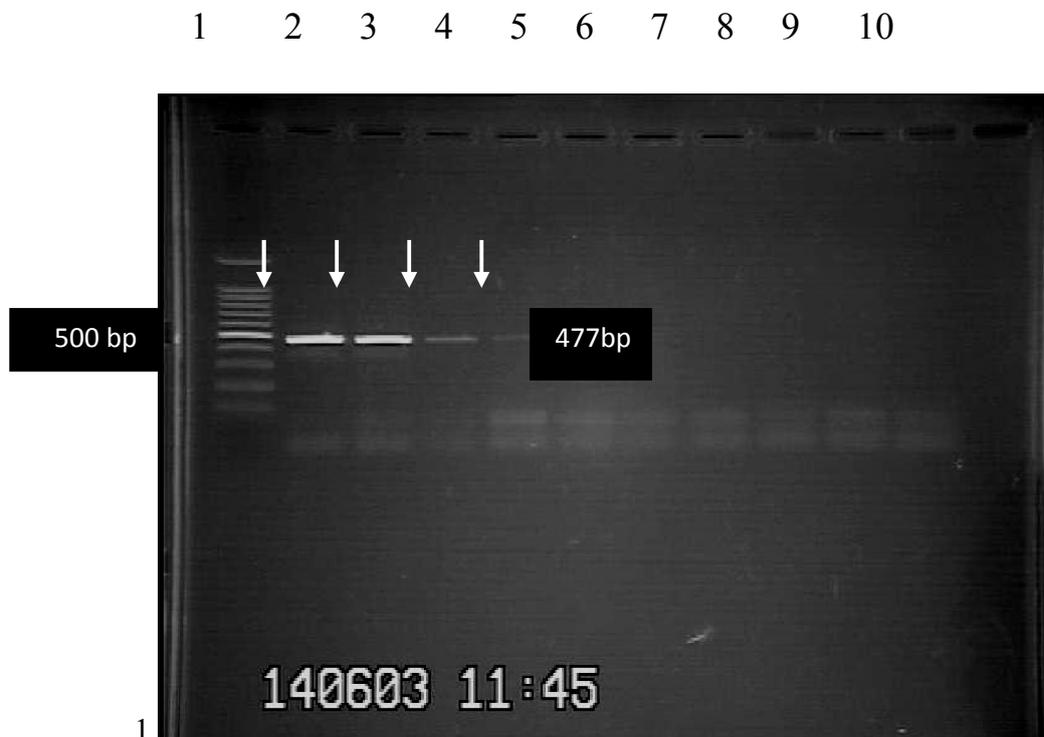


Figure 11: PCR Amplification Product of M gene 477 bp .Lane 1: marker (100-1000) bp; Lane 2:infected trachea ,Lane 3: fifth passage of isolated virus in CEF cell culture, Lane 4: first passage of isolated virus in CEF cell culture, Lane 5: infected chicken embryos(infected chorioallantoic membrane). Lane 6, 7, 8, 9 and 10: samples from AL-Haditha Company showed negative result.

DISCUSSION

Infectious laryngeotracheitis virus is viral respiratory tract infections of chickens which may result in sever economic losses due to mortality and/or reduction of egg production (10). This study is the first study in Iraq was performed to isolate infectious laryngeotracheitis virus from chickens. The clinical and post-mortem findings of infected chickens were severing in agreement with (11, 12).

The virus was isolated from infected larynx and trachea agreed with other studies (13).Chicken embryo fibroblast was used for virus isolation. CEF cell culture was sensitive and rapid for ILT virus isolation disagreed with (14) which have been shown that chicken embryo liver cells is more rapid and sensitive in compare with other cell culture. CEF cell culture showed characteristics syncytial cytopathic effects agreed with other studies (14). Chicken embryos by CAM inoculation was sensitive for virus isolation and showed characteristics pock lesions in agreement with (13). PCR technique was very sensitive to detect virus before isolation (prepared samples of Larynx and Trachea) and after isolation in CEF cell culture agreed with (14).

CONCLUSION

Infectious laryngeotracheitis virus was isolated from infected chickens for the first time in Iraq. Chicken embryo fibroblast cell culture was very suitable for virus cultivation and propagation, also chicken embryos was suitable by chorioallantoic membrane inoculation. PCR technique was very sensitive and rapid for detection of ILT virus in compare with other techniques.

التحري عن فايروس التهاب الحنجره والرغامى المعدي في حقول الدجاج العراقيه

شوني ميخائيل اوديشو عائده برع علاوي سلامه برهوم

فرع الاحياء المجهرية، كلية الطب البيطري، جامعة بغداد، بغداد، العراق

تم فحص عشرون عينه اخذت من الحنجره والقصبه الهوائيه من الدجاج البياض لحقلين من حقول الدجاج مشكوك باصابتها بفايروس التهاب الحنجره والقصبه الهوائيه المعدي للكشف عن الفايروس المسبب باستخدام طريقة العزل في الخلايا الليفيه لاجنة بيض الدجاج النامي وحقنها في اجنة البيض النامي على الغشاء اللقائقي المشيمي كما استخدمت تقنية تفاعل سلسله البلمره لتشخيص الفايروس. تم عزل الفايروس من ثلاث عينات شملت الحنجره

والقصبه الهوائيه من اصل عشرة عينات اخذت من حقل للدواجن في مدينة الصويره في الخلايا الليفيه لاجنة بيض الدجاج كما اعطى الفايروس المعزول بقعا على الغشاء اللقائقي المشيمي عند حقنه في اجنة البيض النامي.

اظهرت نتائج تقنية تفاعل سلسلة البلمره نتيجته موجبته لتشخيص الفايروس في ثلاث عينات(الحنجره والقصبه الهوائيه) والفايروس المعزول في الخلايا الليفيه لاجنة بيض الدجاج واجنة بيض الدجاج عند حقنها على الغشاء اللقائقي المشيمي.

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