

Rapid detection of infectious bronchitis virus in broilers in Al-Diwaniya governorate by using Real-Time reverse transcriptase Polymerase Chain Reaction

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Abstract

Infectious Bronchitis (IB) is currently one of the most important viral diseases in poultry flocks all over the world caused by infectious bronchitis virus (IBV), and it causes huge economic losses in poultry industry. Infectious bronchitis virus has many serotypes that do not confer cross protection against each other. This study was conducted to detect Infectious bronchitis virus in broilers chicken farms in Diwaniya governorate. Tracheal swab from 30 infected chicken flocks located in different areas of Diwaniya governorate were collected to make Rapid immunochromatography test for IBV by using Anigen Rapid IBV Antigen Test Kit and tissue samples were collected from flocks which showed positive for rapid test to make rRT-PCR. Twenty eight (93.33%) flocks were positive for IBV by rapid immunochromatography test. The molecular detection of IBV showed that all flocks (100%) were positive for IBV. In conclusion the IBV was the most important cause of respiratory diseases in this study. The real-Time RT-PCR was found very efficient and rapid in detection of IBV in infected chickens.

Key words: Infectious bronchitis virus, broilers, Real-Time RT-PCR.

التحري السريع عن فايروس التهاب القصبات المعدي في فروج اللحم في مدينة الديوانية باستخدام اختبار تفاعل سلسلة البلمرة المنعكس ذي الوقت الحقيقي

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الخلاصة

يعد التهاب الشعب الهوائية المعدي (IB) حاليا واحدا من الأمراض الفيروسية الأكثر أهمية في قطعان الدواجن في جميع أنحاء العالم ، يتسبب عن فايروس التهاب القصبات المعدي (IBV)، ويسبب خسائر اقتصادية هائلة في صناعة الدواجن ، أن فايروس التهاب الشعب الهوائية المعدي يملك العديد من الأنماط المصلية التي لا تمنح الحماية الكاملة ضد بعضها البعض، وقد أجريت هذه الدراسة للكشف عن فايروس التهاب الشعب الهوائية المعدي في حقول فروج اللحم في مدينة الديوانية ، وقد تم جمع (30) مسحة من القصبة الهوائية لقطعان الدجاج المصابة لحقول تقع في مناطق مختلفة من محافظة الديوانية وقد تم فحصها عن طريق الترحيل المناعي السريع للتحري عن التهاب القصبات المعدي وذلك باستخدام عدة اختبار مستضد فايروس التهاب القصبات المعدي وجمعت عينات الأنسجة من القطعان المصابة التي أظهرت نتيجة إيجابية للاختبار السريع وذلك لعمل اختبار تفاعل سلسلة البلمرة المنعكس ذي الوقت الحقيقي ، وقد تم استخلاص الحامض النووي الرايبوسومي من عينات الأنسجة ، وأظهرت 28 (93.33%) من القطعان نتيجة موجبة لمرض القصبات المعدي بواسطة اختبار الترحيل المناعي السريع ، في حين أظهر التحري الجزيئي للمرض أن جميع القطعان (100%) كانت موجبة لمرض التهاب القصبات المعدي ، يستنتج من الدراسة أن مرض التهاب القصبات المعدي من أهم مسببات أمراض الجهاز التنفسي في الطيور ، وان اختبار تفاعل سلسلة البلمرة المنعكس ذي الوقت الحقيقي يعد فحصا كفوءا و سريعا في التحري عن المرض في الدواجن المصابة .

الكلمات المفتاحية : التهاب القصبات المعدي ، فروج اللحم ، تفاعل سلسلة البلمرة المنعكس ذو الوقت الحقيقي

Introduction

Infectious Bronchitis disease (IB) is an acute, highly contagious upper respiratory disease of chickens caused by infectious bronchitis virus (IBV) belongs to group III of the genus *coronavirus* of the *coronaviridae* family. IB affects chickens of all ages, causing respiratory, reproductive and renal diseases (1). The IBV is an envelope, non-segmented, single stranded and has positive-sense RNA; the RNA is the largest of all groups of RNA virus, approximately (27.7 kb) in length (2). The virus causes significant economic losses throughout the world and is able to spread very rapidly (3). Vaccination is only partially successful due to continual emergence of antigenic variants and requires the application of multiple vaccines at many sites due to the simultaneous presence of multiple antigenic types with less cross protection between them (4). Virus isolation is a sensitive technique but can be laborious, time consuming (5). Reverse transcriptase quantitative real-time PCR has high sensitivity and ability to quantify IBV in sample and highly specific and fast assay that provide rapid detection and less labor intensives as compare to other conventional methods used for laboratory diagnoses such as virus isolation (6).

Materials and methods

Samples were collected from 30 infected broiler flocks which suffered from severe respiratory signs with high mortality, signs were gasping, ocular and nasal discharge, grossly there were severe congestion of trachea, cast plug in the bifurcation of the trachea, affected kidneys were swollen and pale and urate deposit observed in the kidney tissues and in the ureter. Tracheal swabs were collected from 5 chickens per flocks by using sterile cotton swab for rapid test to IBV. Tissue samples of trachea, lung, kidney and cecal tonsils were collected in sterile plastic test tubes and stored in deep freeze at (-42C°) in Najaf veterinary hospital until used for PCR .

Immunochromatography assay

Anigen Rapid IBV Antigen Test Kit (BIONOTE, Incorporation, Korea) Who contains: rapid IBV antigen test devices,

sample collection tubes containing 1ml of assay diluents, sample collection swabs and disposable droppers. Tracheal swab was used by inserting the swab inside the trachea several times then insert the swab into the sample collection tube containing assay diluents. Then mixing until the sample has been dissolved in the assay diluents, and the tube was left until the large particles have settled down in the bottom of the tube. Then four drops of supernatant were taken by disposable dropper and added to the sample hole on the test device. As the test begins to work, the purple color was observed moving across the result window in the center of the test device and the test results were read at 10 minutes.

Reverse transcription real time PCR

preparation of tissue samples: after thawing the samples, 100 mg of tissue was put in sterile mortar and started grinding by pestle with 1ml of phosphate buffer saline (PBS) to obtain homogenous suspension (10%). After that the suspension was transferred into an eppendorf tube and centrifuged at (1200 rpm) in cooling centrifuge at (5°C) for (15 min.). Then the supernatant fluid was collected then filtered in (0.22µm) mile pore filter, after that stored in deep freeze at (-70 °C) in the central laboratory of the general state of veterinary services in Baghdad until used .

RNA extraction

The viral RNA was extracted from tissue samples by using (RNase Mini kit, QIAGEN, USA) according to manufacturer's instructions. IBV was detected using one step Real-Time PCR kit (IBV Real-Time detection kit, QIAGEN, USA).

Master mix

Real- Time PCR master mix was prepared by Real-Time PCR Detection Kit (Gen-Kam, Germany) which composed from three reagents (A,B and Y) and control positive (D1), control negative (D2). Then all these reagents were mixed together on vortex with total volume 18 µl for each well of 96 PCR microtiter plate, then 2 µl of control positive, control negative and sample were added. The amplification was carried out in Applied Bio system 7300 Real-Time PCR device thermal

cycler (USA). For cycling conditions: reverse transcription for synthesis of cDNA was performed at (42 °C) for (60) minute, followed by (70 °C) for (10) minute to inactivate RT-enzyme and activate the taq polymerase. Then followed by extension at (95 °C) for (15 second) and final extension at (60 °C) for (1 minute), 40 cycles.

Results

Clinical signs

The result of clinical examination showed that there were rales, sneezing, gasping during breathing, coughing, ocular and nasal discharge, sometimes accompanied by lacrimation, conjunctivitis and facial swelling, loss of appetite, weakness, depression, and ruffled feather, Figure (1).



Fig. (1): Respiratory signs (gasping).

Gross lesions

The results of gross lesions examination showed that there was tracheal congestion; exudates in trachea and sometime there were hemorrhage, cast plug in the bifurcation of the trachea figure (2), caseated pus in the lungs, air sacculitis, hemorrhages of cecal tonsils and nephritis.

Results of rapid immunochromatographic assay

The results of rapid immunochromatographic assay showed that out of 30 flocks were 28(93.33%) positive for IB.

Results of Real-Time PCR

The results of Real-Time PCR showed that out of 30 flocks were all (100%) positive for IBV. Table (1), figure (3).



Fig. (2): Cast plug in the bifurcation of the trachea.

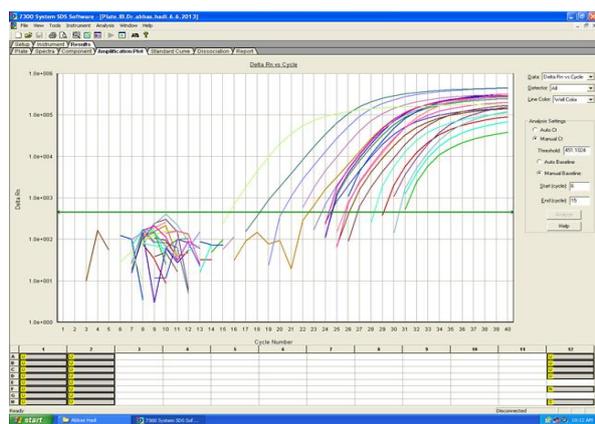


Fig. (3): Graph of Real-Time PCR for IBV showing the Threshold cycle (CT).

Discussion

Infectious bronchitis is a highly contagious viral disease in chicken with significant economic losses throughout the

world and is able to spread very rapidly in non-protected birds (3). The result of clinical examination showed that there were rales,

Table (1): Show the percentage of IB infection (by rTPCR) according to the area.

rTPCR Results IB	No. of infected flocks	Area
2	2	Al-Salahiyia
5	5	Al-Saniyia
3	3	Al-hamza
6	6	Al-bdeer
3	3	Nufer
2	2	Ghumas
4	4	Dughara
3	3	City center
2	2	Al-Shamiyia
30 (100%)	30	Total & %

sneezing, gasping during breathing, coughing, ocular and nasal discharge, sometimes accompanied by lacrimation, conjunctivitis and facial swelling, loss of appetite, weakness, depression, and ruffled feather. These results are companionable with (7) who found that sneezing, coughing and dyspnea was characteristic clinical signs of infectious bronchitis and avian influenza (H9 subtype). While (8) who found tracheal rales, coughing, poor weight gain, and reduced feed efficiency in broilers and a decline in egg production in layers were important clinical signs for IBV. Whereas (9), (10) recorded gasping, coughing, sneezing, tracheal rales, and nasal discharge. While (11) reported that inoculated birds with IBV experimentally showed severe conjunctivitis, associated with abundant lacrimation, edema, and cellulitis of the periorbital tissues at 48 hours following challenge with IBV. The results of gross lesions examination showed that there were tracheal congestion, exudates in trachea and sometime there were hemorrhage, cast plug in the bifurcation of the trachea, airsacculitis, hemorrhages of cecal tonsils and nephritis. This result is agreement with (12) who found post-mortem lesions of dead birds revealed increased trachea mucus, severe renal

congestion, urates filled ureters as well as congestion in liver and spleen in the infection with nephropathogenic strain of avian infectious bronchitis virus. While (7) found tracheal congestion, Lung hyperemia, air sacculitis and swollen kidney, tubular cast formation in tracheal bifurcation were observed in all dead birds, these casts extended to the lower bronchi. The results of rapid immunochromatographic assay showed that out of 30 flocks were 28(93.33%) positive for IBV. The test is characterized by rapidity which can be done in the field, cheap test as compared with other tests and also consider one of most screening test which gave picture about of the presence of viral antigen (13). The results of Real-Time PCR showed that out of 30 flocks were all (100%) positive for IBV. In molecular technique survey of IBV in Iran by RT-PCR and type specific nested PCR, the viral RNA was detected in 42.8% of broiler flocks from different regions of Iran (14). This result were less than our results of IBV percentage by RT-PCR and this may due to the differences in vaccination program and application of other control measures and correction of management in addition to the geographical distribution and climatic factors.

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