



## Evaluation of the prevalence of viral bronchitis infection in broiler chicken by using ELISA Technique

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### Abstract

The study was conducted to evaluate the seroprevalence of Infectious Bronchitis virus (IBV) in broiler chickens farms in Kirkuk Governorate. The technique has been used in the study, Enzyme Linked Immunosorbent Assay (ELISA). Nine hundred serum samples were collected from (10) broilers farms, During the period from April 2016 to May 2016, including (90) samples from (1) symptomatic, non-vaccinated broilers farms. (90) samples from one asymptomatic, vaccinated broilers farm, and another (630) samples from one asymptomatic, non-vaccinated broilers farm, the remaining (90) samples from one symptomatic, vaccinated broilers farms, and were tested for the presence of IBV antibodies by ELISA kit. One hundred and ninety six serum samples were positive ( 65.3% ) : 78/90 (86.6%) samples were symptomatic, non-vaccinated, another 63/90 (70%) were asymptomatic, vaccinated broilers farm, and 432/630 (68.5%) were asymptomatic, non-vaccinated broiler farm, the remaining 15/90 (16.6%) were symptomatic, vaccinated broilers farm. The remaining samples (312) were negative by ELISA.

### Introduction

Domestic chickens are estimated at over 18 billion in the world, the majority of chicken industry are commercial farms, while in developing nations are dominated by village (local) chickens [1], there are many diseases infect chickens and respiratory diseases, such as avian influenza virus, infectious bronchitis virus, Newcastle disease virus and *Mycoplasma gallisepticum*, are very importance because it can cause disease alone or association with others viral or bacterial pathogens[2]. Avian infectious bronchitis (IB) is an acute, highly contagious disease with severe economic losses in poultry industry around the world [3]. It mainly affects the respiratory tract, and frequently causes damage to reproductive systems and kidneys, and when affects proventriculus, the mortality may reach 75% to 100% in chicks [4,5]. The strains typing of IBV is necessary for understanding the evolution and epidemiology of IBVs [6]. The reason of difficult of accurate classification of isolates are high mutation of RNA genome, multiple subtypes, insertion, deletions and recombination among IBVs [7], more than 50 variants and serotypes of virus have been registered around the world [8]. In

Iraq IB becomes endemic, and found in layers and broiler flock, IBV have been reported in Sulaimanayah [9], Duhok [10], Erbil [11], Mosul [12], Baghdad [13], Diyala [14], Hilla, Najaf, Muthane, Theqaar [15, 16], Al-Diwaniya [17], Basrah [18].

The ELISA is a convenient test for checking the viral infection and immune level in chicken flocks [19]. The aim of this study to evaluate the prevalence of IBV infection in broilers via serological Technique in Kirkuk governorate.

### Materials and methods

**Chickens:** for this study, a total of 900 broilers, (24-42) days old (chicken farms) Broiler, were randomly selected, During the period from April 2016 to May 2016, the sera samples were collected to ELISA test from (10) Broiler Farms located to south, east and west of Kirkuk city , the broilers consist of (7 farms) (1farms were located in Laylan region, 2 farms from Daquq region, 2 farms from Taza region and 2 farm from Yaychi region) were suffering from respiratory signs, none of these farms were vaccinated against IBV according to the supervisor instructions of each farm, (1farm located in Altun kupri region) without

respiratory signs and vaccinated with different vaccination program , the remaining (1farm from Dibis region), not vaccinated, without respiratory signs, (1farm from Laylan region), were suffering from respiratory signs and vaccinated. All flocks were contact with specialized veterinarians and all owners was agreed to participate in the test.

**Sample collection:** Blood samples consist of (3ml) obtained from wing vein (brachial vein) by sterile syringes from each bird, and poured in clean plane tube without anticoagulant and centrifuged at (3000 rpm) for (5-7 minutes), the serum was separated and stored in multiple marked tubes at (2-8°C) for ELISA test.

**Enzyme-linked Immunosorbent Assay (ELISA):** IBV ELISA Kit(symbiotic-USA), used to measured IBV antibodies in individual sera of chickens based on the manufacture's instruction, Briefly, all serum samples, positive and negative serum control were diluted by Dilution Buffer (1:50 dilution). 50 µl of Dilution Buffer were added to all wells on the test plate, and 50 µl of diluted IBV positive control serum were added to wells(A1, A3 and H11), 50 µl of diluted IBV negative control serum were added to wells(A2, H10 and H12), then 50 µl of each diluted serum samples were transfer to the corresponding wells of IBV coated test plate, and incubated plate for (30 minutes) at room temperature. Liquid from each well were tapped out into vessel containing decontamination agent such as bleach. 300 µl of Wash Solution were filled to each well, then wash procedure were repeated two more times. 100 µl of Anti-Chicken IgY(G) Peroxides conjugate were added into each well, and incubated plate for (30 minutes) at room temperature. After washing procedure, 100 µl of Chromagen substrate reagent was added into each wells and incubated plate for (15 minutes) at room temperature. 100 µl of stop solution were added into each well. The results of the tests read by ELISA Plate Reader based on optical density at 405-410 nm. The antibody levels of sample to positive ratio (S/P), the endpoint titers was calculated depending on the equation described by manufacturer, the S/P ratio equal or less than to (0.2) were considered as negative and samples greater than 0.2 (titer > 396) were consider as positive.

**Results**

Seroprevalence of IBV in broiler in some regions of Kirkuk province is given in Table(1,2). Overall Seroprevalence of IBV in present study was 78.33%, 62% respectively. The clinical signs on broilers characterized by respiratory signs such as coughing,

rales and gasping (figure 1). Conjunctivitis with Wet frothy eyes, cold and depression were also noticed. Post-mortem examination showed congestion, hyperemia in trachea and caseated plugs at trachea were also seen (figure 2). Kidneys displayed swollen and filled with urates material. Five hundred and eighty- eight out of 900 sera were positive : 78/90 (86.6%) without respiratory signs and vaccinated, another 63/90 (70%), suffering from respiratory signs and vaccinated in (Table 1). 432/630 (68.5%) samples were suffering from respiratory signs, non-vaccinated, others 15/90 (16.6%) , not vaccinated, without respiratory signs In (Table 2).



Figure 1: Broiler, 25 days old, infected with IBV. note.sever gasping.



Figure 2: Trachea of broiler, infected with IBV. note. Caseated plug (black arrow), congestion and hyperemia (blue arrow).

Table (1): IBV ELISA testing samples in different areas of Kirkuk governorate in vaccinated broilers, and Presence of Respiratory signs.

Group No.	Respiratory signs	Regions	Total No. Of Samples	Positive Samples	Prevalence %
Group1	No	Altun kupri	90	78	86.6%
Group2	Yes	Laylan	90	63	70%
Total			180	141	78.33%

**Table (2): IBV ELISA testing samples in different areas of Kirkuk governorate in non-vaccinated broilers, and Presence of Respiratory signs.**

Group No.	Respiratory signs	Regions	Total No. Of Samples	Positive Samples	Prevalence %
Group1	Yes	Laylan	90	75	83.33 %
	Yes	Daquq	180	126	70 %
	Yes	Taza	180	114	63.3%
	Yes	Yaychi	180	117	65%
Group 2	No	Dibis	90	15	16.6%
Total			720	447	62%
Total (G1+G2+G3+G4)			900	588	65.3%

## Discussion

In present study, try to made a seroprevalence of IBV from broiler chickens farms in Kirkuk Governorate. One farm located in Altun kupri region, (without respiratory signs and vaccinated) were detected for antibodies of IBV in this study, 78 out of 90 (86.6%) were positive to ELISA, the percentage of infection is lower than that detected in Duhok, a survey conducted in broilers revealed 100% farms were seropositive [10]. A study used hemagglutination inhibition test to find seroprevalence of 83.3% to 3 IBV strains (M-41, 4/91, D274) in broilers chickens farms free from respiratory disease and all farms were vaccinated against M-41 strain, which is close to our result [20]. In Egypt, had examined 19 broilers farms, for presence of IBV by RT-PCR and the virus has detected in (65.4%), this percentage was lower than the results obtained by this study [24], the reason of low percentage maybe, because low efficacy of vaccines used in mentioned region [9].

In one farm from Laylan region, (suffering from respiratory signs and vaccinated), (70%), of the samples sera were positive against IB virus. [15] had detect IBV by RT-PCR in 43.6% of symptomatic vaccinated broiler chickens flocks in Middle Euphrates. In Jordan, were examined RT-PCR and 16/25 (64%) were positive [20], similarly another study in Fars province in Iran, use RT-PCR, IBV demonstrated in 72% [28], both studies near to results of this study. Tracheal swabs, were collected from 30 infected broiler farms in Diwaniya governorate, by Rapid immunochromatography test, showed that 28 out of 30 farms (93.3%) were positive for IB [17]. A different seroprevalence in various regions of the world, which showed variety viral exposure rates to broilers, which should take into consideration presence of different strains of the virus, and should be remember Massachusetts type are mostly only the vaccine in use [29].

The high titer in table (1) may be due to mistake with either vaccination person education or commitment

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with good bird management practices or with proper timing and routes of vaccination or with vaccine efficacy [30].

seven broilers farms (Laylan region, 2 farms from Daquq region, 2 farms from Taza region and 2 farm from Yaychi region), (suffering from respiratory signs, not vaccinated) were examined for antibodies of IBV, 144 out of 210 (68.5%) were positive to ELISA, the reason may to the nature of disease, which was highly contagious and viral spread by airborne route and mechanical spread [21]. The percentage of infection were detected in this study is higher than that seen in previous studies from other parts of the world. In Duhok, 41.6% of the farms were seropositive for antibodies[10]. A low seroprevalence of 17.2% was reported in chickens in Middle Euphrates[15]. In study done in bangladesh 79.38% of non-vaccinated broilers, is higher than the results obtained by this study[22]. In a serological survey, collected during the period from February 2010 to September 2010 in Iran, the roles of IBV, Newcastle (ND) and Avian influenza H9 subtype (AIV H9) were studied in outbreaks of respiratory diseases of broiler farms, seroprevalence of IB, ND and AIV H9 subtype were 82.43%, 31.2%, 18.47% respectively [23].

The remaining, In farm from Dibis region, (not vaccinated, without respiratory signs), 16.6% were tested Seropositive for IBV, which is close to the results obtained by [25]. The prevalence rate is low, when compare with previous studies from other countries, in [26] were reported that 68% of the local chickens, seropositive for IBV antibodies, as chickens had not vaccinated, the result indicate that broilers exposure to low-attenuated and field strains of IBV, locate in the studied areas[27].

The high titer in some farms like in Altun kupri and Laylan, may be due to increase immunosuppression factors or exposure the birds to high doses of infectious agents[30].

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## انتشار اضرار داء التهاب القصبات المعدي في محافظة كركوك باستخدام تقنية الاليزا

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### الملخص

أجريت هذه الدراسة لتقييم مدى انتشار المصلي المرض التهاب القصبات الفيروسي في حقول افراخ الدجاج في محافظة كركوك باستخدام تقنية الاليزا. تم جمع (900) عينة مصل دم من (10) حقول فروج لحم، أجرى عليها فحص الاليزا لمعرفة وجود الاجسام المضادة، للفترة من شهر نيسان الى حزيران 2016 وجمعت كالاتي: (90) عينة من حقل مصاب بالمرض وغير ملقح، (90) عينة من حقل واحد غير مصاب بالمرض وملقحة، و(7) حقول أخذ منه (630) عينة من حقول غير مصابة وغير ملقحة، والحقل المتبقي جمعت منه (90) عينة من حقل واحد مصابة وملقحة. وكانت النتيجة (588) عينة موجبة وبنسبة 65.3% كالتالي: (90/78) وبنسبة (86.6%) للافراخ المصابة وغير ملقحة، (90/63) وبنسبة (70%) للافراخ الغير مصابة والملقحة، (630/432) وبنسبة (68.5%) للافراخ الغير مصابة وغير ملقحة، (90/15) وبنسبة (16.6%). العينات المتبقية (312) كانت سالبة لأختبار الاليزا.