

## INVESTIGATION OF THE ANTIBODIES OF AVIAN LARYNGEOTRACHEITIS VIRUS IN UNVACCINATED LAYING HENS WITHIN BAGHDAD PROVINCE

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### ABSTRACT

Avian infectious laryngeotracheitis is one of the important diseases which was newly emerged in Iraq, therefore this study was aimed to detect specific antibodies against avian infectious laryngeotracheitis in unvaccinated layer flocks within Baghdad province for the period between March to May 2017, to highlight the epidemiology of disease and its spread progress within the region. Enzyme linked immunosorbent assay (ELISA) technique was adopted as the main methodology in this study. Blood samples were collected from chicken aged 3-80 weeks were received from different avian flocks located within the investigated area. The results showed positive (312 samples) from 455 in total with a percentage of infection 68.75%. The study also indicates a highest percentage of infected (78.98%) birds aged (11-40 weeks). However, birds with 3-10 and 41-80 weeks of age showed less pronounced infection status. The results also showed that only two laying hens were infection free among all 31 examined flocks when 14 flocks were fully infected, the percentage of infected flocks reached up to 93.45%. The infection appears mostly in Abu-Ghrab and Al-Tagi.

### INTRODUCTION

Avian infectious Laryngeotracheitis (ILT) is an acute contagious disease of chickens that affect upper-respiratory tract. The disease could contribute to major economical lost due to high mortality and or severe decrease on egg production [1]. The causative agent is pneumotropic virus of the family *Herpesviridae* genus *Iltovirus*. This virus is classified as a *Gallid herpes virus 1* (9). Peracute form spread suddenly with high morbidity and mortality may exceed 70% (10).

Subacute form shows high morbidity but lower mortality than peracute form between (10-30%) (8). Chronic and mild forms seen among surviving above forms or as an outbreak with low morbidity and mortality (8).

Clinical signs characteristic of ILT include nasal discharge, moist rales, coughing and gasping (4). The most active replication of ILTV will occur within the tissues of the trachea. Active virus replication occurs only during the first week after infection, although low levels of ILTV infectivity can be detected sporadically up to ten days post infection (14). The virus can invade the trigeminal nerve during the lytic phase of infection, resulting in a latent infection that may remain throughout the life of the bird, and some stressors, such as placement with other birds and the onset of egg laying, can cause reactivation of replication and viral excretion (5).

There are several procedures for the diagnosis of ILT such as observation of clinical signs, detection of gross and histopathological lesions, and the use of molecular techniques, including Restriction Fragment Length Polymorphism

RFLP, polymerase chain reaction (PCR), real-time PCR, and loop-mediated isothermal amplification (11). ELISA shown to be highly sensitive in detection of ILT antibodies (13).

This study aims to highlight the epidemiology of disease and its spread progress within the region thereby implementing (ELISA) technique as the main methodology.

## MATERIALS AND METHOD

Four hundred fifty five blood samples were collected from 31 laying hens within target area. The blood was collected from wing vein of chickens aged 3 to 80 weeks, used ELISA kits (ProFlock®) for the quantitative detection of antibodies against Infectious Laryngotracheitis in serum was supplied by (Synbiotics Corporation\U.S.A). The results were read obtained using ELISA micro plate reader at 405nm wavelength and the titers were calculated at compared with the reference classification key titer of different level of antibodies. This study aimed to cover as many unvaccinated layer flocks as possible located within Baghdad province. Therefore, examined flocks were treated according to their location in the region; North, South, east and West (Table 1).

Table 1: the number of examined flocks, their collected blood samples and their infection status.

Percentage of infected blood samples (%)	Percentage of infected flocks (%)	Number of blood samples	Number of examined layer flocks	Region
68.25	100	63	4	Baghdad South (Al-Lateefia, Hoor Rijab & Al-Wihda)
56.55	88.88	122	9	Baghdad North (Al-Taaaji)
76.71	100	189	11	Baghdad West (Abu-Guraab)
67.9	85.7	81	7	East Baghdad (Al-Rashedia)
-	-	455	31	total

## RESULTS AND DISCUSSION

Figure one compares the number of examined laying hens and their corresponding percentage of infection within four different regions. In spite of different number of examined flocks, those in the South and West clearly demonstrate 100% infection. In contrast, flocks infection was less pronounced over those flocks within the North and East of Baghdad. Figure (1) explain the suggestion indicated that the disease was actually developed in the South-West Province of Baghdad and transferred to the North-East region.

The Number of received blood samples and associated percentage of infection are well defined in figure (2). The results indicate that samples have antibodies vary from sever to moderate. This has been well distinct over all collected samples from different regions. It indicates that the disease appears and develops through different stages of infection; lower titer related to chronic infection while higher onest related to acute or subacute infection (12). Antibodies were detected after 5-7 day post infection. It reaches peak at 21days post infection and may turn down in the next month (7) or persist for a year or longer (1).

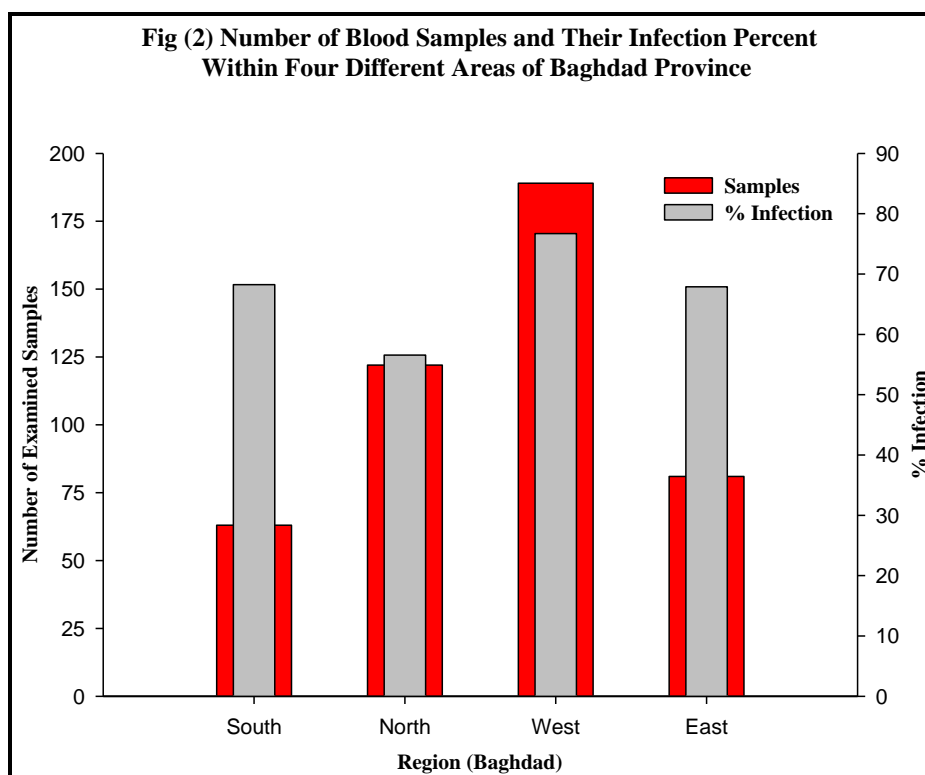
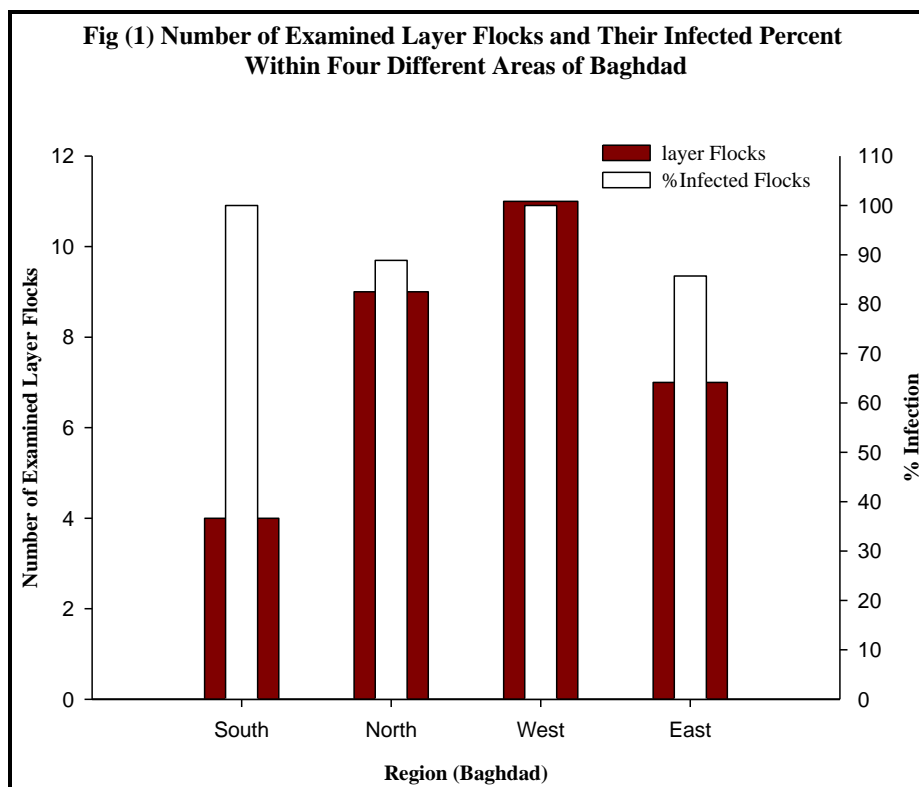


Figure (3) displays the number of received blood samples along with the percentage of infection at various birds' age. Obviously, birds aged from 11 - 40 weeks showed the highest level of infection, followed by 41 - 80 weeks and 3 -10 weeks was the least group. If the number of the received blood samples was taken in to account, it would be worth to suggest that the disease tends to spread faster within birds aged 11- 40 weeks which appeared as more sensitive (13).

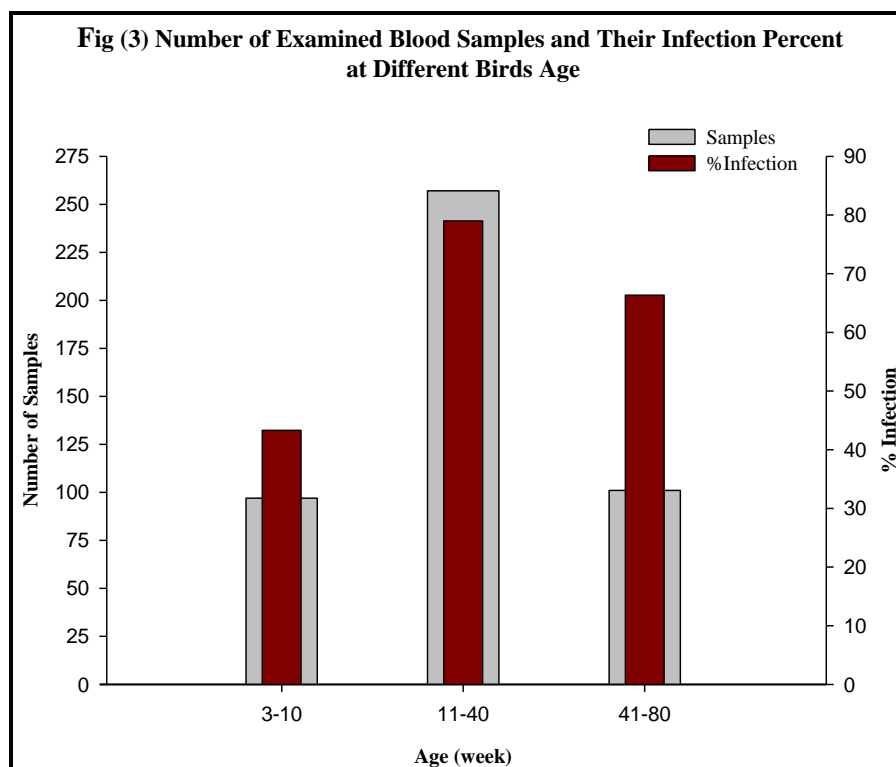
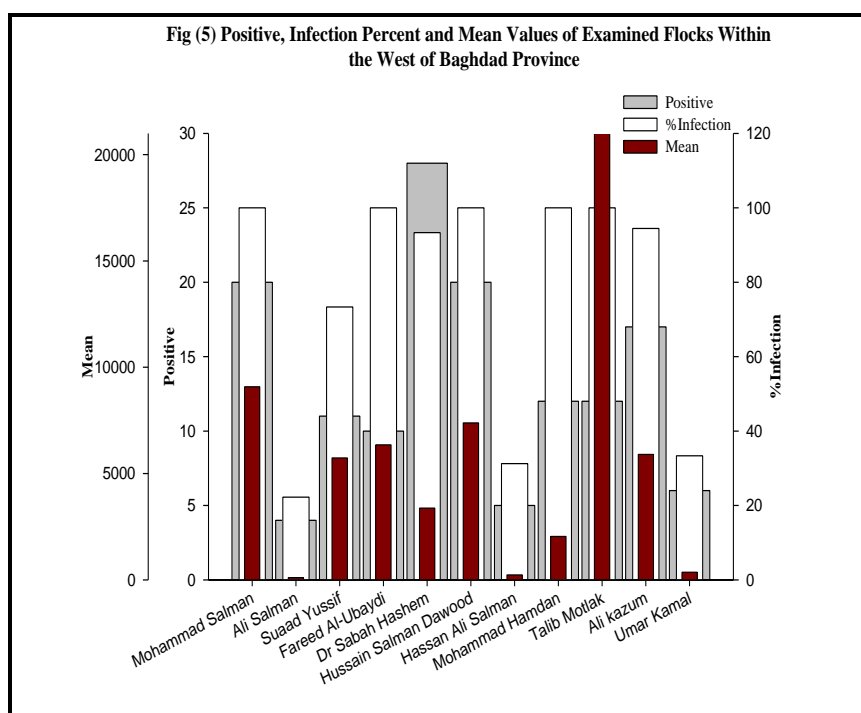
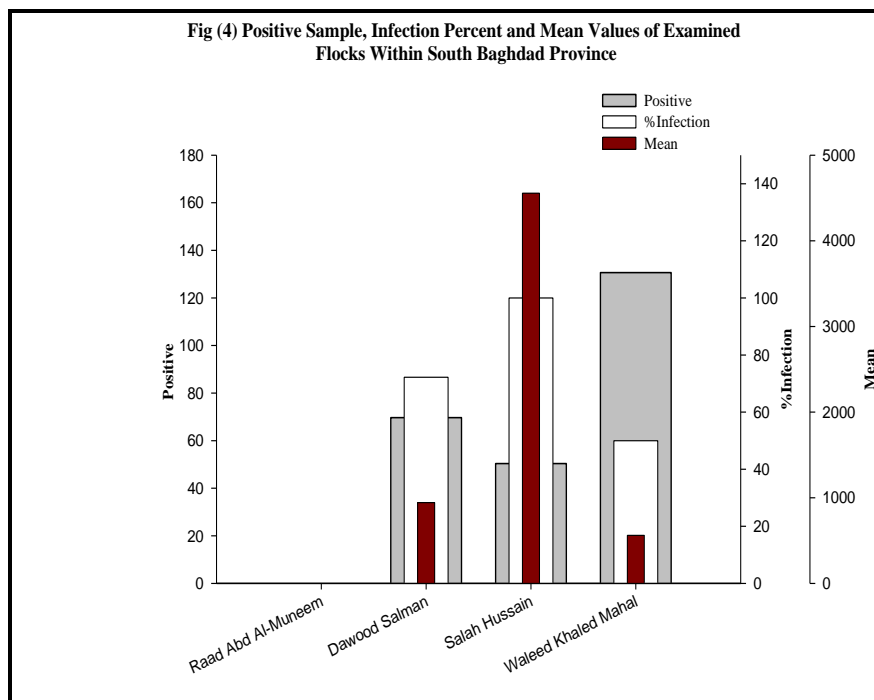
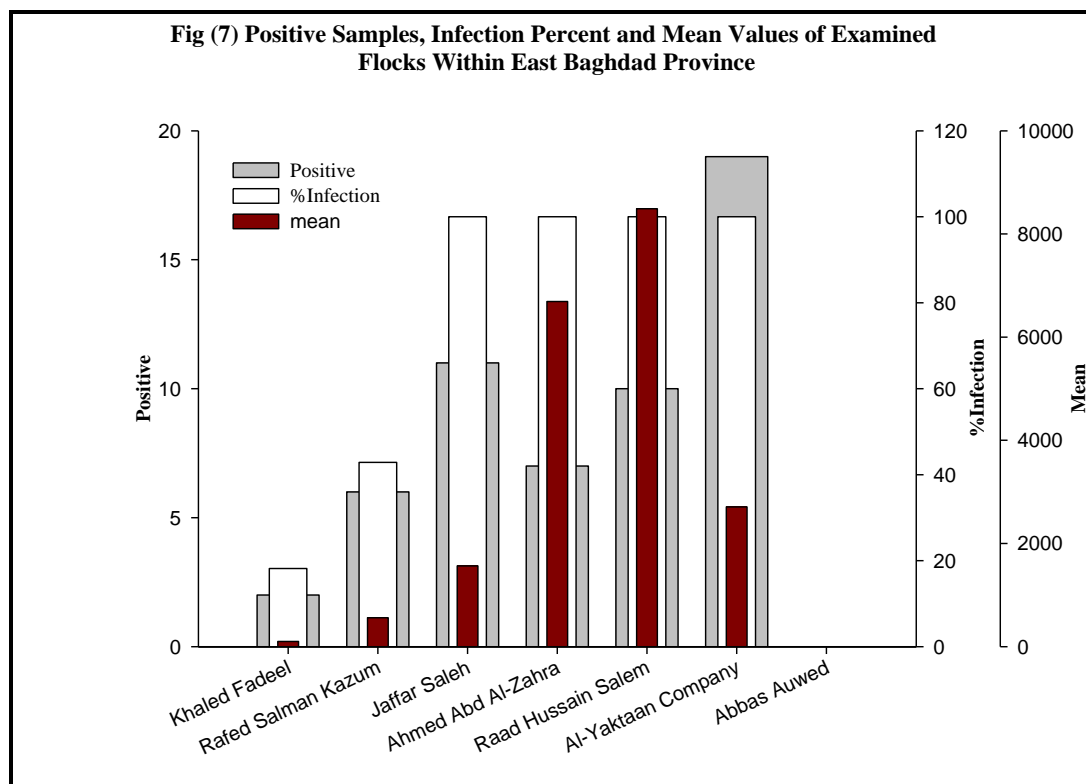
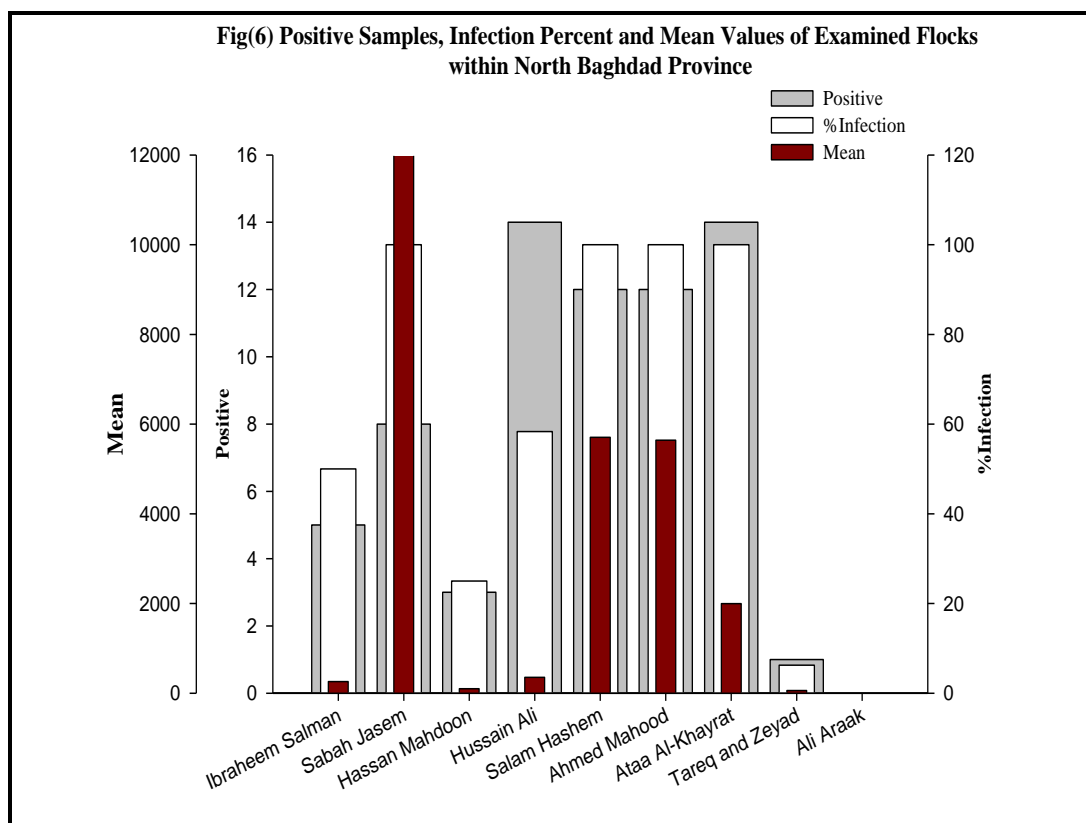


Figure (4) demonstrates the number of blood samples with positive indication and percent of infection along with their associated with antibodies titer Mean values over the examined flocks within the South region. Data shows that all the four examined flocks were infected and the degree of infection are actually ranged from 100% on Salah Hussain flock to the least 16.66% recorded for Raad Abd Al-Muneem. However, due to the fact that flocks infection status were less than 100% in almost all examined flocks, The suggested that the disease is not yet spread to all the birds within the flock. Antibodies titer Mean values were different from one flock to another, ranged from 511 with Raad Abd Al-Muneem flock to 4556 as highest recorded with Salah Hussain flock. The results from flocks located in West region are clearly displayed in Figure (5).

A compared to date from Fig (4), it could be obse in the West region that flocks infection status was more prominent since seven out of eleven examined flocks scored a high percentage of infection  $\geq 93.33\%$ . The antibodies titer Mean values also fluctuated among 105 with Ali Salman flock to as high as 21048 recorded at Talib Motlak flock. Table (2) illustrates a comprehensive data collected out of flocks in both South & West regions. The number of blood samples with positive results and their percent of infection along with their antibodies titer Mean values received from the examined flocks within the North and East regions are illustrated in Figures (6 and 7 respectively). In spite of number of blood samples with positive signs, it would seem that four flocks were fully infected according to their percentage approaching 100% in both two examined regions. However, data also showed that there is single flock with infection free in each region. On the other hand, antibodies titer Mean values from the North flocks fluctuated between 60 and 12403 which appeared more volatile than those with East flocks that ranged between 101 and 8489. Table (3) displays all data collected from flocks in both North & East regions.





**Table 2: Date collected from south and west of Baghdad**

Region	Flock's Name	NO. of Blood Samples	Positive	Negative	% Infection	Mean Value
South Baghdad	Raad Abd Al-Muneem	12	2	10	16.66	511.00
	Dawood Salman	18	13	5	72.22	943.00
	Salah Hussain	23	23	0	100.00	4556.00
	Waleed Khaled Mahal	10	5	5	50.00	561.00
West Baghdad	Mohammad Salman	20	20	0	100.00	9082.00
	Ali Salman	18	4	14	22.22	105.00
	Suaad Yussif	15	11	4	73.33	5735.00
	Fareed Al-Ubaydi	10	10	0	100.00	6353.00
	Dr Sabah Hashem	30	28	2	93.33	3377.00
	Hussain Salman Dawood	20	20	0	100.00	7382.00
	Husain Ali Salman	16	5	11	31.25	234.00
	Mohammad Hamdan	12	12	0	100.00	2041.00
	Talib Motlak	12	12	0	100.00	21048.00
	Ali kazum	18	17	1	94.44	5902.00
	Umar Kamal	18	6	12	33.33	361.00

**Table 4: Date collected from north and east of Baghdad**

Region	Flock's Name	NO. of Blood Samples	Positive	Negative	% Infection	Mean Value
North Baghdad	Ibraheem Salman	10	5	5	50.00	260.00
	Sabah Jasem	8	8	0	100.00	12403.00
	Hassan Mahdoon	12	3	9	25.00	99.00
	Hussain Ali	24	14	10	58.33	354.00
	Salam Hashem	12	12	0	100.00	5707.00
	Ahmed Mahood	12	12	0	100.00	5642.00
	Ataa Al-Khayrat	14	14	0	100.00	1998.00
	Tareq and Zeyad	16	1	15	6.25	60.00
	Ali Araak	14	0	14	0.00	0.00
East Baghdad	Khaled Fadeel	11	2	9	18.18	101.00
	Rafed Salman Kazum	14	6	8	42.85	561.00
	Jaffar Saleh	11	11	0	100.00	1566.00
	Ahmed Abd Al-Zahra	7	7	0	100.00	6691.00
	Raad Hussain Salem	10	10	0	100.00	8489.00
	Al-Yaktaan Company	19	19	0	100.00	2709.00
	Abbas Auwed	9	0	9	0.00	0.00

It should be mentioned that the high fluctuation of antibodies titer Mean values of blood samples of a single flock and others within the same region when it compared with those from antibodies control titer that used in the analysis, emphasis the existence of the disease in different stages in the same flock and others. This is due to the fact that the examined flocks are not vaccinated against such disease. It is well reported that ILTV is quite capable to reproduction during the 1<sup>st</sup> week of infection. However, the recovery period of 7-10 days is required for chickens suffered from primary infection. It has been reported that 10-28 days post tracheal infection, the shedding of the virus may have ceased (1). Thus a latent phase of infection is established through ILTV invasion of nervous tissues (2). It should be mentioned that some recovered chickens become carrier and shed virus for long period of time or much later can shed virus following-induced reactivation of latent infection, thus exposing other susceptible birds (3). According to the collected data from single flock, low antibodies titer Mean value may suggest the existence of primary infection in that particular flock.

In This study a sophisticated analysis was carried out that aims to highlight the epidemiology of disease and its spread progress within Baghdad

Province. The study suggests that the disease spreads much faster within birds aged 11- 40 weeks than those of other ages. Collected data from antibodies titer Mean values of revived blood samples indicate the existence of the disease in different stages in a same flock and others since all examined flock are not vaccinated. It should be mentioned that some recovered chickens become carrier and shed virus for long period of time or much later can shed virus following induced reactivation of latent infection, thus exposing other susceptible birds.

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## التحري عن الأجسام المضادة لفايروس التهاب الحنجرة والرغامي المعدي في حقول الدجاج البياض غير المحصنة في محافظة بغداد

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### الملخص

نتيجة لتزايد القلق اتجاه انتشار مرض التهاب الحنجرة والرغامي المعدي في حقول الدواجن ضمن محافظة بغداد، فقد تم الشروع بالتحري عن المرض في حقول الدجاج البياض في مناطق بغداد للمدة من شهر آذار ولغاية شهر ايار 2017. ولأجل توضيح الصورة الوبائية للمرض ومدى إنتشاره. اعتمدت الدراسة تقنية انزيم المناعي الممتز ELISA كطريقة رئيسة للتحري في هذا البحث. تسلمت عينات الدم من الطيور وبعمر من 3-80 اسبوعاً للحقول الواقعة ضمن منطقة الدراسة. اظهرت النتائج وجود 312 عينة موجبة من مجموع 455 عينة ونسبة إصابة 68.75%. أشارت الدراسة الى ان أعلى نسبة إصابة 78.98% كانت ضمن الطيور التي تتراوح أعمارها من 11-40 اسبوعاً. ل سجلت للطيور التي تتراوح أعمارها بين 3-10 و 41-80 اسبوعاً مستويات أوطأ من الإصابة. كما اظهرت الدراسة ايضاً حقلين فقط كانت خالية من الإصابة ضمن المجموع الكلي للحقول المفحوصة وعددها 31 على الرغم من وجود 14 حقلاً مصاباً بنسبة 100% مسجلة نسبة إصابة 93.54% التي تركزت في أبي غريب و التاجي.