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# Pathological investigation of local infectious laryngotracheitis virus isolated from layers on broiler Chicks

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**Abstract**: In this study, The PCR molecular test was performed on samples gathered from local laying farms that exhibited symptoms of ILT. With the wild strain, ILTV was positive, and subsequently, AI, ND, and IB were tested and all negative findings were found. ECE was inoculated with the prepared positive sample using the CAM method for three separate passages. PCR testing and viral titration, in the same manner, was carried out again once the cytopathic impact occurred. The EID50 is the fourth dilution. An ELISA test for maternal ABs was performed on four groups of broiler chickens (A, B, C, and D), each group containing 50 birds, and an experimental infection was made for three groups, with group D remaining as control negative. Group A was infected at one day, Group B at 15 days, and Group C at 30 days, clinical signs and pathological lesions, both macroscopic and microscopic in nature, were observed. After clinical indicators, affected groups performed the histopathological test. And then all groups had 3 ELISA tests at 1, 2, and 3 weeks following infection. The experiment indicated that group A was the least affected. Group B was more impacted than A, whereas group C had more clinical indications and mortalities, indicating that broiler chickens of all ages are vulnerable to infection, although maternal immunity may offer some protection for at most two weeks. This research was done to provide information to the scientific library of broiler chickens infected with ILT, which has been more investigated in laying hens and breeders.

**Keywords**: ILTV, infectious laryngotracheitis, chorioallantoic membrane (CAM), isolation, PCR, molecular detection.

## 1. Introduction

Infectious laryngotracheitis (ILT), is highly infectious and economically important avian alphaherpesviruses that belong to the genus *Iltovirus* and species *Gallid Herpesvirus 1*(GaHv-1) (Rojas et al. 2021). An upper respiratory tract infection may result in severe losses in production due to mortality, drop in egg production, and weight gain. The virus has a limited host range. It is most commonly described as an infection in chickens, although there are reports of natural infections in pheasants, peafowl, and partridges (Abdul-Aziz and Barnes 2018). Experimental (Gustavohenrique 2016) and natural (Portz et al. 2008) infections were reported in turkeys. Quail and guinea fowl, are refractory to infection (Gustavohenrique 2016). In 1925, the disease was first identified in the region that is now known as the United States of America. Nevertheless, some records suggest that it most likely existed at an earlier time (Menendez et al. 2014). Initially, veterinarians referred to the disease as avian diphtheria; however, the designation "ILT" was chosen in 1931 by the special committee of the poultry diseases of the American Veterinary Medical Association (García, Spatz, and Guy 2013). It would seem that ducks, pigeons, starlings, crows, and sparrows are immune to the disease; nonetheless, all ages of chickens are vulnerable to infection; however, birds that are more than three weeks old are at greater risk (Dufour-Zavala 2008) (Kaur 2021). There are two different epizootic forms that this disease might take. The severe form of the disease is distinguished by a high morbidity rate and a mortality rate that ranges from moderate to high. On the other hand, the mild form of the disease, which is increasingly prevalent in today's developed poultry industries, is the cause of the presence of clinical signs such as expectoration of bloody mucous, tracheitis, sinusitis, conjunctivitis, general depression, watery eyes, and a low mortality rate. Even though it is considered to be a mild form of the disease, it may result in considerable weight loss and a drop in egg production, and it has had a major negative effect on the industry's bottom line during the last decade. In 2000, Iraqi researchers were the first to study pathogenesis, leading to the virus's isolation and identification (Al-Khidr and Al-Hayali 2000). In

ISSN NO: 2771-8840
Date of Publication: 17-09-2022

2018 molecular and phylogenetic study in Al-Diwaniyah province produced a layer hen epidemic (Alaraji et al. 2019). An epidemic of a disease that affects broilers was reported in many different countries, including the country that borders Iraq (Arnaout, Fadel, and Mohra 2010)(Emadi Chashmi et al. 2021) (Razmyar et al. 2021). Not enough information on the potential economic losses caused by the disease in broilers was provided especially in Iraq. Even though pathological infections with acute respiratory signs showed up in the flocks of broiler chickens beside the flocks of laying chickens, which also showed the same pathological signs, this may be a sign of ILT infection. Investigating the merits of the disease and understanding the extent of its economic danger in terms of the mortalities and morbidities it causes, the clinical signs, the gross pathological changes in the organs, as well as the microscopic tissue changes caused by the virus in flocks of broiler chickens after an experimental infection at different ages (one, fifteen, and thirty days), respectively. And a locally isolated strain of laying hens is the main reason for this study, which will undoubtedly shed light on a disease that has been studied in detail on laying hens and breeders, but the scientific library remained poor in information related to the infection of broiler chickens with this disease. The following is a list of the aims of this research, which was carried out in the Waset Governorate of Iraq between the years 2021 and 2022:

1. Molecular detection of the infectious laryngotracheitis virus in layers, followed by virus isolation using embryonated chicken eggs.
2. Study the isolated virus's pathological effects on egg embryos.
3. Study the pathological changes in broilers after the titration of an isolated virus.

## 2. Materials and methods

### 2.1 Samples collection

Twenty tissue samples, including trachea and larynx, were taken from five different strata of flocks in the provinces of Basra, Waset, and Karbala. These flocks exhibited respiratory symptoms, including expectoration of bloody mucus, and showed evidence of respiratory problems. After gathering the case medical history and seeing the gross pathological alterations, tissue samples were obtained, placed in test tubes, and stored at a temperature of -20 degrees Celsius until further use.

### 2.2 DNA extraction and RT-PCR amplification

AniCon Labor GmbH Germany Company's Kylt® RNA/DNA Purification kit was used in the process of removing viral DNA from larynx and tracheal tissue. The following procedure was followed in accordance with the instructions provided by the product as well as the protocol provided by the manufacturer: In a nutshell, a tiny homogenizer was used to do the homogenization on the sample of tissue. 200 ul was the amount that was employed for the DNA extraction process. Two sets of primers were used for the RT-PCR amplification: one set used a FAM-labeled probe to identify a section of glycoprotein G that is largely present in virulent strains, while another set used a Quasar-labeled probe to identify a region of J sequence downstream of glycoprotein G in attenuated ILT isolates (isolates that lack glycoprotein G).

Forward primer: 5'-CAGATCTGGCATCGCCTCAT-3'
Reverse primer: 5'-CCTGGGAACAGAACCTGAACT-3'
Probe: 5' FAM-CTAACCCGTTCGCCGCACTCG-BHQ-3'
Quasar=Cyanine (CY5) replacement
BHQ=Black Hole Quencher
FAM=flurescein amidite

URUK Center is responsible for the importation of all primers, which were all produced by Alpha DNA in Montreal, Quebec, and were made. The samples that returned a positive result for ILTV (wild strain) was then tested for three additional diseases, including infectious bronchitis (IB), Newcastle disease (ND), and avian influenza (AI), in order to confirm that it did not contain any other viruses.

### 2.3 Isolation and determination of viral titers

For the purpose of viral isolation, embryonated chicken eggs (ECE) from Al Ghadeer hatchery in Waset province were obtained from Incubal firm of Belgium. In accordance with the procedure that was approved by (OIE 2021), tissue samples were prepared from positive flocks. Eggs that were 10–11 days old were utilized. Eggs were inoculated into the chorioallantoic membrane using about 0.2 milliliters of supernatant from each of five eggs (Solis 2021), with one egg serving as a control positive. The CAMs that

ISSN NO: 2771-8840
Date of Publication: 17-09-2022

were collected in accordance with (OIE 2021). Using RT-PCR, we were able to confirm the presence of the virus. The second and third passages both took place in the exact same manner, but only with samples that had shown good results in the real time PCR test (Solis 2021). According to (Ravi, Desa, and Madhusudana 2010) 10 day-old embryonated chicken eggs were used to measure the virus' titer. Antibiotics and phosphate buffer solution (PBS) were diluted 10-fold (0.9 ml), PH 7.2-7.4, and 0.1 ml supernatant fluid was inoculated into each of four eggs per dilution through the chorioallantoic membrane route. Sterile PBS was utilized for the control group. Seven days at 37°C incubated the eggs. Embryo infective dose ($EID_{50}$) was calculated according to (Reed and Muench 1938).

## 3. Results

### 3.1 clinical examinations

Five (5) layers flocks from the Waset, Basra, and Karbala regions provided twenty (20) tissue samples (trachea and larynx). The average lifetime of these flocks is between 10 and 30 weeks, showed severe respiratory signs such as difficulty breathing, stretching of the neck and head during breathing, watery eyes (weeping eyes), and the presence of cheesy materials in the oral cavity, decreased in egg production and reduced weight gain, swollen eyelids, nasal discharge, depression, sneezing, with severe conjunctivitis also seen. Disease persisted for around two weeks, with death reaching 10% on average but 25% in certain flocks with acute form, as seen in (Fig. 3.1, 3.2). Most of post mortem lesions were in the trachea and larynx, where they were inflamed, severe hemorrhage with ulceration in their mucosa, mucous with blood clots (mucus plugs) and cheesy material filling the lumen of the trachea, congestion, and edema of the conjunctiva and lungs, there was general body congestion but viscera appeared normal as shown in (Fig. 3.3 to 3.6).

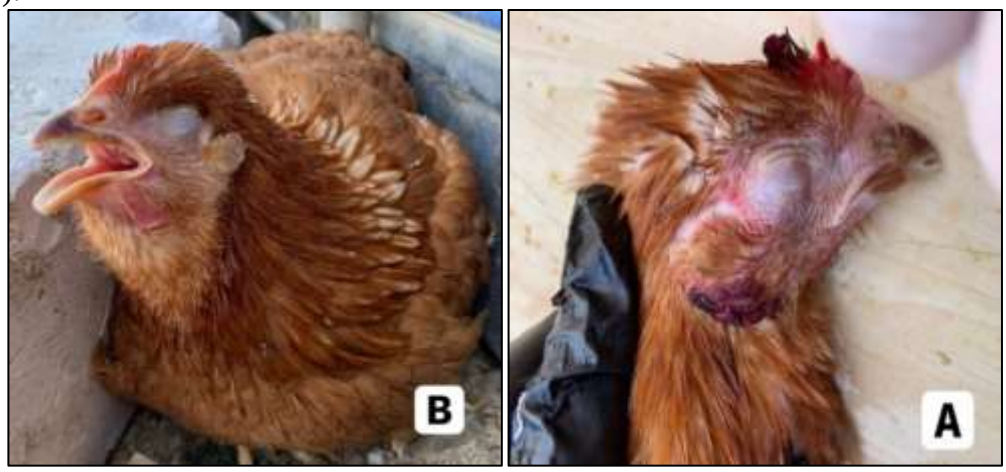



**Figure 3.1: Symptoms of infectious laryngotracheitis in layer birds include (A). the presence of bloody mucus, (B). labored breathing and elongated head and neck.**

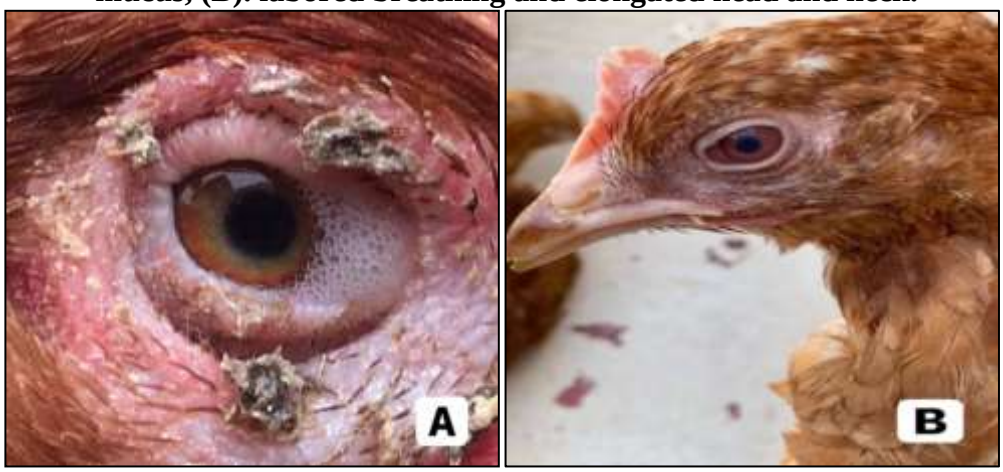



**Figure 3.2: It has been shown that certain layer birds are affected with infectious laryngotracheitis. (A). streaming eyes, B. conjunctivitis**

ISSN NO: 2771-8840
Date of Publication: 17-09-2022

**Figure 3.3: mucous plugs in the lumen of the trachea and larynx**

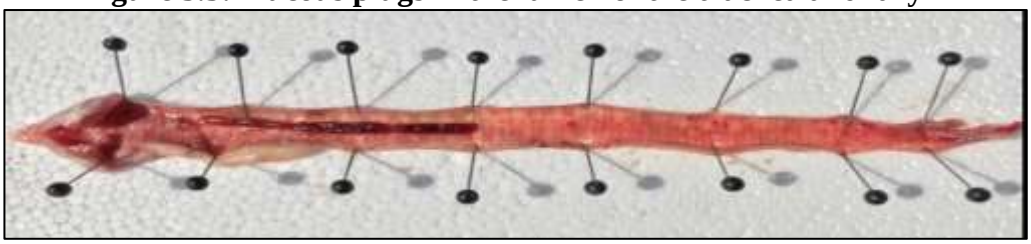


**Figure 3.4: Tracheal and laryngeal thrombus hemorrhage.**

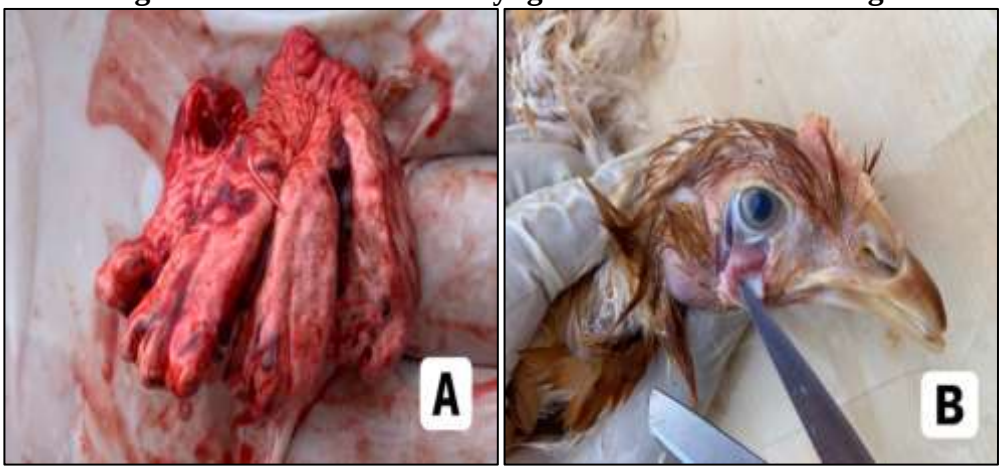



**Figure 3.5: (A) Swelling and congestion in the lungs, (B) eyelid congestion.**

**Figure 3.6: general body congestion (dark muscles)**

### 3.2 Real-time PCR sampling

After subjecting all five samples (farms) to real-time PCR analysis, only one sample tested positive for ILTV (Fig. 3.7), while another sample tested positively (although weakly) and the other three samples tested negatively. As shown by (Fig. 3.8).

ISSN NO: 2771-8840
Date of Publication: 17-09-2022

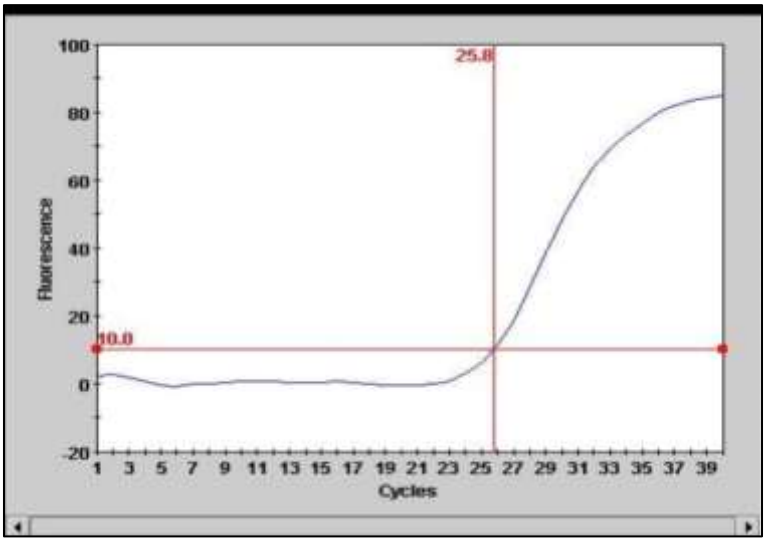



**Figure 3.7: plot of real time PCR shows the ILT virus's G gene was successfully amplified (positive results).**

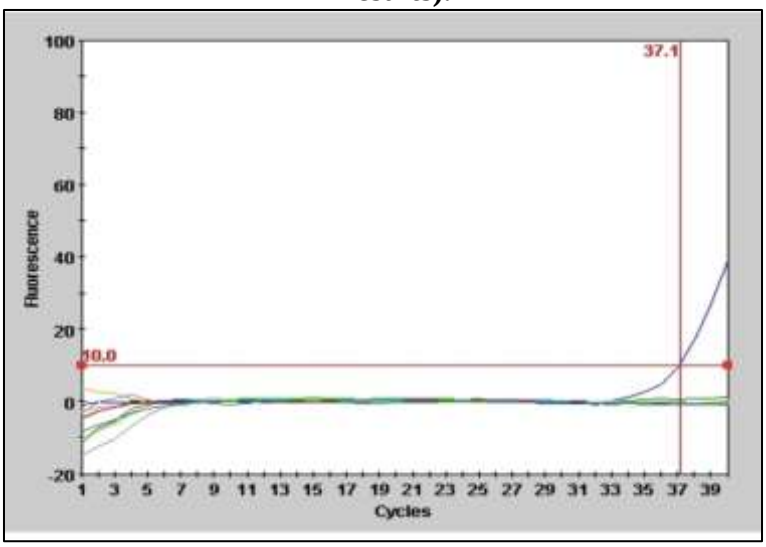



**Figure 3.8: real time PCR plot for the ILT virus G gene indicates a poor and negative result.**

In order to rule out the possibility of a cross-infection, the sample that tested positive for ILT with Ct (25.8) was also tested for ranikhet disease, fowl plaque, and avian corona virus, all of which returned negative results.

**3.3 Results of viral isolation by egg inoculation**

Six days post-inoculation, the infected CAM displayed signs of congestion, an increase in membrane thickness, and growth stunting in the first passage, whereas the control CAM showed no such signs. After 5 days of viral injection, the most typical lesion of ILT virus is white pock lesions, which are shown in the second passage. However, in the third passage, there is increasing thickness and hemorrhage of the CAM along with obvious white pock lesions as shown in the (Fig. 3.9. to 3.11).

ISSN NO: 2771-8840
Date of Publication: 17-09-2022

**Figure 3.9: CAM on the left is congested and thick, and it has pock lesions compared with control positive CAM on right.**

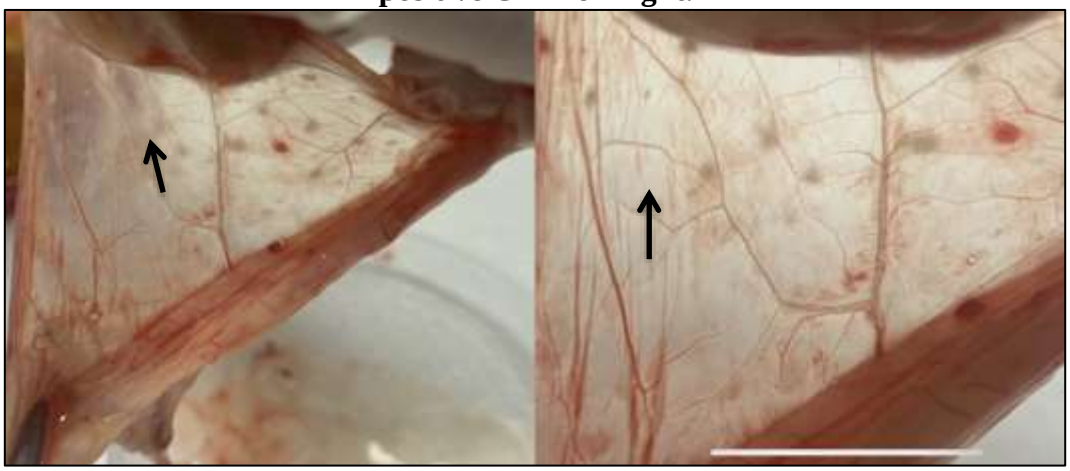


**Figure 3.10: Third-passage CAM lesions manifest as white pocks.**

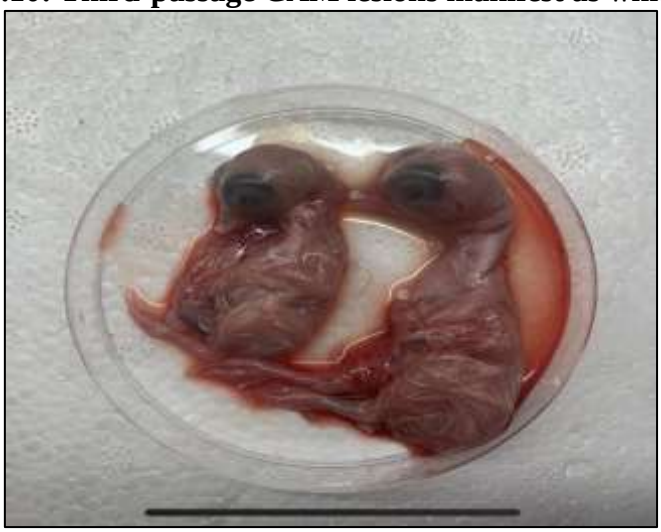


**Figure 3.11: stunting in the growth of left embryo after 6 days post inoculation compared with normal in right.**

### 3.4 Detection of isolated virus in real-time PCR

The harvested chorioallantoic membranes that were infected with ILT virus were examined by real-time PCR to detection of viral DNA after (3rd) passage as shown in (Fig 3.12)

ISSN NO: 2771-8840
Date of Publication: 17-09-2022

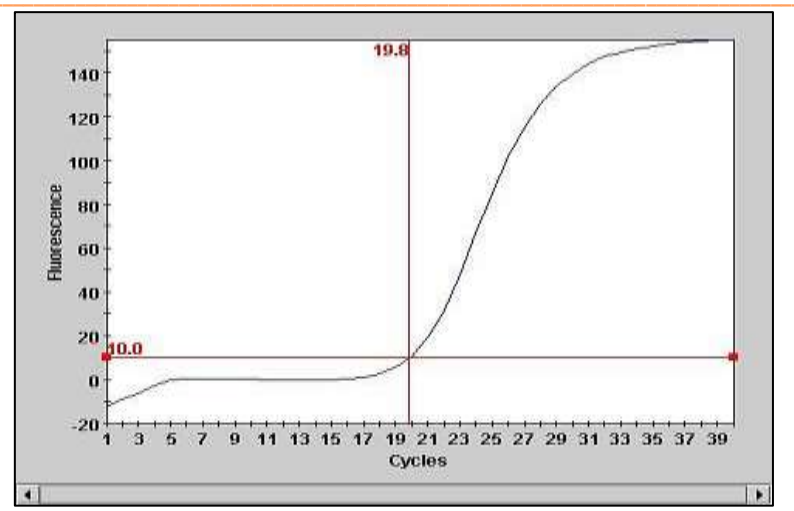



**Figure 3.12: plot depicting the results of real-time PCR amplification targeting the G gene of ILT after (3rd) passage**

### 3.5 Histopathological examination of CAM

The histopathological changes of CAM infected with ILTV show congestion, multinucleated giant cells (syncytial cells) containing inclusions bodies as cytopathic effects appear clearly after the third passage post inoculation shown in (Fig. 3.13)

ISSN NO: 2771-8840
Date of Publication: 17-09-2022

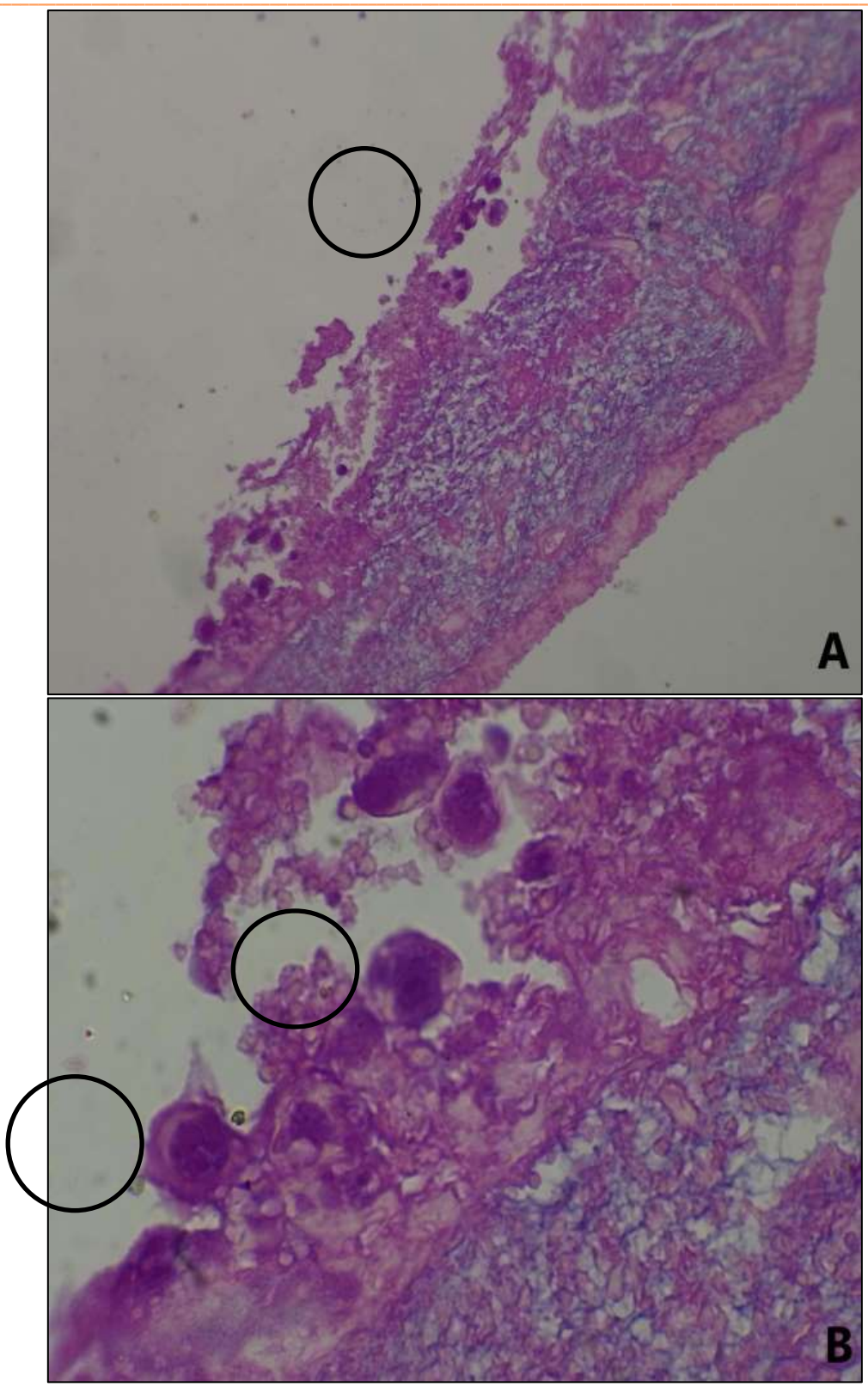



**Figure 3.13 A&B presence of multinucleated giant cells (syncytial cells) containing intranuclear inclusion bodies.**

**3.6 Results of viral titration**

The virus infectivity assay of pooled CAM in ten days old embryonated chicken eggs gave a titer of $10^4$ $EID_{50}$/0.1 ml, following the method of (Reed and Muench 1938).

**3.7 Results of experimental infection in broilers**

**3.7.1 Clinical signs**

ISSN NO: 2771-8840
Date of Publication: 17-09-2022

Clinical indicators were documented daily for each group beginning on day 2 and continuing until day 21 after infection. Breathing patterns, conjunctivitis, and the degree of activity were reviewed and rated daily for all groups of chickens. More specifically, as can be seen in (Tab. 3.1) and in the following: (Fig. 3.14.to, 3.16).

**Table 3.1: displays the clinical indications of all groups over a period of 21 days after the exposure.**

| **Clinical signs** | | **No. of cases/ Total birds** | | |
|---|---|---|---|---|
| **Groups** | | **A** | **B** | **C** |
| Breathing patterns | normal breathing | **31/50** | **25/50** | **12/50** |
| | open mouth breathing | **16/50** | **17/50** | **19/50** |
| | gasping with extended neck | **3/50** | **6/50** | **13/50** |
| | expectoration of bloody mucous | **0/50** | **2/50** | **6/50** |
| Conjunctive | normal | **40/50** | **30/50** | **19/50** |
| | swelling and partial closure of the eyes | **8/50** | **15/50** | **21/50** |
| | complete closure of the eyes | **2/50** | **5/50** | **12/50** |
| Activity | normal | **38/50** | **28/50** | **15/50** |
| | mildly depression | **9/50** | **14/50** | **19/50** |
| | severely depressed | **3/50** | **8/50** | **16/50** |

ISSN NO: 2771-8840
Date of Publication: 17-09-2022

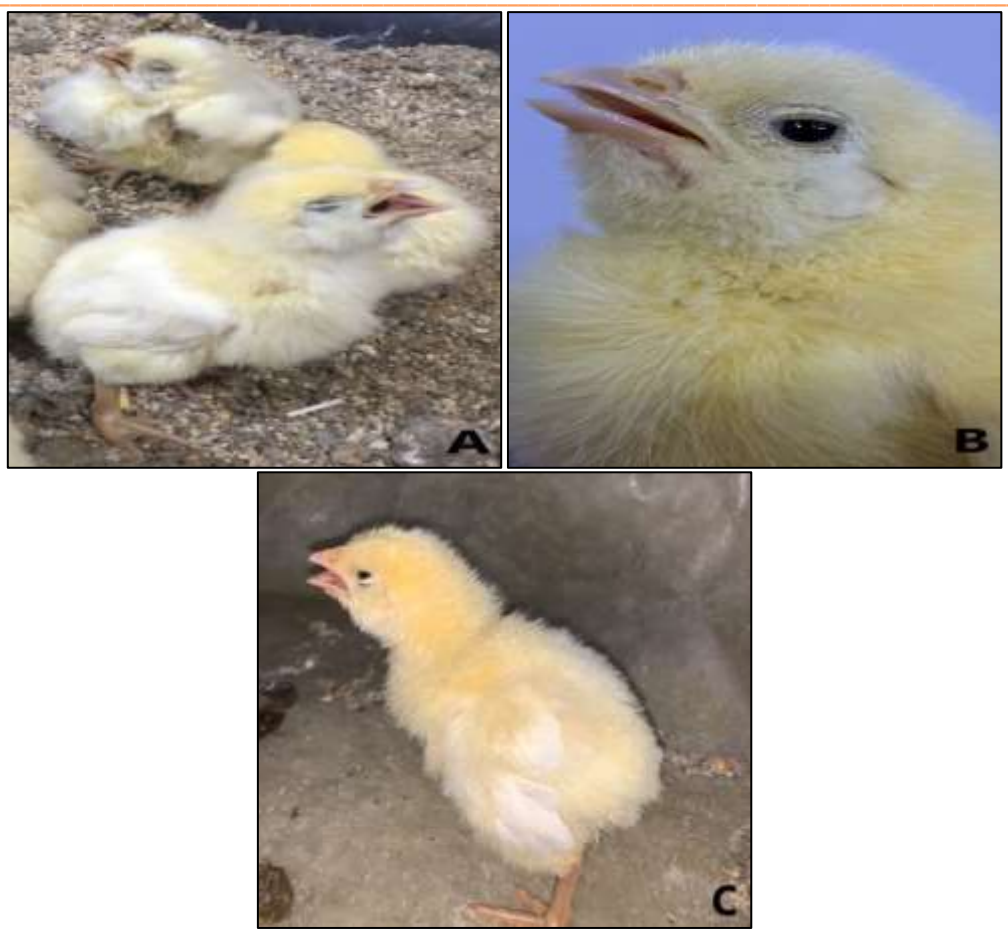



**Figure 3.14: (group A) A. depression and gasping with complete closure of eyes. B. weeping eye C. extends to head and neck.**


**Figure 3.15: (group B) A. watery eye. B. depression and completes closure of eyes.**

ISSN NO: 2771-8840
Date of Publication: 17-09-2022

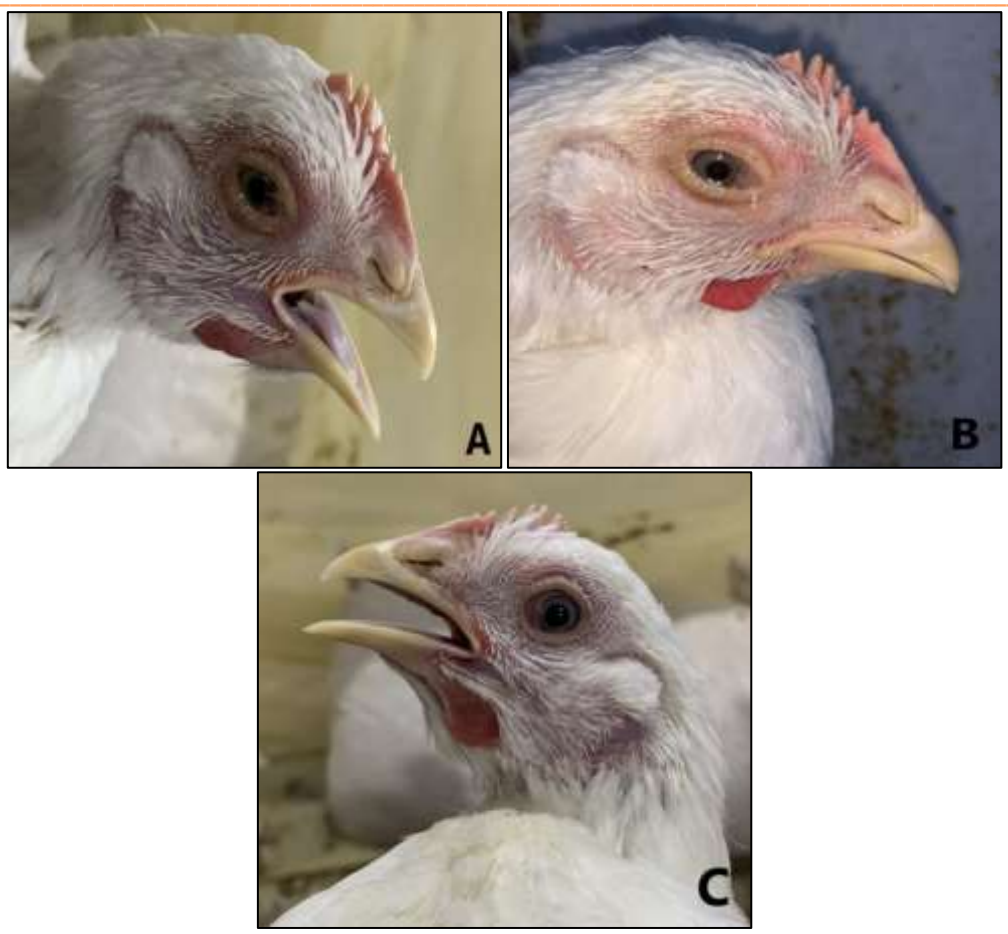



**Figure 3.16 (group C) A. extends to head and neck B. depression and watery eye C. conjunctivitis with difficult breathing.**

### 3.7.2 Mortality rate

The percentage of mortality for each group after 21 days post-infection is shown in (Tab. 3.2)

**Table 3.2: The percentage of mortality that occurred within each group 21 days after infection**

| Groups | No. of death/ total birds | Percentage |
|---|---|---|
| **A** | **4/50** | **8%** |
| **B** | **10/50** | **20%** |
| **C** | **19/50** | **38%** |

### 3.7.3 Postmortem lesions

The most significant pathological lesions that were found are shown in (Tab. 3.3) and (Fig. 3.17).

**Table 3.3: Reported gross lesions and the total number of cases in each group.**

| Gross pathology | No. of cases/total birds | | |
|---|---|---|---|
| **Groups** | **A** | **B** | **C** |
| Catarrhal tracheitis | **1/50** | **2/50** | **4/50** |
| Occluded laryngeal and tracheal lumen (caseated) | **1/50** | **2/50** | **5/50** |
| Hemorrhagic tracheitis | **1/50** | **3/50** | **6/50** |
| Fibrinonecrotic tracheitis | **1/50** | **2/50** | **3/50** |
| lung congestion | **2/50** | **4/50** | **9/50** |

ISSN NO: 2771-8840
Date of Publication: 17-09-2022

| | | | |
|---|---|---|---|
| Airsacculitis | **2/50** | **3/50** | **9/50** |

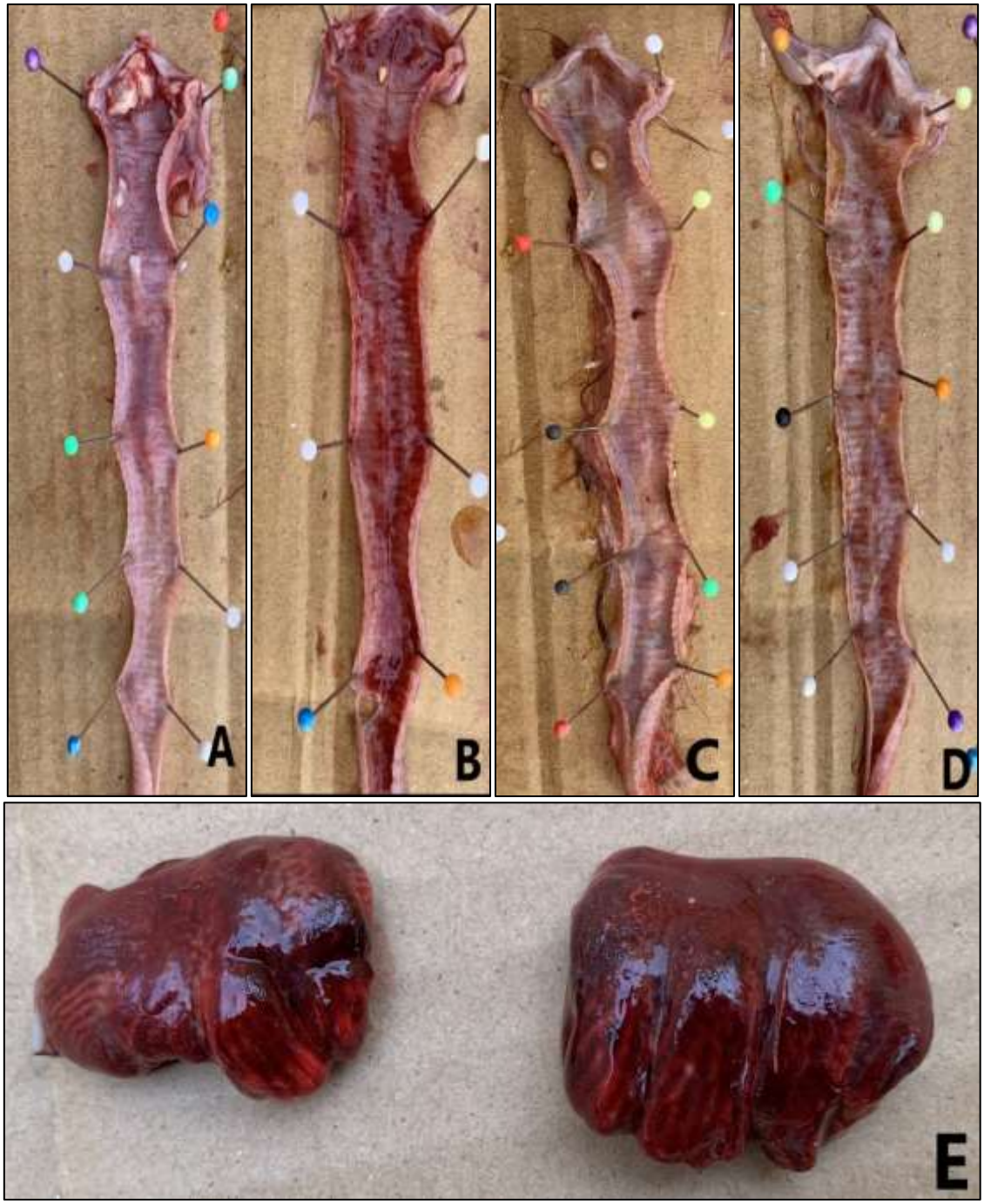



**Figure 3.17: (group C) A. caseated tracheitis B. haemorrhagic tracheitis C. catarrhal tracheitis D. fibrinonecrotic tracheitis E. lung oedema and congestion**

### 3.7.4 Results of histopathological examination

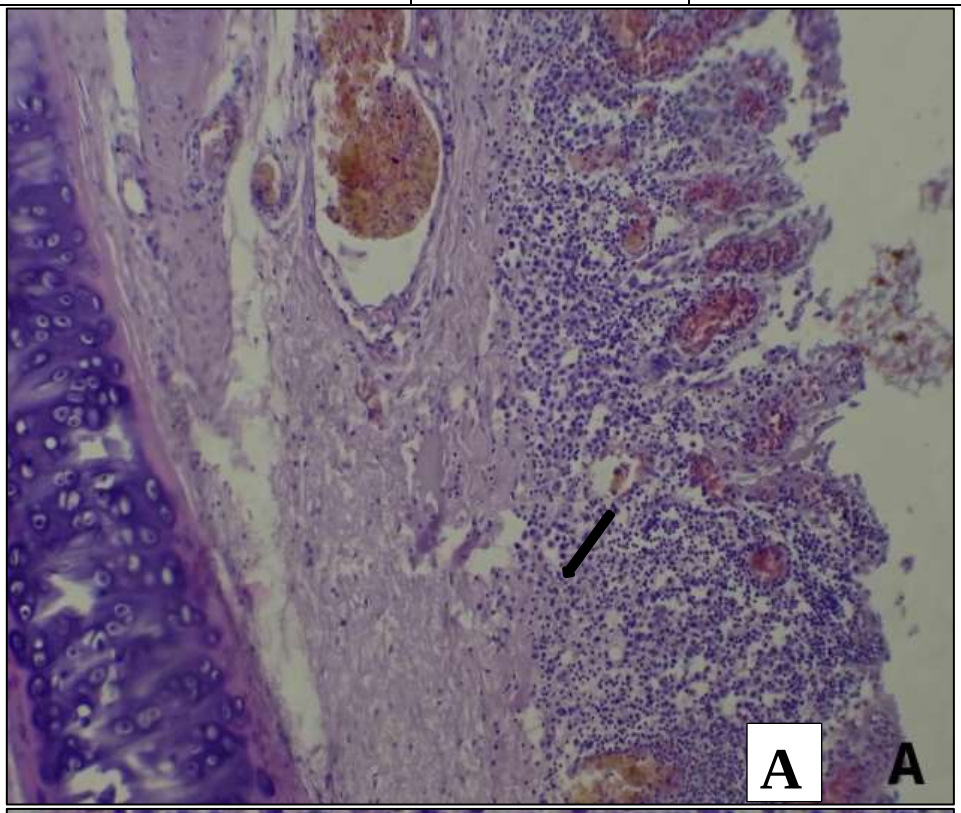
The lesions were scored and summarized in (Tab. 3.4) according to a degree of severity to score1 mild (+), score2 moderate (++), score3 severe changes (+++), and shown in (Fig. 3.18 to, 3.19)

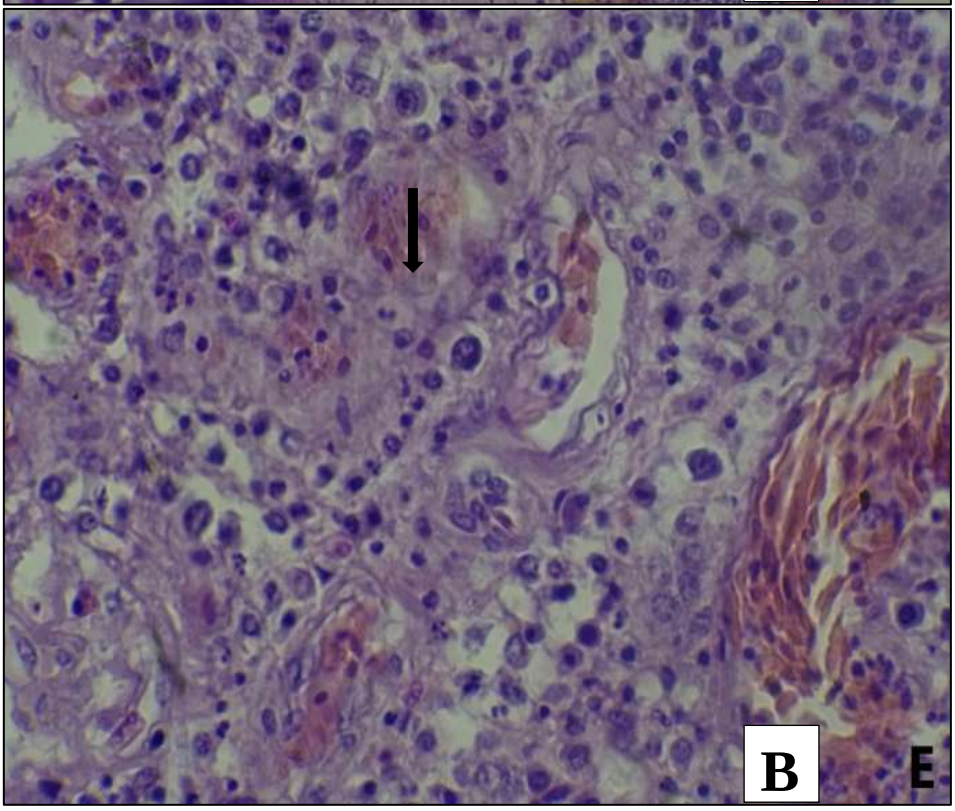
**Table 3.4: Histopathological lesions of varying severities were documented in all study groups.**

| **Histopathological lesions** | **Degree of severity** | | |
|---|---|---|---|
| **Groups** | **A** | **B** | **C** |
| deciliation | +++ | +++ | +++ |

ISSN NO: 2771-8840
Date of Publication: 17-09-2022

| | | | |
|---|---|---|---|
| Tracheal Inflammation | + | ++ | +++ |
| Tracheal necrosis | + | ++ | +++ |
| Tracheal Inclusions | + | + | +++ |
| Pulmonary congestion and edema | + | + | +++ |
| Pulmonary Inclusion | + | + | ++ |



ISSN NO: 2771-8840
Date of Publication: 17-09-2022

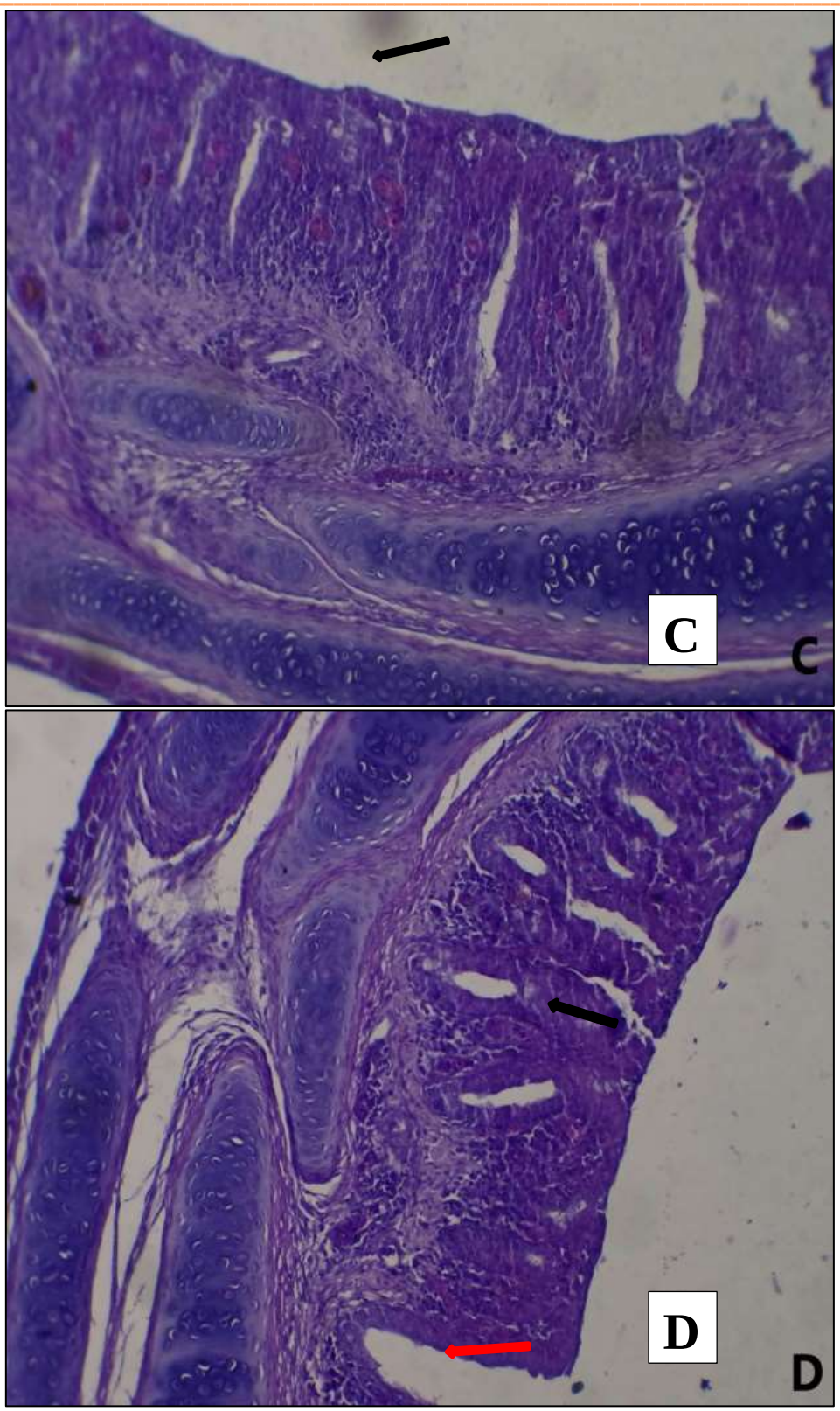
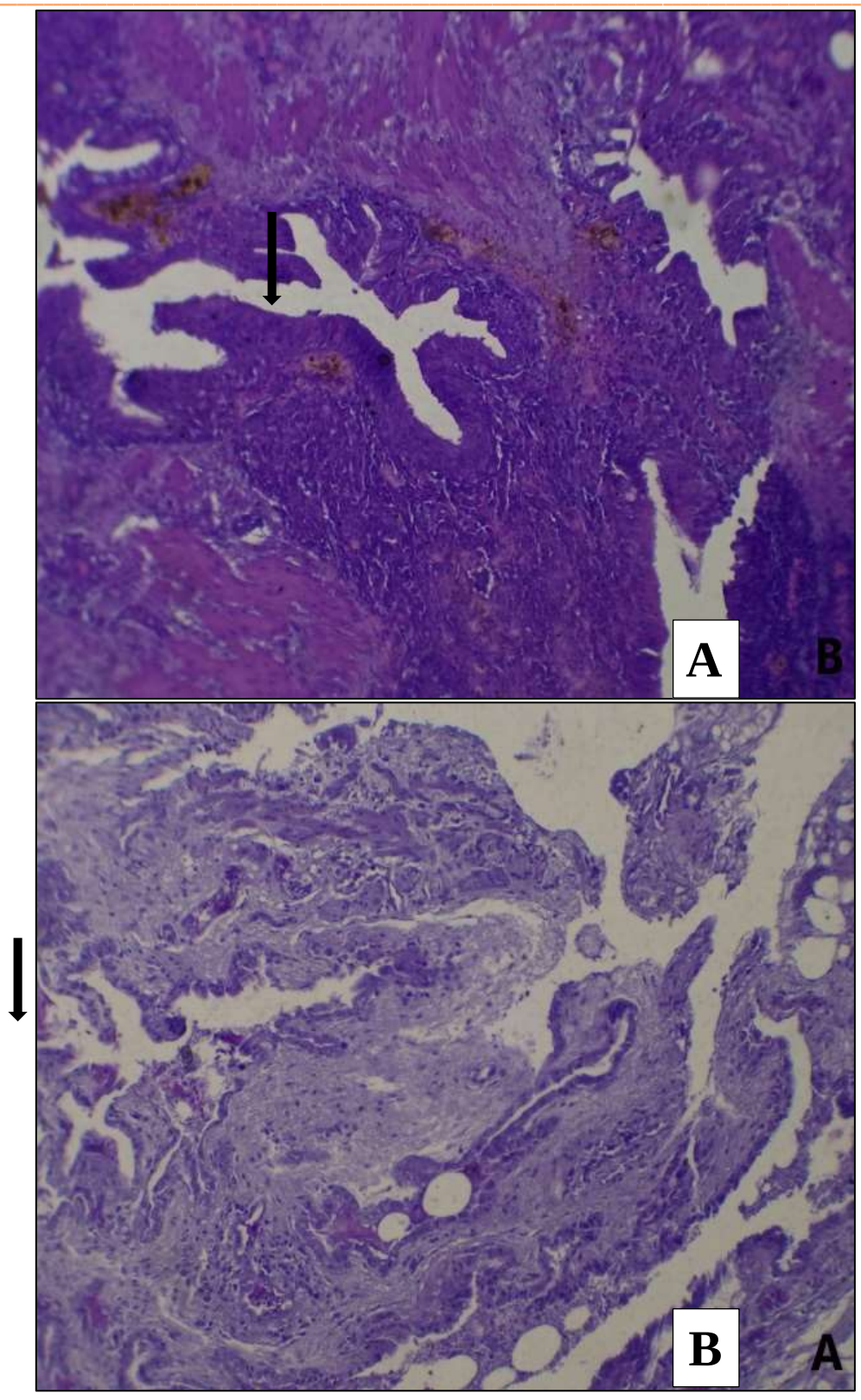



**Figure 3.18: Tracheal microscopic lesions in ILTV infected birds, A. sloughing of tracheal epithelium and hyperemia with inflammatory cells infiltration in tracheal mucosa of (group C) B. sloughing of tracheal epithelium with inclusion bodies (group C) C. hyperplasia, total ciliary loss, and diffuse mononuclear cell infiltrations in tracheal mucosa of (group B) D. cystic dilation of the tracheal gland (red) with moderate mononuclear cells infiltration in mucosa and submucosa (group A).**

ISSN NO: 2771-8840
Date of Publication: 17-09-2022



ISSN NO: 2771-8840
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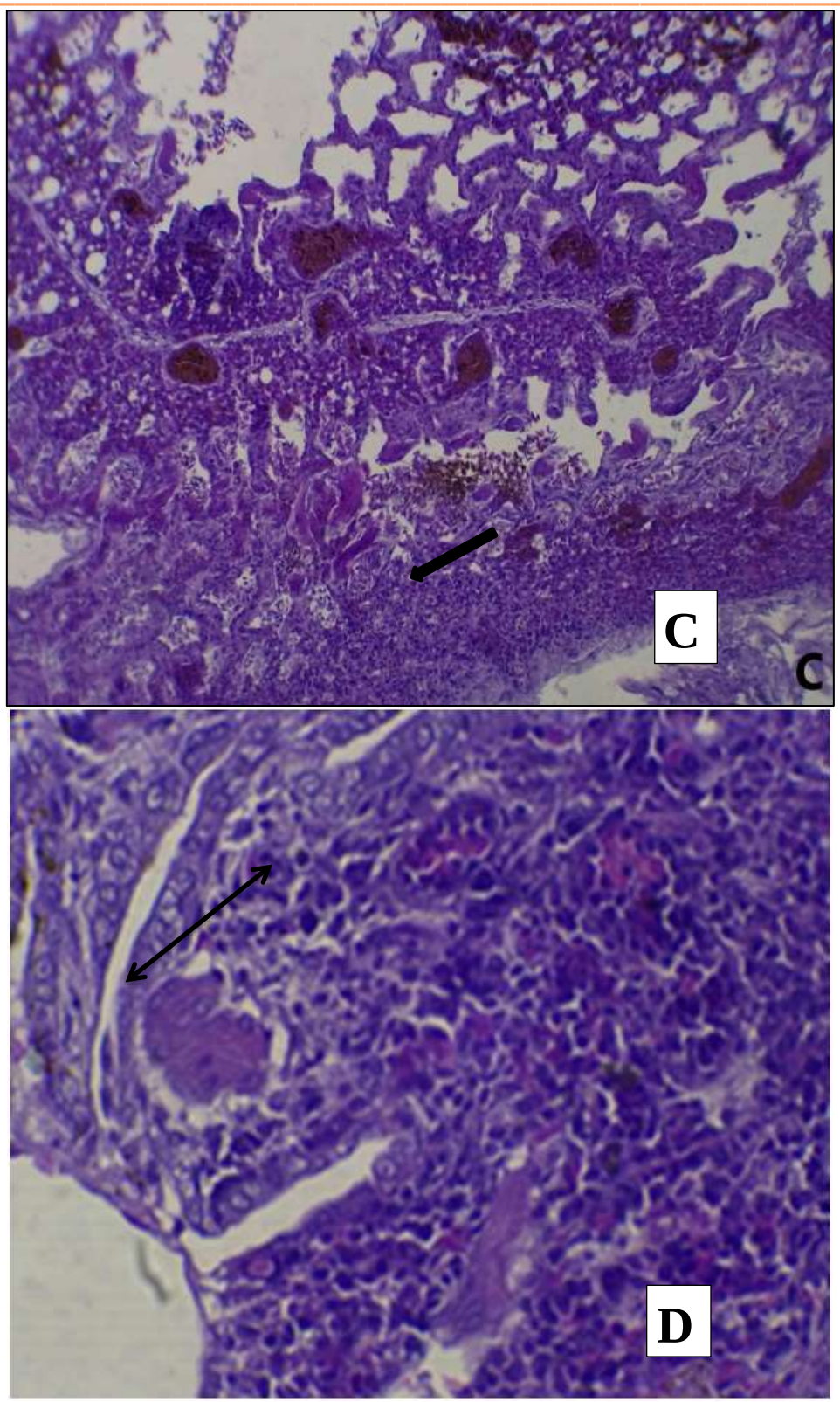


**Figure 3.19: Histopathology of the lung in ILTV infected birds. A. bronchial hyperplasia with a prominence of lymphoid-associated tissue B. fibrenheterophilic exudates in the bronchus lumen with marked destruction of primary bronchi (group B). C. congestion and mononuclear cells infiltration in atrial tissue (group A) D. diffuse mononuclear cells infiltration in parabroncheal tissue with syncytial cells (group C).**

ISSN NO: 2771-8840
Date of Publication: 17-09-2022

### 3.7.5 Results of ELISA test

the results of the ELISA test appeared for all groups starting with the maternal immunity test, which was examined 6 times for comparison with the experimentally infected groups, which in turn were examined after one, two, and three weeks post infection as shown in (Tab. 3.5, 3.6) and (Fig. 3.20, 3.21).

**Table 3.5: showing maternal antibody titers to ILTV for control negative at various ages by ELISA test (Mean ± SE).**

| | Titres of antibodies | | | | | | LSD value |
|---|---|---|---|---|---|---|---|
| | 1 day | 7 days | 15 days | 21 days | 30 days | 37 days | |
| Mean titre | 6042±260 a | 2940±159 b | 1575±113 c | 632±110 d | 61±23 d | 0 d | 882.39 * |

This means having the different letters in the same column differed Significantly.
* (P≤0.05).

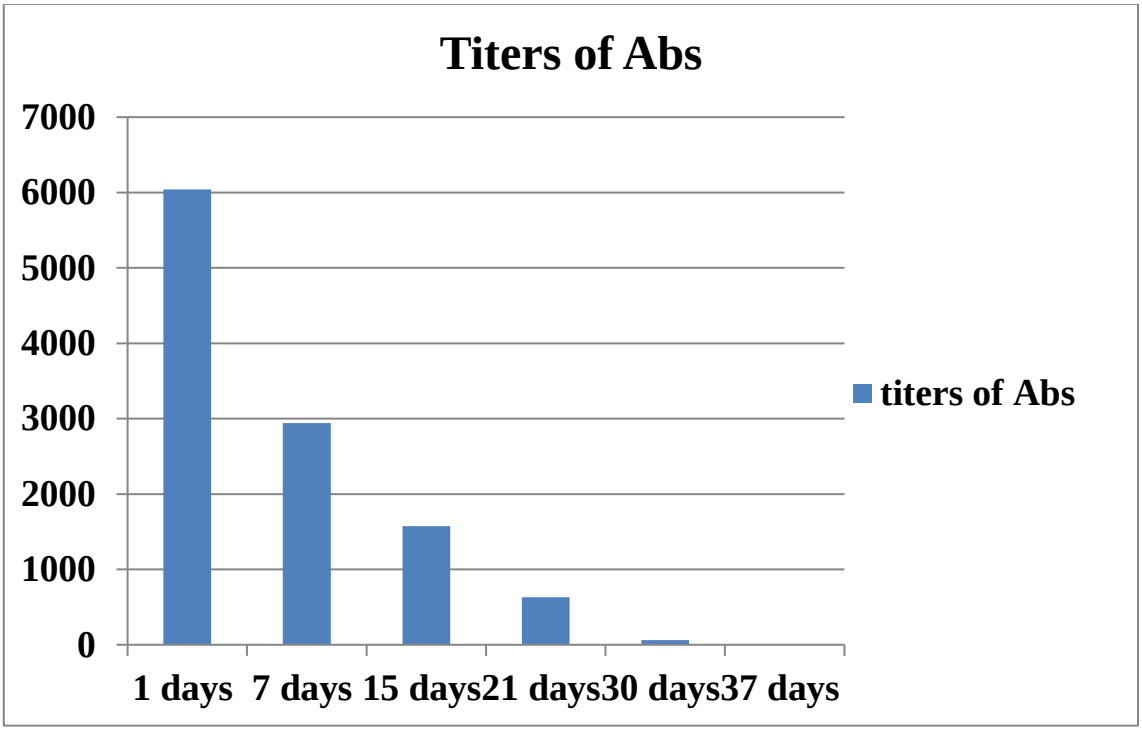


**Figure 3.20: showing maternal antibody titers to ILTV for control negative at various ages by ELISA test**

**Table 3.6: displays the antibody titers for all of the groups after the experience (Mean± SE).**

| Groups | | 7 days post-challenge | 14 days post-challenge | 21 days post-challenge | LSD value |
|---|---|---|---|---|---|
| A | Mean titre | 2568 ±221 c | 4280 ±217 b | 7965 ±226 a | 1507.48 * |
| B | Mean titre | 7330 ±401 b | 10810 ±383 a | 12400 ±498 a | 2066.51 * |
| C | Mean titre | 8550 ±320 c | 16038 ±601 b | 22626 ±1206 a | 2871.56 * |

ISSN NO: 2771-8840
Date of Publication: 17-09-2022

**This means having with the different letters in the same column differed Significantly. * (P≤0.05).**

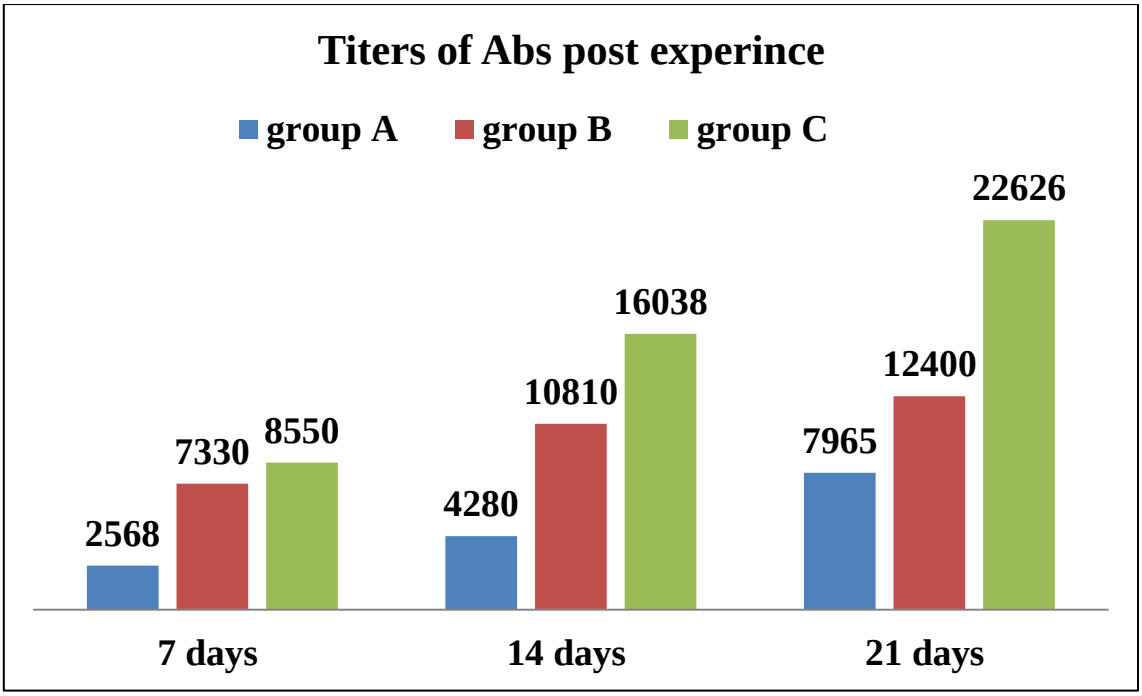


**Figure 3.21: show the antibody levels in each group three times after the experience**

## 4. Discussion

Avian infectious laryngotracheitis (ILT) is a highly contagious viral respiratory disease. That can cause economic losses during repeated outbreaks and has a global spread. Caused by the herpes virus of the order *Herpesvirales*, the family *Herpesviridae*, the subfamily *alphaherpesvirinae*, the genus *Iltovirus*, and the species *Gallid herpesvirus 1 (GaHV1)* (Gopal et al. 2022). ILT had never been documented in Iraq before the year 2000, and a policy of not vaccinating commercial flocks against ILT had been in place for many years after the disease was first reported (Al-Khidr and Al-Hayali 2000). Various studies have been done recently that point to the fact that chickens of any age may get this disease (Abdul-Aziz and Barnes 2018). It is reasonable to declare that the circulation of ILTV among backyard poultry in Iraq still exists at this time after analyzing the data from a scientific study into the epizootic situation with ILT in Iraq, especially on large chicken farms. Possible that the ILTV isolate that was collected from Iraqi layer farms in 2021 and 2022 might serve as proof. Chickens infected with ILT that were gathered from various locations were aged between 10 to 30 weeks showed a wide range of clinical signs, including depression, egg drops, coughing, difficult breathing, gasping, an elongated neck, bloody mucous, and conjunctivitis. These symptoms are typical of the mild and acute forms of the disease and corroborate those mentioned by (Farag and Eissa 2021)(Ponnusamy et al. 2021). The gross pathological lesions of the field cases demonstrated congested tracheal mucosa, the presence of caseated material, and blood clots along the lumen of the trachea. In some instances, there was also a blockage of the laryngeal lumen by caseous material. These post-mortem lesions are very much like those that were discovered by (Bayoumi et al. 2020)(Carnaccini et al. 2022). It is not feasible to diagnose ILTV based just on clinical symptoms and PM, which is why it is vital to use other diagnostic procedures. In light of this, we carried out molecular characterization. The use of the results from many investigations reveals that vaccine-type and wild-type isolates of the virus are present in chicken farms (Thilakarathne et al. 2020)(Veretsun et al. 2021). Molecular diagnostic techniques are now the most effective ways available for distinguishing between vaccines and wild strains (Loncoman 2018). As a result, real-time polymerase chain reaction (RT-PCR) was used in the process of identifying samples obtained from farms that were thought to be affected by the disease. One sample from the five farms that exhibited indicators of disease was positive, one sample was weak, and the

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Date of Publication: 17-09-2022

other samples were negative. The publication of the results of the molecular detection demonstrated that the sample that had been collected was infected with a wild strain of ILT. According to the research that he carried out, there is a chance that birds might be infected with more than one virus at the same time. This is so since the potential of a common viral infection exists (Taher, Amer, and Saad 2017). To rule out the possibility of cross-infection, the sample that tested positive for ILT with a high Ct (25.8) was also examined for the presence of Ranikhet disease, fowl plague, and avian corona infection; all three analyses came back negative. The remaining samples that tested negative for ILTV might potentially harbor ILT-similar viruses such as AIV, NDV, or IBV. The isolation of ILTV on ECE using the CAM pathway was the second stage in determining its presence and for experimental inoculation. Diagnostic virus isolation is still considered to be the golden standard technique for ILTV (Guy et al. 2003). A widespread edema and pinpoint yellowish-white pocks were produced on the CAMs of infected eggs that occurred from the second passage after the viral solution was inserted in ECE through the CAM route. When compared to non-infected CAM, the membranes seem hazy and considerably thicker in the inoculated sample. (Ali et al. 2014) identified a pock lesion after two passes, whereas (Magouz 2015) detected a pock lesion after three passages, and (Islam et al. 2010) detected a pock lesion after four passages. This is in contrast to our data, which exhibited pocks fast on CAM in the second passage. This might perhaps be linked to the high viral concentration as well as the virus's excellent responsiveness to ECE. The findings of stunted embryos and death within four to seven days after inoculation are consistent with those of other investigations (Islam et al. 2010)(Magouz 2015). Histopathological analysis of the infected CAM showed intranuclear inclusion bodies as well as the presence of multinucleated giant cells (syncytial cells) in the infected CAM, which is conflicting with that which was previously described (Ali et al. 2014)(Ponnusamy et al. 2021). After the third passage, the extracted chorioallantoic membranes that had been infected with the ILT virus were subjected to real-time PCR for the detection of viral DNA again and CT was determined to be the correct value (19.8), This indicates an increase in viral load after cultivation which is the equivalent of (Allawe et al. 2016). Titration of the isolated virus to determine the median embryo infective dose (EID50) per milliliter on embryonated chicken eggs that were ten days old produced a titer of $10^4$ $EID_{50}$/0.1 ml. This titer was determined using the statistical approach outlined by (Reed and Muench 1938). This is in accordance with the procedure that was discussed earlier (Reddy et al. 2014) as well as the work that was done by (Nagy et al. 2020)(OIE 2021). Following conducting an experiment in which broiler chickens were intranasally administered an isolated ILT virus from locally layer farms; clinical indications were recorded daily beginning on day 2 and continuing until 21 days after infection for each group. Every day, the breathing patterns, conjunctivitis, and levels of activity of all the groups were observed, evaluated, and compared. The clinical indications reached their highest point between 5 and 6 days PI, and then gradually started to get better between 8 and 10 days PI. Those birds that had varying degrees of clinical symptoms, There were 19 birds out of 50 in group A that were infected at the age of one day, with a mortality rate of only 8 percent of all cases; there were 25 birds out of 50 in group B that were infected at 15 days of age, with a death rate of 10 percent; and there were 38 birds out of 50 in group C, also with death, which is 38 percent of all cases. The catarrhal tracheitis, blocked laryngeal and tracheal lumen (caseated tracheitis), hemorrhagic and fibrinonecrotic tracheitis, lung congestion, and airsacculitis are the most significant pathological gross lesions that were seen in all groups compared to group D (the control), but its appearance varied in degrees; therefore, group A had the least number of pathological lesions compared to group B, which was more affected than group A but less affected than group C; accordingly, group C was the most group in which pathological lesions appeared. As is the case with the macroscopic lesions and clinical symptoms, the microscopic alterations that manifested (deciliation, tracheal Inflammation, tracheal necrosis, tracheal Inclusions, pulmonary congestion and edema, pulmonary Inclusion) themselves may also be categorized into group A (+), group B (++), and group C (+++), respectively. This indicates that group A was the group that was least affected by the infection, whereas group C was the group that was most affected by the infection. These findings are accurate taking into account the maternal ABs that were passed on from the breeders to the chicks show that the mean titer at 1 day age 6042 ± 260 as standard error as shown in (Tab. 4.5), the ELISA tests showed that it offered some degree of protection, particularly for group A, which indicates that group A was the one that was harmed to a lesser extent. There may be an increased risk of sickness and mortality in birds whose antibody levels are lower than the mean value mentioned. We conducted the

ISSN NO: 2771-8840
Date of Publication: 17-09-2022

ELISA test seven days after the infection, and the results showed that the antibody titer had reduced. This was because antibodies and antigens had neutralized each other, which caused the titer to drop. It was until 14 days after infection that the titer started to rise, and this indicates that the plasma cell had begun producing antibodies in response to the antigen, which is proof of an immunological response. The ELISA test shows that the titer has increased significantly, which are solid proof of the immune response to the virus after 21 days of infection. Also because of the long incubation time for the virus in natural infections than in experimental infections, it seems that no early natural infections have been documented. Natural infections may have an incubation time of 6-14 days, whereas experimental infections, as described by (Vagnozzi et al. 2015)(Loncoman 2018)(Solis 2021), have a shorter incubation period. It's possible that the pathological indications would develop after 4 to 5 days in group A, which would explain why early natural infections of the disease aren't recorded. The infection had a greater impact on group B than it did on group A with regard to the number of mortality, clinical symptoms, and pathological abnormalities. This is due to the fact that the maternal antibody titer at the age of 15 days is just a quarter of what it was at the age of one day, which results in the bird being more susceptible to the infection. While group C was the most impacted, the antibody titer shows a considerable increase, and this implies something that indicates the high sensitivity to infection as the bird gets older owing to the full elimination of maternal immunity. Nevertheless, group A was the least affected. But group C most affected. Also, based on what (Beker et al. 2004) said, we can say that as a bird gets older, especially in commercial farms, ammonia gas, even in small amounts, dust, and extremes of heat and cold all affect how well the cilia in the tracheal mucosa work. This makes the bird more likely to get all of the respiratory diseases. This includes ILT. This is in agreement with the findings of a number of studies that said that outbreaks were seen in broiler hens aged between 3 and 4 weeks (Ou and Giambrone 2012) (Vagnozzi et al. 2015) (Tsiouris et al. 2021).

## 5. Conclusions

We conclude the following based on the results of this study:

1. Wild strains of ILTV can cause embryo mortality after 6 days of inoculation.
2. If environmental conditions are favorable, maternal immunity to ILTV may give considerable protection for two weeks.
3. After inoculation, the lowest antibody titers were found in group A, whereas the highest were found in group C.
4. Group C had the most severe clinical symptoms, pathological lesions, and deaths, whereas group A had the least.
5. 1 day old birds (group A) are less likely than 15 day old birds (group B) to infected with ILT, while the latter are less likely than 30 day old birds (group C).

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