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# PATHOLOGICAL STUDY ON LARYNGOTRACHEITIS IN LAYERS

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## SUMMARY

A clinical conditions resembling infectious laryngotracheitis were diagnosed amongst 20,000 , 18,000 , 16,000 and 17,500 respectively, 28-30 weeks old, ISA brown layers.

The hens had nasal discharges, moist rales, coughing and gasping. Hemorrhagic mucous was ejected during sneezing, lacrimation, conjunctivitis with facial swelling with eyes partially or completely closed.

Postmortum examination of dead and affected hens revealed hemorrhagic tracheitis with thin pseudomembrane formation. The larynx, congested with petechia on mucous membrane, Infraorbital sinus contained clear thick fluid.

Histopathological examination of trachea showed hypertrophy, of epithelial cells, loss of cilia and pseudostratification of the mucosal cell surface, extensive hemorrhages and desquamative necrotizing tracheitis with mononuclear cells infiltration. Multinucleated gaint cells in the

ciliated epithelium containing round, oval shaped intranuclear inclusion bodies. The lamina propria shows edema, marked congestion with lymphocytic infiltration.

A presumptive diagnosis of laryngotracheitis was made.

## INTRODUCTION

Infectious laryngotracheitis (ILT) is an acute viral disease occurs primarily in mature or nearly mature chickens although all age groups are susceptible, characterized by marked dyspnea, coughing, gasping and expectoration of blood (1). It is also reported in pheasant and pheasant-chicken crosses and in peafowl (2). ILT was reported by many outhers in different countries ( 3,4,5,6,7,8).

Diagnosis of ILT in chickens can be based on results of histopathology although other methods are widely used (9). Ultrastructral studies of cytopathic changes have been done in Vitro (10), and in Vivo (11). The disease (ILT) is not yet reported in Iraq, in this study we described the histpathological findings of tracheal specimens obtained from layers show signs and lesions of laryngotracheitis.

## MATERIALS AND METHODS

The study was performed on three outbreaks, which occurred in the commercial layer farms of different private sector companies in Baghdad and other Province where layers are raised in large numbers.

The first outbreak occurred amongst 20,000 , 30-31 weeks old of which 20 chickens died on 1996, the second outbreak occurred in other company amongst 18000 , 16000 and 17500 respectively, 28-30 weeks old on 1997.

The hens showed clinical signs of wheezing, gasping and coughing in a few birds. Deaths followed shortly with signs of asphyxiation and thereafter the disease spreads rapidly in susceptible hens. The majority of deaths usually occur within 12-15 days of onset of the disease.

Egg production often declines temporarily and significantly. Individual birds showed signs of coughing, gasping, rales, dyspnea, lacrimation, conjunctivitis, serous nasal discharge and facial swelling with eyes partially or completely closed ( Fig.1).

Postmortum examination was done on dead birds. Tissue samples were taken from larynx, upper and mid-tracheal regions and placed in Bouin's fixation for histopathology. Processing for routine histopathology consisted of fixation, dehydration, clearing, embeding, sectioning and staining with hematoxylin and eosin (H&E). Five to six microns-thick sections were mounted on slides and examined with light microscope at various magnifications.

Fertil chicken eggs were used for growth, isolation and identification of ILT virus by inoculation of 0.2 ml of laryugotracheal tissue suspension onto the chorioallantoic membrane (CAM) of 11-day-old according to the method of (12).

Tracheal and sinus swabs were kept in sterile containers for bacteriological examination.

## RESULTS

1. Gross pathology:

The lesions of ILT are restricted to the respiratory tract and are pronounced in the larynx and upper trachea. The mucous membrane of larynx and tracheas exhibit variety lesions, such as collection of blood-stained mucous, a yellow cheesy plugs or thin psedomembrane formation. Other infected birds showed bloody beak, conjunctivitis and sinusitis (Fig.1). Air sacs were clear. Some birds show only mild congestion with small punctiform hemorrhagic areas. No lesions were detected in all other internal organs examined.

2. Histopathology:

At the onset of the disease, histopathological examination showed hypertrophy, loss of cilia and psudostratification of the mucosal cells surface (Fig.2).

The tracheal epithelium shows extensive hemorrhages and are also evident in the subepithelial layer. The tracheal mucosa showed desquamative necrotizing tracheitis, with mononuclear cells infiltrations. Lesions begins as small foci of cell syncytia and multinucleated gaint cells in the ciliated epithelium (Fig. 3 , 4 ).

Some sections showed rounded, oval shaped intranuclear inclusion bodies within the epithelial cells or in epithelial cells which have recently been desquamated (Fig.5).

The lamina propria showed edema, marked congestion with moderate lymphocytes, monocytes cells infiltrations (Fig.3). Some sections showed deciliation, metaplasia and hyperplasia only with cellular exudate mostly composed of lymphocytes and heterophils with few erythrocytes.

3. Prelminary isolation and indentification:

CAM of the inoculated chicken embryonated eggs had specific ILT pock formation on 3-6 days after inoculation. The pocks were limited to the inoculated areas, they were numerous, thick, grayish, large plaques. Upon the second passage, all CAM of inoculated embryos had pocks dispersed all over, Fig.(6).

4. Bacteriology findings:

Bacteriological examination of tracheal and sinus fluid swabs showed the presence of E. coli in the samples.

## DISCUSSION

Infectious laryngotracheitis is an acute respiratory disease of chickens that may result in reduction of body weight, drop in egg production and lowering hatchability (13).

The characteristic lesions showed in tracheal mucosa were desquamative necrotizing tracheitis and the degenerated epithelia begins as small foci of cell cyncytia and multinucleated gaint cells containing large single oval or rounded shaped intranuclear inclusion bodies (Fig.5), and these are an important differential feature which distinguishes ILT from other respiratory diseases, resembling findings seen by (14,15).

The destructive tracheal mucosa are due to combination effect of the virus and pressure producing by edema and cellular infiltration (16).

Chickens showed respiratory signs and lesions may be easily confused with other respiratory diseases. ILT is only avian respiratory disease that cause a desquamative necrotizing tracheitis of the mucosa (1), other respiratory

disease such as infectious bronchitis (IB), is characterized by greatly thickened tracheal epithelium with diffuse infiltration of the subepithelial and deeper layers of the mucosa with histiocytes, plasma cells and massive infiltration of lymphocytes without any hyperplasia and sloughing (16), whereas in Newcastle disease infection, the most common histopathological reaction of the tracheal tissue is that of moderate to sever edema of submucosa (17).

A thin psudomembrane that may completely blocked the lumen of trachea or larynx should be differentiated from the diphtheretic form of fowl pox. In fowl pox the tracheal lesions are typically ulcerative, yellow patches that are difficult to remove, in contrast to the easily removed exudative pseudomembrane formed with ILT virus. The histological appearance of affected cells are gross hyperplasia of tracheal epithelium with cytoplasmic inclusion bodies (16).

The CAM route is commonly used for ILT virus isolation. It is the most sensitive route of inculation and the simplest and most direct method for primary isolation of ILT viruses (18). The development of CAM lesions in embryos is good evidence of the disease, however, for complete identification this should be comfirmed by microscopic examination for intranuclear inclusions in the CAM or embryos.

Several studies independently confirmed that ILT virus is usually present in tracheal tissues and their pathogenicity is limited (19), and that the death recorded in the field might be due to secondary infection with E.coli .

No any lesions were seen in lungs and air sacs of laryngotracheitis infected chickens. This findings dose not support the observations of (20), it might be due the variation in virus strain, vaccination programs and different predisposing factors.

## ACKNOWLEDGMENT

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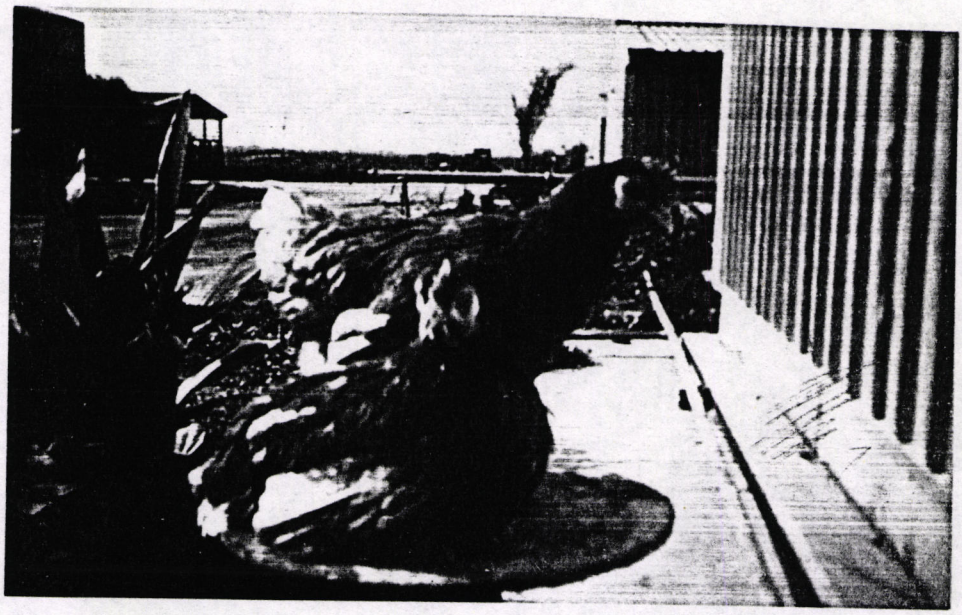

Fig. (1) Hens showed conjunctivitis, swelling of infraorbital sinus and nasal discharge.

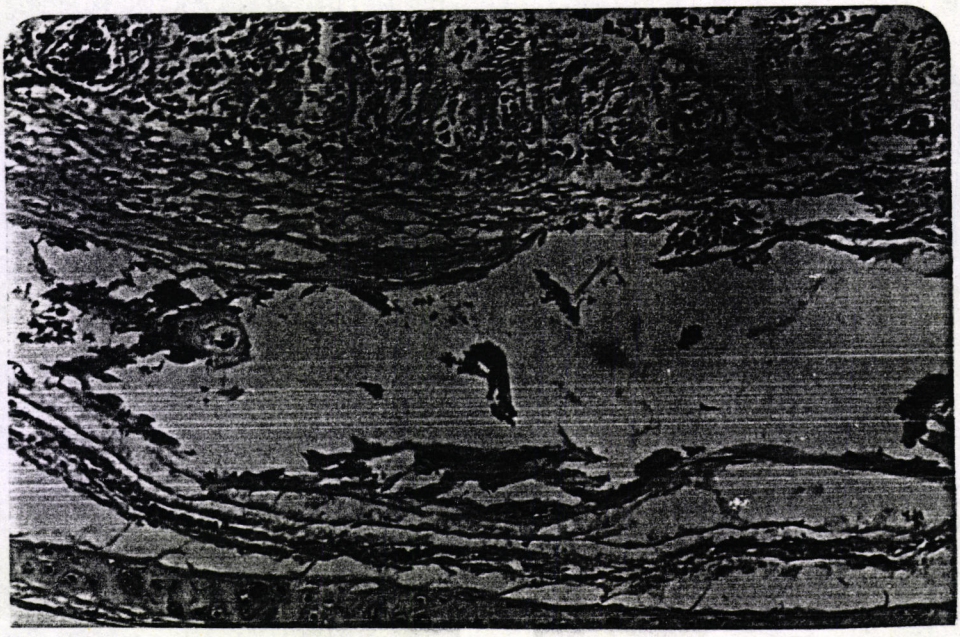

Fig. (2) Trachea of chicken showing loss of cilia and desquamative necrotizing tracheitis with lymphocytic infiltration.

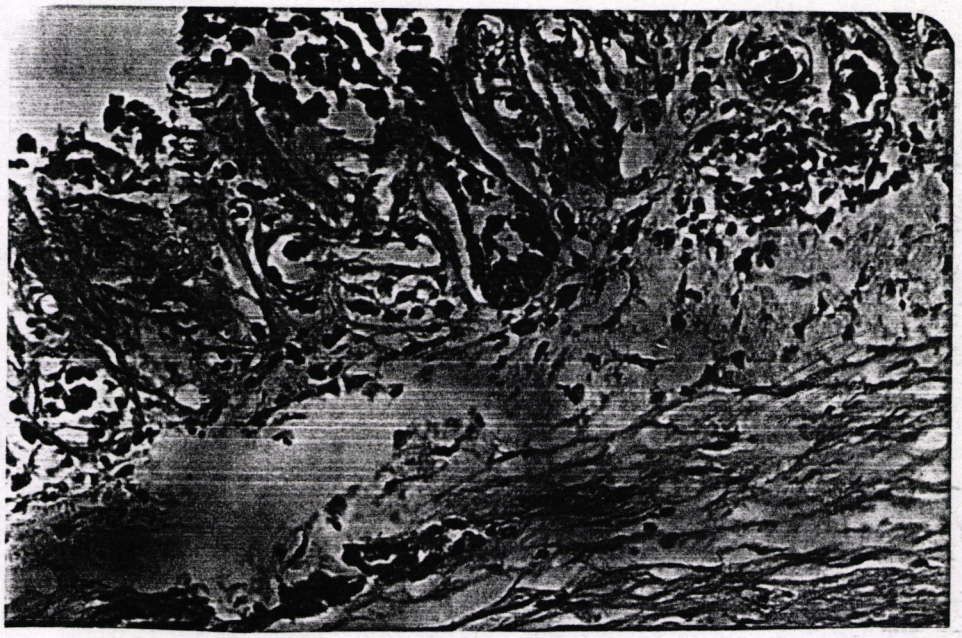

Fig. (3) Trachea of chicken showing hypertrophy of mucosal cells with submucosal edema.

Fig. (4) Trachea of chicken showing numerous cyncytial cells with heavy lymphocytic infiltration of necrotizing mucosal cells.

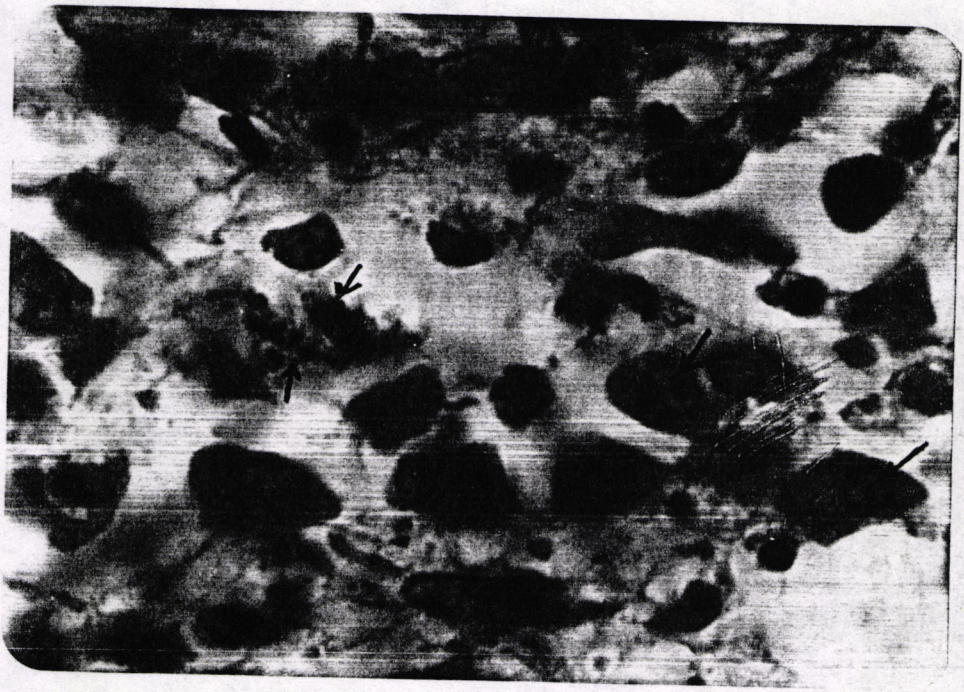

Fig. (5) High magnification of slughed cells showing oval intranuclear inclusion body ( ↑ ) surrounded by hallow space.

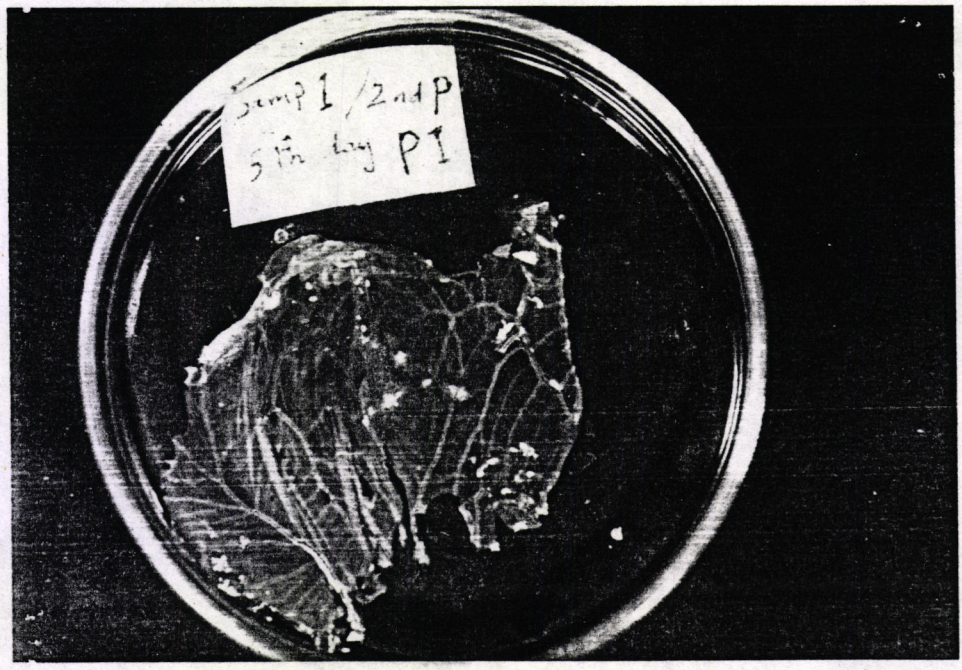


Fig.(6). Chorioallantoic membrane of 11 day-old showed typical pock lesions on fifth day p.i.

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# دراسة مرضية لالتهاب القصبات والحنجرة المعدي في الدجاج البياض

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## الخلاصة

شخصت حالات مشابهة لمرض التهاب القصبات والحنجرة المعدي في دجاج بياض نوع ايسا البني بعمر 28–30 أسبوع. لوحظت اعراض تنفسية على الدجاج المصاب شملت افرازات منخرية ، خرخرة رطبة ، كحة وصعوبة تنفس وعطاس مصحوب بمخاط مصبوغ بالدم ، تدمع والتهاب ملتحمة العين مع انتفاخ الوجه.

تميزت التغيرات المرضية بوجود التهاب القصبات النزفي مع تكون غشاء كاذب على بطانة القصبة، احتقان في الحنجرة مع بقع نزفية في بعضها واحتواء الجيوب الانفية على سائل سميك رائق.

تميزت التغيرات النسيجية بسمك بطانة القصبة مع فقدان الاهداب وتوسف وتموت في ظهارة القصبة ، تخلل خلايا احادية النواة في الطبقة المخاطية مع وجود خلايا عملاقة متعددة الانوية تحتوي علىاجسام اشتمالية نواتية.

اعطي تشخيص مبدئي لمرض التهاب القصبات والحنجرة المعدي في الدجاج البياض.