Pathological Studies On Infectious Laryngotracheitis In Chicken

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ABSTRACT

The clinical signs of infections laryngotracheitis (ILT) were characterized by severe respiratory manifestations in the form of gasping, dyspnea, rales and extention of head and neck with expectoration of bloody tinged exudates. The lesions were observed in Larynx and trachea and in severe cases the lesions were extended to the lungs. The lesions were diphtheritic fibrinous membrane, desquamated epithelium, congestion, hemorrhage, and round cells infiltrations.

The ILT virus was isolated on CAM of embryonated chicken eggs and identified via experimental inoculation of susceptible chickens and agar gel precipitation test.

The study of post vaccinal reaction of one tissue culture adapted vaccine and one chicken embryo adapted vaccine revealed that the post vaccinal reaction induced by the chicken embryo adapted vaccine was more severe than that produced by tissue culture vaccine.

INTRODUCTION

Infectious laryngotracheitis is a viral respiratory disease of chickens that may result in severe production losses due to mortality and / or reduced egg production *(1,2)*. I LT doesn't occur in chicken less than 3 weeks of age due to protective effect of maternally derived antibodies *(3)*.

Signs of respiratory depression, gasping, expectoration of bloody- mucus and high mortality characterize severe epizootic forms of infection. Mild enzootic forms of infection are encountered increasingly in developed poultry industries and manifest variously as mucoid tracheitis, sinusitis, conjunctivitis, general unthriftness and low mortality *(4-6)*.

The lesions were observed in larynx and trachea. Mucoid tracheitis, severe congestion and hemorrhage beside mucus or blood clot in trachea and Larynx were observed. Diphtheretic membranes covering the tracheal mucosa were observed in severe cases. The inflammation may extend down the bronchi into the lungs. Microscopically, the tracheal mucosa showed degenerated epithelium, congestion, hemorrhage and infiltration of the edematous lamina propria by lymphocytes and heterophils. In severe cases, necrosis of epithelial lining with protrude blood vessels into lumen resulted in hemorrhage were observed. The tracheal lumina were filled with fibrin, erythrocytes, mucus, heterophils and macrophages. The lungs showed congested blood vessels, perivascular edema and round cell infiltration *(7-12)* .

Since ILT was introduced to Egypt in 1983, *(13)* prophylactic immunization has been developed in flocks of commercial layers and breeders. Some ILT vaccinated chicken flocks showed severe post vaccinal reaction that extend to complicated cases for 3 weeks *(14)*.

The aim of the present work was to study field outbreaks and pathogenicity of isolated virus to chickens. In addition, study immune response and post vaccinal reaction in vaccinated chickens with both tissues cultured adapted and chicken embryo adapted ILT vaccines.

MATERIAL AND METHODS

Samples

Samples were collected from affected chickens; aged 28 to 40 days, from Sharkia governorate. These chickens showed clinical symptoms suggestive ILT (Table, 1). The clinical signs and postmortem examination were reported. Specimens from Larynx, trachea and lungs were collected for

histopathological examination and virus isolation and identification.

ILT virus isolation

Tracheas from diseased chickens were collected, homogenized in phosphate buffered saline, containing antibiotics. 0.1 ml of the homogenate was inoculated via chorioallantoic membrane (CAM),ten days embryonated chicken eggs and incubated at 37^0C.Five days post - inoculation, chilled eggs showed pock lesions on the CAM which collected, homogenized and stored at -20^0C.Virus titration was carried out and egg infective dose (EID_{50}) was calculated *(15).*

ILT virus vaccines

One tissue cultured adapted vaccine of Schering – Plough Animal health corporation (P20500-12 serial No. : 99288A) and one chicken embryo adapted ILT vaccine of Intervet International B.V. (Batch / Lot: 101007 C) were resuspended in their specific diluents and dropper for intraocular vaccination of experimental chickens.

Experimental infection

Sixty-three, day-old, Hubbard broilers were obtained and housed under good hygienic measures in floor pens and given feed and water adlib.

The experimental chickens at 26 day-old were divided into two main groups (A and B). The group A containing 21 chickens and kept as a negative control. The group B containing 42 chickens and inoculated with 0.1 ml of ILT field isolate that contain 10^4 EID 50/ml.

At 2, 3,4,5,6 and 11 days post infection six infected chickens and three controls were sacrificed and specimens from trachea, larynx and lungs were collected for histopathological examination.

Study of post – vaccinal reaction

Sixty, day-old chicken, were intraocularly vaccinated with Hitchiner B_1, vaccine for NDV at 6, 14, 28 and 35 days of age. At 18 days –old chickens were divided into three equal groups (1, 2&3) each containing 20. The group 1 chickens kept as control, the group 2 chickens vaccinated with tissue culture adapted vaccine and the group 3 chickens vaccinated with chicken embryo adapted vaccine. All experimental chickens were observed daily for 15 days for recording post vaccinal reaction. Specimens from trachea and larynx were collected from birds showed respiratory signs for histopathological examination and serum samples were collected for serological ILT antibody assay.

Agar gel precipitation test (AGP)

Agarose 1% was used for AGP test for ILT identification using hyper- immune serum *(16).*

Histopathology

Specimens from Larynx, trachea and lungs from affected and vaccinated birds were collected and fixed in 10% neutral buffered formalin. Sections of 5 *u* thickness were prepared and stained with H & E stain and examined microscopically *(17).*

ILT antibody titration

ILT antibody titration was proceeding with normal KPL-ELISA test procedure. Optical densities and calculation of titres were carried out using computerized Bio-Tek Elx 800 reader and synobodies profile 2.01 soft ware.

Table 1. Showed diseased flocks in Sharkia governorate :

Flock No	Breed	Locality	Age/days	No. of birds	Morbidity rate	Mortality rate
1	Hubbard	Zagazig	26	5000	50%	8%
2	Isa brown	El Kanaiat	32	5000	60%	12 %
3	Hubbard	Diarb Nigm	28	3500	40%	7%
4	Hubbard	Salhia	35	10000	50 %	15 %
Average	-	-	-	-	50%	10.5%

RESULTS

Field cases

The mild cases of affected birds showed ocular and nasal discharges and mild respiratory signs. Mean while severe affected birds showed gasping, dyspnea, rales and extension of head and neck with expectoration of bloody tinged exudates. The lesions which appeared on Larynx and tracheas were congested mucosa and mucus or bloody mucus as well as caseated material in their lumen. Some affected cases, revealed pseudo-diphtheritic membrane covering the tracheal mucosa.

Microscopically, the mucosa of the trachea and larynx of mild infected birds was intact with few leukocytic infiltrations in lamina propria (Fig.1). In the severe cases, the mucosa revealed metaplasia and desquamation in the lining epithelium with excessive aggregation of leukocytes (Fig. 2). The trachea of vaccinated birds showed mild thickening of tracheal mucosa with Leukocytic aggregations (Fig, 3) and hyperplastic tracheal mucous glands with cystic dilatation of these glands (Fig. 4).

The lung of infected birds showed pneumonia and the air vesicles filled with fibrinous exudates (Fig. 5). Also severe affected birds revealed thickening interlobular septa and blood vessels were congested with aggregations of lymphocytes displaced the pulmonary tissue.

In some cases severe congestion was observed in the pulmonary tissue (Fig. 6) where the erythrocytes were extravasted inside the alveoli and interlobular septa (Fig.7).The lung tissue of vaccinated birds showed fibrinous pneumonia where the air vesicles filled with fibrinous exudates (Fig,8) .

The isolation and identification of ILT

Virus isolation and identification were succeeded and virus titration was carried out and EID_{50} was 10^4 /ml. A positive agar gel precipitation test antibody demonstration in the sera were observed in pooled sera from infected birds.

Table 2. ILT antibody titration proceeded with normal KPL ELISA test for unvaccinated and vaccinated broilers with tissue culture and egg adapted ILT vaccines:

groups	No of birds	Mean of O.D.	Mean of titre
Control group(non vaccinated with ILT vaccine)	20	0.4 ± 0.1	684±171
group vaccinated with tissue culture ILT vaccine)	20	0.93± 0.4	2079±684
group vaccinated with egg adapted ILT vaccine)	20	1.08± 0.3	2757±513

O.D. =optical density

Experimental infection

The induction of ILT disease in susceptible birds with 10^4 EID_{50} resulted in similar clinical signs and lesions to those observed in natural Infection, the signs started 2 days post infection, later on the signs become pronounced.

Post vaccinal reaction

Egg-adapted ILT vaccine induced more post vaccinal reaction than tissue cultured adapted ILT vaccine. Clinically mild respiratory signs, lacrimation, conjunctivitis and swollen eyelids were observed. Also tracheal and laryngeal congestion with increase mucus secretion were observed.

Microscopically, the mucosa of the trachea and larynx was thickened with mild round cell infiltration and hyperplastic tracheal mucous glands. The protection of birds was better with vaccination using egg-adapted ILT vaccine (92%) than that of tissue cultured adapted ILT vaccine (80 %).

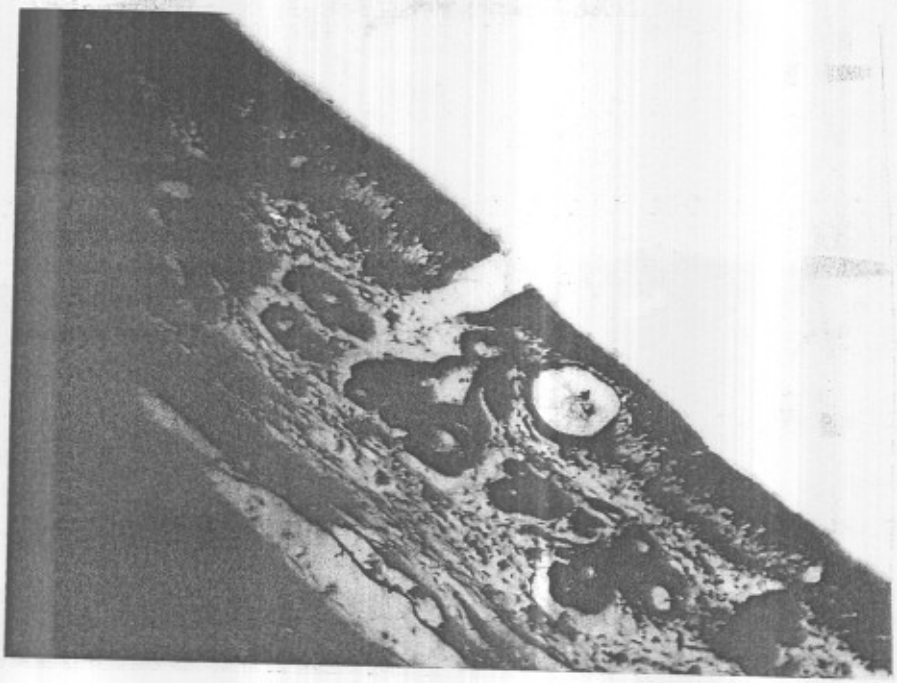

Fig.1. Trachea of infected bird showing leukocytic infiltration in lamina propria H & E X 300 .

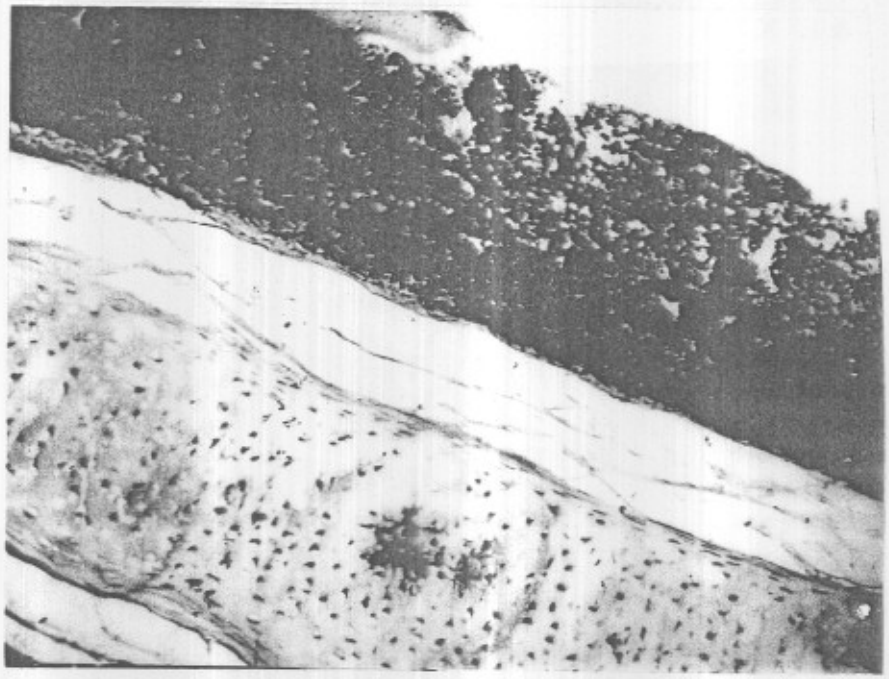

Fig.2. Trachea of infected bird showing desquamation in lining epithelium with severe leukocytic infiltration H & E X 300

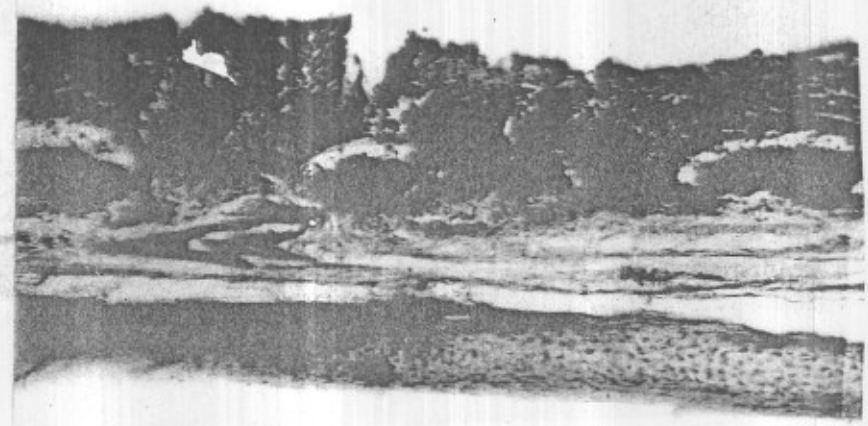

Fig. 3. Trachea of vaccinated bird showing thickened mucosa with leukocytic aggregations H & E X 150 .

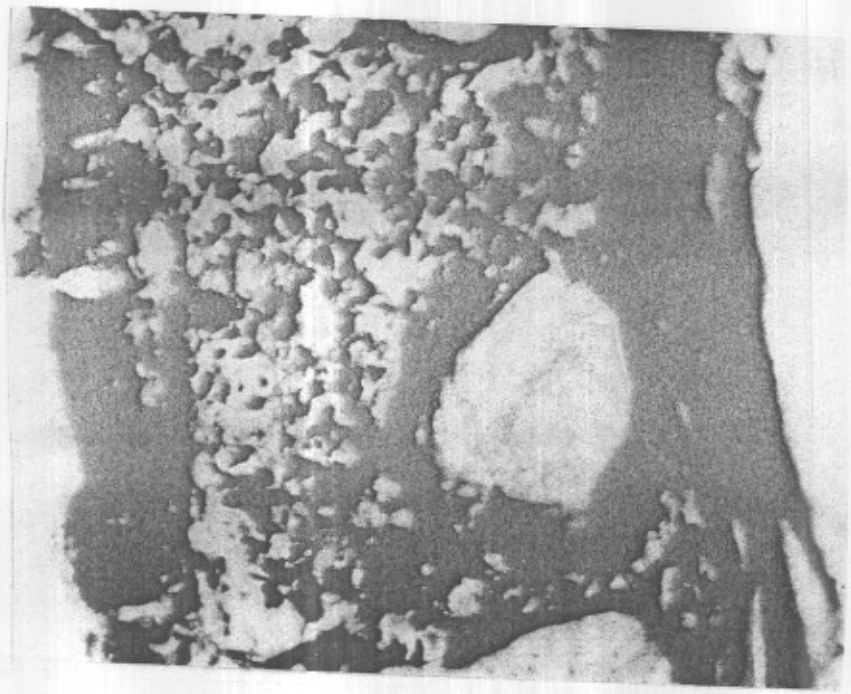

Fig. 4. Trachea of vaccinated bird showing hyperplasia in tracheal mucous glands with cystic dilatation H & E . X 600 .

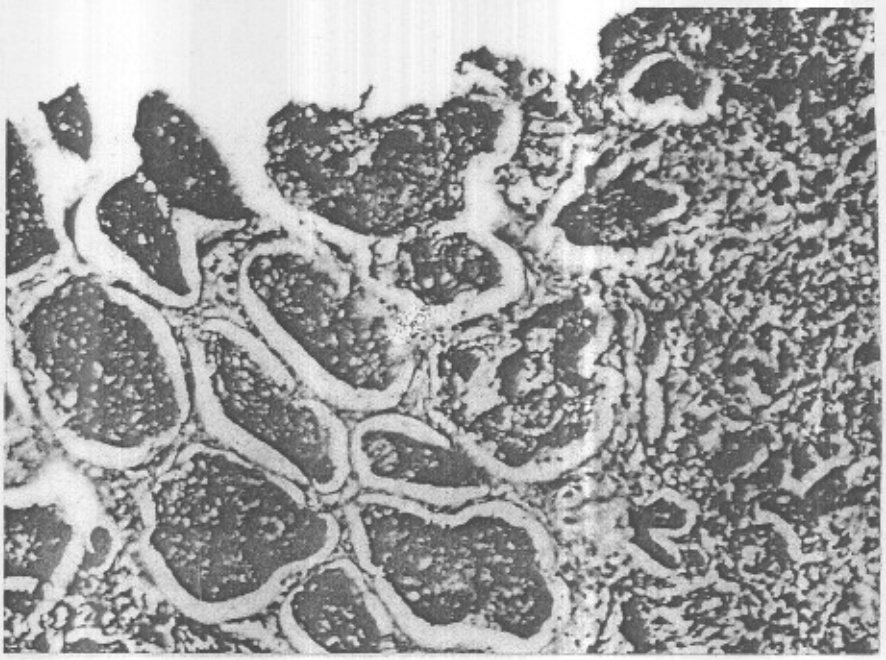

Fig. 5. Lung of infected bird showing pneumonia H & E X 130 .

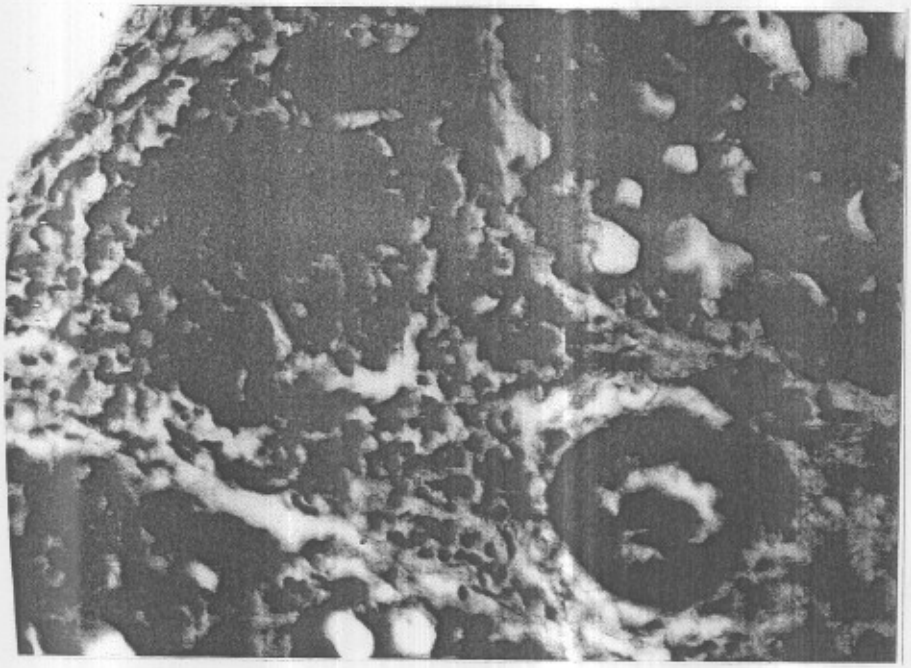

Fig.6. Lung of infected bird showing thickened interlobular septa &congested blood vessels H & E X 300 .

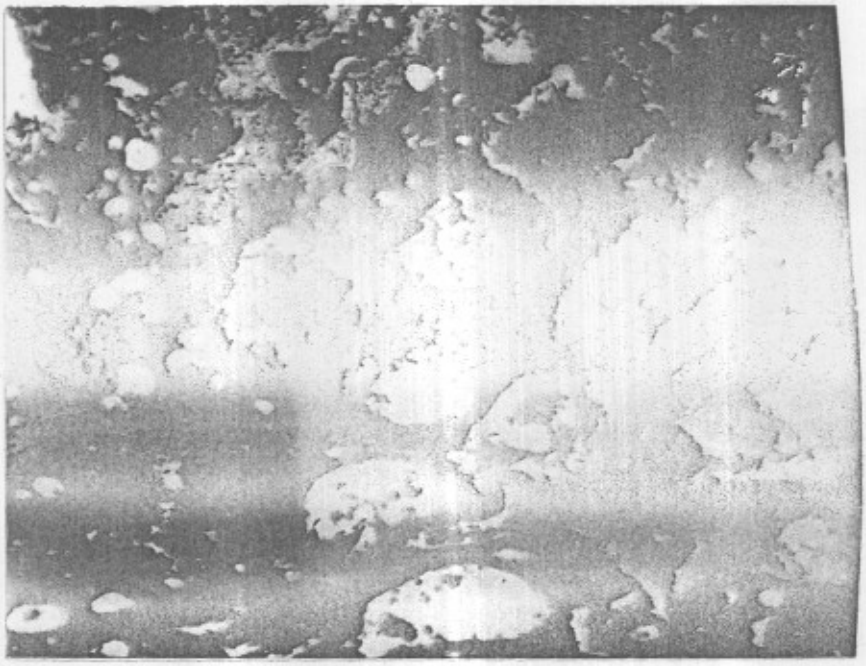

Fig. 7. Lung of infected bird showing severe congestion H & E . X 300 .

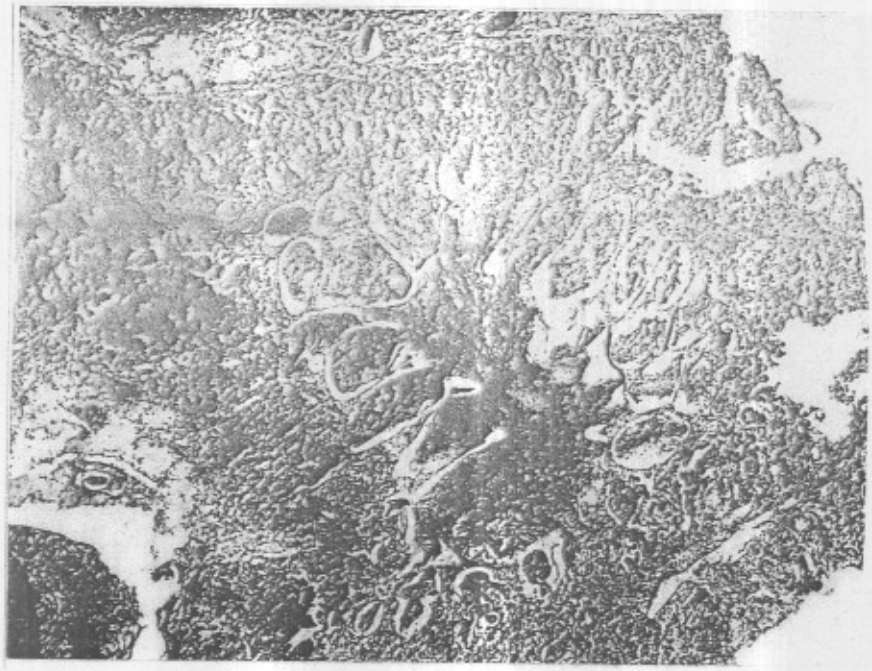

Fig. 8. Lung of vaccinated bird showing fibrenous pneumonia H & E . X 130 .

DISCUSSION AND CONCLUSION

The clinical findings and P.M. lesions of larynx and trachea of field infected birds in our study were similar to those described previously *(5 ,18).*

In our study the diagnosis and isolation of ILT virus was succeeded and similar to those carried out by different investigators *(13, 19)*. In severe cases pseudo – diphtheretic membrane was observed covering the tracheal mucosa; these findings were similar to those previously reported *(4, 5, 12).* The pseudomembrane could be due to secondary bacterial infection associated with ILT infection.

The degeneration and desquamation of the epithelia lining of larynx and trachea and infiltration of lamina propria with leukocytes, these results are consistent to those obtained in several studies *(7, 10 , 20)*. The presence of leukocytes could be due to secondary bacterial infection. The clinical signs and lesions were early appeared (2 days post infection), these could be due to short incubation period after experimental infection , these results were similar to those described by Jordan *(21)*..

In case of vaccinated birds, the chicken embryo adapted vaccine induced more post-vaccinal reaction than tissue culture adapted vaccine. The post vaccinal reaction was in the form of mild respiratory sign, lacrimation, conjunctivitis and swollen eyelids were noticed. Tracheal and laryngeal congestion, and increase mucus secretion were noticed. The field viruses may originate from commercial vaccine strains which increase their pathogenicity in the field by bird to bird transmission *(2,22)*. Protection, judged by antibodies titre, morbidity and mortality, indicated that egg-adapted ILT vaccine produce 92 % protection while tissue cultured adapted vaccine produce 80 % protection, Finally, it could be concluded as explained by different authors *(14,23)* that ILT induce field outbreaks among broiler flocks. Moreover, chicken embryo adapted ILT vaccine induced more post vaccinal reaction and high protection than tissue culture adapted ILT vaccine.

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الملخص العربى

دراسات باثولوجية على الإصابة بمرض إلتهاب الحنجرة والقصبة الهوائية فى الدجاج

صبرى محمد محمد العكشة - سامى أمين أمين عدايل- عاطف أبوزيد

معهد بحوث صحة الحيوان بالزقازيق

لقد تم تشخيص مرض إلتهاب الحنجرة والقصبة الهوائية كمسبب لإصابة الدجاج بأعراض تنفسية فى محافظة الشرقية وكانت الأعراض عبارة عن أعراض تنفسية مع مد الطائر لرأسه وعنقه وإخراج سائل مدمم من الأنف والفم. وكانت الصفة التشريحية هى وجود مواد متجبنة ومدممة فى الحنجرة والقصبة الهوائية .

وبالفحص الميكروسكوبى تبين وجود إلتهاب بالحنجرة والقصبة الهوائية والرئتين مع وجود أنزفة واحتقانات وتجمع لخلايا الدم البيضاء. وتم عزل الفيروس المسبب للمرض وتشخيصه سيرولوجيا بالعدوى التجريبية. وتم تسجيل اختبار رد الفعل لبعض اللقاحات الحية للمرض حيث وجد أن اللقاح المنتج بالتمرير خلال البيض يعطى رد فعل على الطائر المحصن أكثر من اللقاح المنتج عن طريق زراعة الأنسجة مع انتاج مناعة أقوى للوقاية الافضل من المرض.