A study On Infectious Larngotracheitis At Sharkia Governorate

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ABSTRACT

In a trial to isolate the ILT virus from chickens in Sharkia Governorate; Egypt. Twelve flocks with no history of vaccination against ILTV were examined. These flocks were suffering from respiratory signs including dyspnea, gasping, coughing, conjunctivitis and expectoration of mucus admixed with blood. Eight field ILTV isolates showed fine pocks on chorio all antince membrane (CAMS) of embryonated chicken eggs (ECE) beside dwarfing of the inoculated embryos. These field virus isolates were identified by AGPT, VNT. Histopathological findings revealed the characteristic oesinophilic intranuclear inclusion bodies in infected CAM in addition to hyperplastic lymphoid aggregation in the epithelium of trachea.

INTRODUCTION

Infectious Larngotracheitis is a highly contagious disease causes problems ranging from mild respiratory signs affecting chickens, characterized by moist rales, sneezing, coughing, gasping and expectoration of bloody exudate to mortality of an entire flock *(1)*. ILTV classified among members of the Herpes group viruses (alpha herpes), the strains differ in their virulence and pathogenicity, but all isolates belong to one serotype *(2, 3)*. ILT was first described in Rhode Island in America *(4)*. The target organs of infection are the upper respiratory tract. Sometimes, the eyes are affected. Also, pneumonia, sinusitis, conjunctivitis and rhinitis were gross lesions accompanied the disease *(5)*. Economic losses caused by ILTV not only due to the variable mortality rates among chickens, but also reduction in weight gain, drop in egg production, delaying in the start of laying and lower hatchability *(6,7)*. In Egypt; since the first record of ILTV infection among chickens *(8)*, further studies were carried out to investigate ILTV situation until now. Repeated virus isolation and serological studies had proved the wide spread existence of clinical and subclinical forms of the disease among laying and broiler flocks *(9-12)*. Therefore, the aim of this study was to obtain new insights into ILTV through isolation and identification of different isolates from field affected chickens and finally histopathological studies of naturally infected chickens.

MATERIAL AND METHODS

Material

Specimens

Table 1 summarizes the descriptive data of the investigated flocks from different localities at Sharkia Governorates.

Sixty specimens from larynx and trachea were collected from sacrificed and or freshly dead chickens of different breed. All examined chickens were subjected to clinical and /or postmortem examination.

Specimens were subjected to virus isolation and histopathological examination in the early acute phase, 2-7 days from onset of the clinical signs.

Embryonated chicken eggs

Eight hundred, 9-11 day–old embryonated chicken eggs (ECE) were obtained from native breeders and used for virus isolation, propagation, titration and neutralization test.

ILT antigen

Specific ILT AGPT antigen (isolated and titrated) was kindly supplied by Dr. Mohamed Hassen, Animal Health Research Institute, Dokki, Giza, Egypt.

ILTV antisera

Standard ILTV antiserum was kindly supplied by Dr. Susan Tolba, Serum and Vaccines Research Institute, Abbasia, Cairo, Egypt.

Antibiotics: (Pencillin – streptomycin)

Crystalline penicillin and streptomycin were used in dose of 10000 I.U penicillin and 10 mg streptomycin per ml virus suspension.

Stains

Giemsa and Hematoxylin and eosin stains were used for staining of tissue sections for histopathological examination *(13)*

Agar gel medium

Agrose 1% in PBS 7.2 PH containing 8% NaCl, was used for AGP test for ILT virus identification and antibody demonstration *(13).*

Table 1. Descriptive Data of the investigated flocks

Flock* No	Locality	Number of examined flocks	Breed of examined birds	Age of birds (d)	No. of collected samples
1	Awolad Saker	5000	Arbo Acres	35	5
2	Awolad Saker	4000	Balady	70	5
3	Abou Kabir	3000	Avian 43	34	6
4	Abou Kabir.	4000	Avian 43	36	5
5	Awolad Saker	5000	Hubbard	36	6
6	Abou Kabir.	6000	Hubbard	40	4
7	Abou Kabir.	2500	Hubbard	38	4
8	Mena El Kamh	4000	Balady	37	4
9	Mena El Kamh	10000	Balady	40	5
10	Meet Abo Ali	6000	Avian 43	41	4
11	Meet Abo Ali	7000	Avian 43	36	5
12	Anshas El Basal	6000	Arbo Acres	35	7

*All these Flocks were not vaccinated Against ILTV.

Methods

Clinical and postmortem findings

Clinical examination of diseased chickens was recoded and Postmortem examination of both freshly dead and sacrificed birds were carried out.

Virus isolation

The primary isolation of ILTV from prepared specimens was carried out in dropped CAM of 11days-old ECE. Inoculated eggs were candled daily for 5 days PI for observation of inoculated embryos. All inoculated embryos were examined for the presence of pock lesions on their CAMS. Three successive embryo passages were performed before considering the sample negative *(14).*

Virus titration

Titrations of the isolated viruses were carried out in ECE using ten fold dilution of the virus suspension (10^{1}-10^{10}). The Virus infectivity was calculated *(15).*

Virus Neutralization (VN) test

Virus neutralization test was carried using alpha method by mixing equal volumes of serum with each virus dilution and incubating the mixture for 1 h at 37°C. Each dilution was inoculated into CAM of ECE. The EID_{50} was calculated. Neutralization index was estimated *(15.)*

Agar gel Precipitation test (AGPT).

AGPT was carried out *(13).*

Histopathological examination

The collected CAMs and tissue specimens of larynx and trachea from naturally infected chickens were fixed in 10% buffered neutral formalin solution. Five-micron thick paraffin sections were prepared, stained with hematoxyline and eosin, and then examined microscopically for histopathological findings *(16).*

RESULTS

Clinical findings

Clinical examination of affected birds showed respiratory signs as gasping, coughing, rales, conjunctivitis and expectoration of mucous mixed with blood. Sometimes, affected chickens showed extended head and neck forward and upward with partially opened beak with spasmodic coughing leading to expulsion of bloody mucoid discharges.

Post mortem findings

Post mortem examination of both freshly dead and sacrificed chickens revealed gross lesions in the form of congestion of the upper respiratory passage, blood admixed with mucus or diphtheritic membranes in laryngeal and tracheal lumen. The vaying morbidity rates (10-90%) and mortality ranged from 2-35%. (Table 2, Fig. 1)

Virus isolation

The isolation trials of collected specimens revealed only eight virus isolates from examined 12 pools of larynx and trachea after three successive embryo passages. The pock lesions of inoculated CAM of 10-12 days ECE showed different morphology, pock lesions were with opaque edges and depressed central areas of necrosis as early as 3 days PI. The plaques varied from few scattered foci with edema and thickening of CAM with isolate no (1, 2, 5 and 7) to large number of pocks on CAMS with isolate No 3, 4, 6 and 8. Some embryo died 4-6 days after inoculation, survived embryo were dwarfed (Fig. 4) Moreover, infected CAM showed typical lesions of ILTV were subjected to histopathological examination. Virus isolation results were shown in Table 3 and Figs. No 2, 3, 4, 5 and 6.

Virus titration and neutralization

Virus titration and neutralization of suspected isolates of ILT infected samples were shown in Table 3.

Agar gel precipitation test

All eight field ILT virus isolates were positive in AGPT against reference serum. Table 3.

Table 2. Clinical Signs and P.M lesions of examined birds

Flock No	Clinical signs	P.M	Mortalities %	Morbidities%
1	++	++	2	50
2	+	+	1	15
3	+	+	1	18
4	++	+	5	20
5	+	+	2	20
6	+	+	35	50
7	+++	+++	10	90
8	+	+	4	15
9	+	+	3	20
10	+	+	5	20
11	+	+	3	30
12	+++	+++	2	10

Sings , +++=Dyspnea, gasping and blood expectoration, Conjunctivitis,
++ = Coughing, sneezing, rales, gasping,
+ = coughing, sneezing, rales,

P.M. +++ haemorrhagic tracheitis, laryngitis, blood clot and diphtheritic membrane.
++hemorrhagic trachitis and laryngitis and mucus in trachea.
+ trachitis and mucus in trachea.

Histopathological findings

The microscopic changes in trachea in natural infection include desquamared epithelial lining and hyperplastic mucosal lymphoid follicles (fig.7). Moreover, the laynex showed degeneration and necrosis of epithelium with edema and haemorrhages in subepithelial tissues (Fig.8). Histopathological changes of infected CAM showed that the infected epithelial cells become necrosed and degenerated with the presence of esinophilic intranuclear inclusion bodies surrounded by a hallow space (Fig.9).

Table 3. Isolation and Identification of ILT virus isolates

Isolate No	Pock lesions on the CAMS	EID50	Virus Identification	
			AGPT	NI
1	Few No. of specific pocks	6.3	+	2.6
2	Few No. of specific pocks	6.3	+	2.6
3	Fine proliferative necrotic pocks all over CAMS	7.2	+	3.3
4	Fine proliferative necrotic pocks all over CAMS	6.8	+	3.2
5	Few No. of specific pocks	6.5	+	2.9
6	Fine proliferative necrotic pocks all over CAMS	7.2	+	3.3
7	Few No. of specific pocks	6.3	+	2.6
8	Fine proliferative necrotic pocks all over CAMS	6.7	+	3

NI= Neutralization index EID50 = 50% Embryo infective dose.
AGPT = Agar gel precipitation test.

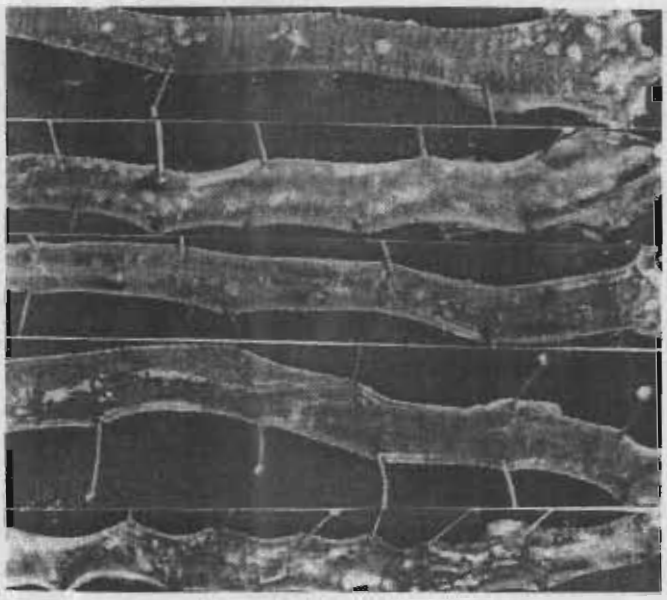

Fig. 1. Layrnx and trachea of naturally infected chickens showing blood – stained mucus blood exudates and diphtheritic membrane.

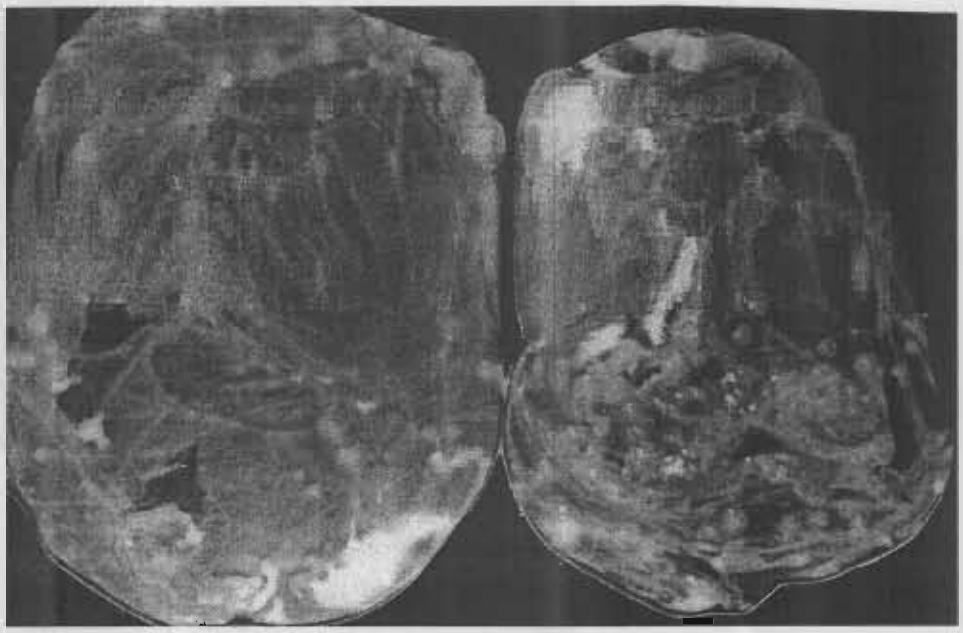

Fig. 2. Pock lesions in inoculated CAM of ECE with field isolates No. (1 and 2).

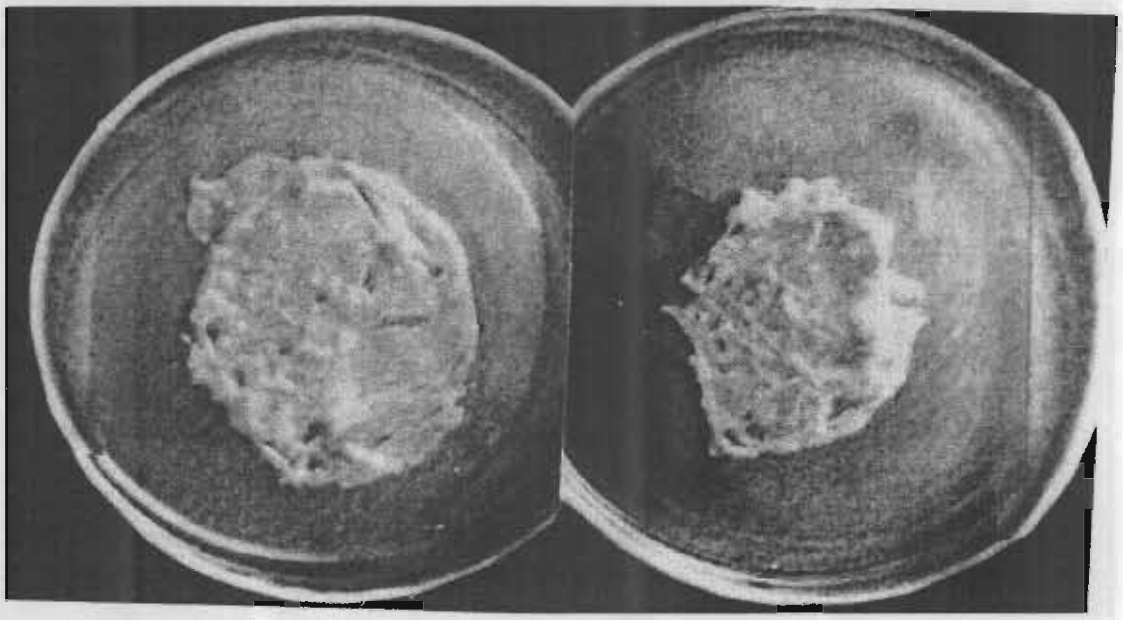

Fig. 3. Pock lesions in inoculated CAM of ECE with field isolates No. (3 and 3).

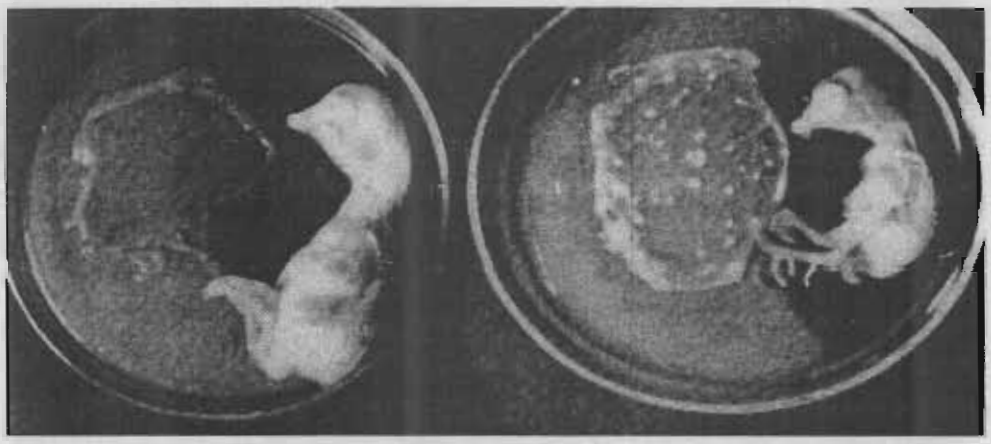

Fig. 4. Pock lesions in inoculated CAM of ECE with field isolates No. 5.

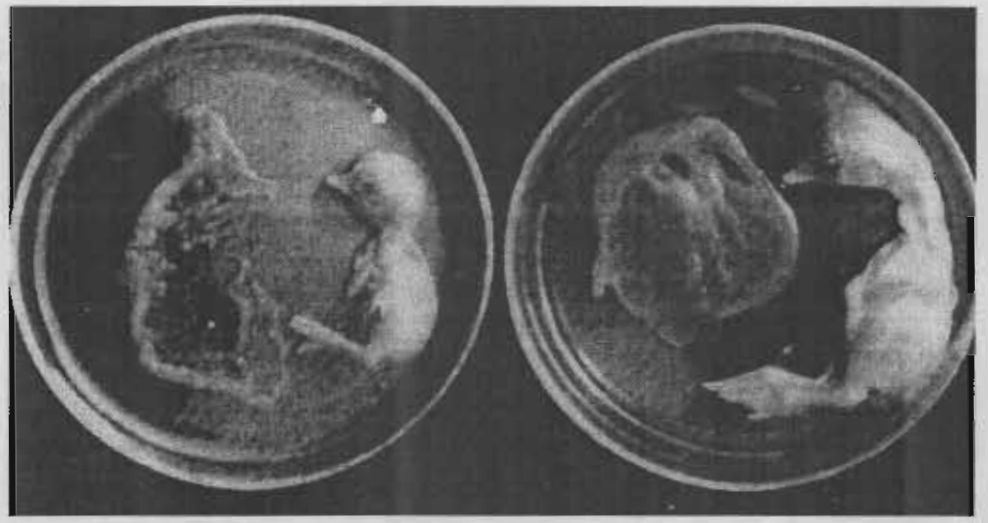

Fig. 5. Pock lesion in inoculated CAM of ECE with field isolates No. 6.

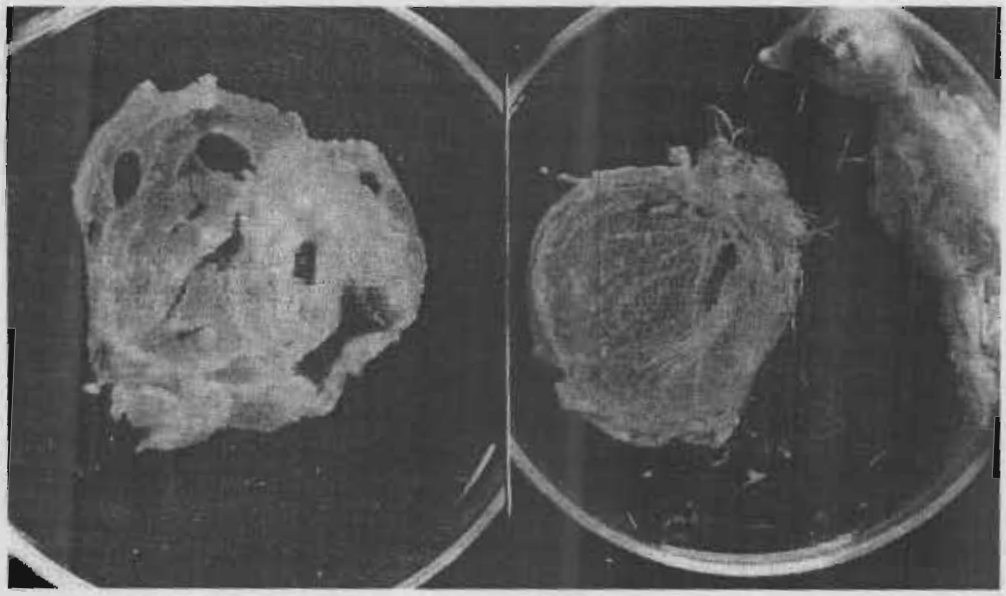

Fig. 6. Pock lesions in inoculated CAM of ECE with field isolates No. (7 and 8).

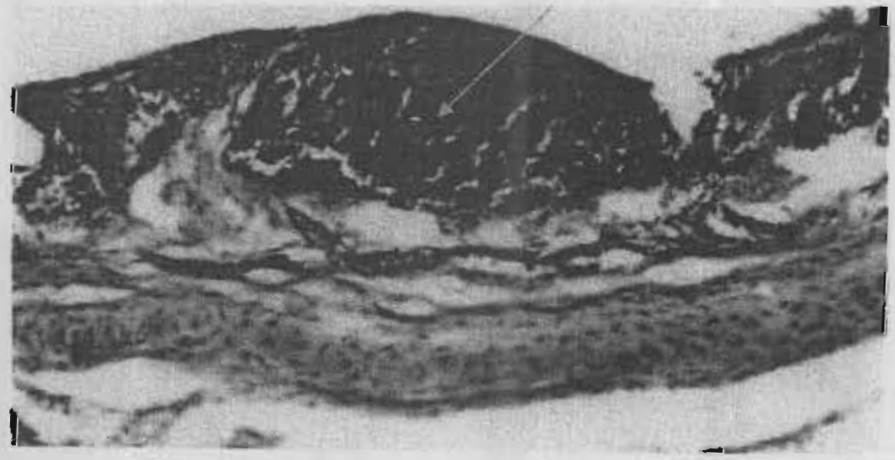

Fig. 7. Trachea of naturally infected chickens showing desquamared epithelial lining and hyperplastic lymphoid aggregation H and E X 300.

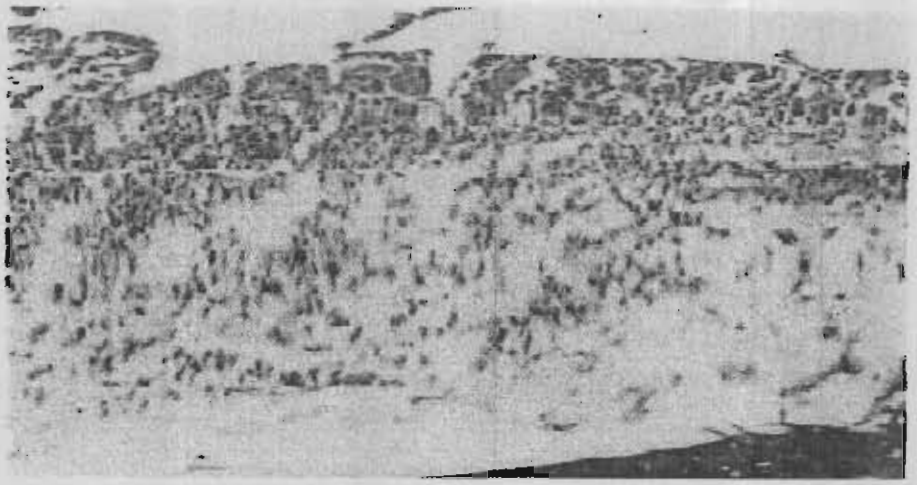

Fig. 8. Larynx of naturally infected chickens showing degeneration and necrosis of epithelium with edema and haemorrhages in subepithelial tissues H and E X 300.

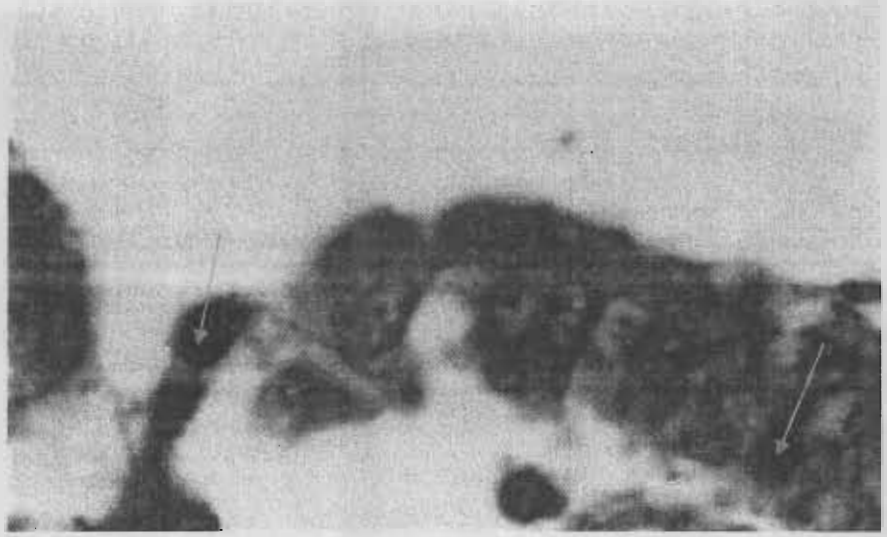

Fig. 9. CAM of ECE infected by field isolated ILT showing characteristic esinophilic intranuclear inclusion bodies within degenerated and necrosed epithelial cell H and E X 300.

DISCUSSION

Respiratory manifestations in chickens still the irritable problem facing poultry industry. Respiratory problems are of economic importance as causing reduction in feed intake and conversion, reduction of body weight and increase mortalities. Several pathogens and management factors are incriminated in these problems. Infectious laryngotracheitis virus (ILTV) exists as a single type with a wide range of disease severity *(17)*.

In a trial to investigate the ILTV infections among chickens at sharkia governorate. Twelve chicken flocks with history of no vaccination against ILTV and suffering from respiratory signs as gasping, coughing and expectoration of mucus tinged with blood. P.M lesions, larynx and trachea contained mucus mixed with blood then diphtheritic membrane formed and finally necrotic tissue sloughs into lumen of trachea. Lungs showed congestion and edema. Eye lesions included conjunctivitis followed by keratitis with caseous exudate adhere to cornea. The mortality (2-35%) and morbidity reached up to 90%. These findings are similar to previously recorded studies *(18-22)*.

The disease was diagnosed in 12 examined flocks and the trials succeeded in isolation of eight isolates from examined flocks. The isolation was carried on CAMS of ECE. The pock lesions of inoculated samples revealed difference in their growth characters indicating that ILT virus isolates varied in virulence. These results are consistent with several previous studies *(2, 18, 22,23)*. Our findings on virus isolation showed pock lesions on CAM as early as in the first passage, which was a characteristic to vaccinal adapted strain virus *(24)*. This may give an explanation that agree with the study which reported that field viruses may originated from commercial vaccine strains specially (C.E.O) after 10 passages from bird to bird in virulence to become a virulent field strain and the pock lesions become more fine, pin headed and widely distributed on CAMS *(19,22)*. The Identification of field ILT isolates was based on the positive results of AGPT and NT *(25)*. All virus isolates showed NI at least $10^{2.6}$ up to $10^{3.3}$ *(8, 12, 22,25)* for locally isolated ILT virus strains.

Concerning the histopathological findings of naturally infected chickens include desquamared epithelial lining and hyperplastic mucosal lymphoid follicles in trachea. Moreover, the laynex showed degeneration and necrosis of epithelium with edema and haemorrhages in subepithelial tissues histopathological findings of CAMS, the infected epithelial cells became disassociated, necrosed and degenerated with the presence of intranuclear inclusion bodies surrounded by a hallow space. Several reports demonstrated similar finding *(1, 12, 18, 22, 26, 27)*.

Finally, it could be concluded that ILT is an endemic disease at Sharkia Governorate in chickens. ILT virus was diagnosed as a cause of sever respiratory disease among chicken flocks at Sharkia Governorate. It should be taken into account that all field outbreaks occurred in summer that the disease is more severe in hot than cooler conditions *(36)*.

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الملخص العربى

دراسة عن مرض التهاب الحنجرة والقصبة الهوائية المعدى فى محافظة الشرقية

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اجريت هذه الدراسة لعزل العترات المسئولة عن مرض التهاب الحنجرة والقصبة الهوائية فى الدجاج بمحافظة الشرقية.

تم فحص 12 قطيع من قطعان دجاج التسمين من سلالات مختلفة غير محصنة ضد مرض التهاب الحنجره والقصبة الهوائية المعدى ذات اعمار مختلفة تتراوح ما بين 34-70 يوم من عدة اماكن مختلفة داخل محافظة الشرقية. هذه القطعان كانت تعانى من اعراض تنفسية (بلغت الدرجه الحاده فى بعض القطعان) مع وجود مخاط او مخاط مختلط بالدم فى منطقة الحنجرة والقصبة الهوائية. وصلت نسبة الاعياء فى هذه القطعان الى 90% ونسبة النفوق من 2-35%

تم تجميع عينات نسيجية من القطعان المصابة وتم وضعها للدراسة فى محاولة لعزل الفيروس على اجنة البيض المخصب وتم اجراء محاولة للعزل على الاقل بثلاث تمريرات متتابعة فى اجنة البيض المخصب لكل عينة حتى يتم عزل الفيروس المسبب للمرض.

تم تشخيص المرض فى 12 قطيع اجريت عليهم الدراسة وتم عزل الفيرس المسبب للمرض من ثمانيه منها وقد سببت العترات المعزولة ظهور بثرات مميزة للمرض ومختلفة فى الشكل تبعا للعترة المعزولة على الغشاء المشيمائى اللقانقى (CAM) لاجنة البيض (من بثرات كبيرة الحجم 2-3 مم قليلة العدد الى بثرات صغيرة الحجم كراس الدبوس ومنتشره على جميع اجزاء النسيج) وبالفحص المجهرى لانسجة هذه البثور تبين ظهور اجسام احتوائيه بالنواة (Ib) مميزه للمرض بالاضافة الى تقزم فى الاجنة المحقونة .

تم اجراء اختبار الترسيب فى الاجار الهلامى ضد سيرم موجب لمرض التهاب الحنجرة والقصبة الهوائيه للعترات المعزولة وادى الى ظهور خط ترسيبى كما تمت معايرة كمية الفيروس المتواجدة فى الاجنة لكل عترة على حدة واعطيت عيارية فيروسية تتراوح من $10^{6.3}$الى $10^{7.2}$ وباجراء اختبار تعادل المصل اظهرت معامل تعادل مصلى يتراوح من 2.6 الى 3.3 .