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VIROLOGICAL AND PATHOLOGICAL STUDIES ON A RECENT ISOLATE OF INFECTIOUS LARYNGEOTRACHEITIS VIRUS IN EGYPT

BY

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SUMMARY

Four groups of 12 weeks old chicken were employed for aerosol, intratracheal and contact infection with ILTV added to a noninfected control group. The aerosol route resulted in the highest morbidity and mortality rate followed by the tracheal ronte, where contact infection resulted in mild tespiratory symptoms. Aerosol infection resulted in higher rate of ILTV virus reisolation . The virus could be reisolated few days later in case of contact birds. The histopathological changes in larynx, tracheas, bronchi, lung and air sacs were described.

INTRODUCION

The world wide distribution of infectious laryngeotracheitis was reported by many authors in different countries (Beach in California, 1931, Gwatkin in Canada, 1925, Dobson in Great Britain, 1935; Seddon and Hart in Australia, 1936; Vivo et al, in Cuba, 1977; El Zein, et al., in Lebanon, 1979). Infectious Laryngeo-tracheitis (ILT) virus isolation and serosurveys in Egypt were reported by Tantawi et al., (1983); Amer, (1984); Shakal, (1986) and Saif Edein et al., (1990).

The disease is obviousely contagious, since it spread comparatively rapidly within a flock and to contact flocks. It was then common for passage through a multiage population resulting in apparent enhancement of virulence. On the other hand, the recovered and vaccinated chicken are considered as carriers for ILT virus for months or years and severity of the disease depends upon the nutrional and hyagenic status of the flocks as well as seasonal variations (Max Burgh, 1982).

As a result of the knowledge about infectious laryngeo-tracheitis disease form in term of severity of clinical signs, postmortem lesions and perfomance of birds, studies on the disease pathogensis of ILT virologicaly and pathologicaly was required. This directed the aim of this work to study the pathogenicity of a recent ILT isolate in experimentally infected chickens.

MATERIAL AND METHODS

1- Experimental chickens:

One hundred and ten chicks of day old layer type (LSL) were reared under hyagenic conditions for 12 weeks and separated on start of experiments.

II- Fertile chicken eggs:

They were used for the virological studies.

III- Viruses:

A recently isolated field ILT virus identified by Abd Elwahd (1994) was used for this study.

IV- Stains:

Haematoxyline and eosine:

Haematoxyline and Eosine (H&E) stains were used for histopathological examination according to Harris, (1898).

V- Histopathological proceedure:

Infected CAM and specimens from nasal sinuses, larynx, trachea, Lungs, air sacs and conjuctiva from experimentally infected chicken were collected and fixed in neutral buffered formaline. Parafin sections of 5μ thickness were prepared and stained by Haematoxyline and Eosin stains then examined microscopically according to Harris (1898).

VI- Experimental Infection:

One hundred and ten 12 week old chickens proved to be free from ILT virus and/or antibodies on testing for infectivity and neutralisation respectivley were divided into four groups named A,B,C and D.

Group A; 30 birds were subjected to aerosol infection with (10^7 EID^{50} /ml) ILTV isolate and kept in closed room for 15 minutes.

Group B: 30 birds were subjected to intratracheal infection with the field virus 0.2 ml/bird (10^7 EID^{50}/ml).

Group C: 20 uninfected birds were in contact with 30 aerosol infected 24 hours previously, those birds were marked.

Group D: 30 birds were kept in a fourth separate room and left as control.

Three birds sacrificed on the 3rd, 7th, 9th and 12th day post infection. Clinical signs were recorded daily in addition to postmortem lesions in dead and sacrificed birds.

Samples for virus reisolation and distribution were collected from larynx, trachea, lungs, air sacs and conjunctiva.

RESULTS

1- Clinical and postmortem findings of the experimentally infected chickens:

The clinical findings started on the 3rd day for both intratracheal and aerosol infection with sneezing turned to gasping on the 5th day where the respiratory signs were less severe in case of tracheal infection rather than the aerosol infection. Contact infection resulted in mild respiratory signs after 5 days.

The morbidity and mortality rate were 100%-90% -60% and 17% -0.5% -0% respectivley in case of aerosol, intratracheal and contact infections.

Postmortem lesions seen in aerosol infection between 1st and 5th day were severe larygnitis and tracheitis with small amount of mucous tinged with blood which turned to haemorrhagic tracheitis and laryngitis extending to larynx and bronchi between 6 and 12 day. Nasal sinuses were sometimes filled with seromucoid or caseous material. Lesions in intratracheal infected birds between 1 and 5th day were less severe only tracheitis and laryngitis with oedematous mucous membrane which turned to diphteritic laryngitis and tracheitis between day 6 and day 12 (Fig. 1).

Contact infected birds showed similar but less severe lesions than those in tracheally infected birds.

II- Virus distribution and relsolation:

Infectious laryngeotracheitis virus recovery from different organs (larynx, trachea, lung, air sac and conjunctiva) on different intervals (3, 5, 7, 9 and 12 days) post infection by aerosol, intratracheal and contact are shown in Table (1).

III- Histopathological findings:

A) 1-5 days postinfections:

Larynx and trachea:

Larynx and Trachea showed mild hyperplasia of lining epithelium containing large single or double oesinophilic oval or rounded intranuclear inclusion bodies (Fig. 2) beside mild lymphocytic infiltration and congested blood cappillaries in the lamina propria. Sometimes revealed coagulative necrosis of superfacial layers of laryngeal mucosa with fibrinous exudate and erythrocytes (Fig. 3).

Table (1): Infectious laryngeotracheitis virus distribution in different organs following experimental infection by aerosol, intratracheal and contact.

Organ	Aerosol infection					Control group
	3 day p.i	5 day p.i.	7 day p.i.	9 day p.i.	12 day p.i.	
Larynx	3/3	3/3	3/3	3/3	3/3	0/3
Trachea	3/3	3/3	3/3	3/3	2/3	0/3
Lung	2/3	3/3	2/3	1/3	1/3	0/3
Air sac	0/3	1/3	2/3	2/3	0/3	0/3
Conjunctiv	0/3	0/3	0/3	0/3	0/3	0/3

Organ	Intratracheal infection					Control group
	3 day p.i.	5 day p. i.	7 day p.i.	9 day p.i.	12 day p.i.	
Larynx	3/3	3/3	3/3	2/3	2/3	0/3
Trachea	3/3	3/3	3/3	2/3	2/3	0/3
Lung	0/3	0/3	0/3	1/3	0/3	0/3
Air sac	0/3	1/3	2/3	2/3	0/3	0/3
Conjunctiv	0/3	0/3	0/3	0/3	0/3	0/3

Organ	Contact infection					Control group
	3 day p.i	5 day p.i.	7 day p.i.	9 day p.i.	12 day p.i.	
Larynx	0/3	1/3	2/3	1/3	1/3	0/3
Trachea	0/3	1/3	2/3	1/3	1/3	0/3
Lung	0/3	0/3	0/3	0/3	0/3	0/3
Air sac	0/3	0/3	0/3	0/3	0/3	0/3
Conjunctiv	0/3	0/3	0/3	0/3	0/3	0/3

Day p.i. : day post infection

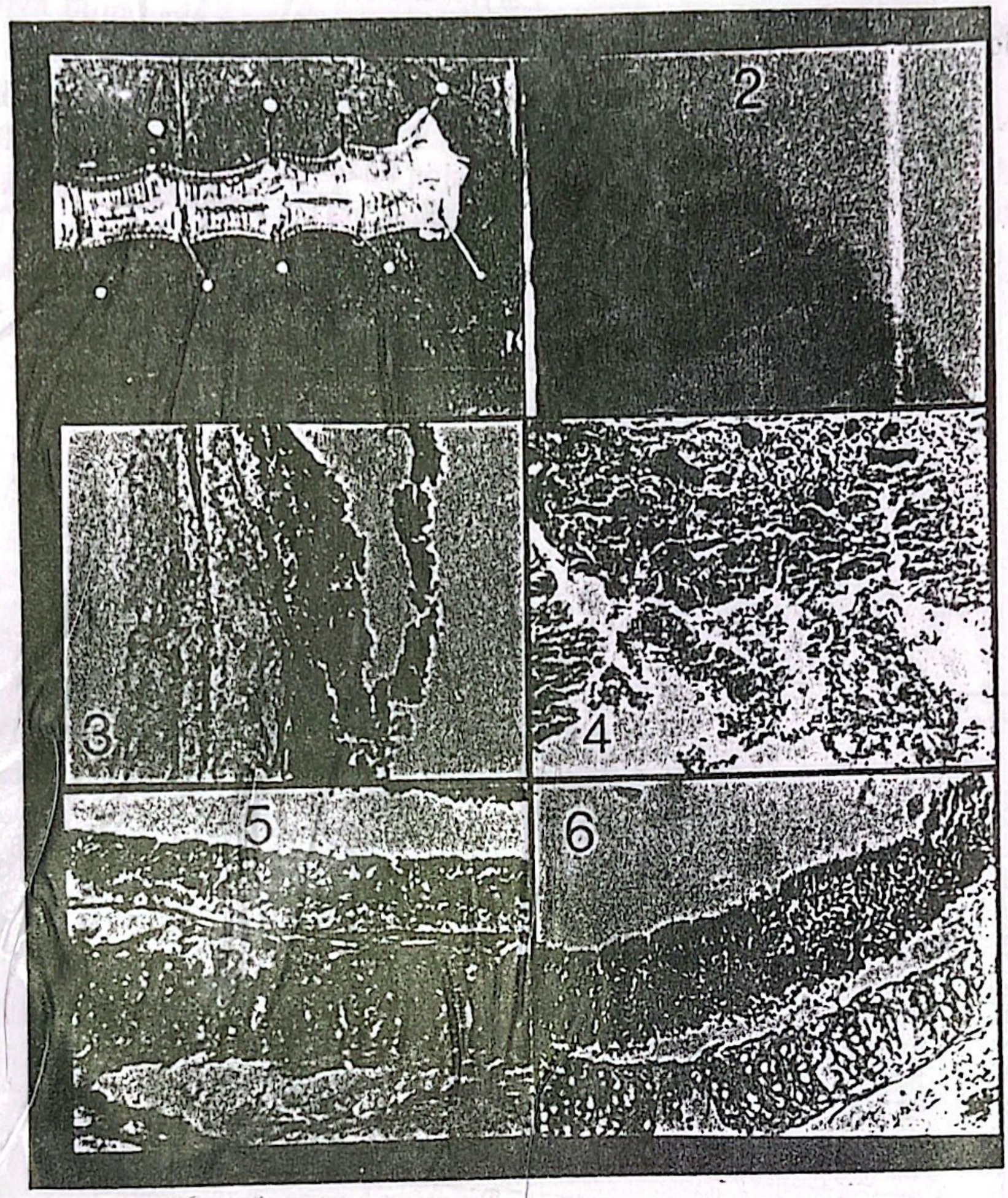

Fig. (1): Larynx and trachea of experimentally infected chickens via the intratracheal route.

Fig. (2): Trachea of chicken showing oesinophilic inctranuclear inclusion bodies surrounded by clear hallow space within tracheal epithelium.

Fig. (3): Trachea of chicken showing focal coagulative necrosis with desquamation of tracheal epithelium and inflammatory reaction.

Fig. (4): Lung of sacrificed chicken (5 dyas post intratracheal infection) showing broncheolitis characterised by presence of inflammatory exudate and desquamate epithelium in the broncheal lumen with thickend wall.

Fig. (5): Nasal sirus showing haemorrahgic exudate in the lumen with thickened mucosa by hyperplastic mucous glands and oedema.

Fig. (6): Tracha of sacrificed chicken (9 days postinfection) showing regenerated ciliated epithelium with basophilic cytoplasm containing few goblet cells and tracheal glands over some inflammatory cells.

Lungs:

Bronchi of experimentally infected chickens by aerosol and intratracheal routes showed haemorrhagic and mucous exudate mixed with extravasated erythrocytes inside their lumen. Their walls were thickened by oedema mixed with lymphocytes, erythrocytes and congested blood vessles (Fig. 4) . No pulmonary lesions were detected in contact birds.

Air sacs:

Chickens experimentally infected by aerosol showed extensive oedema, lymphocytic infiltration, thickening and congested blood vessels of air sacs and no lesions were seen in contact birds.

Nasal sinuses:

The lining epithelium revealed mild degree of hyperplasia and excessive metaplasia to goblet cells (Fig. 5).

B) 6-12 days postinfection:

Trachea and larynx:

The epithelium of both trachea and larynx showed less goblet cells and cell infiltration (Fig 6). Only some epithelial cells appeared degenerated and contained large irrigular oval or rounded intranuclear inclusion bodies.

Lung and air sacs:

Lesions became more mild in intratracheal infection rather than aerosol infection and no lesions were found in contact birds.

Nasal sinuses:

The nasal epithelium in majority of chickens regenerated containing fewer goblet cells.

DISCUSSION

Infectious laryngeotracheitis is a disease of high economic importance due to variable mortality rates among chickens with reduction of body weight drop in egg production and lowering hatchability (Reggi et al., 1961).

An observation of different authors that ILT in its epizootology has waves of outbreaks of acute disease followed by periods of subclinical forms (Shakal., 1986) leaded to the need to study the pathological and virological aspects of localy ILT recent isolates.

Clinical, postmortem and histopathological findings of experimentally infected chickens were less in severity than naturally infected chickens and in agreement with the findings of Chang et al., (1973); Max Brugh (1982) and Tripathy and Hanson (1989).

The different routes of infection resulted in various intensity of clinical signs where aerosol route resulted in the highest morbidity and mortility followed by the tracheal route. Contact infection resulted in mild respiratory symptoms. Mortality in case of aerosol infection was 17% where in case of tracheal infection 0.5%. Those observations are in agreement with Purcell (1971).

Following the virus distribution by reisolation after experimental infection by different routes, it was found that ILT virus is mainly isolated from larynx and trachea. Aerosol infection resulted in higher percentage of reisolation of the virus and from deeper respiration passages. ILT virus was

reioslated few days later in case of contact infection.

In early infection (1-5 days) mild hyperplasia of tracheal epithelium, fibrinous or serofibrinous exudate mixed with desqumated epithelium were seen inside the lumen. Tracheal lesions showed regeneration attempts between day 6 and 12 P. I.

Only some of the epithelia appeared degenerated containing intranuclear inclusions with lymphocytic infiltration in lamina propria resembling findings seen by Hayashi et al., (1985) and El Mahadi et al., (1989).

Bronchi of both aerosol and tracheal experimentaly infected chickens showed mataplasia of epithelial lining to goblet cells with oedema and thickening of the wall. Haemorrhagic and mucous exudate mixed with extravasated erythrocytes were found in the lumen. Intensity of lesion was higher in case of aerosol infection specially in interstitial tissues peribronchial and interstitial lymphocytic infiltration were seen in both cases.

The pulmonry lesions were intense and persistant between 6-12 days P.I. in case of aerosol infection but not in tracheal infection. While no particular lesions were detectable in contact birds from 1 to 12 days similar to the results of Purcell (1971).

Extensive oedema in air sacs was only seen in aerosol infection with proliferation and desquamation of epithelia with lymphocytic and macrophage infilteration supporting the observations of Snocyenbos et al., (1972).

Nasal sinuses between 1-5 days showed hyperplasia of the epithelial lining and excessive metaplasia to goblet cells which turned to regeneration of the nasal epithelium between 6-12 days in agreement with the findings of El Mahadi et al., (1989).

The pathogenesis and virological studies done in this work proved the disease inducing capacity of the recent isolates demanding a regular following up for new infectious laryngeotracheitis virus isolation and possible changes of ILT epizootology in poultry producing areas and their control.

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