

**Comparative Molecular and Pathological Studies in  
Chickens Experimentally Infected with Infectious  
Laryngotracheitis Virus**

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# CONTENTS

	<b>Page</b>
<b>Introduction</b> .....	1
<b>Review of literature</b> .....	5
<b>Material and methods</b> .....	51
<b>Results</b> .....	63
<b>Discussion</b> .....	124
<b>Conclusion</b> .....	137
<b>Summary</b> .....	139
<b>References</b> .....	144
<b>Vita</b> .....	157
<b>Arabic Summary</b> .....	

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## List of Abbreviations

<u>Abbr.</u>	<u>Description</u>
AILTV	: Avian infectious laryngotracheitis virus.
CAMS	: Chorioallantoic membranes.
CCO	: Cell culture origin.
CEO	: Chicken embryo origin.
ECEs	: Embryonated chicken eggs.
EID <sub>50</sub>	: 50% embryo infective dose.
H&E	: Hematoxylin and Eosin.
IB	: Inclusion bodies.
IgA	: Immunoglobulin A.
IgG	: Immunoglobulin G.
ILT	: Infectious laryngotracheitis.
ILTV	: Infectious laryngotracheitis virus.
INI	: Intranuclear inclusion bodies.
ORF <sub>s</sub>	: Open reading frames.
PBS	: Phosphate buffered solution.
PCR	: Polymerase chain reaction.
PI	: Post inoculation.
PM	: Postmortem.
PV	: Post vaccination.
SPF	: Specific pathogen free.
TRG	: Trigeminal ganglion.

## List of Tables

<b><u>Tab.</u></b>	<b><u>Title</u></b>	<b><u>Page</u></b>
1	Sequence and amplified of ALTV primers.....	54
2	Experimental design .....	57
3	Preparation of PCR Master Mix .....	61
4	Steps, temperature, time and number of cycles .....	62
5	Intensity of clinical signs and lesions in both dead and sacrificed chickens among different experimental groups.....	85
6	The lesion scores among different experimental groups.....	86

**List of Figures**

<u>Fig.</u>	<u>Title</u>	<u>Page</u>
1	Chicken experimentally infected with ILTV (group 1) showing ocular discharge leading to closed eye.....	88
2	Chicken experimentally infected with ILTV (group 1) showing open beak for respiration .....	88
3	Chicken experimentally infected with ILTV (group 1) showing congested lung .....	89
4	Conjunctiva of chicken (group 1) 3 <sup>rd</sup> day post infection showing conjunctivitis represented by focal epithelial destruction, congestion of blood vessels (arrow) with fibrinous exudate in subepithelial tissues (arrowhead). H&E X 300.....	89
5	Trachea of chicken (group 1) 3 <sup>rd</sup> day post infection showing diphtheritic membrane containing blood and syncytial multinucleated cells with eosinophilic intra nuclear inclusion bodies (arrow). H&E X 300.....	90
6	Trachea of chicken (group 1) 3 <sup>rd</sup> day post infection showing eosinophilic intranuclear inclusion bodies (arrow). H&E X 1200.....	90
7	Trachea of chicken (group 1) 3 <sup>rd</sup> day post infection showing cystic dilatation of tracheal gland (arrow) and intense inflammatory cells in mucosa. H&E X 300.....	91
8	Trachea of chicken (group1) 3 <sup>rd</sup> day post infection showing metaplasia of tracheal gland to goblet cells (arrow) beside inflammatory cells and erythrocytes in mucosa. H&E X 300.....	91
9	Trachea of chicken (group 1) 3 <sup>rd</sup> day post infection showing thickening of lamina propria by leukocytic infiltration (arrow), congestion and hemorrhage with metaplasia of glandular epithelium to goblet cells. H&E X 300.....	92

10	High power of the previous figure to show heterophilic infiltration (arrow). H&E X 1200.....	92
11	Trachea of chicken (group 1) 3 <sup>rd</sup> day post infection showing partial desquamation of epithelial cells forming sheet inside the tracheal lumen (arrows). H&E X 300.....	93
12	Lung of chicken (group1) 3 <sup>rd</sup> day post infection showing severe dilatation of blood vessels and capillaries (arrow). H&E X 300.....	93
13	Lung of chicken (group 1) 3 <sup>rd</sup> day post infection showing extensive heterophilic infiltration (arrow). H&E X 300....	94
14	Lung of chicken (group1) 3 <sup>rd</sup> day post infection showing vacuolation in wall of blood vessels with hyalinosis and perivascular edema (arrow) and hemorrhage. H&E X 300.....	94
15	Lung of chicken (group1) 3 <sup>rd</sup> day post infection showing congestion (arrow) and focal alveolar emphysema in lung tissue. H&EX300.....	95
16	Nasal sinus of chicken (group1) 3 <sup>rd</sup> day post infection showing extensive leukocytic infiltration with congestion of blood vessels (arrow). H&E X 300.....	95
17	High power of the previous figure to show heterophilic infiltration (arrow). H&E X 1200.....	96
18	Conjunctiva of chicken (group1) 7 <sup>th</sup> day post infection showing hyperplasia of the surface epithelium (arrow) or focal destruction and subepithelial tissue inflammatory cells aggregation. H&E X 300.....	96
19	High power of the previous figure to show heterophilic infiltration (arrow). H&EX 1200.....	97
20	Larynx of chicken (group1) 7 <sup>th</sup> day post infection showing partial desquamated epithelial cells (arrow) with leukocytic infiltration and dilated blood vessels in mucosa (headarrow). H&E X 300.....	97

21	Larynx of chicken (group1) 7 <sup>th</sup> day post infection showing syncytial formation and intranuclear inclusion bodies (arrow) beside intensive heterophilic infiltration and edema. H&E X 300.....	98
22	Larynx of chicken (group 1) 7 <sup>th</sup> day post infection showing eosinophilic intranuclear inclusion bodies in surface epithelium (arrow). H&E X 300.....	98
23	Trachea of chicken (group1) 7 <sup>th</sup> day post infection showing diphtheritic membrane in the tracheal lumen consisted of mucus, fibrinonecrotic tissue, erythrocytes and desquamated epithelial cells (arrows). H&E X 300.....	99
24	High power of previous figure to show diphtheritic membrane and its constituent. H&E X 1200.....	99
25	Trachea of chicken (group1) 7 <sup>th</sup> day post infection showing denuded mucosa (arrow) with dilated blood vessels (arrowhead). H&E X 300.....	100
26	Lung of chicken (group 1) 7 <sup>th</sup> day post infection showing perivascular hemorrhage (arrow). H&E X 300.....	100
27	Lung of chicken (group 1) 7 <sup>th</sup> day post infection showing thickening of interalveolar septa by fibrinous threads containing inflammatory cells (arrow) and edema. H&E X 300.....	101
28	Lung of chicken (group1) 7 <sup>th</sup> day post infection showing perivascular edema (arrow) with focal interstitial heterophilic aggregation (arrow head). H&E X 300.....	101
29	High power of the previous figure to show heterophilic aggregation (arrow) and perivascular edema. H&E X 1200.....	102
30	Nasal mucosa of chicken (group1) 7 <sup>th</sup> day post infection showing thickening of lamina propria with inflammatory cells (arrow) and congested blood vessels (arrowhead). H&E X 300.....	102

31	High power of the previous figure to show heterophils in both mucosa and submucosa (arrow). H&E X 1200.....	103
32	Nasal sinus of chicken (group1) 7 <sup>th</sup> day post infection showing destructed epithelial cells, intense RBCs and inflammatory cells in its lumen (arrow). H&E X 300.....	103
33	High power of the previous figure to show destructed epithelial cells, intense RBCs and inflammatory cells in nasal lumen (arrow). H&E X 1200.....	104
34	Conjunctiva of chicken (group 1) 15 <sup>th</sup> day post infection showing spongiosis (head arrow) and hyperkeratosis. H&E X 300.....	104
35	Larynx of chicken (group 1) 15 <sup>th</sup> day post infection showing numerous goblet cells in the glandular epithelium (arrow). H&E X 300.....	105
36	Lung of chicken (group 1) 15 <sup>th</sup> day post infection showing focal hemorrhage, congestion and compensatory emphysema (arrow) with focal aggregation of lymphocytes. H&E X 300.....	105
37	Lung of chicken (group 1) 15 <sup>th</sup> day post infection showing degenerated and living heterophils beside aggregation of lymphocytes (focal pneumonic area) (arrow). H&E X 300.....	106
38	Lung of chicken (group 1) 15 <sup>th</sup> day post vaccination showing scattered bacterial colonies and inflammatory exudate in the pulmonary tissue (arrow) with inflammatory edema. H&E X 300.....	106
39	Nasal sinus of chicken (group1) 15 <sup>th</sup> day post infection showing extensive leukocytic infiltration with homogenous eosinophilic exudate (arrow). H&E X 300..	107
40	Chicken vaccinated with CEO vaccine (group 2) showing mild respiratory signs with partial closed eyes.....	107
41	Chicken vaccinated with CEO vaccine (group 2) showing foamy eye and swollen lower eyelid.....	108



42	Chicken vaccinated with CEO vaccine (group 2) showing pale comb and completely closed eyes.....	108
43	Conjunctiva of contact chicken (group 2) 3 <sup>rd</sup> day post vaccination showing congestion (arrow), hemorrhage and inflammatory edema. H&E X 300.....	109
44	Larynx of vaccinated chicken (group 2) 3 <sup>rd</sup> day post vaccination showing moderate thickening of laryngeal mucosa with marked heterophilic infiltration (arrow) beside intact ciliated edges. H&E X 300.....	109
45	Larynx of contact chicken (group 2) 3 <sup>rd</sup> day post vaccination showing congested laryngeal mucosa with extensive leukocytic infiltration with beside the presence of fine eosinophilic fibrin threads (arrow) and partially destructed epithelium. H&E X 300.....	110
46	Larynx of contact chicken (group 2) 3 <sup>rd</sup> day post vaccination showing dilated mucosal glands (arrow) and mild lymphocytic infiltrations in mucosa. H&E X 1200...	110
47	Trachea of contact chicken (group 2) 3 <sup>rd</sup> day post vaccination showing cystic dilatation of some tracheal gland containing few inflammatory cells (arrow) beside few leukocytic infiltration in mucosa. H&E X 300.....	111
48	Trachea of contact chicken (group 2) 3 <sup>rd</sup> day post vaccination showing focal desquamation of surface epithelial lining (arrow) and hyperplasia of glandular epithelium. H&E X 300.....	111
49	Larynx of vaccinated chicken (group 2) 7 <sup>th</sup> day post vaccination showing hyperplasia of mucosal glands (arrow) with severe mucosal leukocytic infiltration (arrowhead) and hyperplastic epithelium. H&E X 300.....	112
50	Larynx of vaccinated chicken (group 2) 7 <sup>th</sup> day post vaccination showing focal necrosis of lining epithelium with leukocytic infiltration (arrow). H&E X 300.....	112

51	Larynx of contact chicken (group 2) 7 <sup>th</sup> day post vaccination showing moderate laryngitis characterized by congestion of blood vessels and extensive leukocytic infiltration beside necrotic surface epithelium (arrow). H&E X 1200.....	113
52	Larynx of contact chicken (group 2) 7 <sup>th</sup> day post vaccination showing hyperplastic epithelium with syncytial formation and intra nuclear inclusion bodies (arrow) beside thrombosis of blood vessels (arrowhead). H&E X 300.....	113
53	Larynx of contact chicken (group 2) 7 <sup>th</sup> day post vaccination showing partial desquamation and presence of epithelial sheet (arrow) beside exudate inside lumen (arrowhead). H&E X 300.....	114
54	Larynx of contact chicken (group 2) 7 <sup>th</sup> day post vaccination showing necrotic and folded mucosa (arrow) with congestion and mild leukocytic infiltration. H&E X 300.....	114
55	High figure of the previous figure to show denuded mucosa, congestion (arrow) and lymphocytic infiltration in necrotic mucosa (head arrow). H&E X 1200.....	115
56	Trachea of contact chicken (group 2) 7 <sup>th</sup> day post vaccination showing destruction of epithelial lining, epithelial sheet and RBCs inside tracheal lumen with infiltration of mucosa with large number of leukocytes beside regenerated lining epithelium (arrow). H&E X 300.....	115
57	Lung of contact chicken (group 2) 7 <sup>th</sup> day post vaccination showing focal hemorrhage with thickening of interalveolar septa (arrow), perivascular edema and lumen of air vesicles contained few RBCs. H&E X 300.....	116
58	High power of the previous figure to show thickening of inter alveolar septa by edema, inflammatory cells and proliferative pneumocytes (arrow). H&E X 1200.....	116

59	Lung of contact chicken (group 2) 7 <sup>th</sup> day post vaccination showing vascular endotheliosis (arrow), perivascular edema and hemorrhage. H&E X 300.....	117
60	Lung of contact (group 2) 7 <sup>th</sup> day post vaccination showing depleted lymphoid follicle, edema with leukocytic infiltration (arrow) and numerous goblet cells in bronchial wall (arrowhead). H&E X 300.....	117
61	Conjunctiva of contact chicken (group 2) 15 <sup>th</sup> day post vaccination showing lymphoid aggregation (arrow), edema and mild inflammatory cells (arrow head) under normally epithelium. H&E X 300.....	118
62	Chicken of group (3) vaccinated with TCO vaccine showing watery eye and mild swelling of lower eyelid.....	118
63	Larynx of contact chicken (group 3) 3 <sup>rd</sup> day post vaccination showing extensive infiltration of mucosa with inflammatory cells (arrow). H&E X 300.....	119
64	High power of the previous figure to show extensive lymphocytic infiltration (arrow). H&E X 1200.....	119
65	Trachea of contact chicken (group 3) 3 <sup>rd</sup> day post vaccination showing hyperplasia of epithelial lining (arrow) and mild inflammatory cells besides a few cellular exudate within lumen (arrowhead). H&E X 300..	120
66	Trachea of vaccinated chicken (group 3) 3 <sup>rd</sup> day post vaccination showing thickening of mucosa by inflammatory cells (arrow) and hyperplastic tracheal gland (arrowhead). H&E X 300.....	120
67	Trachea of vaccinated chicken (group 3) 3 <sup>rd</sup> day post vaccination showing desquamation of epithelial sheet (arrow). H&E X 300.....	121
68	Lung of vaccinated chicken (group 3) 3 <sup>rd</sup> day post vaccination showing bronchitis (arrow). H&E X 300.....	121

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69	CAMs of chickens 11-13 day inoculated with CEO vaccinal strain.....	123
70	Detection of AILTV using PCR. AILTV nucleic acid was detected in inoculated, infected as well as vaccinated and contact that was represented by 647 bp but of faint intensity.....	123

## **SUMMARY**

This study was done to evaluate the transmission and replication of live attenuated avian infectious laryngotracheitis vaccinal viruses derived from chicken embryo origin (CEO) and cell culture origin (CCO) into exposed contact chickens. Compare lesions induced post vaccination with that of experimental viral infection. Trial of detection and isolation of AILTV from vaccinated as well as contact chickens. Detection and identification of AILTV transmission and replication into contact as well as experimentally infected chickens using conventional PCR. So in this aspect the work was conducted on using 84 one-day old Sasso chick obtained from private hatchery at Sharkia Governorate and reared under hygienic conditions till reached 44-days old then they were divided into 4 groups. The first group contained 24 chickens, 12 chickens were inoculated intra ocular with 0.1 ml of ILTV isolates and other 12 chicken kept as contact control group. The second group also contained 24 chickens, 12 chickens were vaccinated via eye drop in one eye by CEO vaccine, and other 12 chickens kept as contact to them. The third group contained 24 chickens, 12 chickens vaccinated with CCO vaccine and other 12 chickens kept as contact to them. Four group contained 12 chickens (none infected and none vaccinated chickens) and kept as control negative during the previous work. Chickens in all groups kept under observation for 3 weeks PI and PV and sacrificed (3 chickens of each sub-group per day on each sacrificy) on 3, 7, 15, 21 day PI and PV. All clinical signs and postmortem lesions were recorded. Specimens from

conjunctiva, larynx, trachea and lungs were collected and fixed in neutral buffered formalin 10% and prepared for the routine paraffin histopathological technique. Tissue specimens (larynx, trachea and lung) were taken from both vaccinated and contact chickens of groups (2 and 3) on 7<sup>th</sup> day post vaccination and stored at -70°C until processed for virological analysis.

Clinical finding of group (1) were in form of eye signs represented in lacrimation, swollen eyes, watery eyes (conjunctivitis) and other chickens showed severe reddened conjunctiva. In some severe cases chickens showed ocular discharge sticking to eye lids leading to closed eyes. Respiratory signs of varying degree of severity in the form of nasal discharge, marked dyspnea, gasping and extending head and neck for respiration. Coughing in form of severe cough to intermittent cough, moist rales and expectoration of mucus mixed with blood and chickens showed bloody beak and there was blood in oral cavity, feathers and this was a characteristic sign appeared on infected chickens. Clinical signs, lesions in both contact and infected chickens appeared early and nearly similar due to high virulence of inoculated virus. The course of disease extended till 15 day post infection.

The gross lesions revealed congestion of upper respiratory organs. Hemorrhagic tracheitis and tracheal lumen being filled with mucus mixed with blood. Edema and congestion of epithelium of nasal sinuses were present. Hemorrhagic conjunctivitis and lungs were congested.

Microscopic finding revealed syncytial formation in epithelial lining of conjunctiva, larynx and trachea with eosinophilic intranuclear inclusion bodies. Hyperplasia of surface epithelium of larynx and trachea and mucus gland and some gland appeared cystic and contained inflammatory cells mainly heterophils and other gland showed metaplasia to goblet cells. Tracheal and laryngeal lumina showed desquamated epithelial cells, necrotic debris, exudate and inflammatory cells. Lungs showed congestion, hemorrhage, edema and thickening of inter alveolar septa.

Clinical finding of group (2): Mild to moderate signs were found represented in respiratory signs in form of coughing, gaping, respiratory noises and bloody expectoration and eye reactions (foamy eyes, redden eyes) in both vaccinated and contact chickens. Signs were more sever in contact chickens than vaccinated chickens.

Gross lesions were detected in conjunctiva, larynx, trachea and lungs and represented by moderate congestion of mucosa beside slimy appearance. Diphtheritic lesions in larynx and upper part of trachea were present in some contact and vaccinated chicken in severely affected chickens.

Microscopic finding showed syncytial formation contained eosinophilic intranuclear inclusion bodies in early stage of infection before complete desquamation of epithelial lining.

Clinical finding of group (3): Chickens showed clinical signs 3-4 day post vaccination, milder clinical signs were found in comparison with group (2) in which mild cough and slight eye reactions were

present. The most consistent gross finding was mucus in the trachea and larynx beside mild congested mucosa of larynx and trachea and lungs.

The gross finding nearly similar to group (2) but milder in intensity. Lungs showed congestion. Eyes showed mucoid secretion.

Microscopic finding revealed mild hyperplasia of tracheal epithelium with focal desquamation of epithelial lining. Mild hemorrhage in laryngeal lumen with inflammatory cell infiltration. Laryngeal lumen of vaccinated chickens showed desquamated syncytial cells.

The isolation of virus vaccine was carried on the CAMs of 11-13 day old ECEs. The CAMs of ECEs inoculated with CEO vaccine showed pock lesions. The pock lesions characterized by depressed central area of necrosis with scattered foci all over the CAMs which magnified and intensified at the 3<sup>rd</sup> passage. The pock lesions were associated with thickening, fibrosis as well as hemorrhage widely distributed on CAMs. AILTV nucleic acid was detected in vaccinated and contact chickens using PCR that was represented by 647 bp but of faint intensity due to the replication behavior, the virulence and latency nature of such virus in addition to the virus formulated as a vaccine live attenuated.

It could be concluded that ILT infection caused great losses among experimentally infected and contact chickens. Mortality rate was 41.6% and morbidity rate up to 90%.



Both of CEO and CCO vaccine transmitted from vaccinated chickens to contact exposed chickens. The CCO vaccine was better than CEO vaccine on basis of post vaccinal reaction represented in mild respiratory manifestation, mild eye reactions and number of affected and dead chickens. When compared vaccinal lesions with experimental induced viral infection we found that CEO vaccine made same signs as viral infection so that vaccine when used in vaccination not preferable due to spreading infection inside farm.

Vaccination resulted in carrier birds so vaccination is recommended only in geographic areas where disease is endemic.