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

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A Thesis

Submitted to Zagazig University for the Degree of

Master of Veterinary Medical Sciences

(Pathology)

Department of Pathology

2016

CONTENTS

List of Abbreviations

Abbr.	Description
AILTV	: Avian infectious laryngotracheitis virus.
CAMS	: Chorioallantoic membranes.
CCO	: Cell culture origin.
CEO	: Chicken embryo origin.
ECEs	: Embryonated chicken eggs.
EID_{50}	: 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_S	: 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

List of Figures

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 convential 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 sacrifiy) 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^{th} day post vaccination and stored at -70ºC 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 redden 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 inter-mitten 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 signs 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 intra nuclear 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 3rd 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 were disease is endemic.