VMC 605: Systematic Animal Virology

Online lecture

Topic:
“ Infectious laryngotracheitis virus”

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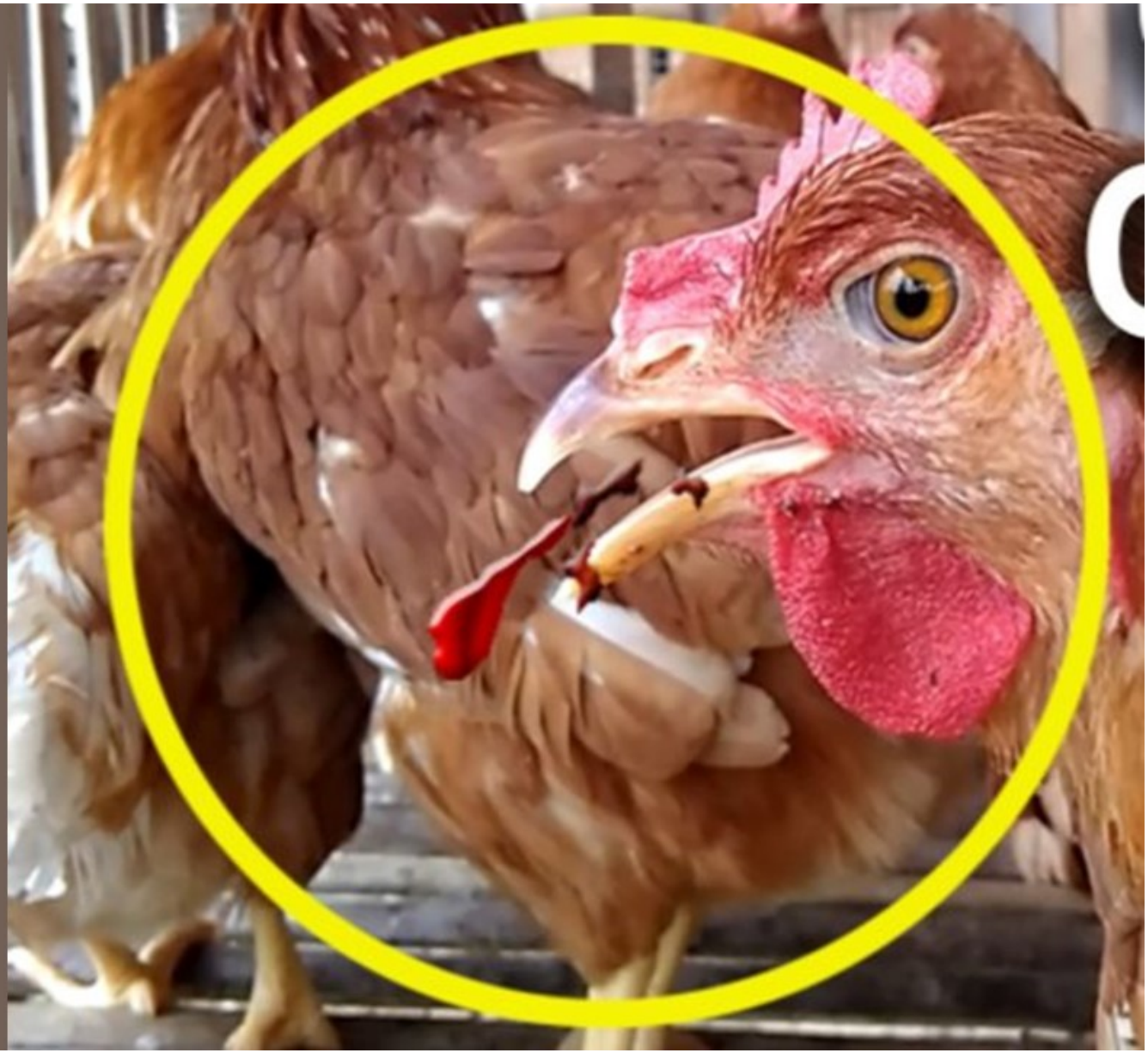


INFECTIOUS LARYNGO TRACHEITIS

A VIRAL DISEASE OF POULTRY

ILTV

A MEMBER OF FAMILY

HERPESVIRIDAE

Infectious laryngotracheitis virus

Belongs to subfamily – *Alphaherpesvirinae*

Placed under the genus *Mardivirus*

Introduction

- Infectious laryngotracheitis (ILT) is an acute, highly contagious infection of chickens and pheasants
- Result in severe production losses due to mortality and/or decreased egg production
- Severe epizootic forms of infection are characterized by signs of respiratory depression, gasping, expectoration of bloody mucus and high mortality
- Mild enzootic forms of infection are encountered increasingly in developed poultry industries and manifest variously as mucoid tracheitis, sinusitis, conjunctivitis, general unthriftiness and low mortality
- Included within List B of the **World Organization for Animal Health (OIE)**

HISTORY

- The disease was first described in 1925 in Canada
- Followed by United States in 1926
- Given several different names including laryngotracheitis, infectious laryngotracheitis, and avian diphtheria
- The name infectious laryngotracheitis was adopted in 1931 by the **Special Committee on Poultry Diseases of the American Veterinary Medical Association**
- The cause of LT was first shown to be a filterable virus by Beaudette
- Laryngotracheitis was the first major avian viral disease for which an effective vaccine was developed

Classification

- Infectious Laryngotracheitis virus (ILTV) is taxonomically identified in the
- **Family:** *Herpesviridae*
- **Subfamily:** *Alphaherpesvirinae.*
- **Genus:** *Mardivirus*
- **Species:** *Gallid herpesvirus 1*

Morphology

- **Shape:** hexagonal
- **Size:** virus particle has a diameter of 195- 250 nm.
- **Nucleocapsid**
  - 80-100 nm in diameter.
  - icosahedral symmetry
  - composed of 162 elongated hollow capsomeres

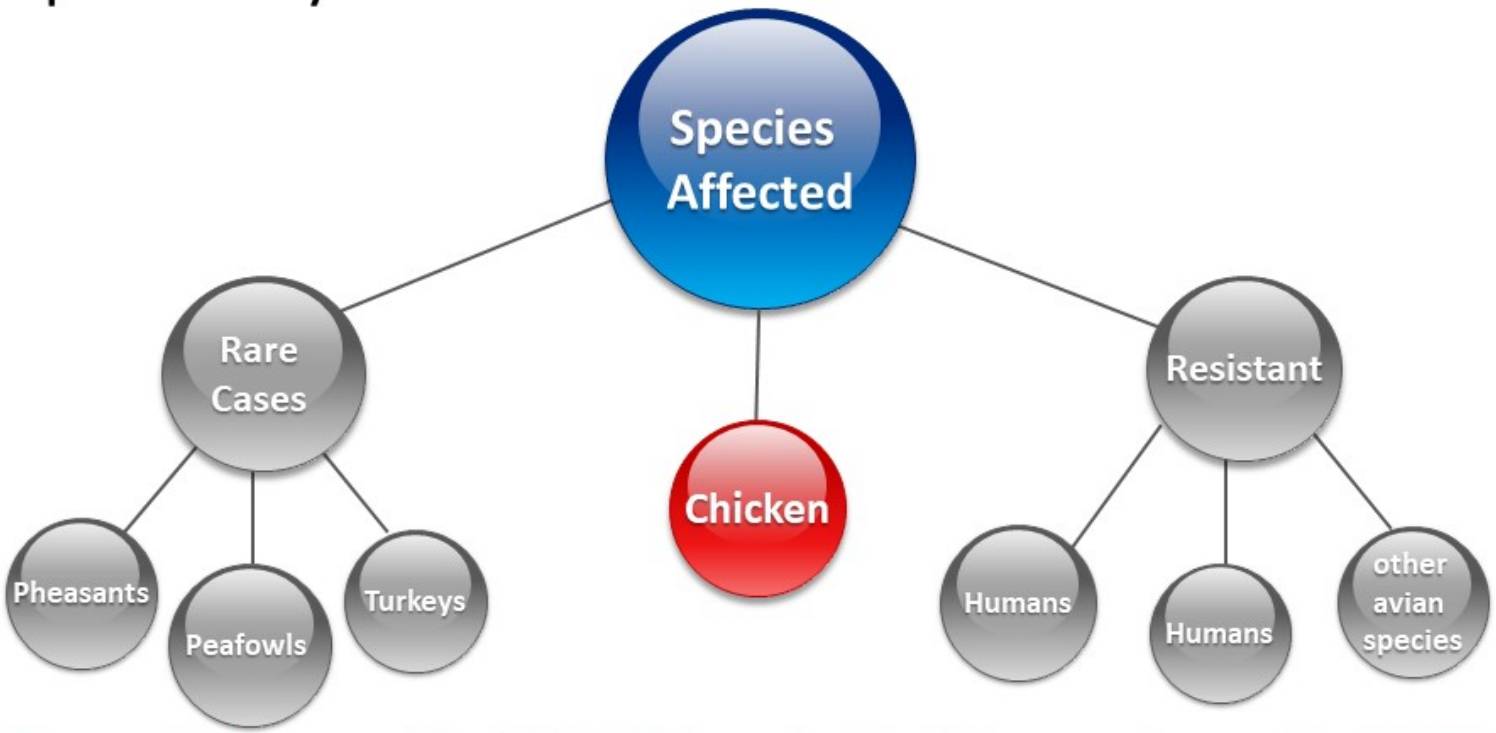
Genome

- consist of a linear 155-kb double-stranded molecule
- comprised of unique long(UL) and short segments(US) flanked by inverted repeats

EPIDEMIOLOGY

- Distributed world-wide
- May be present only in certain localities within a country or geographic region
- The greatest incidence of disease is generally seen in areas of highly intensive
- poultry production
- Route of entry : upper respiratory and ocular routes
- Transmission occurs : acutely infected bird , mechanical transmission (contaminated
- equipment and litter)
- **No evidence** for vertical ILTV transmission to the egg or for shedding ILTV on shells of eggs laid by infected hens
- Incubation period :
    - **6-12 days**, following natural exposure
    - **2-4 days**, following experimental inoculation

Susceptibility



- All ages of chickens are affected, but chickens older than 3 wk are most susceptible to ILTV
- ILTV can infect pheasants, pheasant-bantam crosses, and peafowl
- ILT can infect turkeys at about 100 d of age
- Humans are resistant

ETIOLOGY

Gallid herpesvirus 1

Genus *Iltovirus ;* Subfamily *Alphaherpesvirinae ;* Family *Herpesviridae*

Symmetry Icosahedral

Complete virus particle has a diameter of 195–250 nm and consists of an irregular envelope surrounding the nucleocapsid

DNA genome consists of a linear 155-kb ds molecule composed of **unique long (UL)** and **unique short (US)** regions flanked by **inverted repeats**

Nucleic acid of LTV is composed of DNA with a buoyant density of **1.704 g/mL**

HOST SYSTEM

I. Chicken Embryo

Embryonating chicken eggs are the most common method for propagating ILTVs

In chicken embryos, ILTV forms plaques on the chorioallantoic membrane (CAM).

The plaques can be observed 48 h after infection, and embryos can die in 2-12 d post infection (PI)

HOST SYSTEM

II. Cell Culture

ILTV can be isolated in primary cell cultures, such as chicken embryo liver (CEL), chicken embryo kidney (CEK), and chicken kidney (CK) cell cultures.

The sensitivity of ILTV isolation and propagation from field samples vary depending on the type of cell cultures. CEL was the most sensitive for isolation, followed by CK. The CEK and chicken embryo lung cells were less sensitive.

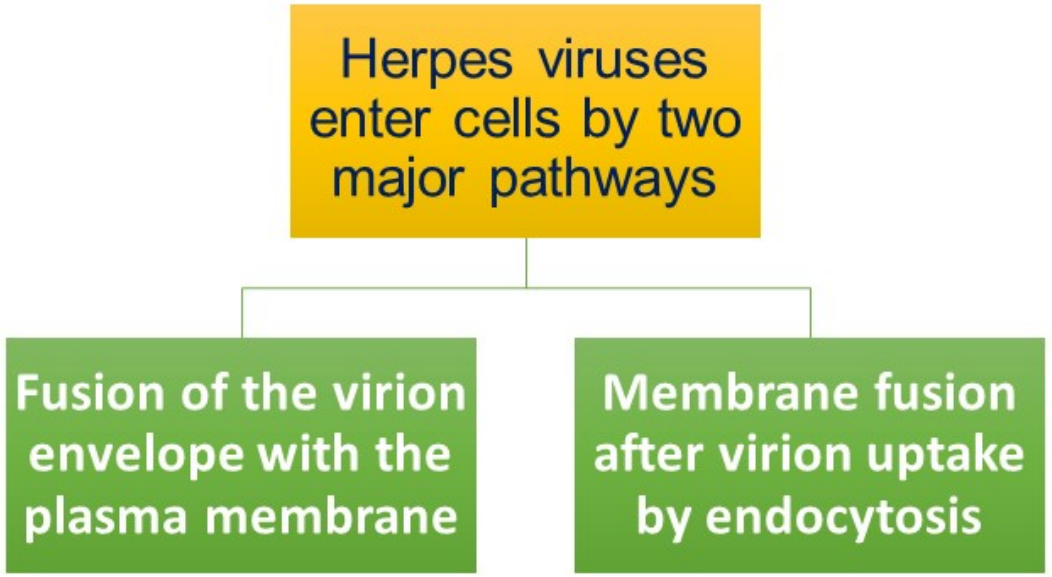
Chicken embryo fibroblasts can also be used

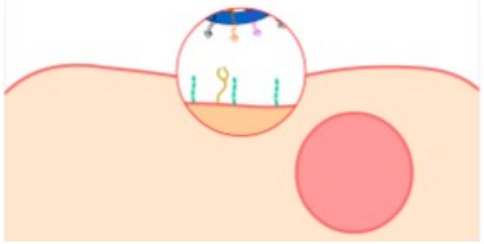
CPE consist of round refractile single cells or small syncytia in focal arrangement.

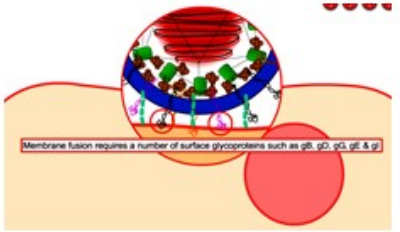
Altered cells were subsequently detached from the flask surface forming plaques and lyse

VIRAL REPLICATION

- Initiation of infection begins with receptor binding through the initial interactions of the virus genome with the host transcriptional machinery in the nucleus





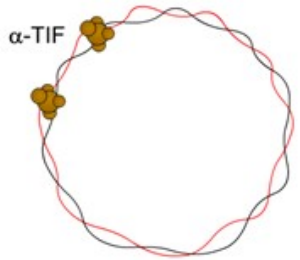


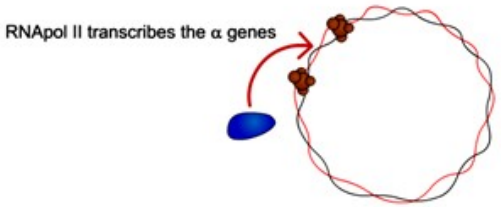
Fusion of virion and cellular membranes is mediated by gB and the gH /gL complex

Attachment of ILTV with host receptor

Transcription & replication

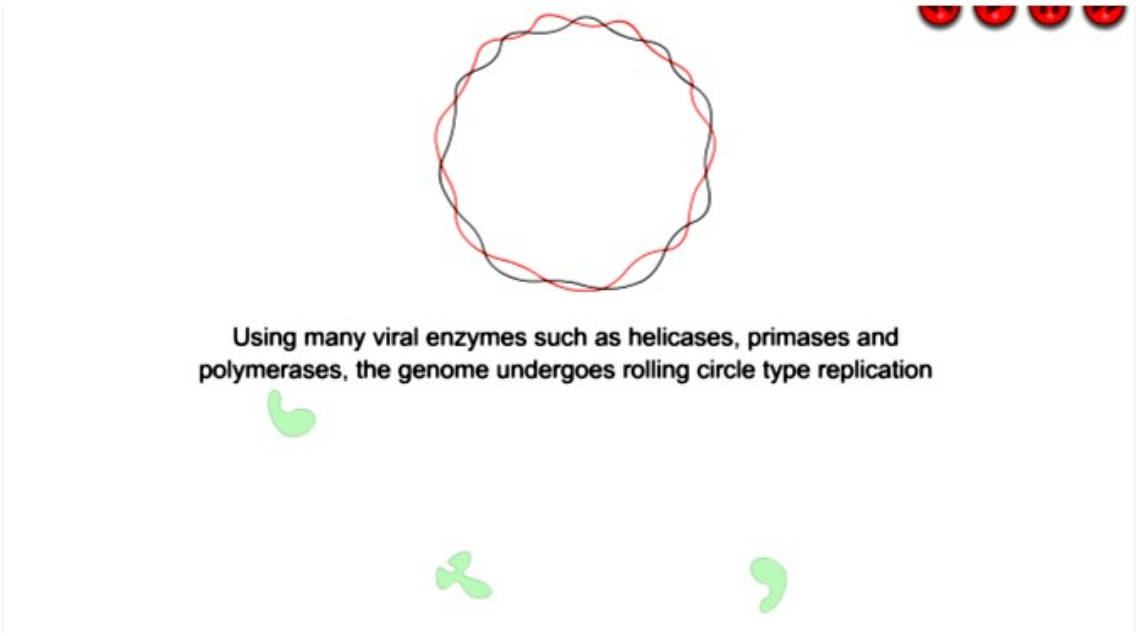
- Three classes of mRNA- **α, ß and ɤ** are transcribed in sequence by **cellular RNA polymerase II**
- **α RNA (immediate early) -** processed appropriately to become mRNAs, are translated to form α proteins
- **ß RNA (early RNA) -** the translation of which produces ß (early) proteins
- **ɤ RNA** (late) mRNAs, which are transcribed from sequences situated throughout the genome are translated into ɤ proteins



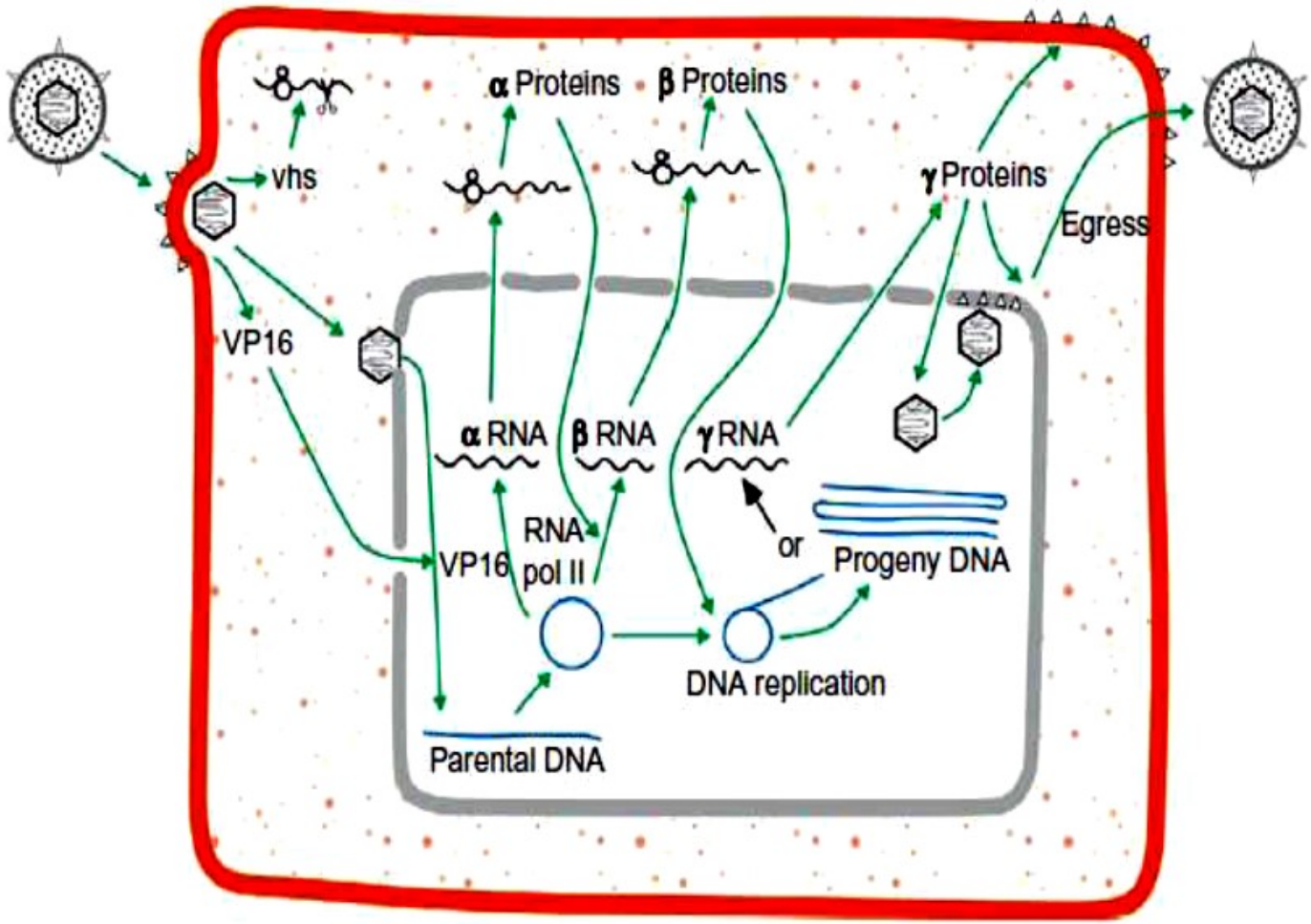


VIRAL REPLICATION

- Viral DNA replication then commences, utilizing some of the viral α and ß proteins, in addition to host-cell proteins.



Replication cycle



VIRAL REPLICATION

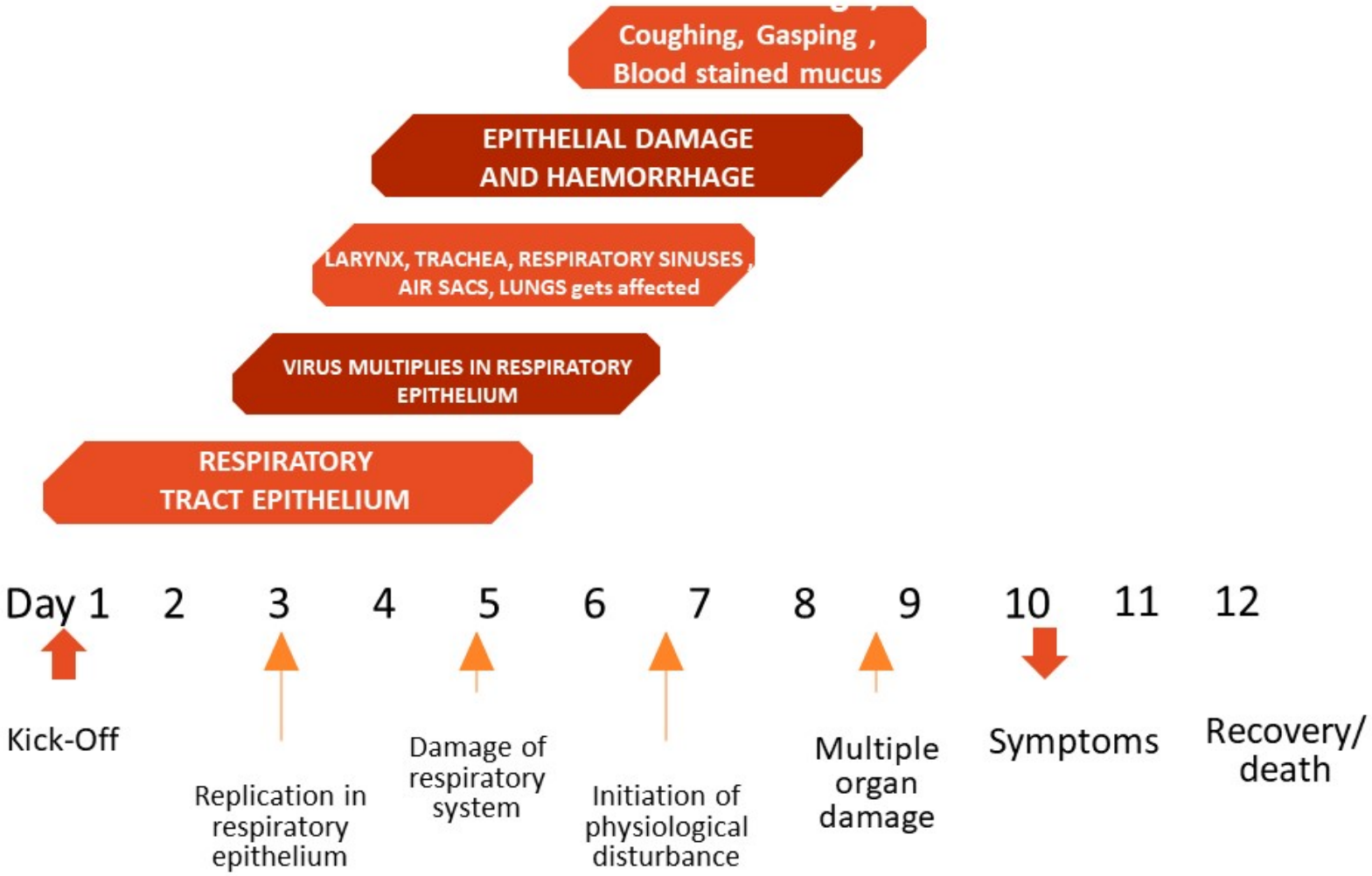
Maturation involves the completion of encapsidation of virion DNA into nucleocapsids and the association of nucleocapsids with altered patches of the inner layer of the nuclear envelope

Complete envelopment occurs by budding through the nuclear membrane

Mature virions accumulate within vacuoles in the cytoplasm and are released by exocytosis or cytolysis

Virus-specific proteins found in the plasma membrane, where they are involved in cell fusion

Pathogenesis Timeline



PATHOGENESIS cont…

Infectious virus usually is present in tracheal tissues and tracheal secretions for **6–8 days PI**

Extratracheal spread of LTV to trigeminal ganglia after 4-7 days of tracheal exposure

Trigeminal ganglion is the principal site of LTV latency

Reactivation of latent LTV from the trigeminal ganglia 15 months after vaccination of a flock has been reported from Germany

IMPORTANCE OF LATENT INFECTION

Rates of shedding of ILTV into the trachea increase by the stresses of either the onset of lay or mixing with unfamiliar birds

In this case, the latently infected chicken can act as an unsuspected reservoir host and enable ILTV to infect further susceptible chickens

CLINICAL SIGNS

Sub-acute form

- Nasal and ocular discharge
- Tracheitis
- Conjunctivitis
- Mild rales

Acute form (Severe form)

- Nasal discharge
- Moist rales
- Gasping **(Pump handle respiration)**
- Signs of respiratory distress (Dysnoea) including gasping and expectoration of bloody mucus
- High morbidity and moderate-to-high mortality
- Expectoration of blood stained mucus

Mild form

- Decreased egg production
- Watery eyes, conjunctivitis, swelling of infra-orbital sinuses,
- Mild tracheitis, persistent nasal discharge and hemorrhagic conjunctivitis mucoid tracheitis, sinusitis, conjunctivitis, general unthriftiness, and low mortality

CLINICAL SIGNS

Course of the infection varies with the severity of lesions

Generally, most chickens recover in 10–14 days

Epizootic forms of the disease cause high morbidity (90–100%) and variable mortality

Mortality can vary from 5% to 70% but usually is in the range of 10–20%

Mild enzootic forms of the disease result in morbidity as low as 5% and very low mortality (0.1–2%)

CLINICAL SIGNS

-RECOVERY

Infected birds that recover often become latent carriers and may intermittently shed the virus into the environment and infect other birds.

These recovered carrier birds are often the source of infection after introduction to naïve birds

Open beak breathing

Chicken is stretching neck and opening beak while breathing

chicken suffer from depression and gasping

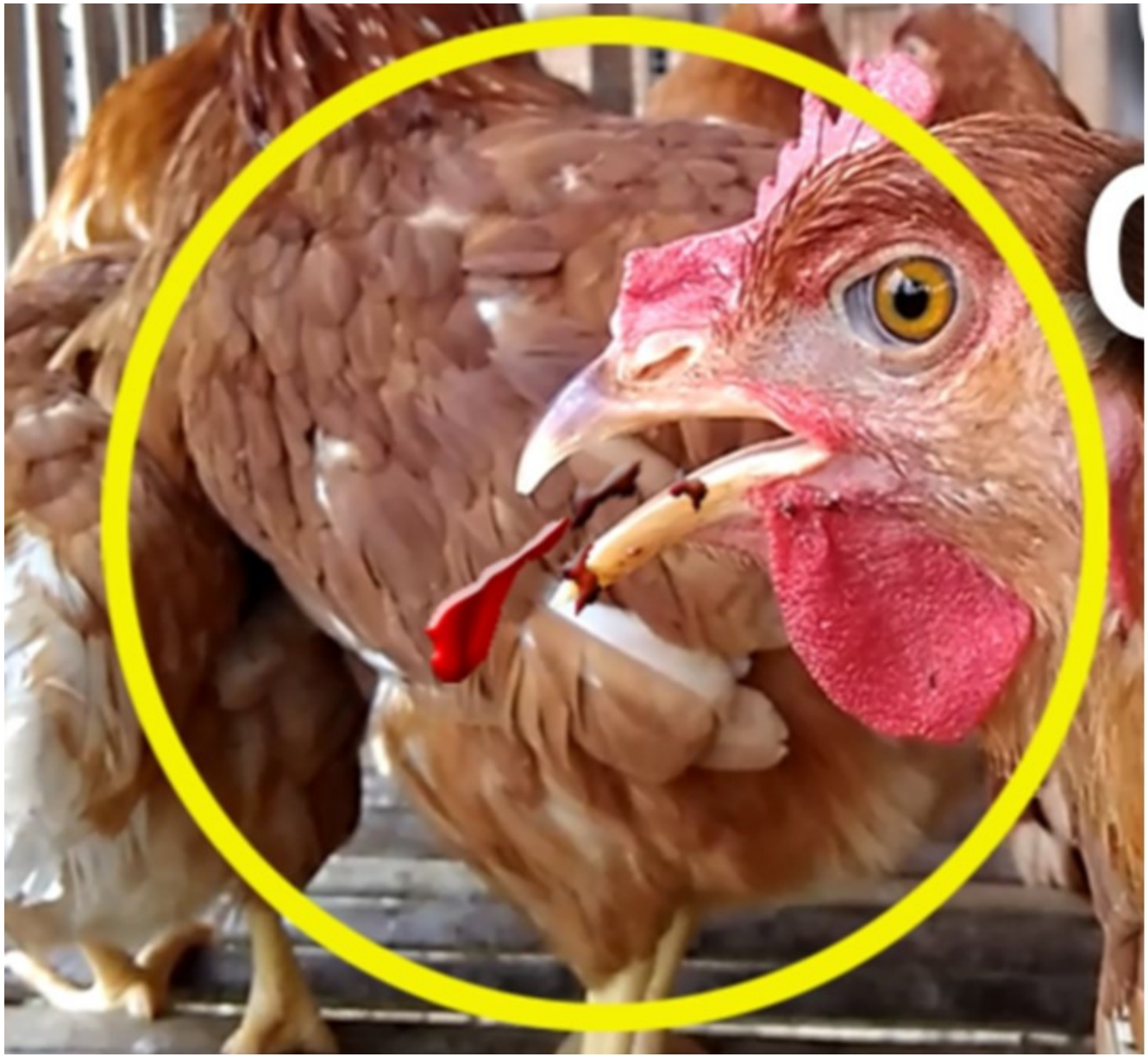

DEPRESSION
AND GASPING


LACRIMATION
ILTV INFECTED BIRD



Swollen eyelid and conjuctivitis


SWOLLEN EYE LID



Blood Coughing

Chicken coughing blood

Lacrimation


Diagnosis

Diagnosis

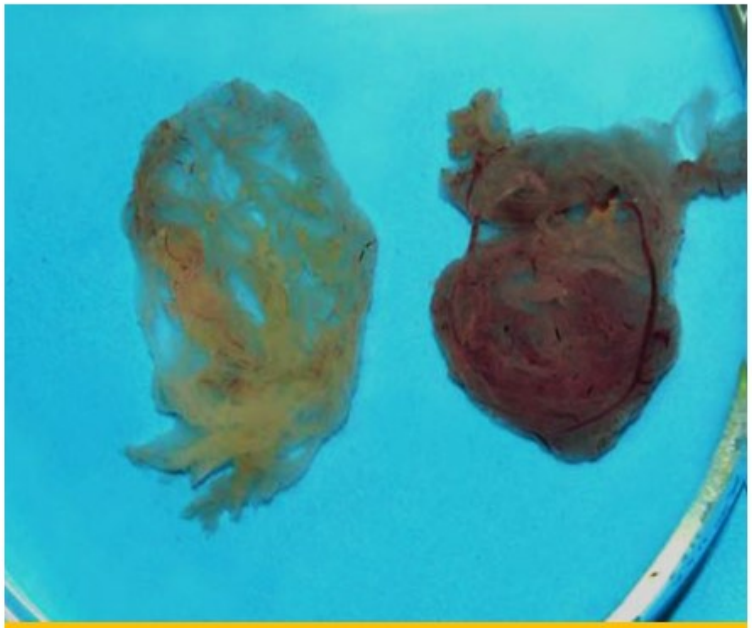
- 1. Clinical signs and symptoms
- 2. Isolation and identification of virus
    - Specimen for virus isolation:
        i. From live birds –Nasal swabs, throat swab, tracheal swab
        ii. From dead birds- lungs and trachea

Contd…..

- **Isolation of virus in chicken embryo**
  - Suspected sample is inoculated into SPF/antibody free embryonated chicken egg (9-11 day old) via CAM route
  - **Lesions:**
    - opaque plaques CAM resulting from necrosis and proliferative tissue with centralized area of necrosis is observed after 4-5 days for identification and confirmation of the virus.
    - embryo deaths
- **Isolation of virus in cell culture**
  - Primary cell culture of chicken embryo fibroblast(CEF) and Chicken embryo Kidney (CEK) cell are employed for isolation of virus
  - **CPE:**
    - Increased refractiveness and swelling of cells
    - Chromatin displacement, and rounding of the nucleoli
    - Cytoplasmic fusion results in formation of multinucleated giant cells.
    - Intranuclear inclusion bodies are detected

Contd….

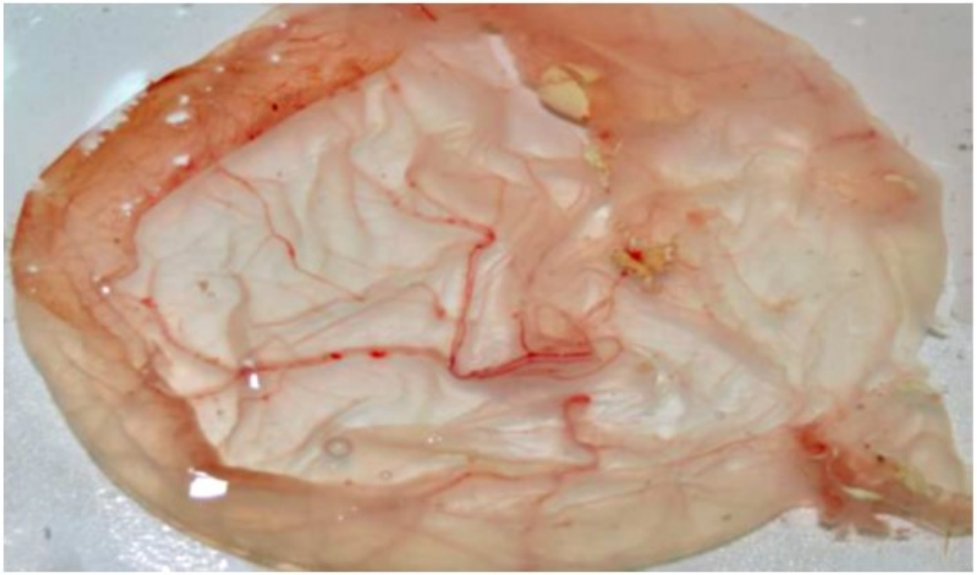
- ILTV identification by immunoprobes using Fluorescent-labelled polyclonal antibodies
- Detection by Immunoperoxidase-labelled monoclonal antibodies
- Electron microscopic examination of tracheal scrapings
- Serology.
  - agar gel immunodiffusion (AGID)
  - virus neutralization (VN)
  - indirect fluorescent antibody (IFA) test
  - ELISA
- DNA detection of ILTV
  - dot-blot hybridization assay



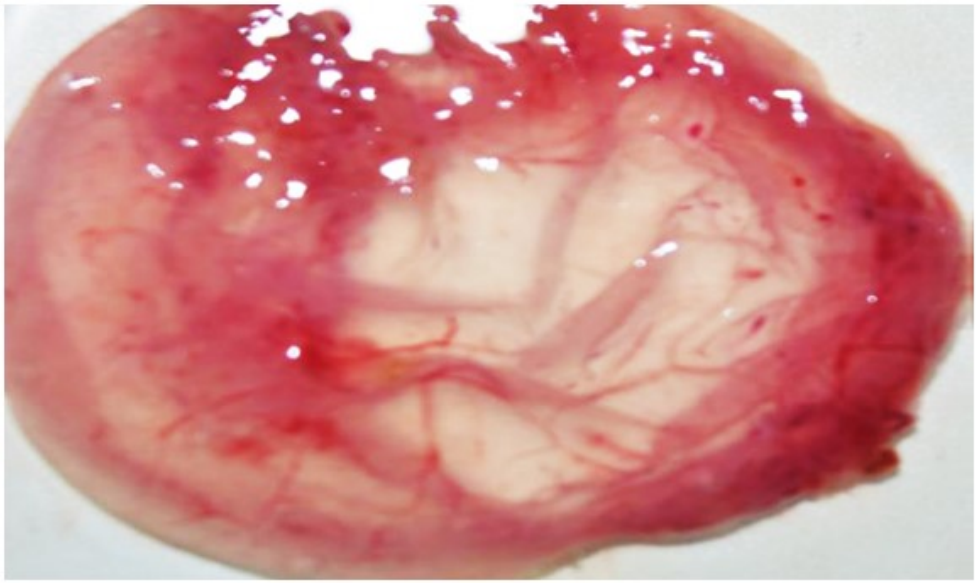
Left : infected CAM
Right: normal CAM

Left one showing stunted growth
Right one normal growth of embryo

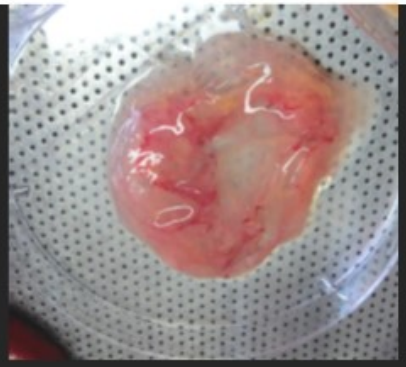
Isolation of ILTV in chicken embryo



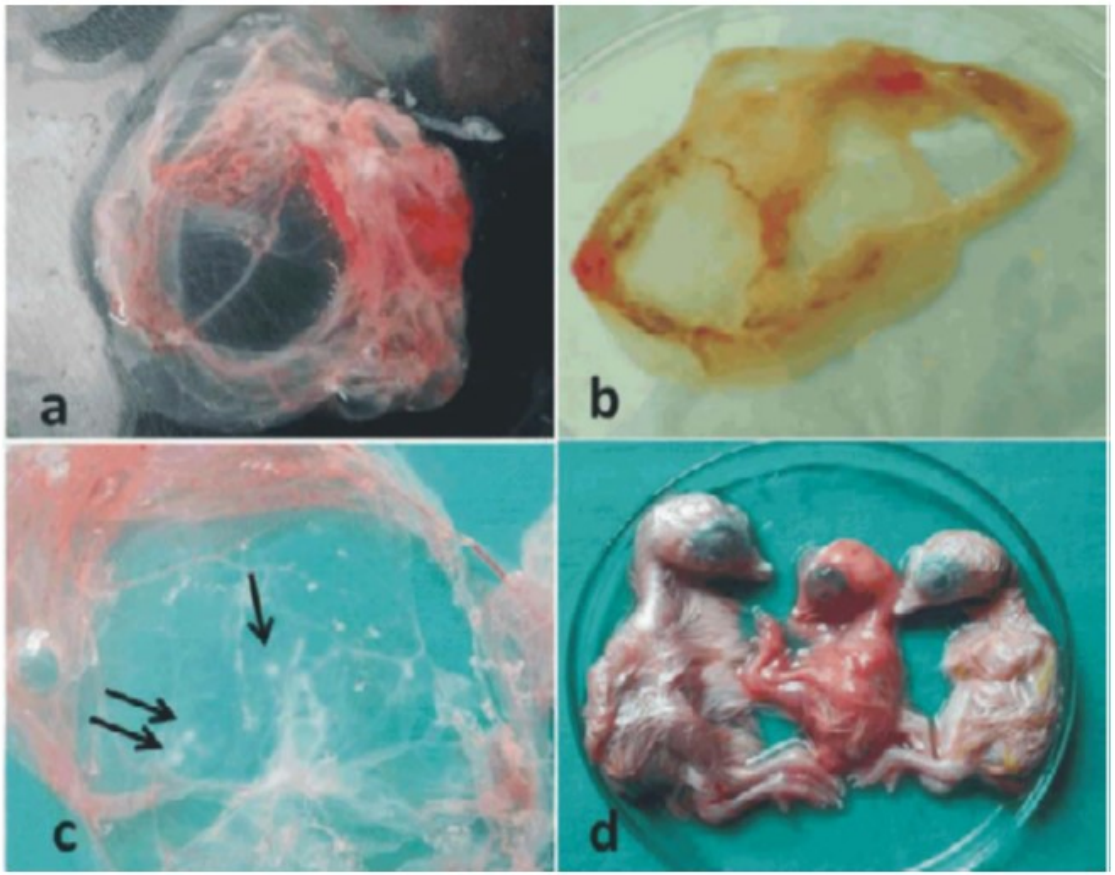
Noninfected CAM



Infected CAM with ILT virus



Isolation virus on Chorioallantoic membrane(CAM)

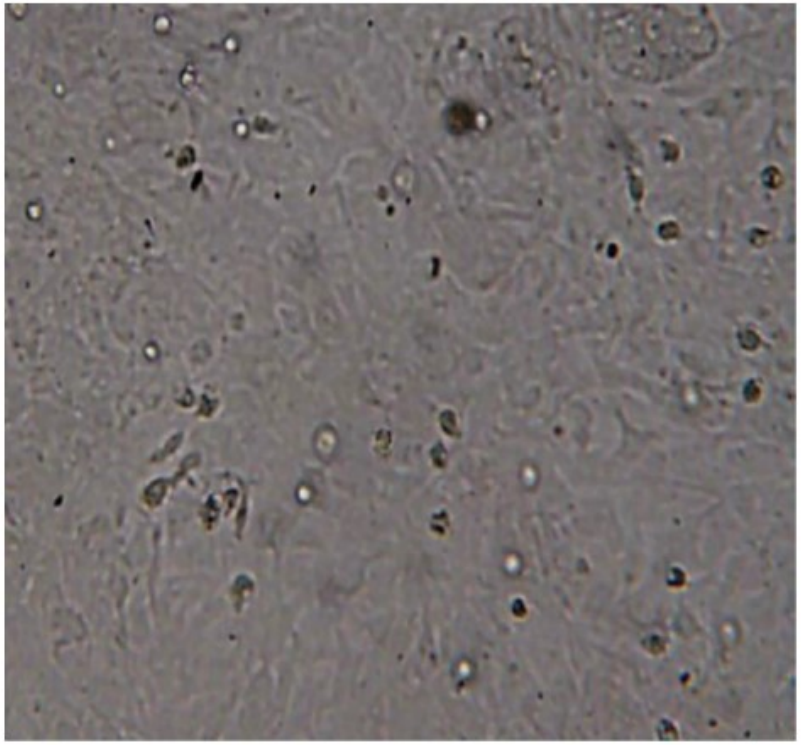


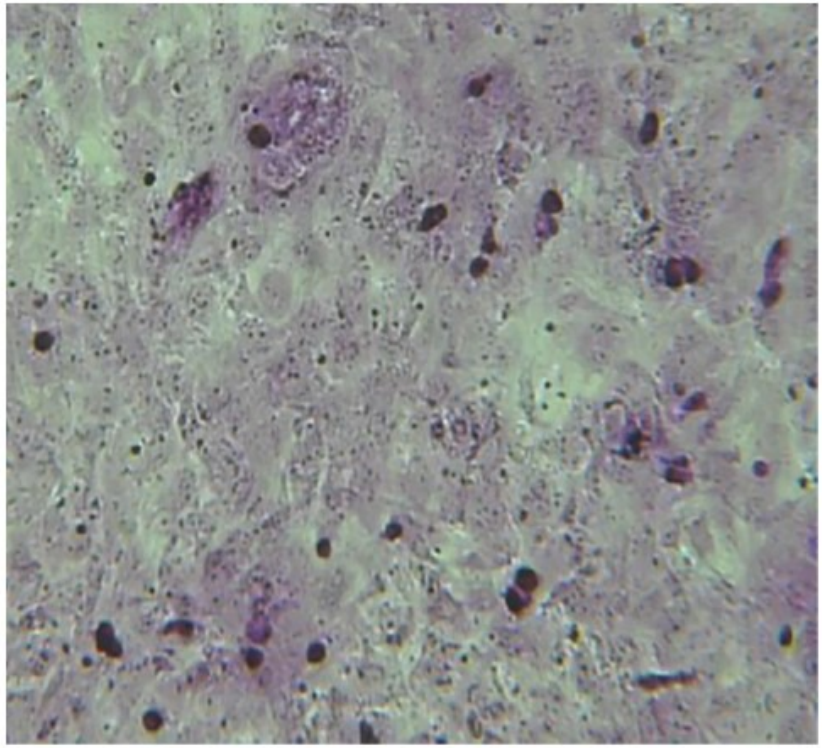
CAM Route isolation

- **a**. Normal CAM of non-infected embryo.

b. CAM of ILTV infected embryo showed thickening and cloudy appearance.
c. CAM of ILTV infected embryo showed white pock lesion after 3rd passage inoculation.
d. Normal non infected embryo (left) congested and stunted embryos (right).

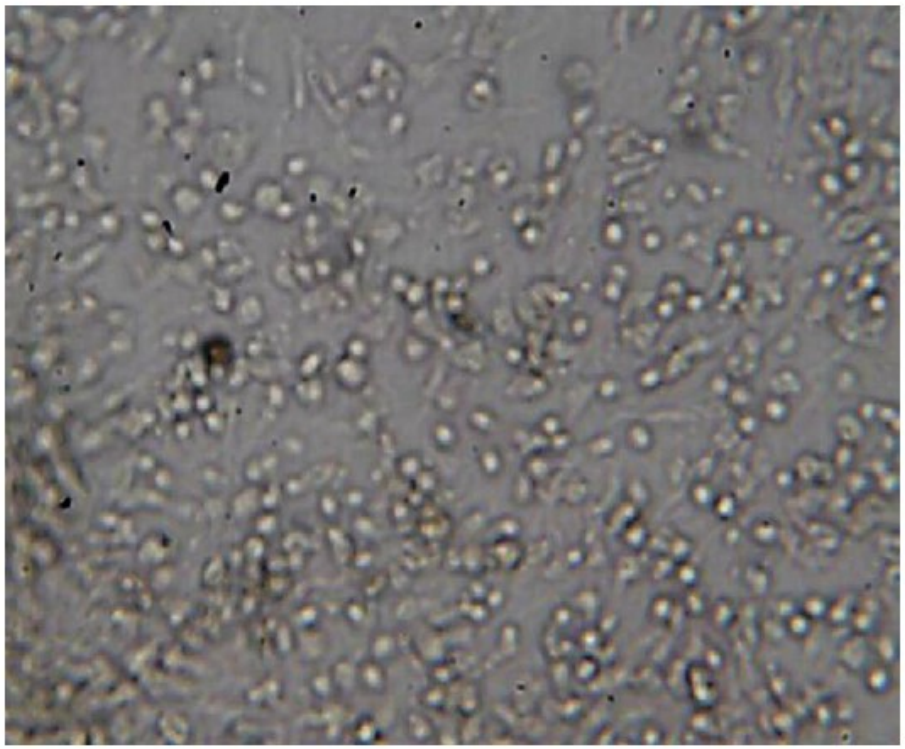
Isolation of ILTV on Cell culture



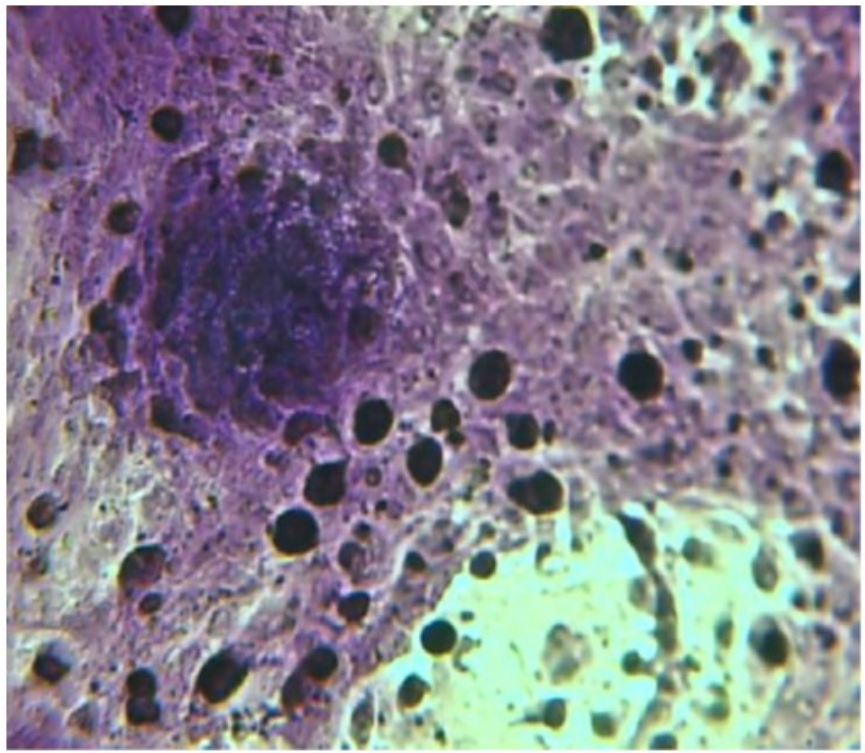


Normal chicken embryo cell cultures inoculation fibroblast cell culture

Isolation of ILTV on Cell culture

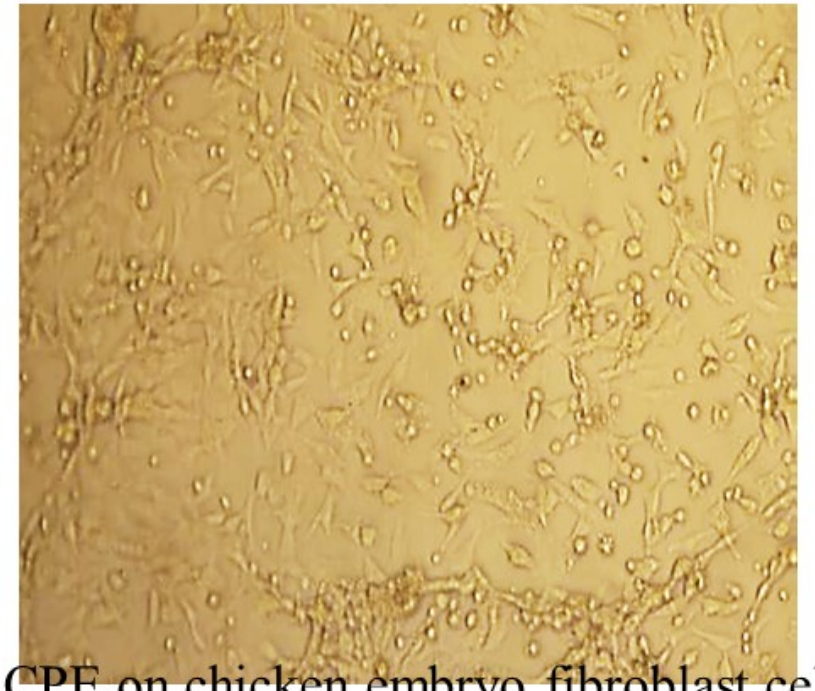


CPE on Chicken Embryo Fibroblast



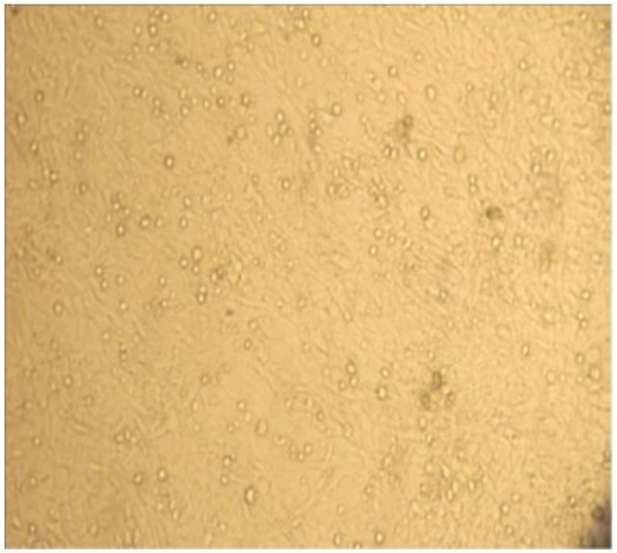
Explanation: Focal & Rounding of Cells

None infected CEF cell culture



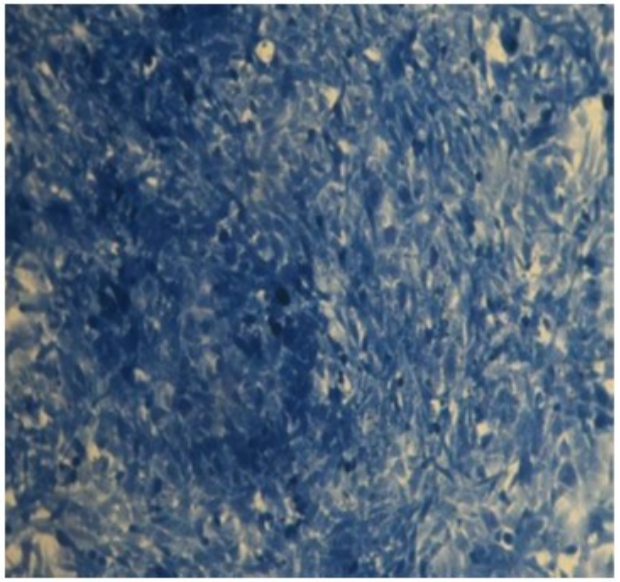
CPE on chicken embryo fibroblast cell culture after 5 days post infection

Isolation of ILTV on Cell culture



CPE of isolated virus on chicken embryo fibroblast cell

None infected chicken embryo fibroblast cell culture

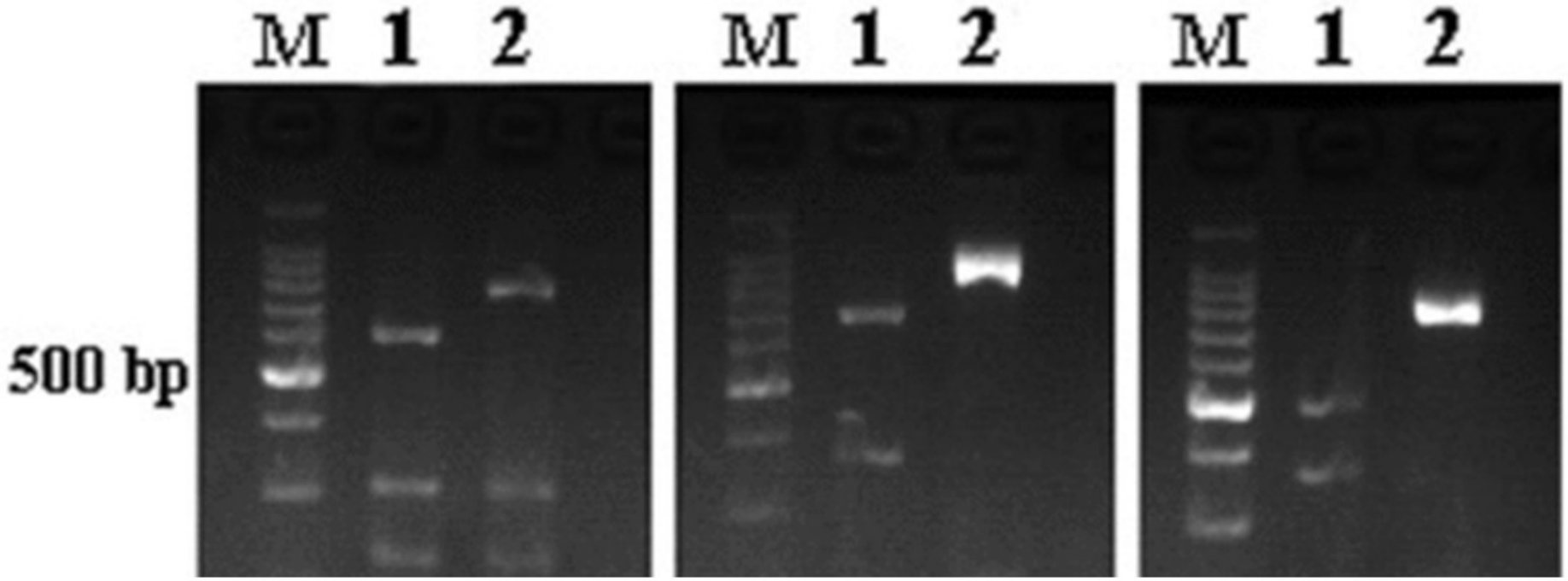


None infected chicken embryo fibroblast cell culture

Isolation of ILTV on Cell culture

CPE in cell culture

CPE of isolated virus on chicken embryo fibroblast cell culture after 5 days post infection with isolated ILT virus

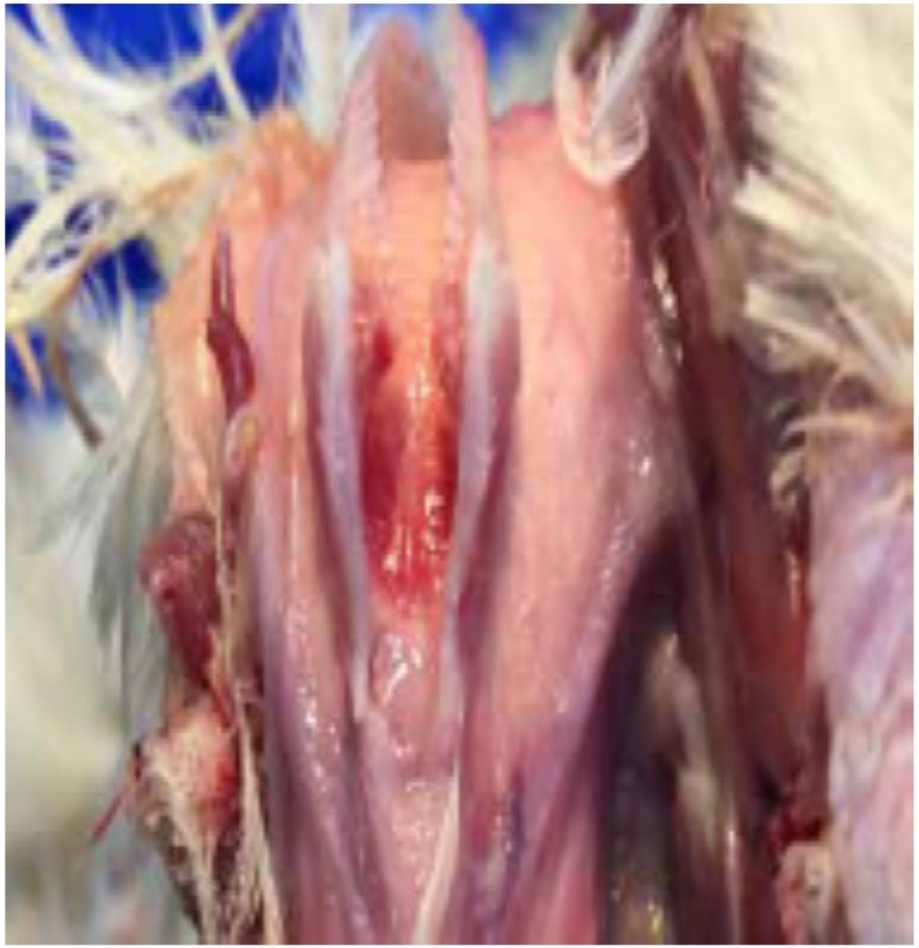


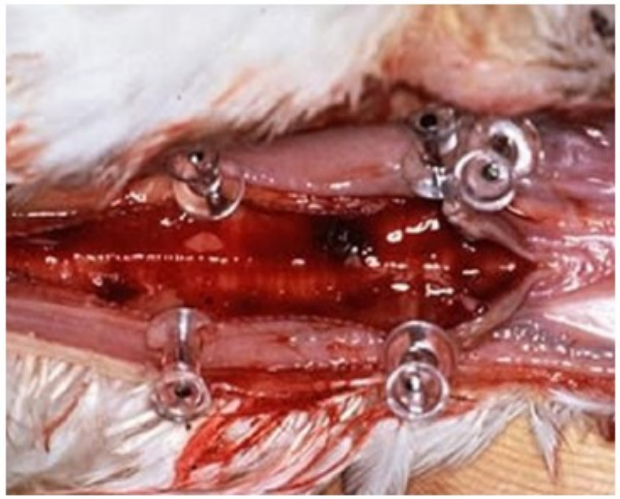
- The TK gene of infectious laryngotracheitis virus (ILTV) field strains and vaccine (tissue culture origin and chicken embryo origin) strains digested with *Sau*96I (**a**), *Nci*I (**b**), and *Hae*III (**c**). Lanes M: molecular size marker (100-bp DNA ladder); lanes 1: strain Guangdong-10; lanes 2: strain Guangdong-1. Guangdong-10 represented for strain Guangong-12, and Guangong-1 represented for the other 16 field ILTV strains and 6 vaccine strains.


INFLAMMATION OF SCIATIC NERVE

Fibrinohaemorrhagic tracheitis characteristic of ILT. This plug may occlude the trachea and cause death from asphyxiation.

Inflammation of the larynx and trachea.





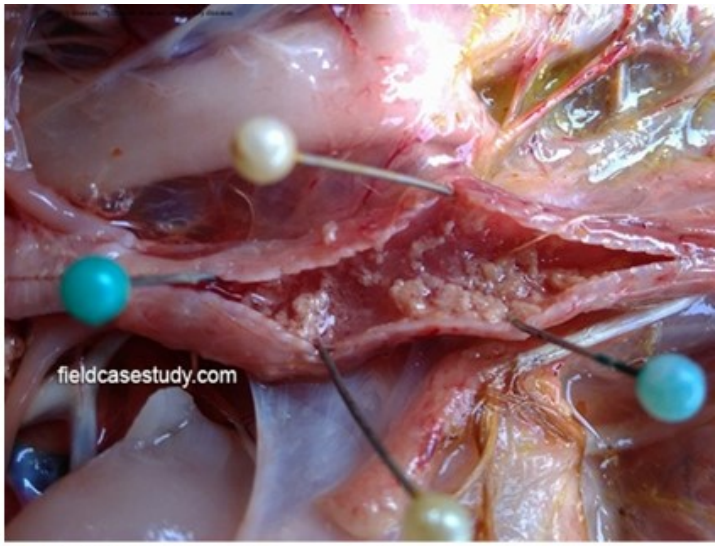


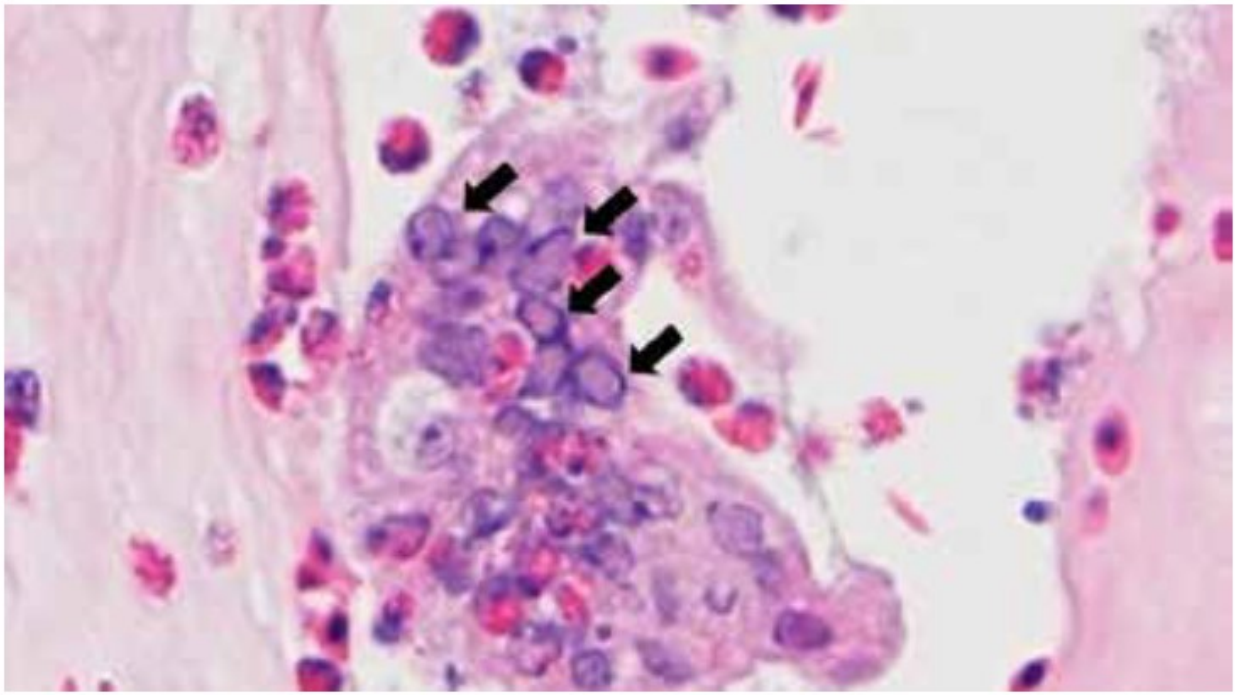
Fig. 2. Larynx and trachea, 35-week-old chicken, naturally infected by ILTV. Abundant caseous fibrinous exsudate blocking the lumen, characterizing the typical gross lesion of infectious laryngotracheitis.

Haemmorhage within the trachea – a common sign of infection.


TRACHEITIS DUE TO ILTV INFECTION

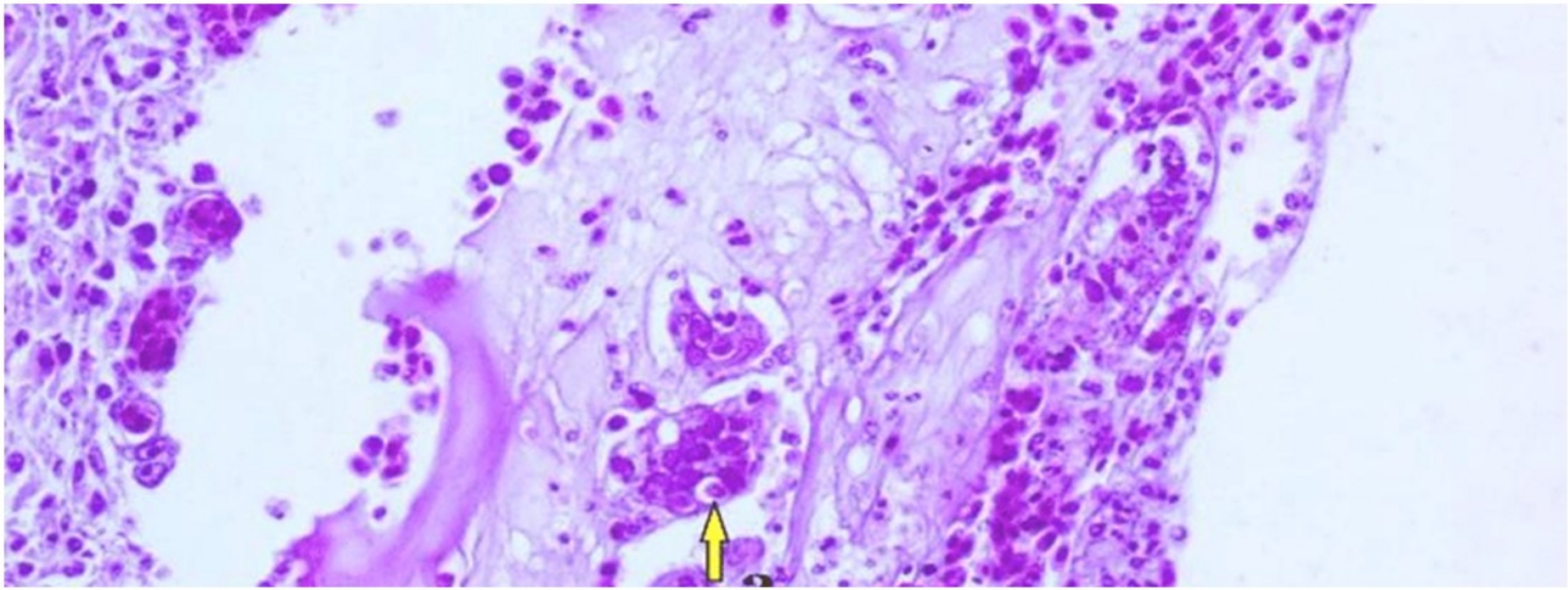
Hemorrhagic (1) and Fibrinous (2) Tracheitis in Layers Suspected of ILT


HAEMORRHAGIC ANDFIBRINOUS TRAHEITIS
2



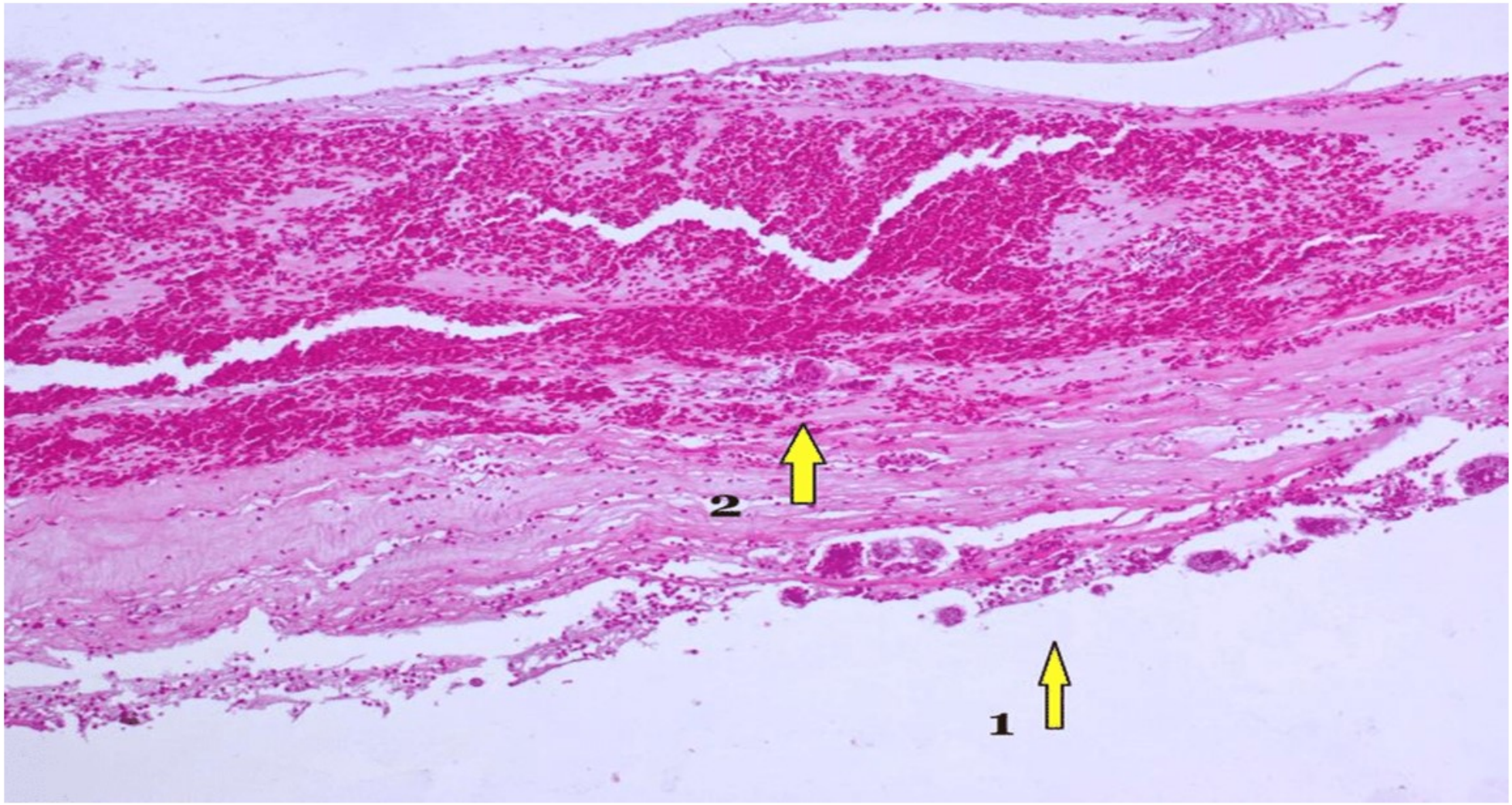
Histopathology

- *Microscopic view of affected tracheal mucosa.*
- *Large multinucleated syncytial cell showing many intranuclear inclusion bodies (arrows).*

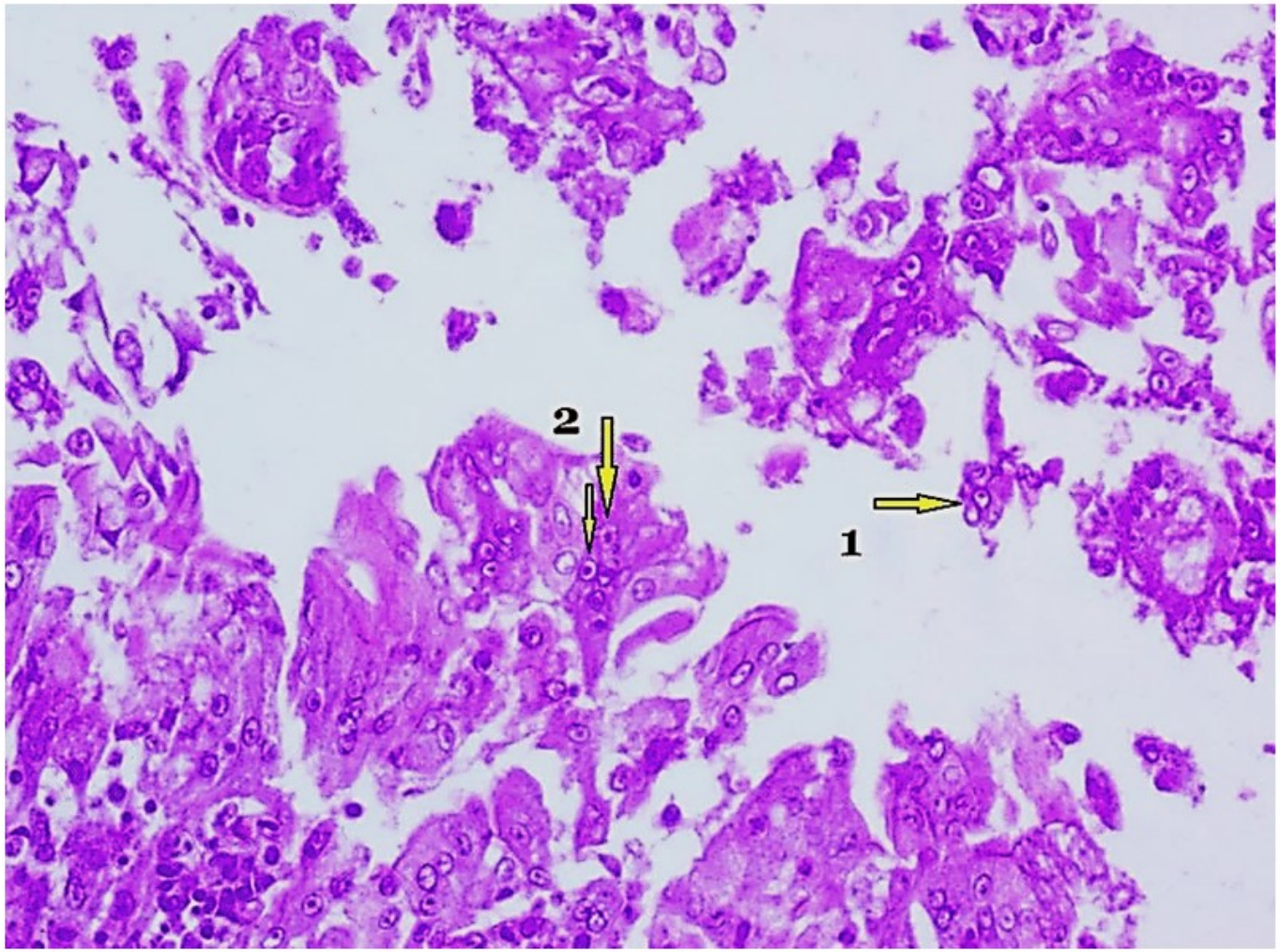


Histopathology

- Trachea of a layer hen suspected with ILT (H&Ex400)
- [1: Syncytium ++; 2: intranuclear inclusion bodies ++]
-



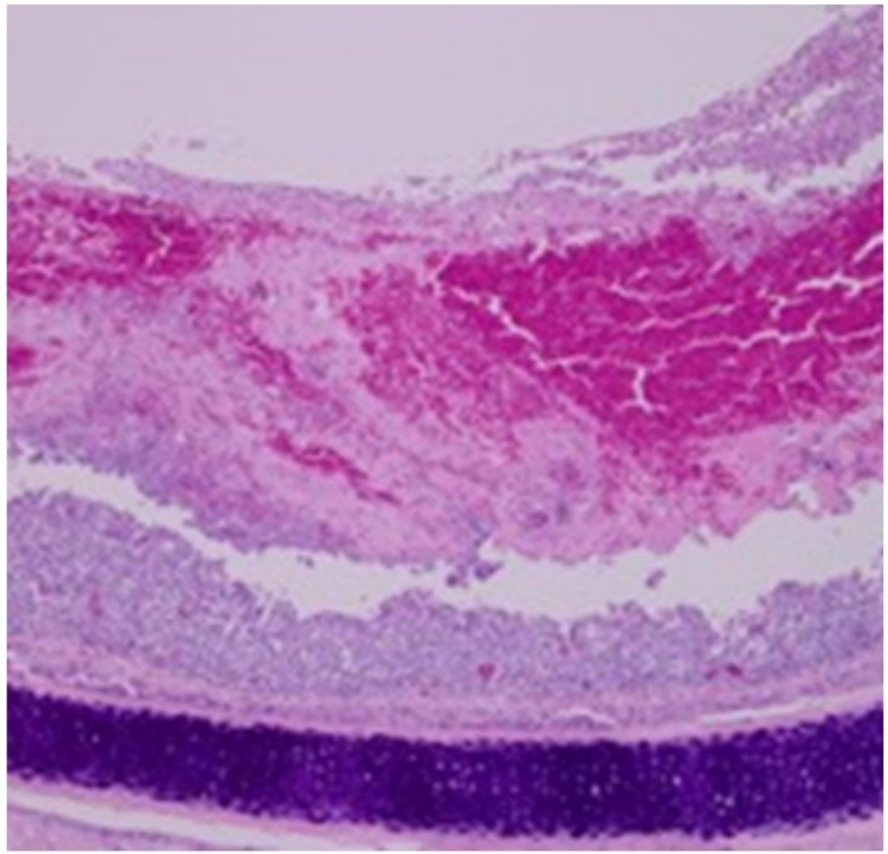
Hemorrhagic laryngitis in a layer hen suspected with ILT (H&Ex100)
[1: Necrosis of the epithelium surface +++; 2: hemorrhagic infiltrate of the chorion +++]



Larynx of a layer hen suspected with ILT (H&Ex200)
[1: Syncytium ++; 2: intranuclear inclusion bodies ++]

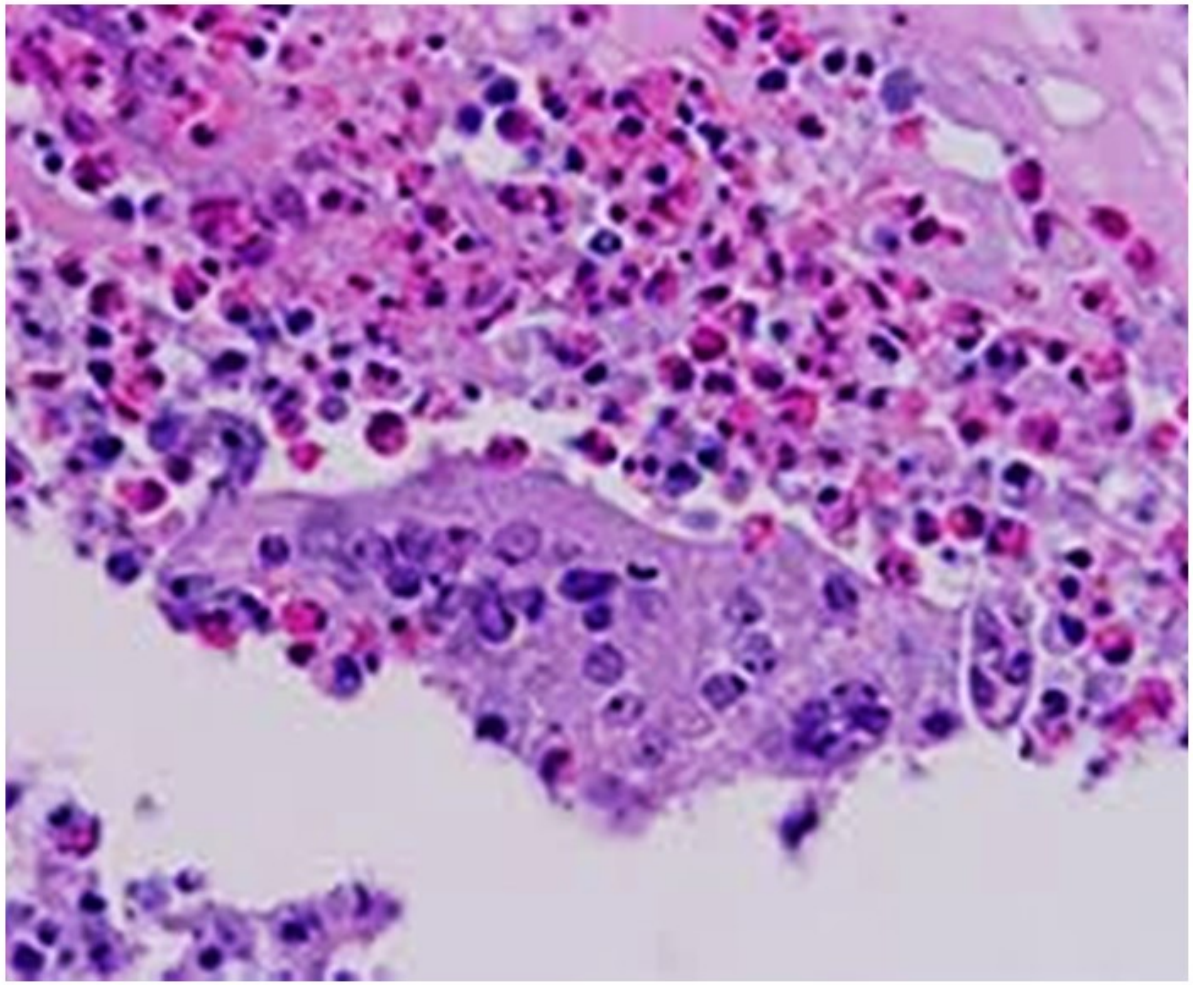
Trachea

- Extensive exudation of hemorrhage, fibrin, heterophils, macrophages, and sloughed epithelial cells into the tracheal lumen.



Trachea

- Numerous intranuclear inclusion bodies in sloughed tracheal epithelial cells.
- Many degenerating epithelial cells contained eosinophilic intranuclear inclusions. The lamina propria contained large numbers of lymphocytes and plasma cells.




Prevention
and control

INTERVENTION STRATEGIES

Vaccination for ILT cannot prevent infection

Vaccines can minimise the clinical effects of the disease, including production traits.

Vaccination

Type	Administration Route	Age
Modified Live Chick Embryo Origin (CEO)	✓ Eye Drop (preferred method) or Drinking Water or Coarse Spray	~ • Dose 1: 3–8 weeks of age. • Dose 2: 9–14 weeks of age, prior to moving to lay house/entering lay.
Modified Live Tissue Culture Origin (TCO)	✓ Eye Drop	~ • Dose 2: 10 weeks after dose 1, and prior to moving to layhouse/entering lay.
Vectored Pox-ILT	✓ Subcutaneous Injection	~ • Day of Hatch. May need to follow with CEO or TCO prior to entering lay if in high ILT challenge area.
Vectored Pox-ILT	✓ Wing-Web	~ 7–8 weeks of age.

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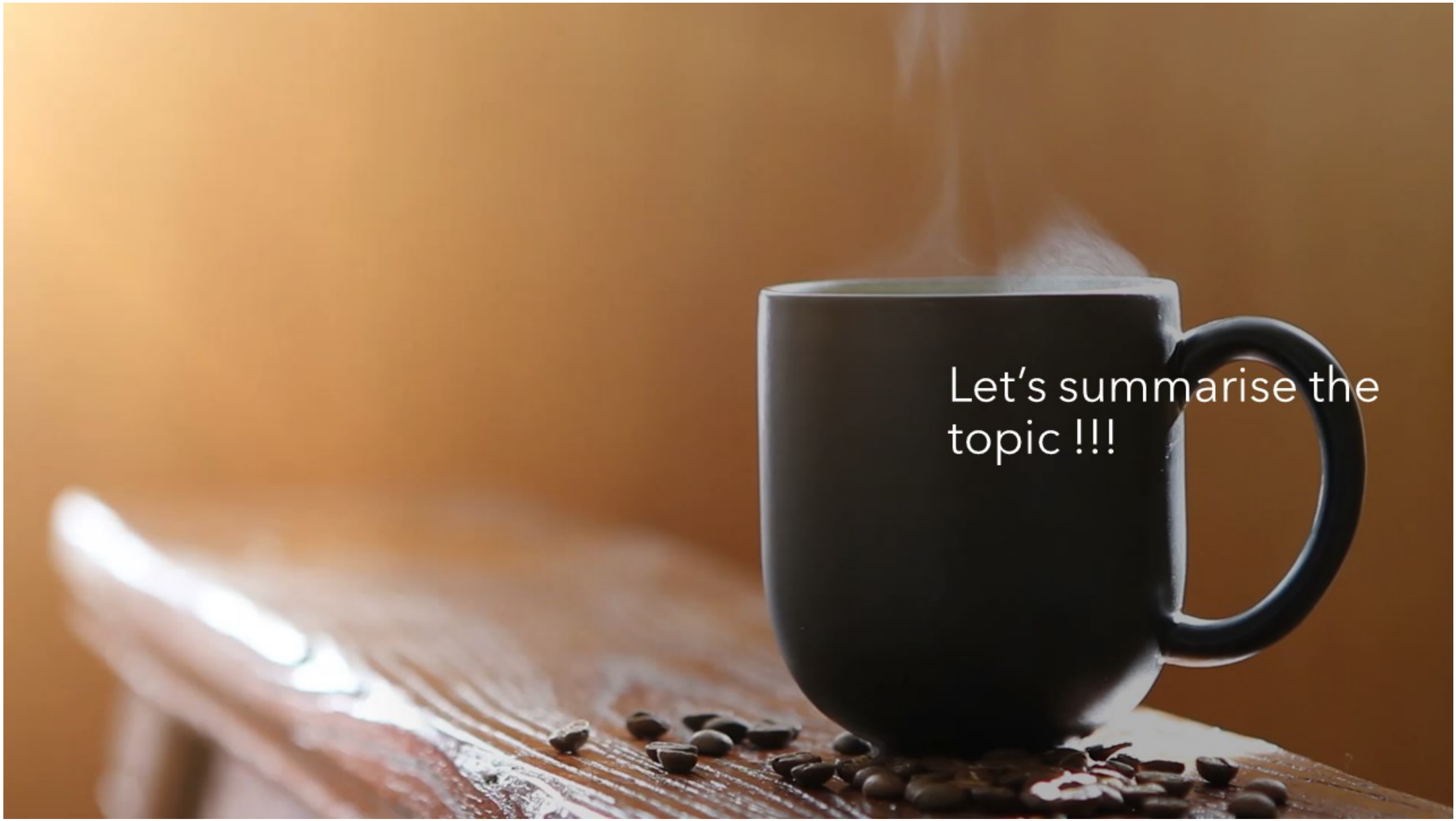
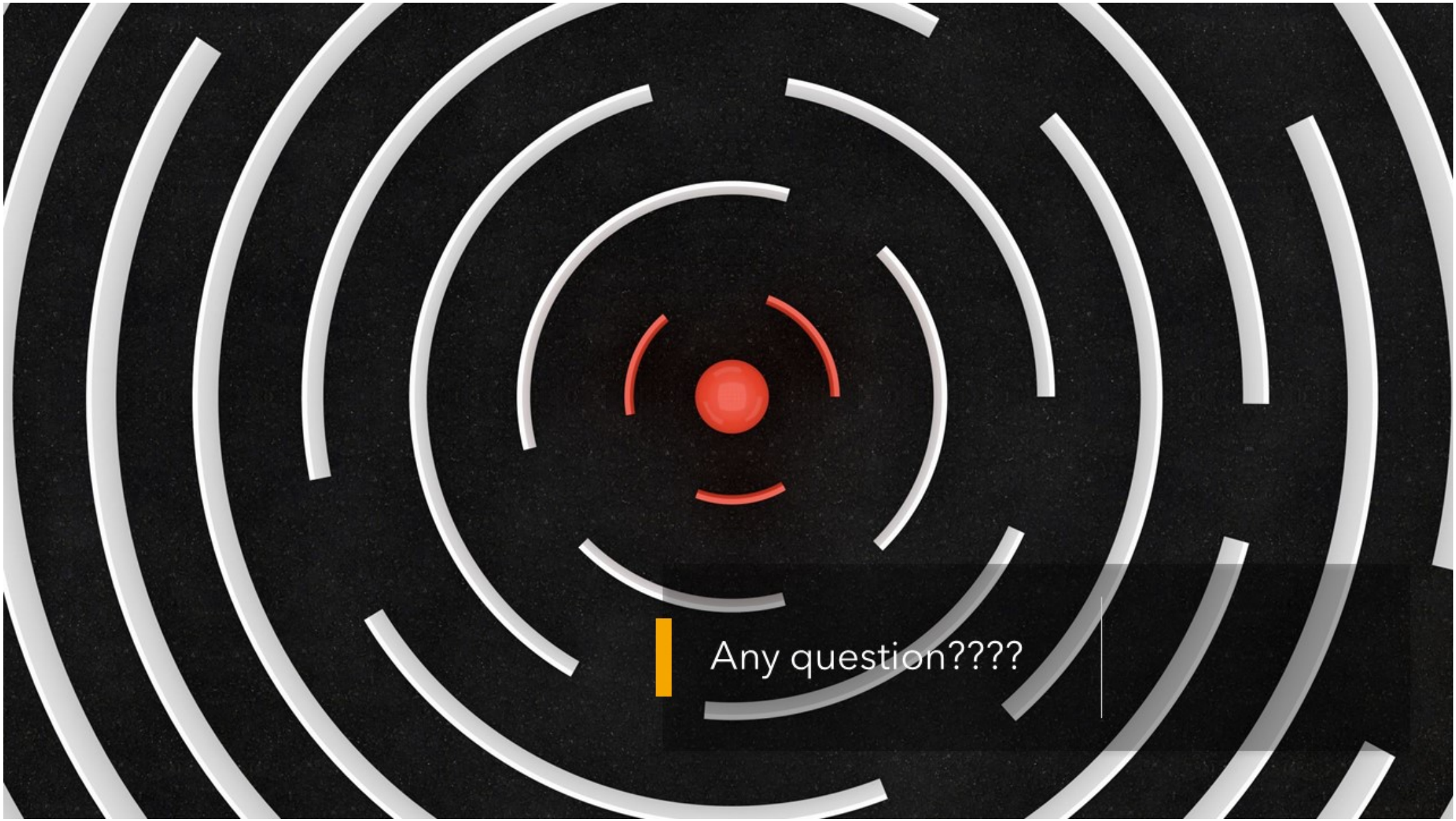
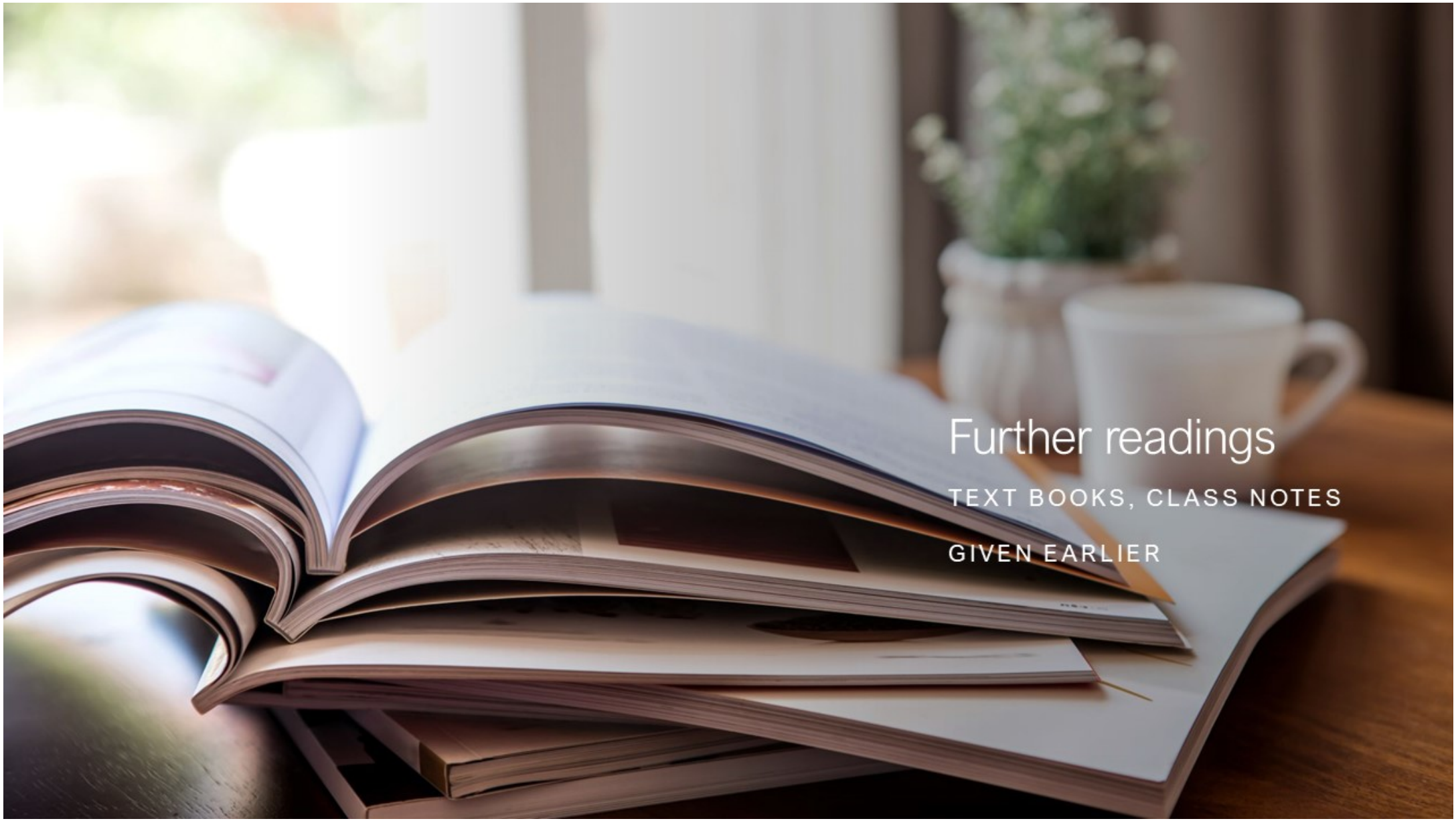
Modified Live Tissue Culture Origin (TCO)	✓ Eye Drop	~ • Dose 2: 10 weeks after dose 1, and prior to moving to layhouse/entering lay.
Vectored Pox-ILT	✓ Subcutaneous Injection	~ • Day of Hatch. May need to follow with CEO or TCO prior to entering lay if in high ILT challenge area.
Vectored Pox-ILT	✓ Wing-Web	~ 7–8 weeks of age.


FieldVet Channel : ILT


Let’s summarise the
topic !!!


Any question????


Further readings
TEXT BOOKS, CLASS NOTES
GIVEN EARLIER


Next topic: Poxviridae


FieldVet Channel : ILT
Thank you

ACKNOWLEDGEMENT

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