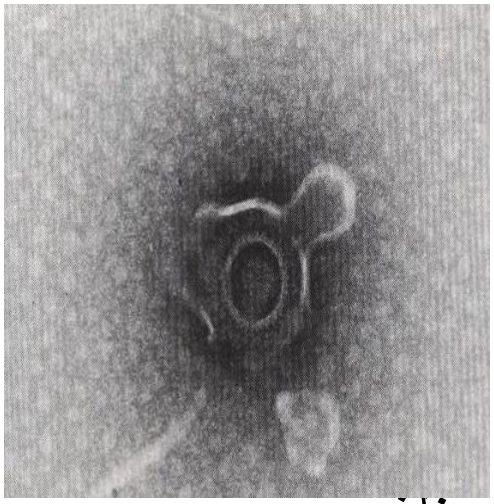
Infectious Laryngotracheitis **(ILT)**

مرض حاد فيروسي تنفسي معدي يصيب الدجاج بشكل أساسي وخاصة الجهاز التنفسي العلـوي.

يؤدي إلى خسائر اقتصادية ناتجة عن النفوق وانخفاض إنتاح البيض.

ILT is an Acute contagious herpes viral respiratory tract infection in chickens

- Gasping, neck extension and conjunctivitis (inflammation of the membrane around the eye).
- Production losses. (Growth and Egg)
- High Mortality,
- It can also be a subacute disease with nasal and ocular discharge, tracheitis, conjunctivitis, and mild rales.
- النهاب ملنحمة



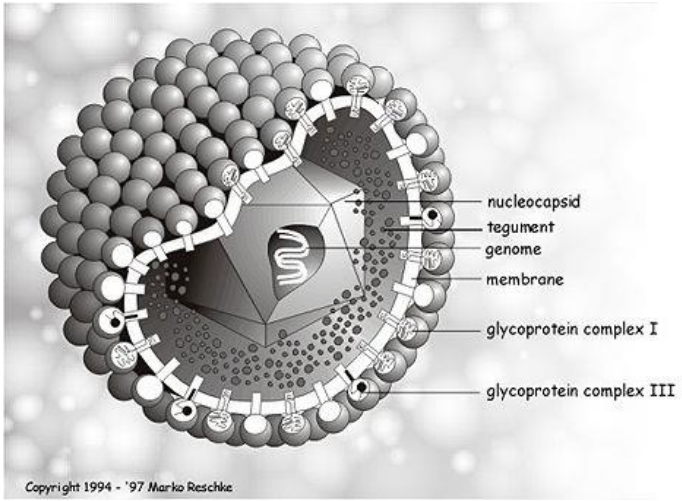
Etiology

ينتمي فيروس ILT إلى عائلة الحمّات الحلئية Herpesviridae .

- وتدعى هذه الأنواع Galled Herpes virus1
- يملك معظم الصفات الشكليائية لفيروسات الهربس مغلف ، سلسلة مضاعفة من DNA
- حساس للمؤثرات الخارجية in Vitro 55 درجة مئوية 15 د ، in Vivo 10-15° مدة 10 أيام.

Etiology

- **Herpes virus - Herpesviridea family –**
- **Alphaherpesvirinae subfamily.**
- **Enveloped Double- stranded DNA 190 – 250 NM**
- **May live for 8 to 10 days in droppings,**
- **up to 70 days in carcasses,**
- **80 days in throat exudates if not disturbed.**
- **Sunlight, heat and drying appear to be the natural enemies of the ILT virus.**
- **One percent Lysol or three percent cresol will inactivate ILT virus in less than a minute.**



Etiology 2

- The virus may be isolated in :
- Chicken embryo liver ***
- Chicken embryo kidney
- Chicken kidney cell cultures.
- CEF is not suitable
- CAM of 10-12-day-old Embryonated chicken eggs

- **ينمو الفيروس على أجنة الدجاج بعمر 9 – 12 يوم مؤدياً إلى تشكيل بثرات على CAM ونفوق وتقزم الجنين بعد 2 – 8 أيام و الأجنة الباقية تبقى على قيد الحياة مع استمرار الحضانة.**
- **كما يؤدي إلى نفوق وتقزم جنين الدجاج**

الاختلاف بين العترات Strains differentiation

- تعتبر عترات ILTV متماثلة أنتجينياً إلى حد كبير بالاعتماد على اختبار التعادل الفيروسي أو اختبار التألق المناعي
- وجدت بعض العترات التي تعادلت بشكل ضعيف مع الأضداد النوعية .
- ذراري شديدة وأخرى متوسطة أوضعيفة الضراوة تسبب أخماج شديدة إلى أخماج غير ظاهرة الأعراض
- يشكل الفيروس في خلايا الجهاز التنفسي العلوي أجساماً احتوائية داخل النواة تدعى أجسام سيفرد الاحتوائية تشاهد بعد 1-5 أيام من بداية الإصابة في ظهارية رغامى الطيور المصابة.

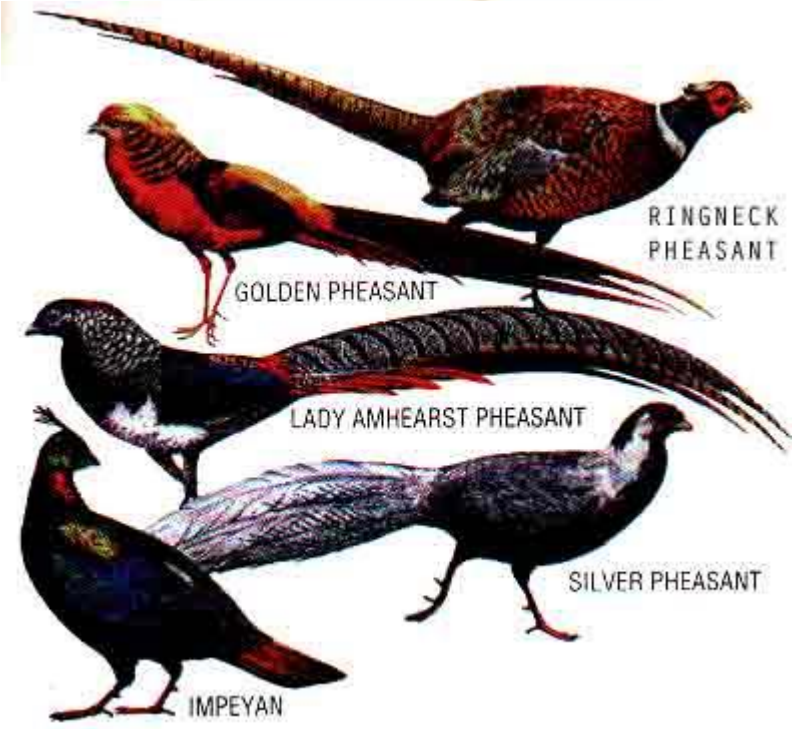
- **AGP- VN – FA - ELISA**

HOSTS

- **Species susceptibility:**
- Most frequently associated with *chickens*, but can also cause disease in related birds such as the *pheasants*, *peafowl* . *Duck and Geese* *are* *not affected*
- Chicken 2 – 10 months Could be less than 2 months
- fowls and turkeys.
- Wild birds may act as carriers.
- The ILT virus does not cause infection in humans.
- The disease often persists 2-6 weeks in the flock,

  a course longer than that of most respiratory viral diseases of chickens

Pheasant


RINGNECK
PHEASANT
GOLDEN PHEASANT
LADY AMHEARST PHEASANT
SILVER PHEASANT
IMPEYAN

- ينتقل المرض أفقياً عن طريق التماس الماشر مع مخاطية الجهاز التنفسي العلوي.
- لم يثبت انتقال المرض عمودياً عن طريق البيض أو اختراق قشرة البيضة.

- هناك شكلين تمييزين للمرض:

- 1 - الشكل الحاد
- 2 - الشكل المعتدل

Transmission

- Rapid *airborne* transmission among birds in close contact
- The virus enters the bird through the *eye*, the *nose* or the *mouth.*
- The coughed - up mucus and blood contains virus and is another means of quick spread of the disease.
- Movement of poultry, people and equipment. ***
- a major means of disease spread is by introduction of *affected birds* and latent carrier usually defined as *recovered birds or vaccinated* chickens, become carriers and can shed the virus for *long periods*

- *People in contact* with infected birds and on the same day contacting susceptible flocks may transfer the disease if suitable precautions are not taken.
- Small feathers and shed dust as well as litter and manure are ideal transporting agents.
- The virus is *not transmitted* through the egg so chickens are not affected at the time of hatching.

Signs

- أعراض عامة :الخمول وارتفاع حرارة الجسم وفقدان الشهية .
- سيلانات أنفية وإفرازات دمعية ، ومع تقدم الاصابة تزداد كثافتها من مصلية أو مخطية الى قيحية.
- صعوبة تنفس
- يؤدي اختلاط العلف مع الإفرازات إلى انسداد فتحات الأنف يرافق ذلك سعال ولهاث وعطاس ،
- **The disease often persists 2-6 weeks in the flock,**

Symptoms 3

Egg production (5 – 15%) with no apparent eggshell abnormality.

- Great economic losses can occur in broilers due to decrease *growth rate.*
- Disease may affect 5 to 80% of an exposed flock, taking 2 to 4 weeks to go through an entire flock.

Infected birds usually die but can recover within 7 to 10 days.

- Latent, infectious nature of the virus.

Signs summary

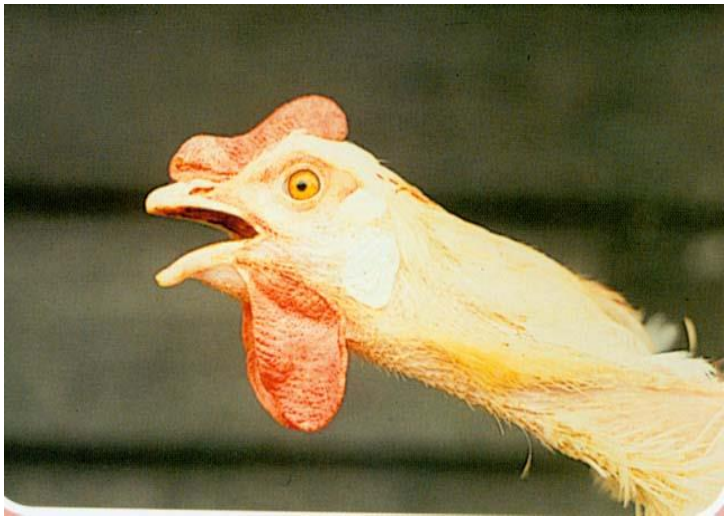
- ***Mild Form "ILT"***
- **Conjunctivitis**
- Ocular and Nasal discharge
- **Swollen sinuses**
- **Nasal discharge**
- **Egg loss / layers**
- **Growth loss / broilers**

- ***Severe Form "ILT"***
- **Severe coughing**
- **Bloody exudates**
- **Neck extended**
- **Labored breathing**
- **High Mortality -**
- **Persists for 2 - 6 weeks unlike other respiratory diseases**

Mild Form “ILT”

* Conjunctivitis
* Swollen sinuses
* Nasal discharge

ILT Respiratory signs



Losses

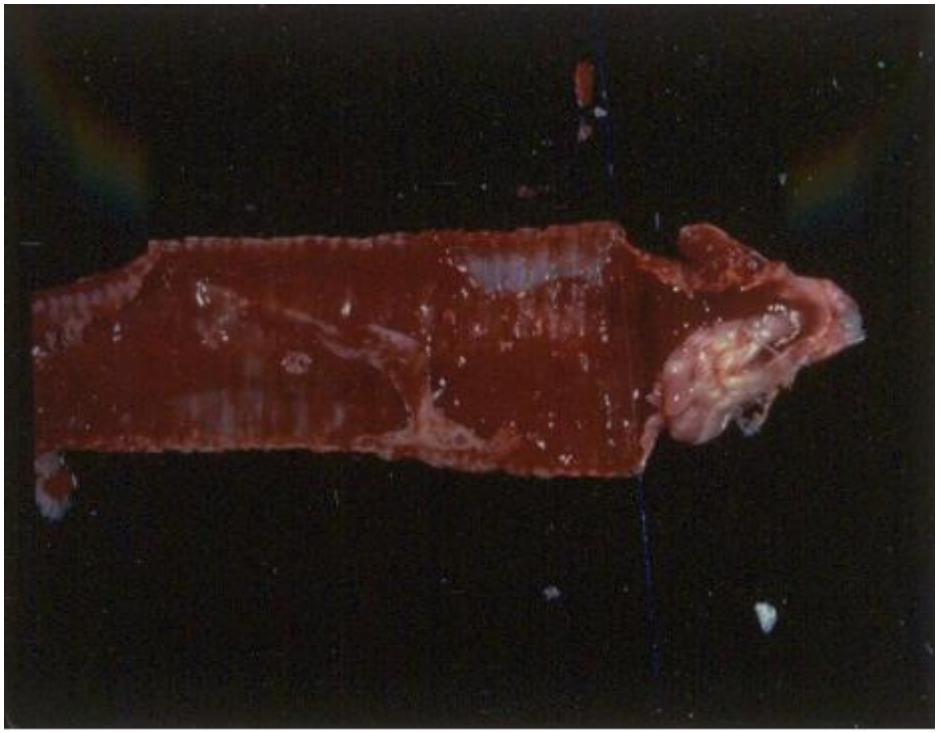
- In Delmarva in 1998, approximately 200 cases of ILT resulted in losses in excess of $1 million US due to vaccination, medication, carcass condemnations, mortality and decreased production.

Gross Lesions

- Hemorrhages in the trachea and larynx
- Trachea lumen is often filled with blood clots, mucus, caseous yellowish exudate
- Or tracheal plug which may cause death from asphyxia
- Airsacculitis and pneumonitis may be detectable in severe cases
- In the subacute form, punctiform hemorrhagic areas in the trachea and larynx and mild conjunctivitis with lacrimation may be detected

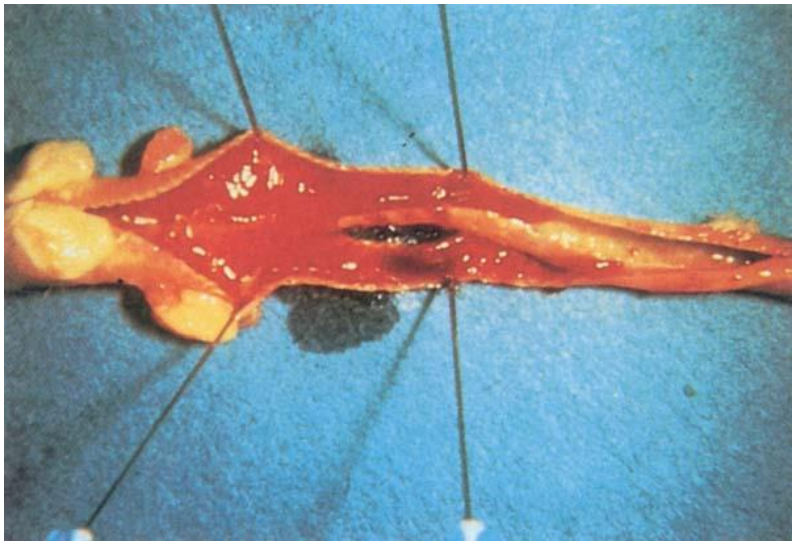
ILT Bloody exudates

- Ilt Trachea



ILT Fibrinous exudates

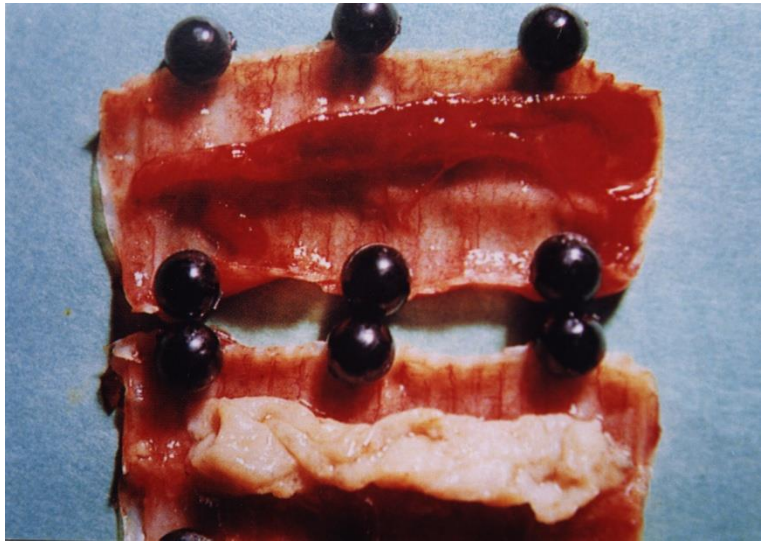
- **Caseous plug in the trachea.**

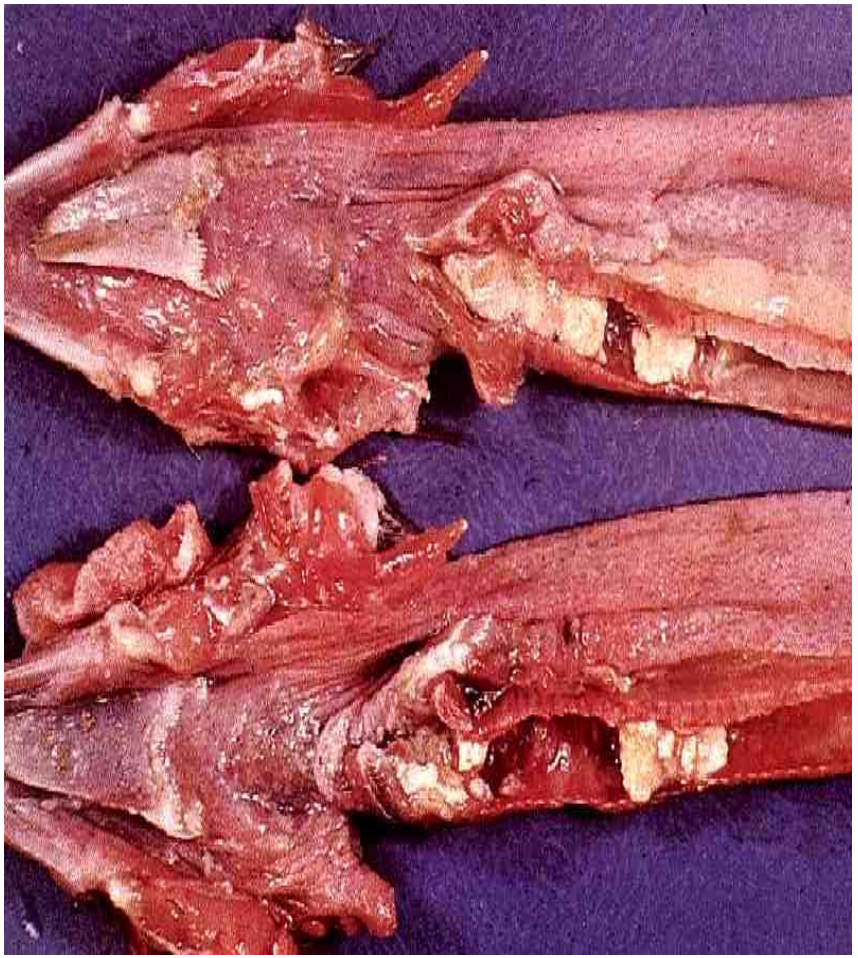


ILT

ILT- Severe hemorrhage into the tracheal lumen.

- ILT mucoid casts





DIAGNOSIS

- Clinical signs in acute form (Bloody Exudates).
- Mild Form, similar to ND, IB, Box:

- أخذ العينات:

أنسجة الحنجرة و الرغامى .

- سوائل الحنجرة و الرغامى .
- ملتحمة العين أو الجيوب تحت الحجاجية .
- الرئتين.

Diagnosis

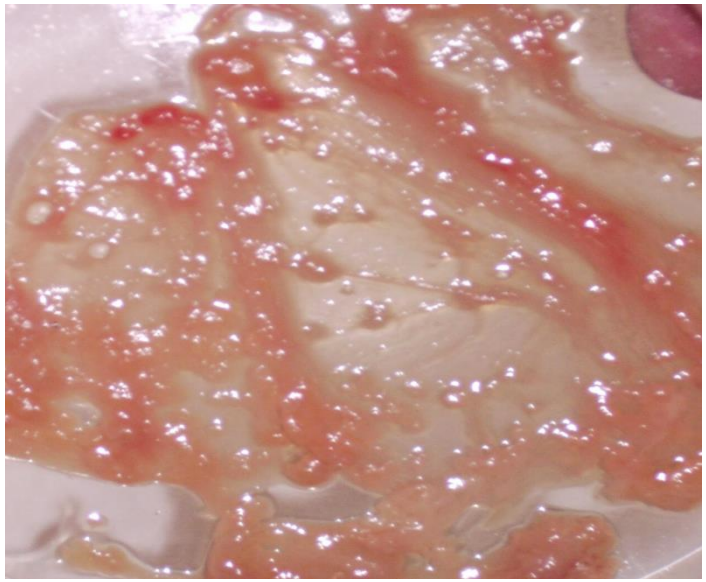
- Embryo Inoculation:

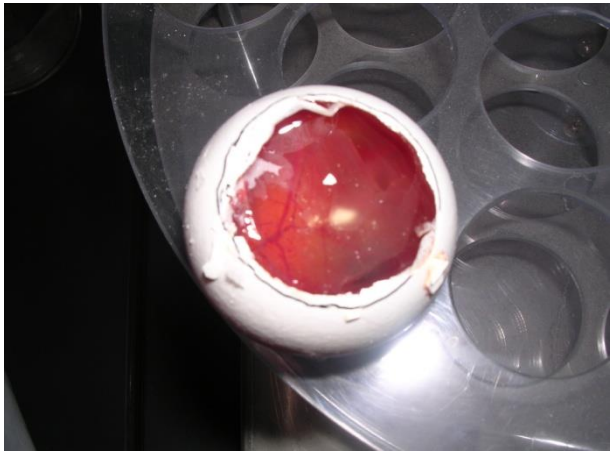
- Suspension of tracheal exudate or tracheal or lung tissues
- 9-12 days old chickem embryo on CAM
- 3 days after inoculation, plaques on CAM

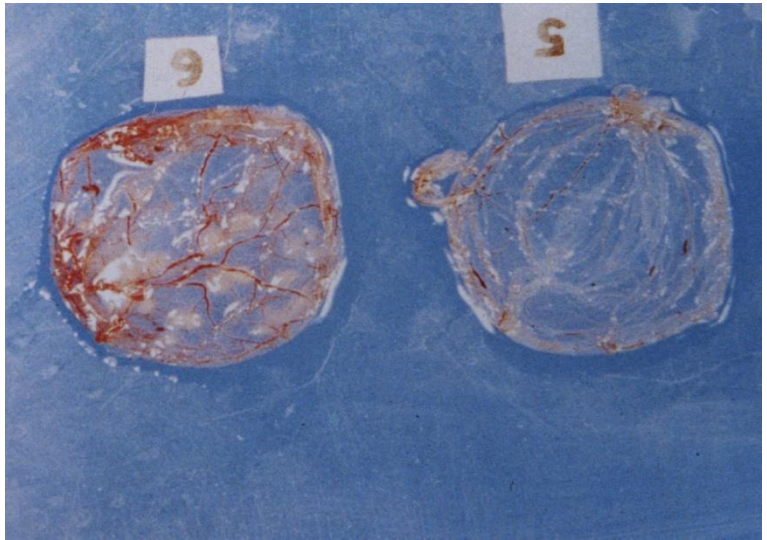
  (Proliferation and necrosis of affected cells)
- Some embryos die 2 to 8 days PI,
- Size of surviving embryos is often reduced

Plaques on CAM -ILT

- ILT







Laboratory Diagnosis

- The causative herpesvirus may be demonstrated directly in tracheal exudate by *electron microscopy* .
- Viral antigens may be detected by immunofluorescence , agar gel immunodiffusion (AGID)
- Enzyme-linked immunosorbent assay (ELISA), using tracheal mucosal scrapings
- Histopathological examination of the trachea for typical herpesvirus *intranuclear inclusions* may also be helpful .
- Polymerase chain reaction (PCR) have been described and PCR has been reported to be generally more sensitive than virus isolation .

Histopathology

1- Tracheas for histopathological examination placed in formol saline immediately after removal from the birds
2- Embedded in paraffin wax.
3- Staining by haematoxylin and eosin.
4- Intranuclear inclusions may be seen in the epithelial cells in longitudinal sections .

Classical Cowdry type A inclusions of herpesviruses, may be present for only 3-5 days after infection.

In severe cases where most infected cells have detached from the tracheal lining, inclusions may be seen in intact cells among the cellular debris in the lumen of the trachea.

VIRUS ISOLATION

- The virus may be isolated in
- Chicken embryo liver ***
- Chicken embryo kidney
- Chicken kidney cell cultures.
- Chorioallantoic membrane (CAM) of 10-12-day-old embryonated chicken eggs

Immunofluorescence

In immunofluorescence tests for viral antigens, epithelial cell scrapings from the trachea are smeared on to a glass slide. Alternatively, snap-frozen 5 µm thick sections of trachea may be used. The preparations are fixed in acetone at room temperature for 10 minutes. These can be stained directly by applying chicken anti-ILT virus immunoglobulin labelled with fluorescein isothiocyanate (FITC) for 1 hour, followed by rinsing for 15 minutes in a bath of phosphate buffered saline (PBS), pH 7.2, agitated with a magnetic stirrer. Otherwise, they can be stained indirectly by applying an appropriate dilution of chicken anti-ILT serum for 1 hour. The slide is rinsed thoroughly with PBS for 15 minutes as above, and an FITC-labelled anti-chicken immunoglobulin is applied for 30 minutes. After a final rinse, cover-slips are applied over nonfade mountant. The preparations are examined for specific intranuclear fluorescence in the epithelial cells using a microscope with epifluorescent ultraviolet illumination. Suitable controls include the use of known uninfected specimens and, for the indirect method, the application of nonimmune chicken serum. Particular care should be taken in the reading of indirect immunofluorescence preparations, as endogenous chicken IgG in the trachea may cause unwanted attachment of FITC-labelled anti-chicken IgG.

Diagnosis cont..

- **Agar gel immunodiffusion**
  ILT viral antigens may be demonstrated by AGID tests on tracheal exudate, infected CAMs and infected cell culture material using hyperimmune ILT virus antiserum. The gel is made with Noble agar (1.5%) containing sodium chloride (8%) and sodium azide (0.02%) - as preservative - in distilled water. The ingredients are autoclaved at 15 lb/sq. inch (2.4 bar) for 15 minutes; 5 ml of the molten agar is poured into a 5 cm diameter Petri dish. When the agar has set, a pattern of wells is punched in the agar, consisting of a central well and six surrounding wells. The wells are usually 8 mm in diameter and 4 mm apart. The hyperimmune serum is pipetted into the central well, while the surrounding wells are filled with suspect virus samples under test, but with at least one well containing positive viral antigen. Dishes are incubated in a humid atmosphere at room temperature or at 37°C, and examined 24-48 hours later by oblique illumination for lines of precipitation (reactions of identity). Tests should include uninfected material as negative antigen and known negative antiserum as controls. For economy of materials, the test can be done on a microscale - the agar being poured in a thin layer on to a microscope slide and holes punched of 4 mm diameter and 2 mm apart.

VACCINES

- **اللقاح الضاري :**
- استعمل هذا اللقاح في الماضي بطريقين :
- 1- طريق خدش مخاطية فتحة المجمع : .
- 2- التنقيط على الغشاء المخاطي لفتحة المجمع .
- في كلا الحالتين : يظهر تفاعل إيجابي تورم وانتباج شفاه المجمع
- إن مدة المناعة الناتجة عن هذا اللقاح تستمر لمدة عام تقريباً .

- **اللقاح ضعيف الضراوة :**
- وتعطى عن طريق ماء الشرب
- أو عن طريق التقطير في الأنف أو ملتحمة العين ،
- وتعطى بعمر يتراوح بين 8 – 12 أسبوعاً ، وتكون مدة المناعة الناجمة عن هذا اللقاح تتراوح بين 6 – 12 شهراً .
- أو 4-6 أسبوع يعاد 14-18 أسبوع

Vaccine

- Chicken Embryo Origin (CEO) vaccines can revert back to virulence.
- CEO vaccines passage 20 times in specific pathogen free chickens revealed an increase in virulence after the 10th passage.

Vaccination programs

Most popular and accepted methods are via eye drop.

- The first vaccine is usually eye drop, followed by the second via the eye drop route or strategic water application.

Vaccine cont…

- There is only one approved vaccine for LT in some countries - modified tissue culture,. -
- Chickens vaccinated with CEO vaccine are considered to be infected.
- CEO-vaccinated birds can become infected with LT and shed the virus without showing clinical signs of the disease.
- The shed vaccine virus can cause illness in unvaccinated chickens.

Inactivated and Genetically Engineered Vaccines

- Inactivated Vaccines and purified glycoproteins, stimulate immune responses and varying degrees of protection
- High costs
- Gernetically Modified Vaccine (promising)

FOWL LARYNGOTRACHEITIS - MAREK'S DISEASE VACCINE

- **Serotype 3, Live Marek's Disease Vector**
- **INNOVAX®-ILT is a frozen, cell associated, live virus vaccine that contains the recombinant serotype 3 turkey herpesvirus with genes from larygotracheitis virus.**
- **The vaccine ampules are inserted in metal canes, stored and shipped in a liquid nitrogen container.**
- **Subcutaneous Vaccination of Day Old Chickens**

Broiler Vaccination ??

- Frankly, broilers should not be vaccinated.
- It is a plague that needs to be controlled.
- Can vaccine do it? Yes, it can, but the industry needs to be willing to pull out all measures as explained, to reduce the shedding and spread of the vaccine virus.
- If vaccination needs to be implemented, then every bird should be picked up and dosed with one drop of diluted vaccine per bird. Impractical due to time, unavailability of vaccine crews; cost of application and frustration to be vaccinated NOW scenario.
- Hence, drinking water or coarse spray application is selected.

- From the reading of countless articles, the preferred method in an outbreak area, besides eye drop, is drinking water.

  All research points to drinking water as a preferred route to coarse spray. However, no product is licensed for drinking water, therefore a professional, such as a veterinarian should be employed.

Broiler Vaccination ??

- Literature and communication points to a CEO product, high titered and given at 14 days of age.
- Remember that CEO vaccines may affect performance and possibly responses to other respiratory vaccines.
- Complications with other respiratory vaccines run a risk, beware of this interference.
- Do not mix different respiratory vaccines together with ILT, this being IBV/NDV.
- Most companies using broiler vaccines at 12 – 14 days of age suffer excessive vaccine reactions, interference with IBV/NDV programs, condemnations, and performance shortfalls when they have to vaccinate.
- The older the birds, the worse the problem. Severe weather (very hot or very cold) exacerbates the vaccine reactions.

•One relatively new tool for managing ILT in broilers is a recombinant vector vaccine based on the turkey herpes virus (HVT) that causes Marek's disease. The vaccine, Innovax-ILT, was developed by scientists at Intervet/Schering-Plough Animal Health and is administered subcutaneously to day-old chicks. The vaccine was first launched in the United States in June 2007. Controlled studies show the vaccine protects 97 per cent of chicks from ILT.

• When Innovax-ILT vaccine is injected into birds, the Marek's portion of the vaccine produces protection against Marek's disease. However, the vaccine also expresses the immunogenic proteins of the inserted genes of the donor virus, ILTV. Those ILTV proteins induce antibodies, which then trigger protection against ILTV.