Avian Leukosis Complex (ALC)

Avian Leukosis Sarcoma Virus Group (ALSV)

Dr./ Wafaa Abd El-ghany
Assistant Professor of poultry dis.,
It comprises a variety of transmissible benign and malignant neoplasms of semi-mature and mature chickens and caused by members of genus of avian retroviruses belonging to the family Retroviridae.

Under natural conditions the most common form of neoplasms is the lymphoid leukosis (LL).
Economic losses from ALS group of diseases come from two sources:

First mortality ranged from 1 to 2% and may reach to 20% or more.

Second, subclinical infection by ALSV produces a depressive effect on performance especially egg production and quality.

In addition, their suppressive effect on the immune response leading to increase the susceptibility to many diseases and vaccination failure.
Etiology

- Avian leukosis viruses are members of the leukosis / Sarcoma (L/S) group of avian retroviruses.
- Thermolabile, RNA virus, the envelope has composition of outer cell membrane from which the virus was derived.
- The virus has its reverse transcriptase enzyme used for virus replication.
- ALVs. has a common group antigen.
There are 6 groups in chickens: A & B, C & D, E and J. This classification is based on properties for viral envelope glycoproteins. A, B, C, d and J are exogenous (encogenic viruses).
1. Acute transforming virus: REV have its viral oncogene → onset of neoplastic transformation.

2. Slowly transforming virus: LLV have no oncogene → indirect transformation of cell.

3. Defective virus:
   A. Defective transforming mutants: some acute RS transforming lost their oncogene.
   B. Defective replication mutants: LV defect in replication gene and need HELPER VIRUS.
Laboratory host system

1. **Chicks:**
   - RSV induce tumors 3-5 days from S/C or I/M injection and contact.
   - LLV intra abdominal injection in 1 day old → tumors after 200 – 270 days.

2. **Cell culture:**
   - RSV in rapid neoplastic transformation.
   - LLV in EEF multiply without cytopathic effect.

3. **Embryo:**
   - RSV on CAM after 11 days induce pock lesion.
   - LLV I/V in 11 day old embryo induce tumors at 2 weeks after hatching.
RSV-pock lesion on CAM
Transmission

- Chickens are the natural host. Rous sarcoma virus has the widest host range.

A- **Transmission of exogenous virus:**

- It occurs in two ways:
  - **Vertically.** Four serologic classes occur in mature chickens in relation to ALV infection:
    - No viremia, no antibody (V-A-); (genetic resistant, no infection).
    - No viremia, with antibody (V-A+); (not transmit infection, Ab from previous infection) (immunotolerant, as when the Abs decreased in blood & activate virus and become V+A-).
    - With viremia, with antibody, (V+A+); (transmit the infection).
    - With viremia, no antibody (V+A-). (transmit the infection).
  - Congenitally infected embryos develop immunologic tolerance to the virus and after hatching make up the V+ A- class, with high levels of virus in the blood and tissues and absence of antibodies.
  - **Horizontal** infection (Shedding of virus from saliva, faces and semen of cock) to other birds.
B- **Transmission of endogenous ALV:**

- Endogenous ALVs are usually transmitted genetically in germ cells of both sexes (vertical transmission). Endogenous viruses have little or no oncogenicity but may influence response of the bird to infection by exogenous ALV.
- Blood sucking insects as the chicken red mite, the fowl tick, and mosquitoes.
- Live virus vaccine prepared from so-called “normal embryos” (commercial eggs not SPF ones) are potential source of spread of infection.
Exogenous Virus
Horizontal

♀ or ♂ → Infectious Virus → Transient Viremia
Immunity to exogenous Virus
Lymphoid leukosis rare

Congenital

♀ → Infectious Virus → Chronic Viremia
Immune tolerance to exogenous Virus
Lymphoid leukosis common

Endogenous Virus

Genetic

♀ or ♂ → Viral DNA integrated in gamete DNA

♀ or ♂ → Viremia or antigen expressed
Immune tolerance to endogenous Virus
Lymphoid leukosis very rare

Horizontal and vertical transmission of exogenous LLV and genetic transmission of endogenous virus. (Crittenden, Avian Pathol)
Clinical Forms

I. Non Neoplastic:

II. Neoplastic:

1. Lymphoid leucosis
2. Erythroblastosis
3. Myeloblastosis
4. Myelocytomatosis
5. Osteopetrosis
7. Other tumors
Lymphoid leukosis (LL) (Big liver disease)

- It is the most common ALSV in birds.
- In field outbreaks, LL cases can occur any time after 14 wk of age; however, incidence is usually highest at about sexual maturity.
Lymphoid leukosis (LL)

- Pale comb and wattle (anaemia).
- Inappetence and progressive emaciation.
- Low egg production.
- Feather are soiled with ureate.
- Enlarged abdomen (penguin like position) due to enlargement of the liver.
- Enlarged liver, bursa and kidneys can be palpated from out side.
- Diarrhea.
- General weakness.
Lymphoid leukosis (LL)

- Milliary (nodular) or diffuse soft smooth glistening tumors in liver, spleen, lungs, gonads, heart, kidneys, heart, bursa of fabricus, bone marrow and mesentery.
- In diffuse form, the liver and other organs showed uniform enlargement, gray colour and friability.
- Both nodular and diffuse form can occur in the same case.

**Haematology:**
Blood films reveal the presence of immature lymphoblasts and budded or pseudopoded lymphocytes in large amounts
### Differential diagnosis between lymphoid leucosis and Marek's disease

<table>
<thead>
<tr>
<th>Feature</th>
<th>Lymphoid leucosis</th>
<th>Marek's diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of onset</td>
<td>weeks &lt; 6</td>
<td>weeks or older ≥ 4</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Absent</td>
<td>Frequently paralysis or paresis</td>
</tr>
<tr>
<td>Incidence</td>
<td>% Seldom above 5</td>
<td>% Usually above 5</td>
</tr>
<tr>
<td>Gross Lesions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral nerve enlargement</td>
<td>Absent</td>
<td>Usually present</td>
</tr>
<tr>
<td>Bursa of Fabricius</td>
<td>Nodular tumors</td>
<td>Diffuse enlargement or atrophy</td>
</tr>
<tr>
<td>Skin, muscle or proventriculus tumours</td>
<td>Usually absent</td>
<td>May be present</td>
</tr>
<tr>
<td>Microscopic Lesions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral nerve infiltration</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Cuffing in white matter of cerebellum</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Tumour in the liver</td>
<td>Focal or diffuse</td>
<td>Frequently per vascular</td>
</tr>
<tr>
<td>Bursa of Fabricius</td>
<td>Intra-follicular tumour</td>
<td>Inter-follicular tumors or atrophy</td>
</tr>
<tr>
<td>Follicular patterns of lymphoid cells infiltration in the skin</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Cytology</td>
<td>Uniform lymphoblasts</td>
<td>Polymorphic mature and immature cells including lymphoblast, small medium and large lymphocytes and reticulum cells</td>
</tr>
</tbody>
</table>

**Note:** The table above provides a differential diagnosis between lymphoid leucosis and Marek's disease based on various features such as age of onset, symptoms, incidence, gross lesions, microscopic lesions, and cytology.
Erythroblastostosis (EB)

- It is usually seen in growing birds (over 3 months of age).
- Affected birds showed weakness, slight to severe paleness or cyanosis of comb, emaciation, diarrhea, hemorrhage from feather follicles and severe anemia.
- The course of the disease vary from days to several months.
- Stained blood smears reveal a variable number of erythroblasts, and immature cells of the myelocytic series (anaemia).
- Hemoglobin content is reduced, plasma ratio is increased with severe low erythrocytes count.
Erythroblastosis (EB)

- General anemia with petechial hemorrhages in all organs and viscera.
- S/C edema, ascitis and hydropericardium.
- Diffuse enlarged chary red to dark mahogany soft and friable liver, spleen and kidneys.
- Bone marrow → soft watery, hyperplastic, cherry red and has hemorrhages (grayish red, Jelly like).
- Atrophy of immune organs (bursa and thymus).
- The blood is watery, light red, slow clot and shows increase the number of immature RBCS (11%) (erythroblasts), high plasma (88%) and low PCV and Hb.
Immature RBCS
Erythroid leukosis. The liver of this fowl is greatly enlarged and cherry red in colour. Similar lesions may occur in the spleen. The disease is rare.
Myeloplastosis (MB)

- It affects also growing birds.
- Signs are similar to erythroblastosis.
- The disease may result in a secondary anemia, in which severe decrease of erythrocytes and plasma ratio with highly increase of leukocytes or granulocytes ratio (leukemia).
- It is characterized by a spectacular leukemia. In the peripheral blood, myeloblasts may be found in a large number, may compose 75% of all blood cells (thick buffy coat).
Myeloplastosis (MB)

- The parenchymatous organs (liver, spleen and kidneys) are enlarged and friable but in chronic cases the organs may be firm and show nodular or diffuse tumor.
- Bone marrow is reddish gray to gray.
- In advanced cases, parenchymatous organs appear mottled or even granular.
- The course is variable, but it is longer than that for erythroblastosis.
Mylocytomatosis (MC)
ALV-J (skeletal tumors)

- It has a longer incubation period than Eb and Mb, but shorter than LL.

General signs:

- Are similar to those of Mb and Eb.
- In addition, skeletal growth of myelocytes may result in abnormal protuberances of the head (skull), thorax (sternum at the osteochondral junction), and shank.
- Cause stunting due to hypothyroidism (affect metabolism).
- It affects broiler chickens. The course is highly variable and usually prolonged.
Mylocytomatosi (MC)

- Characteristically, they occur on the surface of bones in association with the periosteum and near cartilage.
- Tumors (small, white and solid) often developed at the osteochondral junctions of the ribs, inner sternum and cartilaginous bones of mandible and nares.
- Flat bones of the skull are also often affected.
- Moderate to great enlargement of liver and skeletal myelocytomas affecting vertebrae.
- In some cases, tumors in spleen, thymus, gonad and kidney.
- Atrophy of the bursa and thymus.
Mylocytomatosis (MC)

Haematology:
- The disease is usually aleukemic (meleocytes) but occasionally is associated with erythroblastosis (severe anaemia).
- Sometimes a heterophilic leukocytosis is present.
Myeloid leukemia (ALV-J)

- It causes predominantly myeloid leukemia (Mylocyto-matosis: ML) in broiler breeders and commercial broiler flocks at 4 weeks of age and older.
- The frequency of tumors varies considerably between lines of chickens.
- It has slow tropism for bursal follicle cells (destruct it causing immunosuppression), then metastasis in pituitary gland causing stunting in chicks.
- Signs and lesions are similar to mylocytomatosis but skeletal deformity mainly in sternum and skull.
Myloid leukosis (ALV-J)

- ALV-J spreads vertically through the embryo and horizontally by contact, producing tolerant viremic birds and immune birds, respectively. All birds of the former class are shedders and transmitters of ALV-J to their progeny, as are a few birds in the immune class.

- Meat – type birds infected soon after hatching are particularly prone to becoming tolerant also.

- Shedder can be identified by testing vaginal swabs and egg albumen for ALV groups-specific antigen by ELISA, allowing ALV-J eradication protocols to be designed.
Myeloid leukemia. Chalky white myelocytes are present on the sternum and ribs of this fowl.

Substernal lesions (arrows) in an adult fowl.
The disease is most commonly seen in birds 8-12 wk of age or may develop any time after 1 month of age.

It is seen sporadically in the field and more often in males than in females.

**Haematology:**

The blood picture is ordinarily a leukemic and there is often a secondary anemia (as a result of reduction of the marrow) with relative lymphocytosis.
Osteopetrosis

- Long bones of the limbs are most commonly affected.
- Jerky gait or movement with uniform or irregular thickening of the diaphyseal or metaphyseal regions were observed.
- Birds with advanced disease have characteristic “boot-like” shanks.
- Affected birds are usually pale and show secondary anaemia.
Osteopetrosis

- Marked thickening in the diaphysis of the tibia and/or tarsometatarsus. Alterations soon are seen in other long bones and bones of the pelvis, shoulder girdle ribs.

- Marrow cavity is narrowed or completely obliterated by newly formed bone tissues formed hyperplasia of periosteum and abnormal bone is spongy.
202 Osteopetrosis. This cross-section of an affected tarsometatarsus in a fowl shows the great increase in thickness of the cortical bone.
Diagnosis of ALC

- Signs and gross lesions are not indicative methods.
- The samples are plasma, serum, tumors, whole blood
- Tissue of embryo at 10 days old, or egg albumin from female or shell glands, semen from males.
- Samples must be kept at – 70C due to virus is highly thermo-labile.
- Isolation of the ALSV on ECE or cell culture (ECF cells) is not easily applied as routine methods.
- If the dose of the virus increased, the pathogenic reaction (cytopathic effect) of the virus will disappear due to super infection.
- A number of biologic assays can be used for the detection of endogenous and exogenous ALVs.
Diagnosis of ALC

- Indirect biologic assays such as

**RIF-test (resistant inducing factor):**

a. Six tissue culture plates inoculated with prepared sample.

b. After incubation each inoculated with one type of RSV, the related groups interfere with each other.

○ (COFAL).

○ (ELISA) for ALV (ELISA-ALV),
Diagnosis of ALC

- The most sensitive procedure for differentiating between endogenous and exogenous ALVs is the virus isolation and ELISA test.
- Phenotypic mixing resistance-inducing factor (RIF) are used for the detection of ALVs.
- The most specific test for detection of antibody to ALVs is the virus neutralization (VN) test, indirect immunoperoxidase test and ELISA tests.

For detection of exogenous ALV, samples are inoculated on CEFs that are genetically resistant to subgroup E ALV. Seven to 9 days later, cell lysates are tested for the presence of ALV gs antigen by ELISA.
COFAL test

- Complement Fixation Of Avian LeuKosis (COFAL).
- **Samples:** Whole blood during viraemia. Neoplastic tissues.

**Procedure:**
1. Put unknown Ag (samples) in six test tubes.
2. Add known antisera of different groups to the tubes.
3. Add complement → agglutination.
4. Inoculate TC (CEF cells) from positive tube → No cytopathic effect.

**Result:**
If antigen is specific to the antisera antigen, antibody reaction induces fixation.
COFAL test

- This test is used for detection of common group specific antigen (common or major antigen) which present in leucosis sarcoma viruses.

- This test not differentiate between different sub-groups, but identify only leucosis sarcoma viruses through identification of the common antigen.
RIF test

- Resistant Inducing factor test.
- Used for detection and differentiation between different sub-groups of leucosis sarcoma viruses (6 sub-groups from A to J) by using Ross sarcoma viruses of different sub-groups.
- The test depends on that when CEF cells are infected with a leucosis virus it become resistant to super infection with Sarcoma virus of the same sub-group.
- In general, leucosis viruses don’t induce alteration of the cultures.
Only viruses of the same sub-group interfere with one another in this way.

The property of interference used for assay of leucosis viruses and detection virus sub-groups by RIF test.

In RIF test known susceptible ECF cell culture is inoculated with suspected material.

The culture is tested for susceptibility to Ross-Sarcoma virus of different sub-groups.

Several different challenge viruses one for each sub-group requires a separate cell culture plate for testing.
For example, presence of sub-group A in cell culture in plate 1 is indicated by absence or reduction of cytopathic effect by standard RSV of sub-group A when compared with challenge control cells.
Prevention and control

I- Immunization: No vaccines that protect chickens and turkeys from infection with ALV are available commercially.

II- Eradication: depends on breaking the vertical transmission of virus from dam to progeny. The program is based on the elimination of dams that test positive for ALV gs antigen by COFEL or ELISA.

- Sanitation and isolation rearing of susceptible chicks is the only practical means available at present.
- Ectoparasites should be kept in check at all times.
- Live virus vaccines should be prepared from leukosis free-eggs.

(III) Genetic resistance: breeders free from ALC.
THANK YOY