Retroviridae

Equine infectious anemia virus, Bovine leukemia virus
Lymphoid leucosis virus,Visna/maedi virus

Latin *retro*, "backwards", refers to the activity of reverse transcriptase which transfers genetic information from RNA "back" to DNA

A diverse group of RNA viruses, which replicate via **reverse transcription** (synthesize a DNA copy of their RNA genome during their replicative cycle)

Group VI: RNA Reverse Transcribing Viruses

Family: ***Retroviridae***
Subfamily: *Orthoretrovirinae*
Genus:

Alpharetrovirus	**Avian leukosis virus**
Betaretrovirus	***Pulmonary Adenomatosis***
Gammaretrovirus	
Deltaretrovirus	**Bovine leukemia virus**
Epsilonretrovirus	
Lentivirus	**Maedi/Visna virus**
	Equine infectious anemia
	HIV
	Bovine Immunodeficiency Virus

Properties of Retrovirus

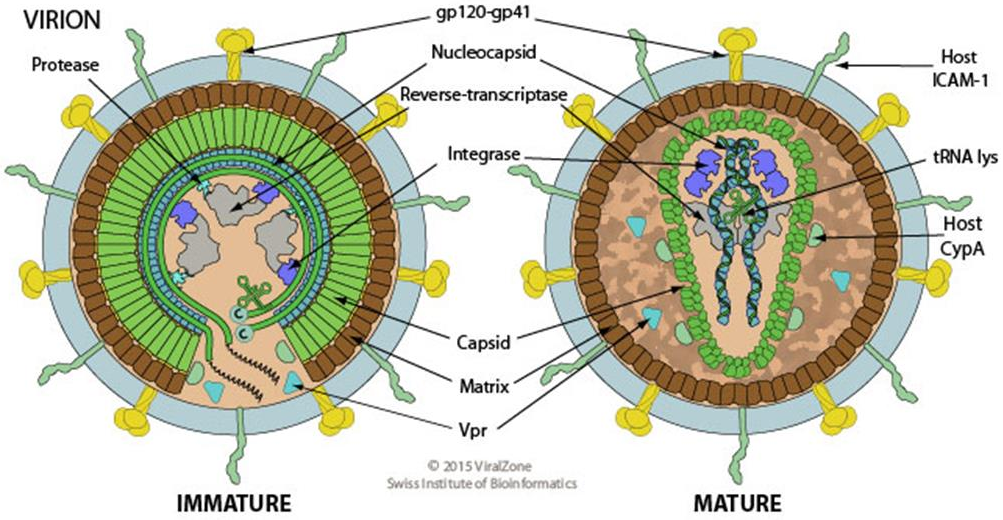
- Virions are enveloped, 80–100 nm in diameter, and have a
- Three-layered structure: an innermost genome–nucleoprotein complex with **helical symmetry**, surrounded by an icosahedral capsid, in turn surrounded by an envelope with glycoprotein spikes
- The genome **is diploid, consisting of a dimer of two molecules of linear positive-sense, single-stranded RNA** each 7-11 kb in size
- Virus transcription and **replication take place in the nucleus**
- All non-defective retroviruses have *gag*, *pol*, and *env* genes; some acquire an oncogene and are usually defective in their own replication as a consequence.

Viral reverse transcriptase transcribes DNA from virion RNA following the formation of long terminal repeats**; linear and circular double-stranded DNA is formed** and **linear DNA forms integrate into cellular chromosomal DNA** as a **provirus**.
Retroviruses require **reverse transcriptase for their replication**.
Among its many functions,

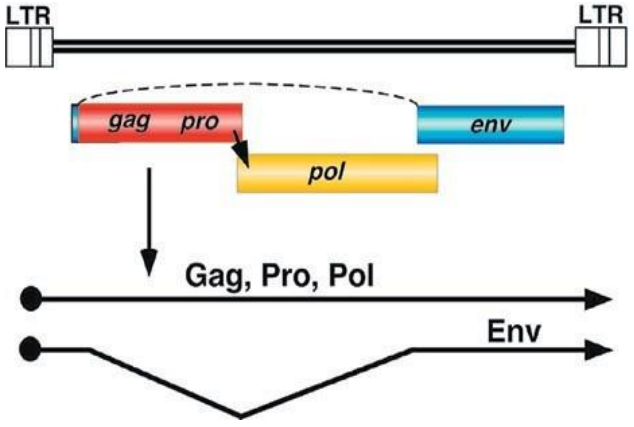
Reverse transcriptase serves as
RNA-dependent DNA Polymerase,
a **DNA- dependent DNA polymerase,**
and a **RNase**,

with each distinctive function being carried out by a different part of the protein molecule

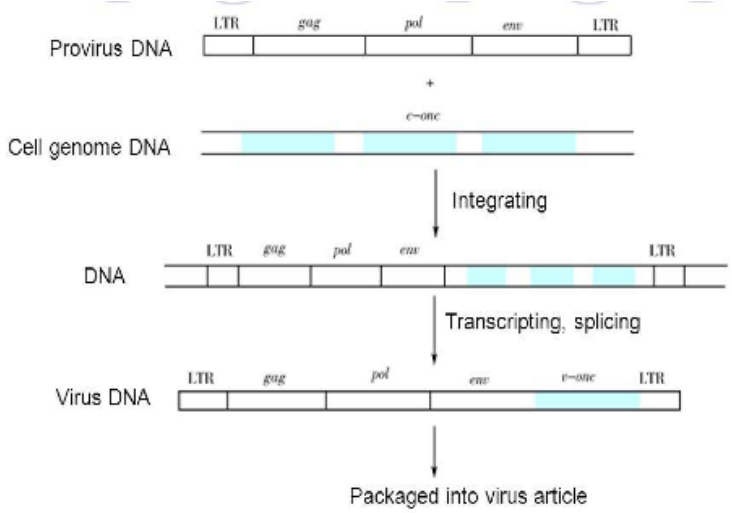
GENOME: is diploid, consisting of a dimer of two molecules of linear positive-sense, single-stranded RNA
REPLICATION: NUCLEAR (Translation in Cytoplasm)



Arrangement of the Retroviral Genomes :
All retroviruses have gag, pol, and env genes
Some acquire an oncogene, and are defective in their replication
Gag gene – group specific antigen – encodes the virion core/capsid proteins
Pol gene – polymerase – encodes the reverse transcriptase
Env gene – envelope – encodes the envelope glycoprotein peplomeres.



Obtaining of V-Onc gene



Properties

Viruses that are capable of acute cellular transformation , retro- viruses typically contain **viral oncogenes** (v-*onc*).

The presence of the v-*onc* gene, which was originally acquired from a host genome, is often associated with deletions elsewhere in the viral genome as a result of a "trade" of a viral gene for a host-cell gene ("*onc*") during recombination. This trade of a viral gene for a cellular one reflects the packaging constraints imposed during replication and the viral *env* gene is usually exchanged.

Thus most **v-*onc*-containing viruses are unable to synthesize a complete envelope**, are **replication defective** and **must associate with non-defective viruses** that are replication competent that act as helper viruses to accomplish replication and spread to other hosts. **Rous sar- coma virus is an exception; its genome contains the viral oncogene, v-*src*, but it also contains complete *gag*, *pol*, and *env* genes** and is therefore **replication competent**

Chronic, also referred to as slowly transforming, retroviruses induce neoplasia by insertional mutagenesis through random inte- gration into regions of the host genome that influence cell division, activating host oncogenes.

Oncogenic Retroviruses

Major cause of leukemias, lymphomas, and sarcomas in many animal species

Acute- transforming viruses –

Viral genome has **v-onc +**

This is associated with deletions elsewhere in the genome – most v-onc+ viruses are replication defective, EXCEPT **ROUS SARCOMA VIRUS**

The viruses produce tumors shortly after infection in a high percentage of infected hosts

– e.g. feline sarcoma virus

Chronic transforming viruses

V-onc- viruses that cause cancers late after infection and in only a relatively low percentage of infected hosts.

Replication

1. Adsorption, penetration by fusion or receptor-mediated endocytosis, penetration and uncoating.
2. Viral RNA is released into the cytoplasm
3. Parental RNA is copied to single stranded DNA - RNA:DNA hybrid – by reverse transcriptase. Single stranded DNA is made DOUBLE STRANDED again by reverse transcriptase
4. **Double stranded DNA moves to the nucleus, becomes circularized, and then is integrated as a provirus at different sites in the cellular DNA**
5. **Provirus** is replicated with host genome and is passed to daughter cells – type III spread
6. **Needs host cell polymerases –** provirus is a template for making mRNA for protein synthesis and positive sense RNA molecules which are encapsidated into progeny virions
7. Maturation of virions occurs by budding through plasma membrane.

NATURAL HOST : Vertebrates

TROPISM : Bovine Leukemia Virus- B cells Varies with the species
HIV – T cells

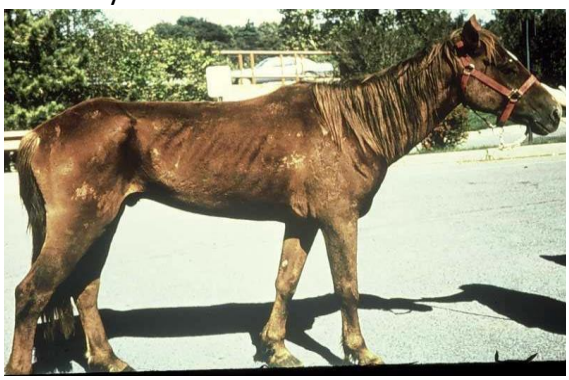
ASSOCIATED DISEASES

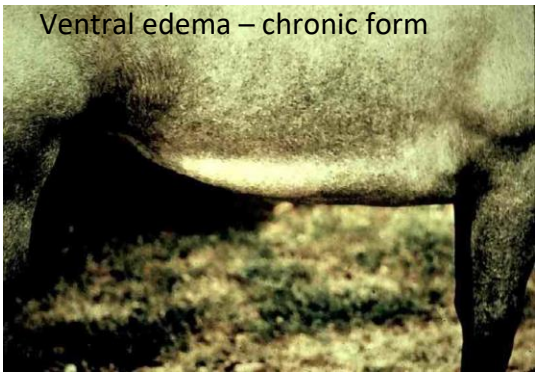
Alpharetroviruses	Avian leukosis virus,	Chicken	Leukemia, Avian Leucosis
	Avian sarcoma, Rous sarcoma virus		lymphomas, sarcoma, sarcoma
Betaretrovirus	Pulmonary adenomatosis	Ovine	Pulmonary adenomatosis
Gammaretrovirus	FeLV and sarcoma	Cats	FeLV, feline sarcoma, sarcoma,
Deltaretrovirus	Bovine leukemia virus	Bovine	Bovine leukemia
Lentivirus	Maedi/Visna virus	Sheep/goats	Maedi/ Visna
	Caprine arthritis- encephalomyelitis virus Equine infectious anaemia	Goats Horses	Arthritis Equine infectious anaemia
	HIV 1 and 2	Humans	AIDS
	FIV	Cats	Feline immunodeficiency virus
	SIV – Simian immunodeficiency Virus	Monkeys	Immunodeficiency
	BIV - Bovine Immunodeficiency Virus	Bovine	Immunodeficiency

Equine Infectious Anaemia – Swamp Fever

EIA in Equidae is characterized by **a long relapsing illness** after an initial acute attack
Etiologic agent – **Equine lentivirus** – one serotype
Transmission – occurs via transfer of blood cells from an infected horse
Mechanical transmission – **by tabanids, stable flies, mosquitoes and probably Culicoides**
Virus is infective for up to 30 minutes on a fly's mouth parts
Vertical transmissions via placenta and through colostrum and milk

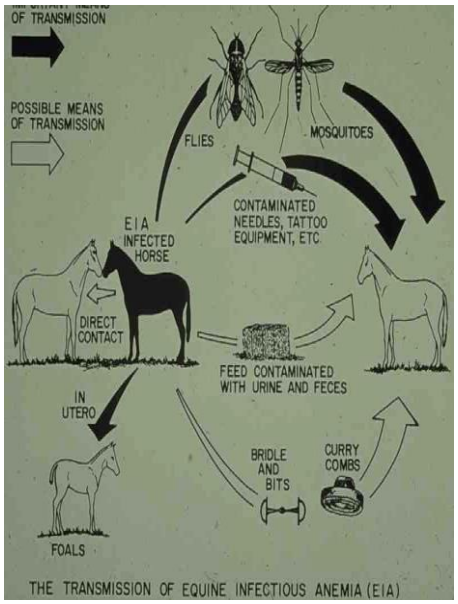
Pathogenesis – replicates in macrophages and lymphocytes
Lifelong, cell associated viremia develops in all infected horses
Persistent Ag-Ab complexes – immune complex hypersensitivity – result in damage to vascular endothelium – vasculitis – followed by inflammatory changes in parenchymatous organs– LIVER
Vasculitis in the CNS results in ataxia, spinal leptomeningitis and encephalomyelitis
Anaemia – viral antigens adsorb to RBC, bind with EIA antibody, and trigger erythrophagocytosis by mononuclear phagocytes; complement-mediated hemolysis – type II hypersensitity




Ventral edema – chronic form

Glomerulonephritis
Results from immune complex hypersensitivity

Clinical Features – IP 2-43 weeks but can be up to 3 months
Acute – fever, severe anemia, jaundice, blood stained feces, tachypnea and petechial hemorrhages of the mucosae
Mortality rate is **80%**
Subacute – moderate fever – recovery, may recur
Chronic – mild signs and failure to thrive and episodic persistent fever, cachexia and ventral edema.



Diagnosis
Coggins test /Agar gel immunodiffusion test – AGID.
Coggins test detects antibodies to the major group specific antigen of EIA virus.
Results are valid for 1 year from the date the blood is collected
Detects all infected animals except those in early incubation period, the first 2-3 weeks after infection**. A suspected horse should be retested in 4-6 weeks.**

Foals that have been nursed by infected dams may be temporarily positive, however, they should be **negative by 6 months of age if not infected.**

Control and Prevention-EIA

Use disposable needles and syringes, one per horse, when administering vaccines and medications.

- Sterilize dental tools and other instruments before using them on another horse.
- Test all horses for EIA at least annually.
- Test horses at the time of purchase examination.
- Stable owners, horse show and event managers should require and verify current negative Coggins certificates for all horses entering the premises.
- New horses should be quarantined for 45 days and observed for any signs of illness, including elevated temperatures, before introducing them to the herd. They should be retested if exposure to EIA is suspected at a 45-day interval.
- All stable areas should be kept clean, dry and waste-free. Good pasture management techniques should also be practiced. Remove manure and provide adequate drainage to discourage breeding sites for pests.
- Horses at greater risk, such as those in frequent contact with outside horses or who live or travel in geographic regions known for EIA outbreaks, should be tested more frequently, every 4 – 6 months.

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Bovine Leukemia / Enzootic bovine leukosis

Bovine Leukemia Highly fatal, systemic, **malignant neoplasia of the reticuloendothelial system** of cattle Characterized by the development of **aggregations of neoplastic lymphocytes** - various organs
Worldwide.

Etiologic agent – DELTA RETROVIRUS – *V-ONC*-gene

Transmission – close, prolonged contact; primarily by transfer of blood lymphocytes between animals – found in blood, milk, tumor masses
Blood volumes capable of transmission = 0.1mL
Transfusions, trauma, needles, mechanical vectors - arthropods

Lymphosarcoma of the mammary gland ⇨

Vertical transmission occurs in utero or through colostrum and milk, accounts for a small proportion of infections – less than 10%.

Pathogenesis – **B-lymphocytes** are the major targets
Four possible outcomes of infection
1.Genetic resistance – no infection
2.Asymptomatic infection – antibody production, seropositive 4-12 weeks later
3.Permanent infection, persistent lymphocystosis – benign proliferation of B lymphocytes – 33% of cases
4. **Infected, seropositive animals –** lymphosarcoma **LESS THAN 1-2%; occurs in cattle between 4-8 years of age.**


Mediastinal lymph nodes associated with virus

Bovine Leukemia
Clinical Signs, Rx, Prevention and Control
Clinical signs vary depending on organ/tissues affected.
Weight loss, anorexia, decrease in milk production are common.

Diagnosis – BLV infection must be distinguished from clinical disease?
Clinical hx and histopathologic examination of affected tissues
Serology – persistent infection leads to marked increase in antibody – detected easily
Agar gel immunodiffusion test – AGID, ELISA, Western Blot, IFA are used

Prevention and control – EBL can be eradicated from a herd by repeated serological testing of animals over 6 months of age at 2-3 months intervals.

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Avian Leukosis

Avian Leukosis

Avian sarcoma leukosis virus is characterized by a wide range of tumors, the most common of which are lymphomas. Lymphoid leukosis is the most common form of this disease and with typical presentation of gradual onset, persistent low mortality, and neoplasia of the bursa. The disease is also characterized by an enlarged liver due to infiltration of cancerous lymphoid cells. In addition, other abdominal organs and the bursa of Fabricius are often infected.

The viruses causing avian leukosis are endemic in all chicken populations worldwide.
Incidence is 3-20% in most chicken populations.

Etiologic agent – Alpharetrovirus

Virus is v-onc-gene

Transmission

- congenitally via the egg – **vertical** – or horizontally with the first few days of life (less than five days)
- The chicken becomes **viremic for life** because of the development of immunological tolerance.
- The **birds excrete virus in the saliva and feces**
- If horizontal transmission occurs beyond 5/6 days after hatching, the chicken develops neutralizing antibodies
- The viremia is transient, and the chicken is **unlikely to develop leukemia**

Pathogenesis

The primary target cells are lymphoblasts with B lymphocyte markers in the bursa of Fabricius ;

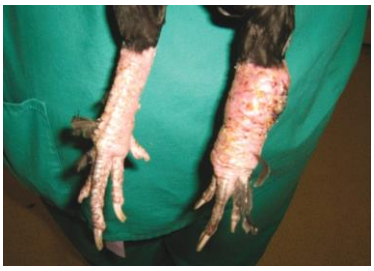
Clinical Features – Disease occurs sporadically in birds over 14 weeks of age

1. Lymphoid leukosis – synonym **– visceral lymphomatosis – Big liver disease**

Observed in chickens 14-30 weeks of age

Lymphoid cell infiltrations of various organs e.g. liver, spleen, kidney, bursa of Fabricius etc.

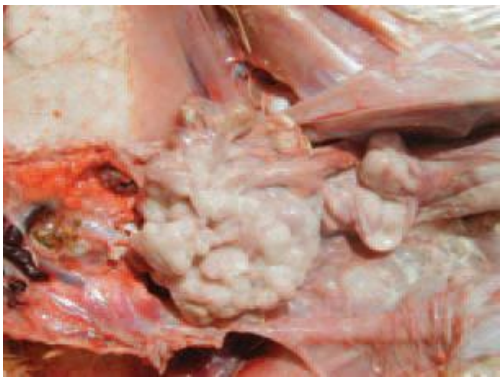
2. Osteopetrosis – thick leg – characterized by a proliferation of the periosteal osteoblasts of the long bones of the limbs. Non neoplastic, bilateral thickening. The neoplastic changes begin always from the bursa of Fabricius, where various-sized lymphomas are detected (transverse section through neo-plastically grown bursa -fixed preparation).



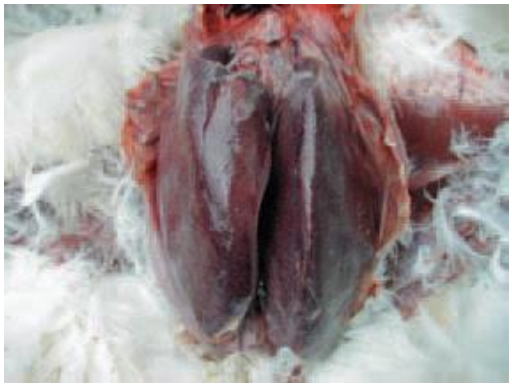
Clinically, pale comb and wattles, sometimes swelling of the abdomen because of the highly enlarged liver are observed.

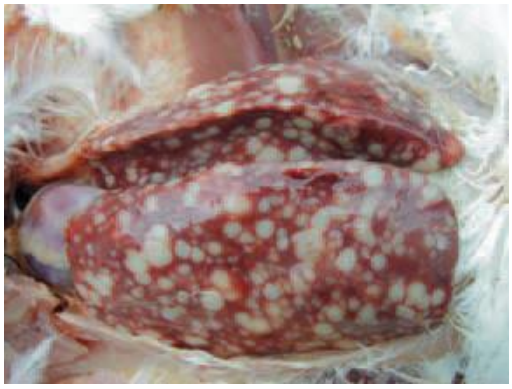
Diffuse or **nodular neoplastic growths could be detected in many organs**, but they are more common in the liver, the spleen, the kidneys, the heart and the ovary.

Neoplastic Bursa



Neoplastically transformed ovary in LL





Avian Leukosis – visceral lesions

Diagnosis-ALC

1. Flock history and tumors in multiple organs without nerve and ocular involvement,
2. RT-PCR, 3.ELISA.

Control - ALC

- No Vaccine, No treatment
- Terminate / Cull the breeder flock

J Virus: A strain of avian leukosis virus (ALV) belonging to a new envelope subgroup J (ALV-J) emerged in 1988 as a new subgroup of ALV and spread rapidly throughout the world. (Wu L, *et al.,* 2022).

Avian leukosis virus (ALV) belongs to the genus *alpharetrovirus* of the family *retroviridae* and causes avian leukosis as the first known virus-related tumor diseases, leading to great economic losses. Up to now, 11 different subgroups of ALV (designated A to K) have been determined based on host range, envelope properties as well as cross-reactivity, and those in subgroups A, B, C, D, E, J and K are capable of infecting chickens. Among them, the strains of subgroup E are endogenous and non-pathogenic, while that of subgroup J (ALV-J) is the most prevalent and causes the myelocytoma, hemangioma and multiple other malignant tumors. In China, ALV-J was first detected in 1999 and then spread rapidly throughout the country, which used to be a major disease endangering China's poultry industry and posed a huge threat to the supply of eggs and chicken products for a long time. (Li, H., *et al.,* 2021).

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Maedi Visna

(Maedi=**progressive pneumonia** and Visna=**wasting**).

Maedi Visna (MV) is a highly infectious viral disease affecting goats and sheep. It is mainly **transmitted through the ingestion of milk from a virus infected sheep**, although disease can be spread within flocks through **direct contact or contamination**.

Maedi refers to the **progressive pneumonia** presentation of the disease; ***visna*** (Wasting) refers to a **slow progressive encephalomyelitis** of sheep.

Ultimately fatal, most of the **economic loss attributed to** this disease is due to **decreased milk production; lowered weaning weights; increased incidence of severe arthritis and wasting**; higher than average number of respiratory infections; and decreased ewe fertility.
There is no cure or treatment for the disease, however, there is a reliable blood test that can identify infected animals.

Transmission Maedi Visna

The route of transmission is related to body fluids, mainly respiratory exudates and milk or colostrum, which may contain infected **monocytes and macrophages**.
The main transmission routes are airborne, favored by overcrowding (stabling), and milk borne. Regardless of the route of transmission, the viruses cross the mucous barriers and infect resident **macrophages** and dendritic cells.

Clinical Signs - Maedi Visna

The tissues mainly infected by MVV are located at the lungs, the mammary gland, the nervous system, and the joints.

Pneumonia and mastitis are the predominant clinical manifestations of MVV in sheep.
Less frequently, lesions such as lymphoidtissue hyperplasia may be apparent in kidneys, liver, and heart (possible target organs). Multiple-organ infection may be observed in the progression of the disease, but the severity of lesions varies among the affected organs .

Respiratory clinical signs include **dyspnea** and **increased respiratory rate**, caused by the characteristic lymphocytic interstitial pneumonia.

Symptoms from the nervous system include ataxia, paresis, weakness in hind limbs, incoordination or, in heavier cases, total paralysis, due to meningoencephalitis, astrocytosis, microgliosis, and focal secondary demyelination in the brain and the spinal cord.
In mammary gland, MVV can cause an indurative **non-suppurative interstitial mastitis**.
The udder is hard but not painful, with decreased milk production mainly noticed the first days postpartum, a situation usually described as "**hard udder syndrome**". The lymphocytic inflammatory pattern, caused by the replication of virus in macrophages and mammary gland epithelial cells,provokes the destruction of the acinar structure and the reduction of milk production.

Arthritis can also be the outcome of MVV infection, although it is less common in sheep .
The affected joints are usually the carpal and tarsal, but metatarsal, metacarpal and vertebral joints can also be affected. **Finally fibrosis, mineralization, and necrosis of synovium and joint capsule**. In advanced arthritis cases, the cartilage is destructed and the articular capsule is fibrotic. In the majority of cases, **arthritis is progressive causing lameness** and involuntary culling of the animal.

Diagnosis Maedi Visna

1. AGID Test

AGID test is commonly used as a diagnostic tool in MV control programs due to its simplicity. It is a highly specific diagnostic method but less sensitive than

2. RIPA Radioimmunoprecipitation assay

RIPA is as old as WB and both of them are considered as the reference standards. They have similar sensitivity and are mainly used as confirmatory assays . RIPA is not frequently used due to its high cost and its difficult application.

3. Western Blot

WB is a confirmatory laboratory test which has been used to detect antibodies in serum that recognize viral proteins. In general, WB is more sensitive than the ELISAs but more cumbersome and with lower throughput. Cross-reactivity with non-specific cellular proteins is also a problem. unreliable for a definitive diagnosis.

4. ELISA

ELISA is the most commonly used test in population screening and for the surveillance.
It detects antiviral antibodies with satisfactory sensitivity and specificity, indicating the occurrence of infection and seroconversion at some point [6 ,20]. Seropositivity is not necessarily followed by clinical disease and a seronegative animal cannot be safely considered to be free of infection.

5. PCR

PCR can directly detect proviral DNA in fluids and tissues across the animal body (lungs, milk, peripheral blood, mammary gland, synovial membranes etc.). The most reliable cells for the detection of virus are the peripheral blood mononuclear cells.
The most significant advantage of PCR is its ability to detect infection before seroconversion. However, PCR is not a reference method
and it is suggested to be combined with serological testing to overcome the problem of selective specificity associated with the lack of reliable universal .
As neither antiviral treatment nor vaccination is available, diagnostic tests are the backbone of most of the schemes implemented to prevent the spread of MVV.

Preventive and Eradication Measures Maedi Visna

1. Annual, biannual, or more frequent blood sampling from the breeding stocks and serological and molecular testing for the diagnosis of the infected animals.

2. Post-lambing management primarily based on the application of artificial suckling and the use of colostrum and milk substitutes or pasteurized colostrum/milk.

3. Immediate removal of animals with apparent clinical signs and positive laboratory diagnosis. (i.e., culling of the remaining seropositive animals).

4. Keeping the replacement animals, post-weaning, in separate housing facilities to avoid horizontal transmission of MVV through the contact with adult animals of the remaining flock.

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Ovine Pulmonary Adenomatosis (Jaagsiekte Disease)

Ovine pulmonary adenocarcinoma (OPA), also known as ovine pulmonary adenomatosis and jaagsiekte, is a **contagious tumour of sheep and, rarely, of goats**. It is a **progressive respiratory disease**, principally affecting adult animals. The issue is always fatal. Caused by **Betaretrovirus**.

Susceptible species : Sheep, rarely goats

Distribution

The disease is not widely spread, it is reported by some countries in Europe, America, Africa and Asia. It has never been identified in the Pacific Region.

Clinical signs

The period of incubation exceeds months and the clinical signs only appear when the tumours are sufficiently developed. Usually it affects sheep of 3-4 years old and signs include:

-**Increased respiratory rate** (tachypnea),
-**Loss of weight despite normal appetite**,
-**Wet cough**,-**Eyes and nose discharge**, Death after a few months, usually associated with secondary pasteurellosis.

Post-mortem findings

Lungs may appear firm, grey and flat. They sink in water. Bronchi are full with abundant white and frothy fluid.

Differential diagnosis

Maedi-Visna, Parasitic respiratory diseases, Other chronic respiratory diseases

Specimens required for diagnosis

Tumours are solid, grey or light purple with a shiny translucent sheen and often separated from the adjacent normal lung by a narrow emphysematous zone. The presence of frothy white fluid in the respiratory passages is a prominent feature and is obvious even in lesions as small as a few millimetres.

In advanced cases, this fluid flows out of the trachea .

Samples should be taken at necropsy for histopathology, immunohistochemistry or PCR for The diagnosis can only be done by histological examination of lungs. PCR is the latest technique can be applied.

Transmission

The virus is shed in lung secretions and is transmitted by aerosols.

Control / vaccines

No treatment and no vaccine.

Reference

Li, H., Tan, M., Zhang, F. *et al.* Diversity of Avian leukosis virus subgroup J in local chickens, Jiangxi, China. *Sci Rep* **11**, 4797 (2021). https://doi.org/10.1038/s41598-021-84189-7

Wu L, Li Y, Chen X, Yang Y, Fang C, Gu Y, Liu J, Liang X, Yang Y. Isolation and characterization of avian leukosis virus subgroup J associated with hemangioma and myelocytoma in layer chickens in China. Front Vet Sci. 2022 Sep 23;9:970818. doi: 10.3389/fvets.2022.970818. PMID: 36246325; PMCID: PMC9555167.

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