



LECTURE 12: RETROVIRIDAE II

Russ Fraser, Fall 2023

Housekeeping

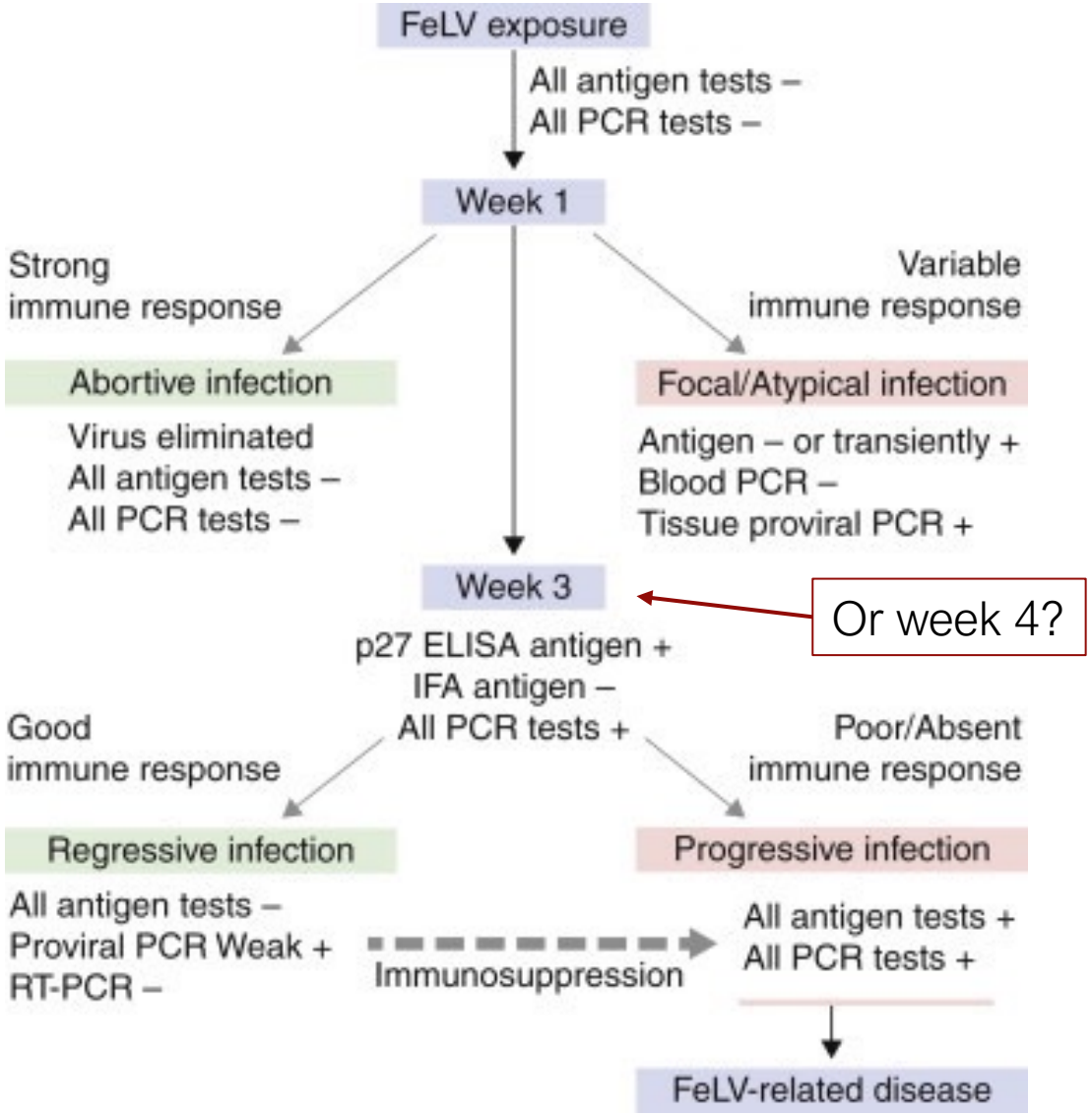
- Questions?

- When do you re-introduce a cat that has recovered from parvo, considering they can shed for up to 6 weeks post-infection?

- How soon after exposure can you detect FeLV?

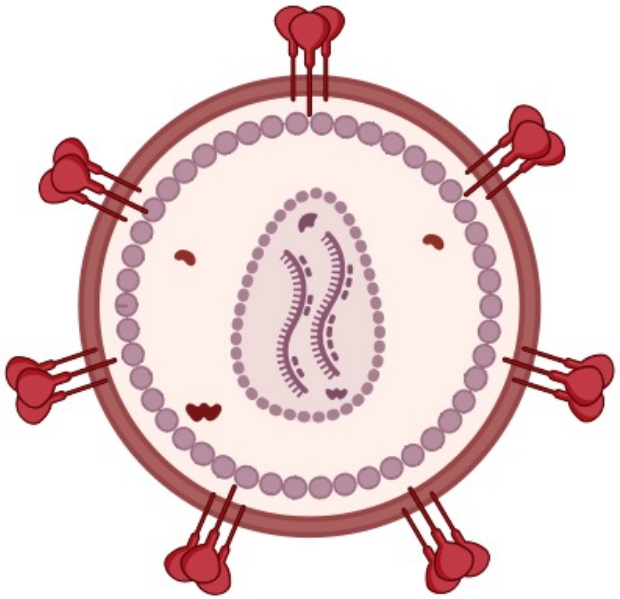
FeLV: diagnostic testing

Outcomes of infection with FeLV. In the first week of infection, cats test negative with all antigen assays (i.e., p27 ELISA and IFA assays), RT-PCR for viral RNA, and PCR for proviral DNA. Rarely, cats exposed to low levels of virus eliminate the infection before or at this time point *(abortive infection).* Soluble antigen assays and proviral PCR assays become positive from about the third week after infection, and virus is shed in saliva, tears, urine, and feces. After this time point, productive viral infection is suppressed by the host immune response *(regressive infection)* or the virus multiplies rapidly in the bone marrow and a progressive infection occurs, which is characterized by persistently positive antigen ELISA, IFA, viral RNA, and proviral DNA assay results. Cats with *focal or atypical infection* are never or transiently antigen positive and test negative using PCR assays on blood or bone marrow, but some tissues (such as the mammary gland or bladder) are positive with proviral PCR. The latter occurs in only a small percentage (e.g., < 5%) of infected cats.



Sykes JE, Hartmann K. Feline Leukemia Virus Infection. Canine and Feline Infectious Diseases. 2014:224–38. doi: 10.1016/B978-1-4377-0795-3.00022-3. Epub 2013 Aug 26. PMCID: PMC7152252.

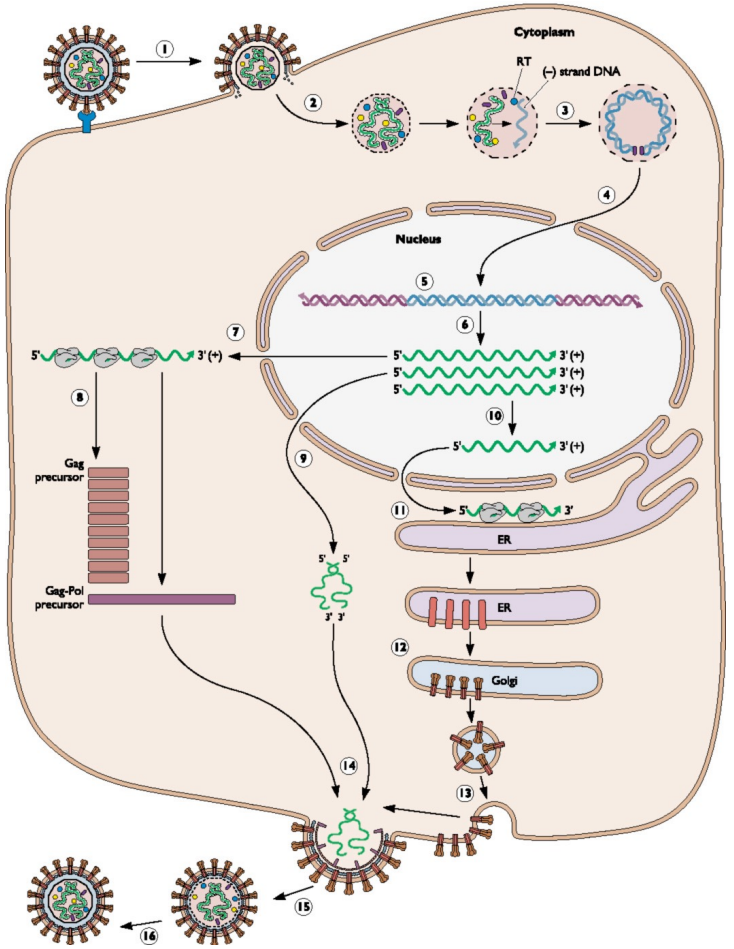
FeLV: diagnostic testing



Genome of FeLV is <u>ssRNA</u>

PCR

- PCR requires <u>DNA.</u>
- Thus, 'regular' PCR will **not** detect circulating FeLV, but **will** detect provirus.
- However, we can add in a step, in which we isolate **only RNA**, and then reverse transcribe it into DNA. This will **not** detect provirus, but **will** detect circulating virus.
  - This type of PCR is called 'RT-PCR'



Provirus is <u>DNA</u>

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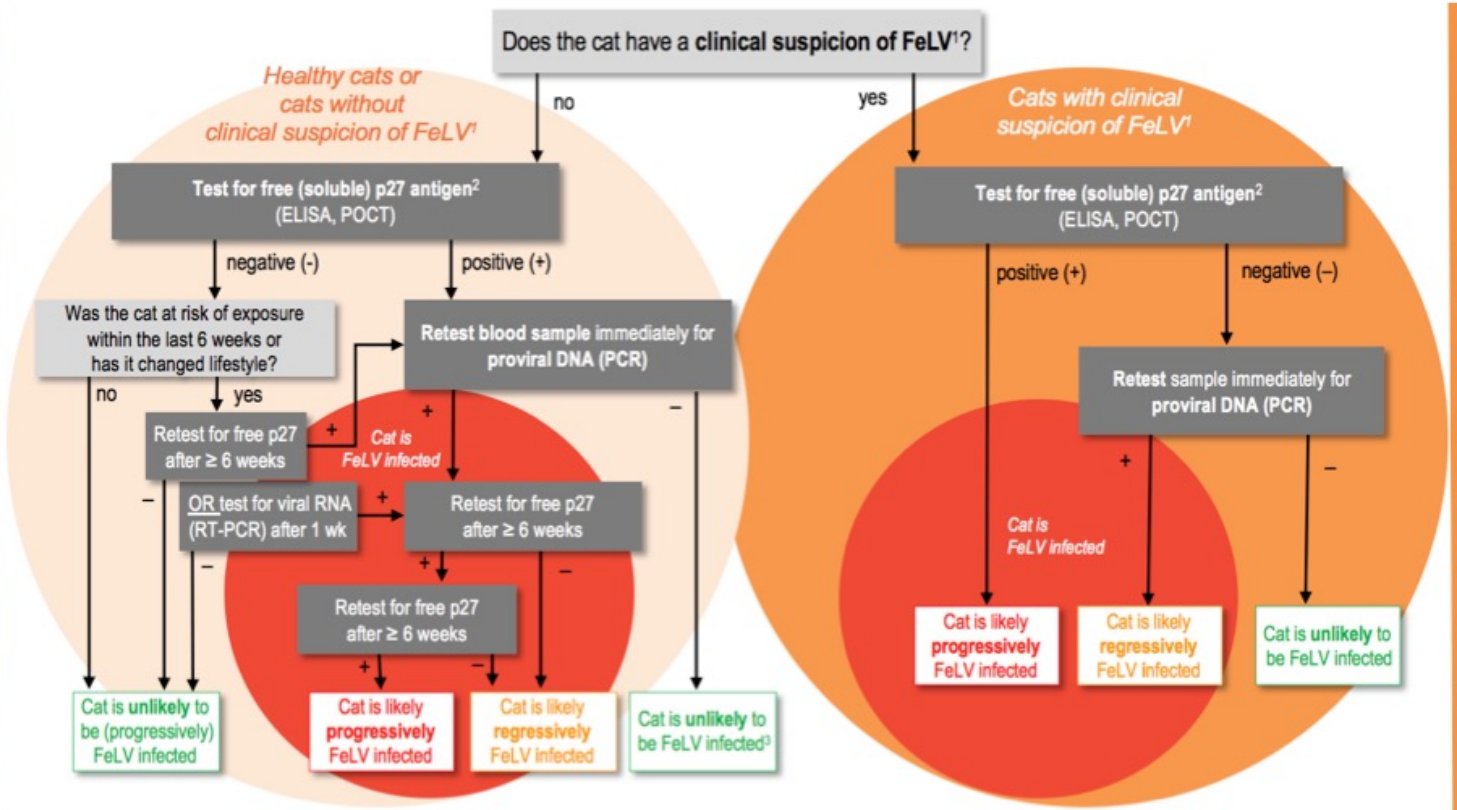
ABCD

ABCD FeLV diagnostic tool

ABCD TOOL





Risk factors and clinical problems that can be associated with FeLV infection
Mixed breed, free-ranging or feral cat, cat from household with FeLV-positive cat(s)
Cats from an area with high FeLV-prevalence
Presence of neoplasia (lymphoma, leukaemia, other neoplasia)
Bone marrow suppression (non-regenerative anaemia, thrombocytopenia, neutropenia, pancytopenia)
Chronic or recurrent infections suggesting immunosuppression
Chronic gingivostomatitis
Immune-mediated haemolytic anaemia
Neurological signs (peripheral >> CNS)
Reproductive disorders
Fading kitten syndrome
Rarely, other disorders such as immune-mediated uveitis or erosive polyarthritis

When to test?

- FeLV testing is not recommended in cats that have never been exposed to FeLV with certainty.
- It is recommended in all other situations.
- Screening for FeLV is particularly recommended:
  - *At the time cats are first acquired*
  - *Prior to initial vaccination against FeLV*
  - *If there is concern that a naïve cat has been exposed to infected cats*
  - *If clinical signs are present*

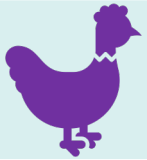
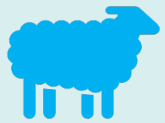
Notes on testing

1. For risk factors or clinical disorders associated with FeLV, see table above.
2. Whenever testing for free FeLV p27 antigen of blood samples is suggested (ELISA, POCT) in any of the boxes in the figure, alternatively testing for viral RNA of saliva samples (RT-PCR) can be used.
3. In very rare cases, a focal FeLV infection can be the reason for such a result: positive in free p27 antigen and negative in provirus-PCR, both from blood samples.

POCT = point-of-care test

https://www.abcdcatsvets.org/wp-content/uploads/2022/11/TOOL_FeLV_Feline_leukemia_virus_2020_EN.pdf

Retroviruses: important diseases

	Avian leukosis virus
	Feline leukemia virus
	Feline immunodeficiency virus
	Bovine leukemia virus
	Maedi/Visna virus
	Caprine arthritis-encephalomyelitis virus
	Equine infectious anemia virus !



Feline immunodeficiency virus

FIV

Feline immunodeficiency virus: general

- Infects domestic and wild cats (and hyenas!)
- Around 3.6 % of cats are positive in NA
  - Prevalence is higher in sick cats (because some cats with FIV eventually become sick…)
- Intact, feral/outdoor males are at ↑ ↑ risk



FIV: transmission

- Horizontal is the major route
  - Saliva and blood
  - “Injection” of saliva via bites is one of the most common methods
  - “Fighter’s” virus

- Vertical transmission is possible but not considered to be important in natural settings



FIV: pathogenesis

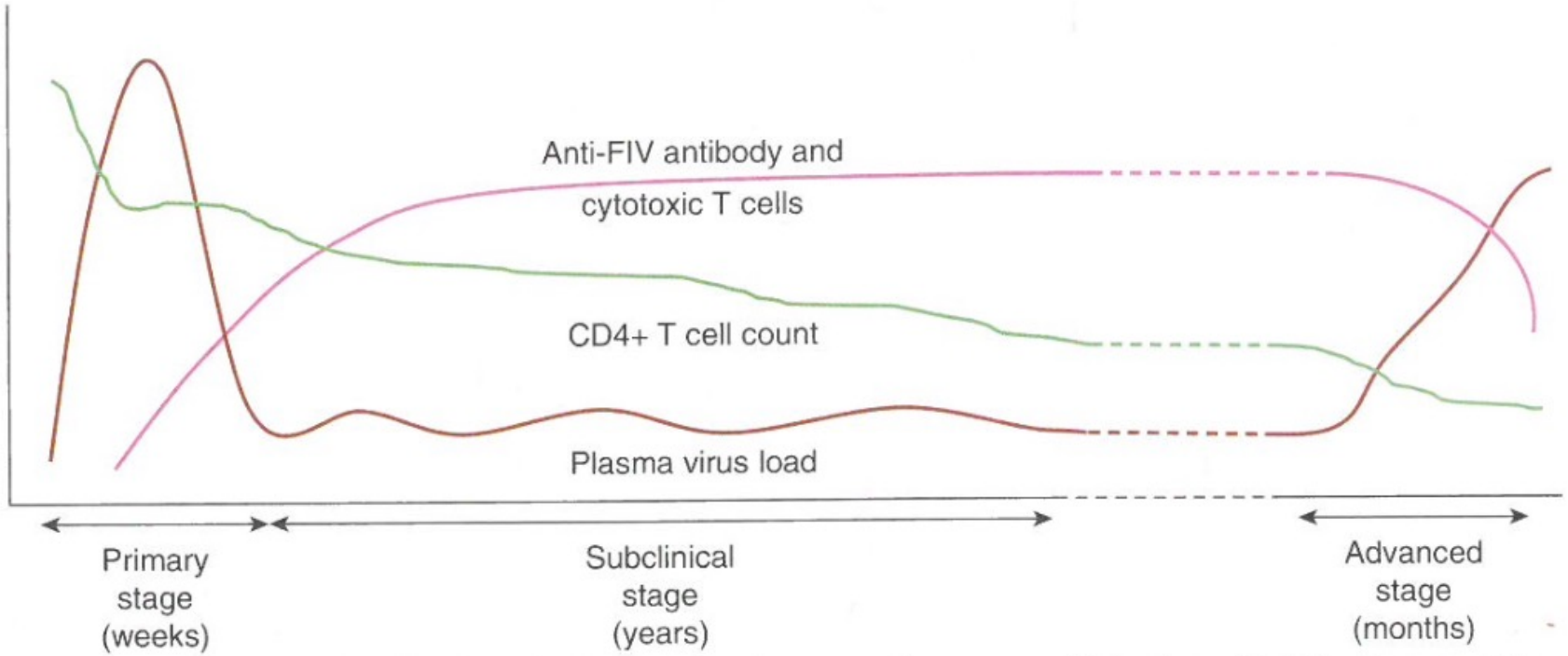
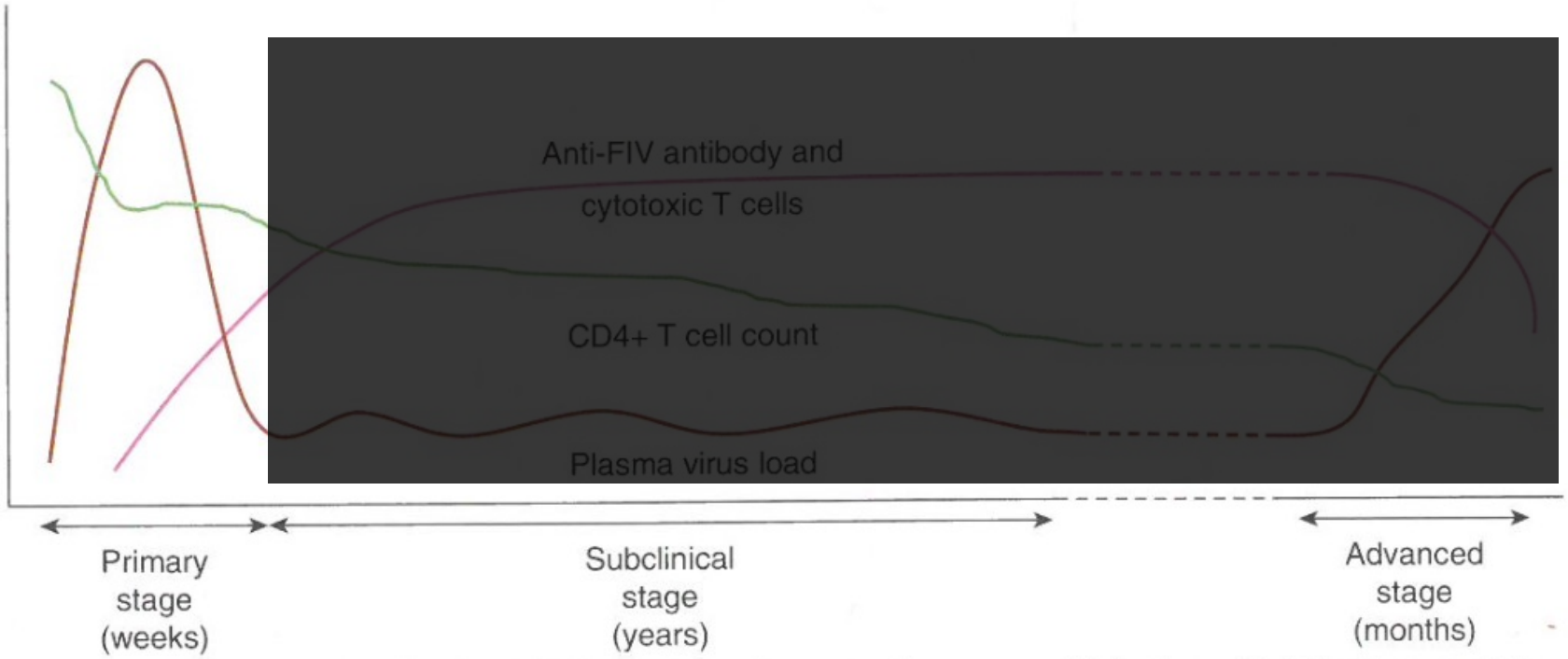


Fig. 33.3 Changes in virus load and CD4+ T-cell numbers over the course of infection with feline immunodeficiency virus (FIV). Viremia is detected early after infection and may be accompanied by systemic signs (primary stage). Plasma viremia then falls to very low levels and remains this way for many years (subclinical stage). Although this period is referred to as "clinical latency," the virus itself is not latent; there is ongoing production of virus and a steady decline in CD4+ T-cell counts. The rate of progression of the subclinical stage depends on factors such as the virus strain, co-infections with other agents that activate virus transcription, and host immunity. Some cats progress to the advanced stage of infection where signs of immunodeficiency develop, and plasma viremia increases. Advanced FIV infection is eventually terminal. Antibody production declines, and antibody tests may be negative in cats with advanced-stage disease, but polymerase chain reaction assays are usually positive.

FIV: pathogenesis



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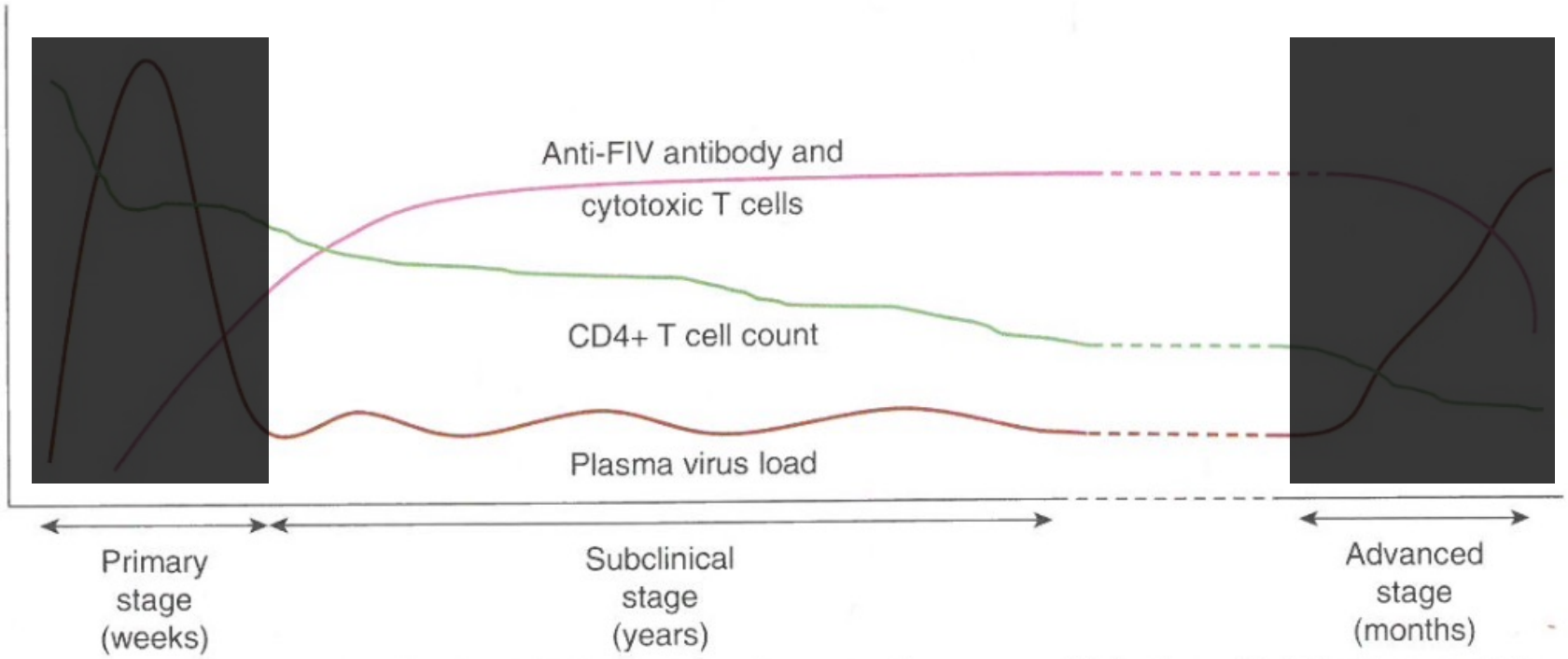
Primary stage

- Rapid viral replication
- Initial decline of CD4 T-cells
- Rise of CD8 T-cells

- Variable clinical signs:
  - Lymphadenopathy
  - Fever
  - Lethargy
  - Anorexia

FIV: pathogenesis



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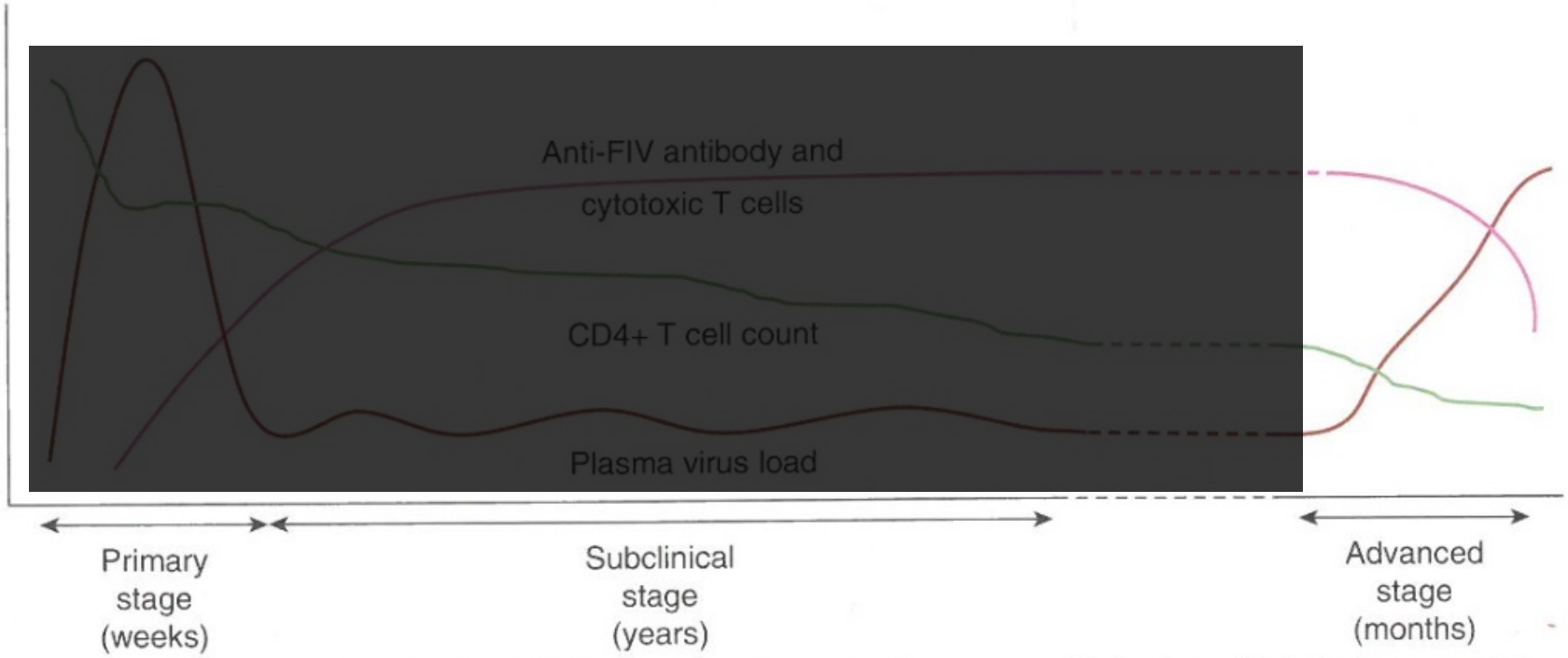
Subclinical stage

- Replication is contained, but continues in LN
- Virus is <u>not cleared</u>
- <u>Viremia is low to absent</u>
- CD4:CD8 ratio is still skewed
- Clinical signs are generally absent
- May have impaired immunity to opportunistic pathogens
- Lasts for years, and <u>can last for the cat's entire lifetime</u>

FIV: pathogenesis



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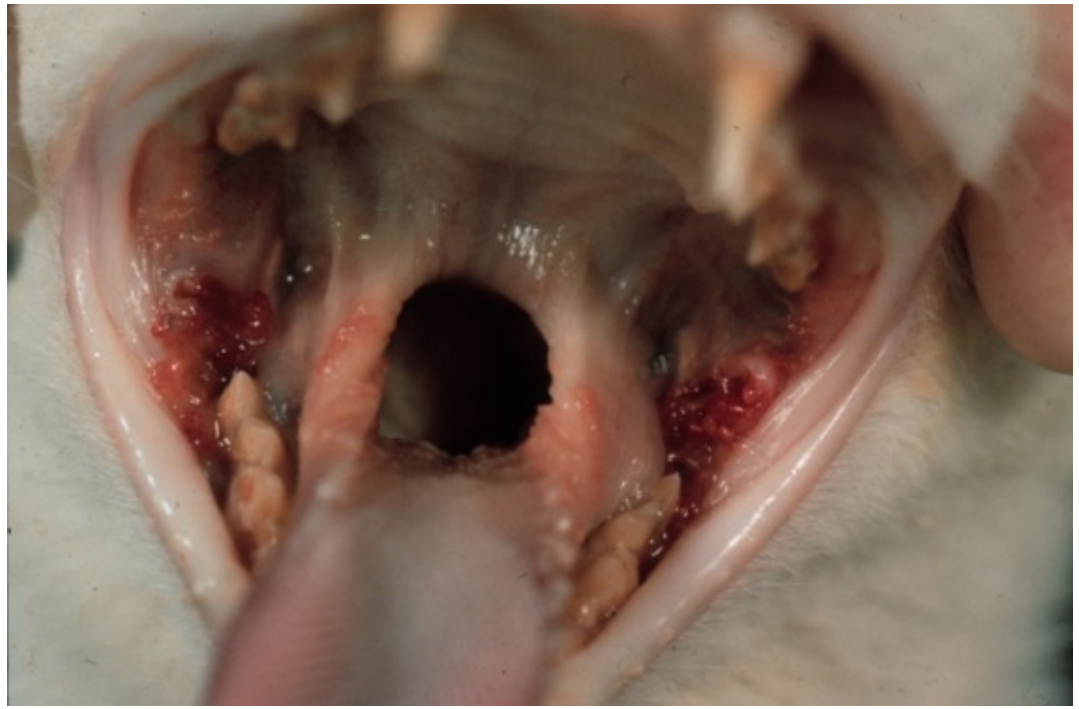
Advanced stage

- Profound lymphoid depletion
- Viremia
- May have:
  - Frequent opportunistic infections
  - FCGS
  - Neoplasia
  - Myelosuppresion
  - Neurologic disease
  - These are often less responsive to treatment

FIV: clinical signs

- Acute phase
  - Occurs shortly after infection (2 – 4 weeks)
  - Duration is variable: weeks to months
  - Clinical signs vary, are subtle, and are often missed:
    - Transient fever
    - Diarrhea
    - Generalized lymphadenopathy
    - Stomatitis
- Latent phase: viremic but asymptomatic* for months to years.
    - *mostly asymptomatic. Stomatitis can occur at any stage. Similarly opportunistic infections are more frequent.
    - This stage can be so long that some cats will die from other causes, and never develop the advanced stage.
- Advanced phase (feline AIDS): depletion of CD4+ lymphocytes resulting in opportunistic infections, declining health, ± neoplasia.



FIV: Diagnosis

- DDx: FeLV

- No distinct clinical signs

- Laboratory diagnosis is based on detection of **antibodies** by ELISA
  - IDEXX SNAP does **NOT DIFFERENTIATE** between infected and vaccinated cats
  - Antibodies are usually present by 60 days post-infection, but some cats can take longer
  - False positives – more likely with whole blood or in areas of ↓ prevalence
  - Positives should be checked (esp. in healthy or low-risk animals)
  - Careful in kittens < 6 mos – maternal Abs!

FIV: treatment and control

- Control:
  - Keep cats indoors (to avoid cat fights)
  - Keep FIV+ cats indoors
  - Test using FIV antibody tests:
    - It is recommended that veterinarians should know the FIV status of all cats.
  - An inactivated vaccine (Fel-O-Vax®) is available
    - Some antibody tests (e.g. IDEXX SNAP) cannot differentiate between infection or vaccination.
    - Previous vaccination does not rule out infection
    - Vaccine efficacy is not clear (protected 82% of vaccinated cats in experimental challenge; performance in real world is not known).
    - If the decision is to vaccinate, then cats should test negative immediately before vaccination.
    - For these reasons, in Canada, the use of this vaccine is generally not recommended.



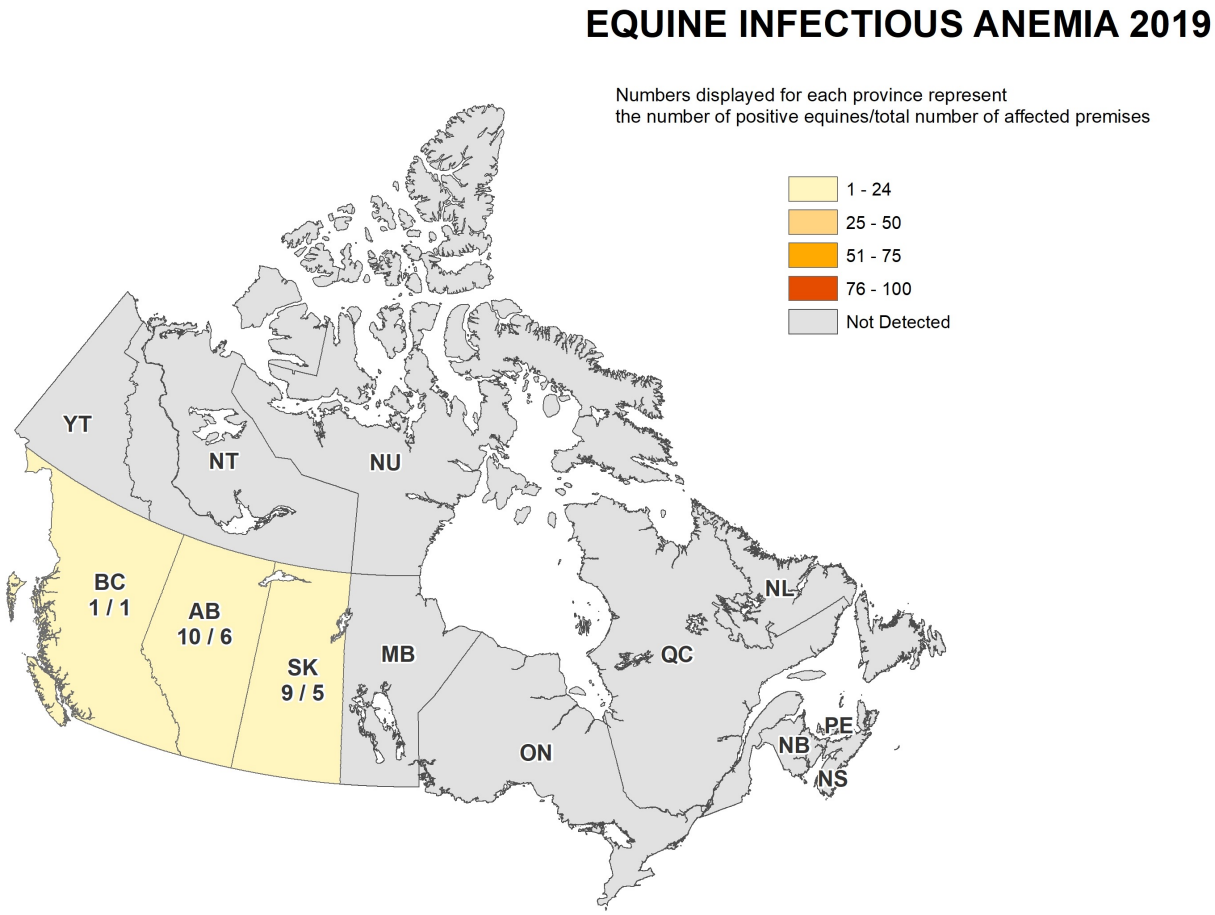


Equine infectious anemia virus

EIA, ‘swamp fever’

EIAV: general & transmission

- Worldwide distribution
- Horses, mules, and donkeys
- <u>Reportable (Canada and the US)</u>



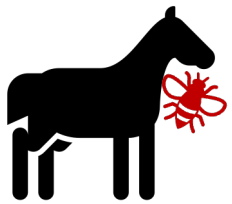
EIAV: general & transmission

- <u>Horizontal transmission by insects is most important</u>
  - Tabanid and stable flies
  - Note that EIA virus does not replicate in insects; this is mechanical transmission
  - Via equipment contaminated with blood
- Vertical transmission is possible but considered less important.
- Incubation period: 5 days to several months (it is unusually short for a lentivirus).



EIAV: pathogenesis and clinical signs

Infection and replication in cells of the monocyte-macrophage system



Acute

- Usually 1-3 weeks post-exposure
- Viremia and High fever
- Thrombocytopenia
- Anemia
- Lethargy, anorexia, depression

Chronic

- Waxing and waning episodes similar to acute
- May be separated by intervals of days, weeks, or months
- Due to antigenic escape by the mutation-prone virus
- Some will not progress past this point

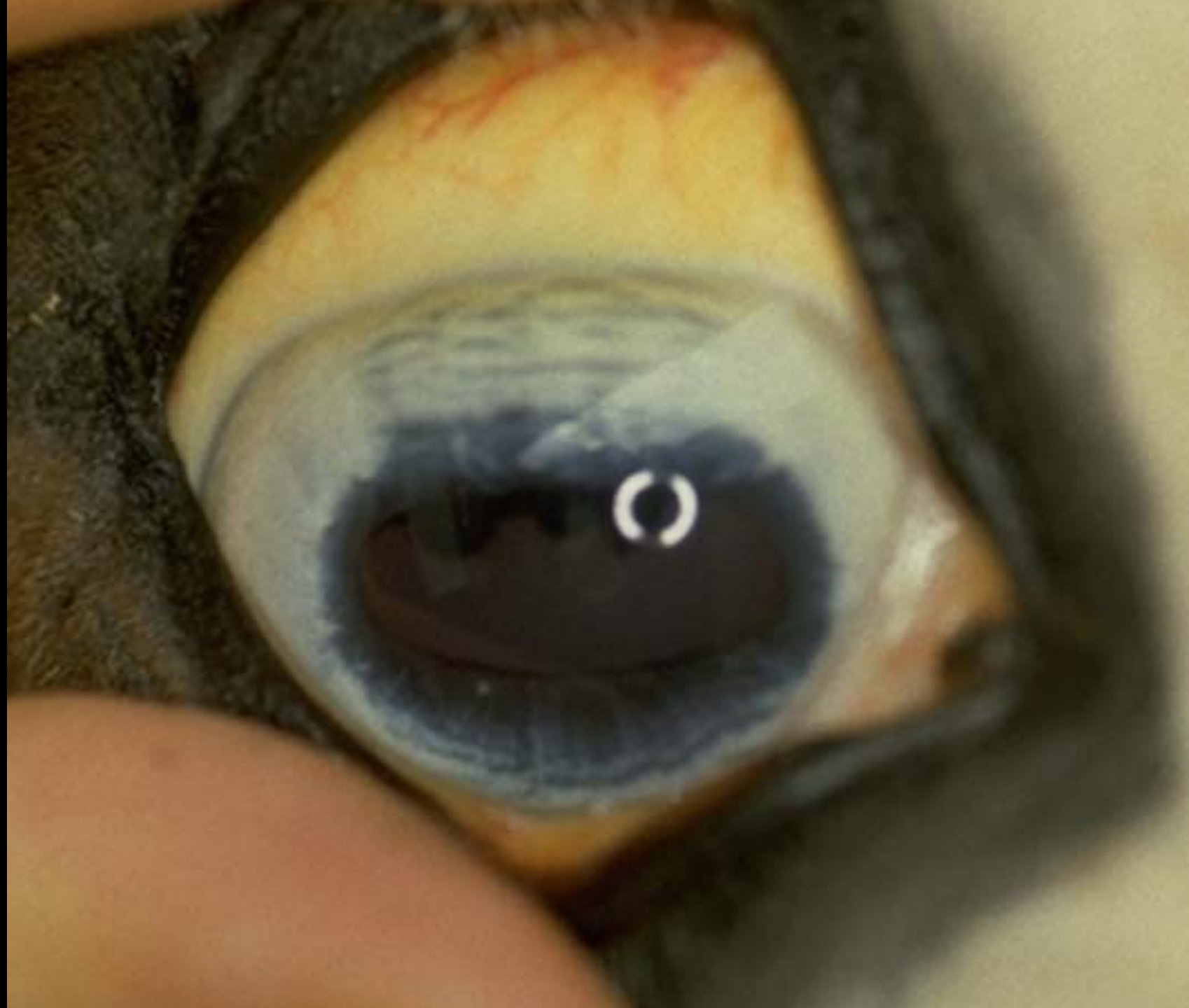
Inapparent

- MOST horses will progress to this stage
- Clinically normal
- Reservoirs of the virus



EIAV: clinical signs

- Severe anemia → pallor and jaundice (immune-mediated)
- Thrombocytopenia → petechia
- Fever
- Weakness
- ± death



EIAV: clinical signs

- Severe anemia → pallor and jaundice (immune-mediated)
- Thrombocytopenia → petechia
- Fever
- Weakness
- ± death

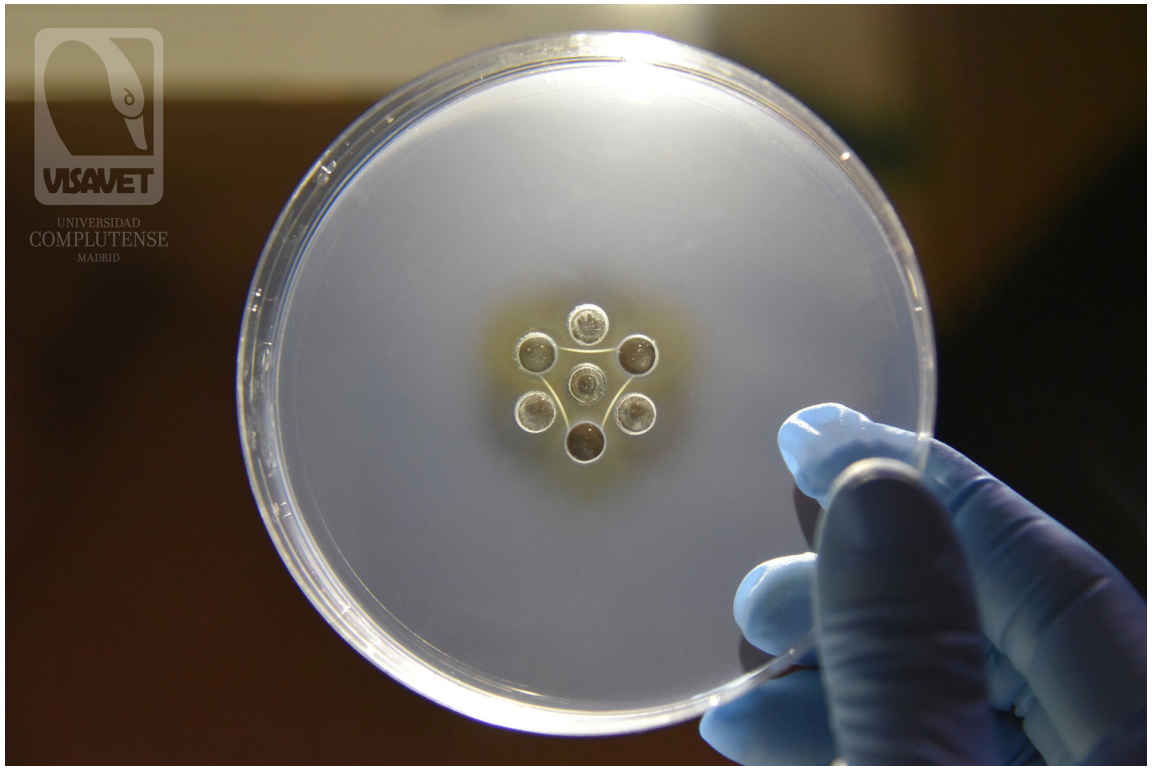


EIAV: diagnosis and control

Equine infectious anemia virus is a federally reportable disease

EIAV: diagnosis and control

- Diagnosis revolves around the detection of antibody
  - What sample should you take?
  - Various ELISAs are available
  - Coggin's test (technically agar gel immunodifussion assay)



EIAV: diagnosis and control

- No vaccine
- Horses that are being moved or attending shows, etc., require testing.
- If positive, animal is destroyed and owner compensated
  - Max $2000!
  - In contact animals are tested (x2 45 days apart)
  - Premises are quarantined
- Vector control:
  - 200-yard segregation from positive horses.
  - In summer, stable horses at night.
- Good management:
  - “One-needle, one-horse”
  - Avoid use of common equipment among horses (e.g., bridles, brushes, etc.).



Bovine leukemia virus

‘enzootic bovine leukosis’ (EBL), BLV

BLV: general

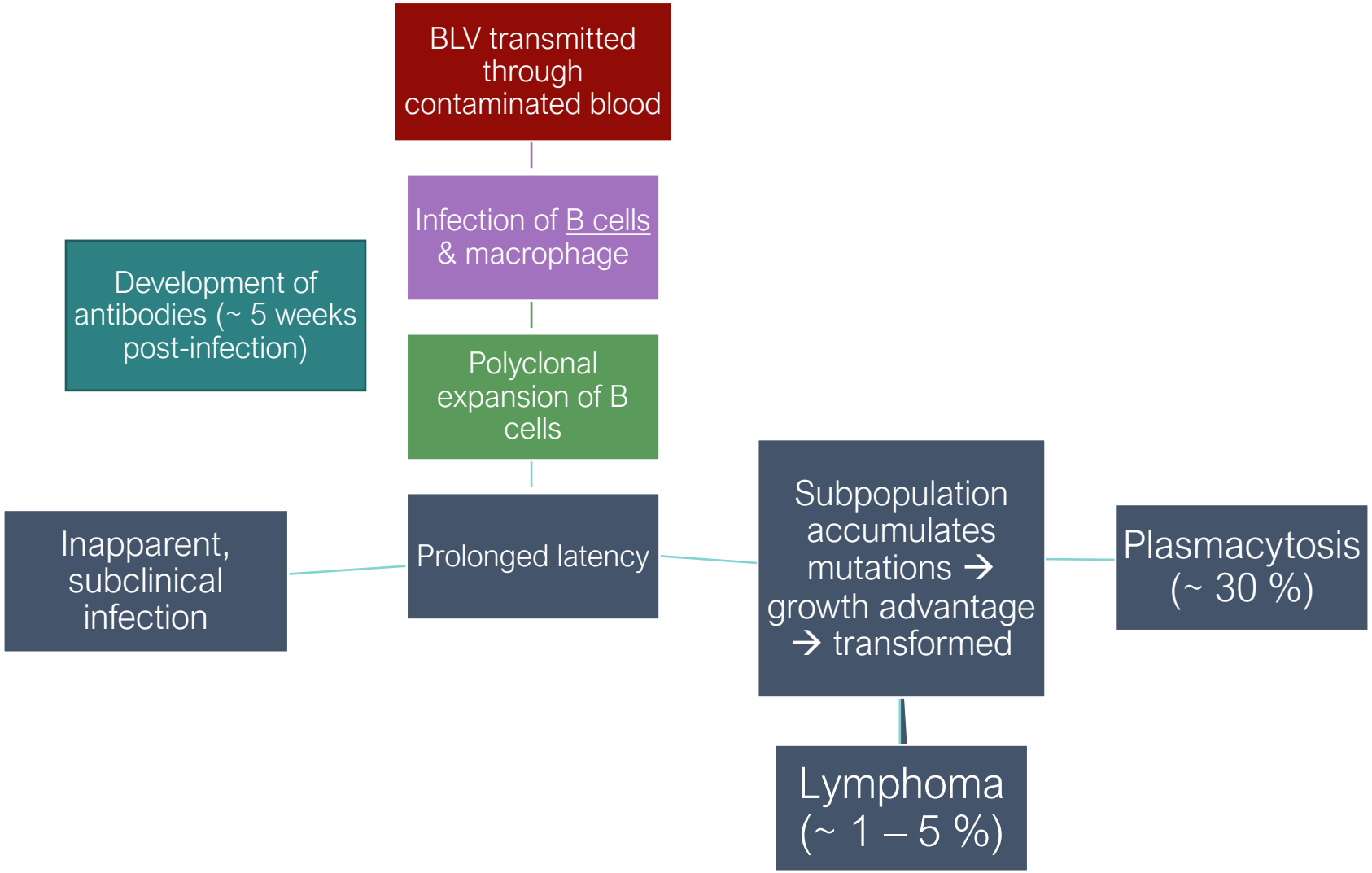
- Widespread in North America
- Has no oncogenes, but has additional genes (tax) that upregulate gene expression
- Long incubation period (disease only seen in older animals)
- Disease incidence is low, but economic importance is high:
  - Trade restrictions
  - Production loss
- Disease typically presents in older cattle (> 2 years, 4-8 years is the peak)

BLV: transmission

- Horizontal is the most important
  - Transfer of **blood**
    - Mechanical vectors (tools, instruments, insects)
    - Rectal palpation w/out changing sleeves
  - Other fluids can experimentally transmit virus, but are not thought to be as important in a natural setting

- Vertical transmission can occur
  - Either *in utero*, during parturition, or consumption of milk

BLV: pathogenesis



BLV: clinical signs

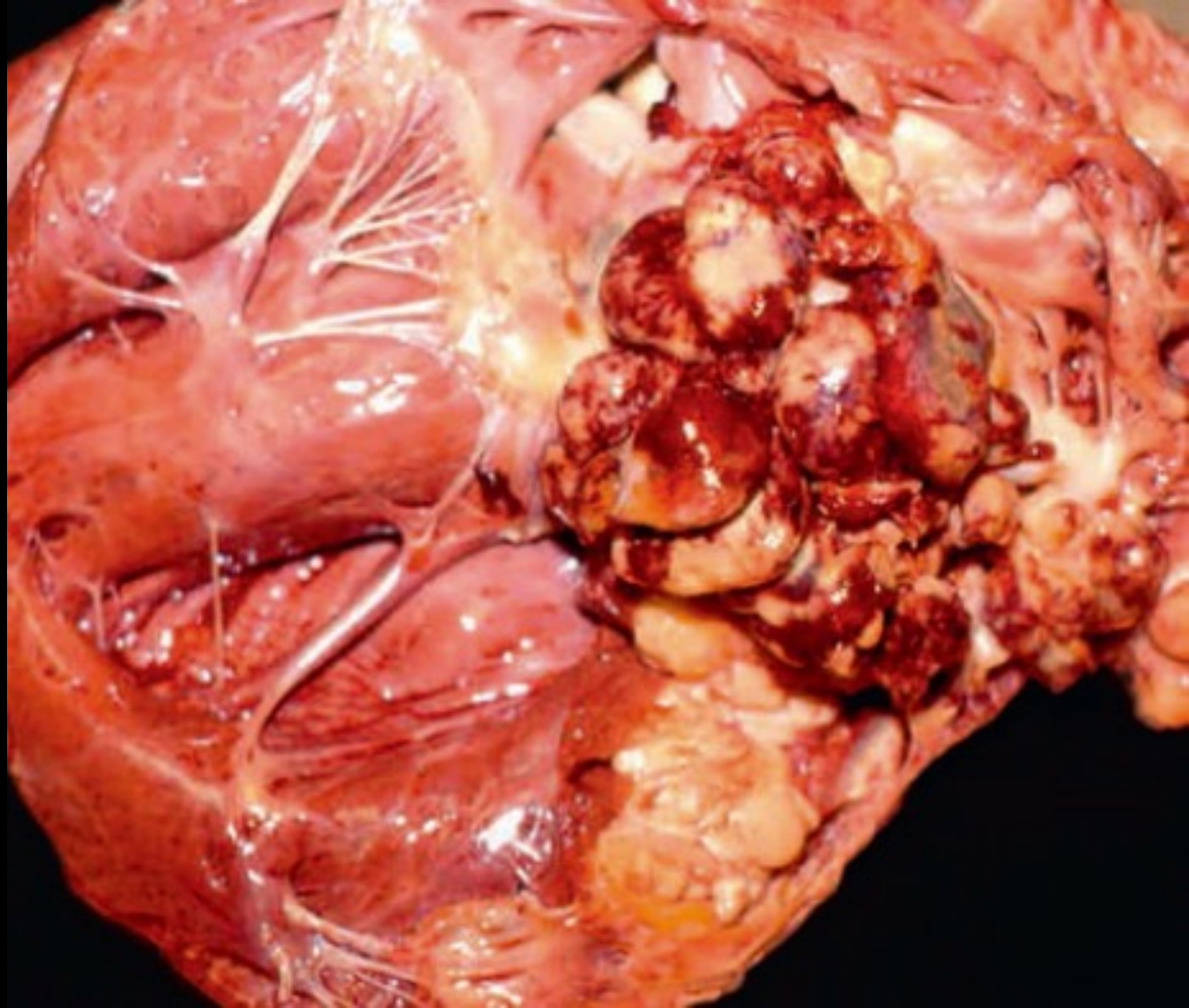
- The majority of cattle are asymptomatic

- Cattle with plasmacytosis are clinically normal (but a source of virus)

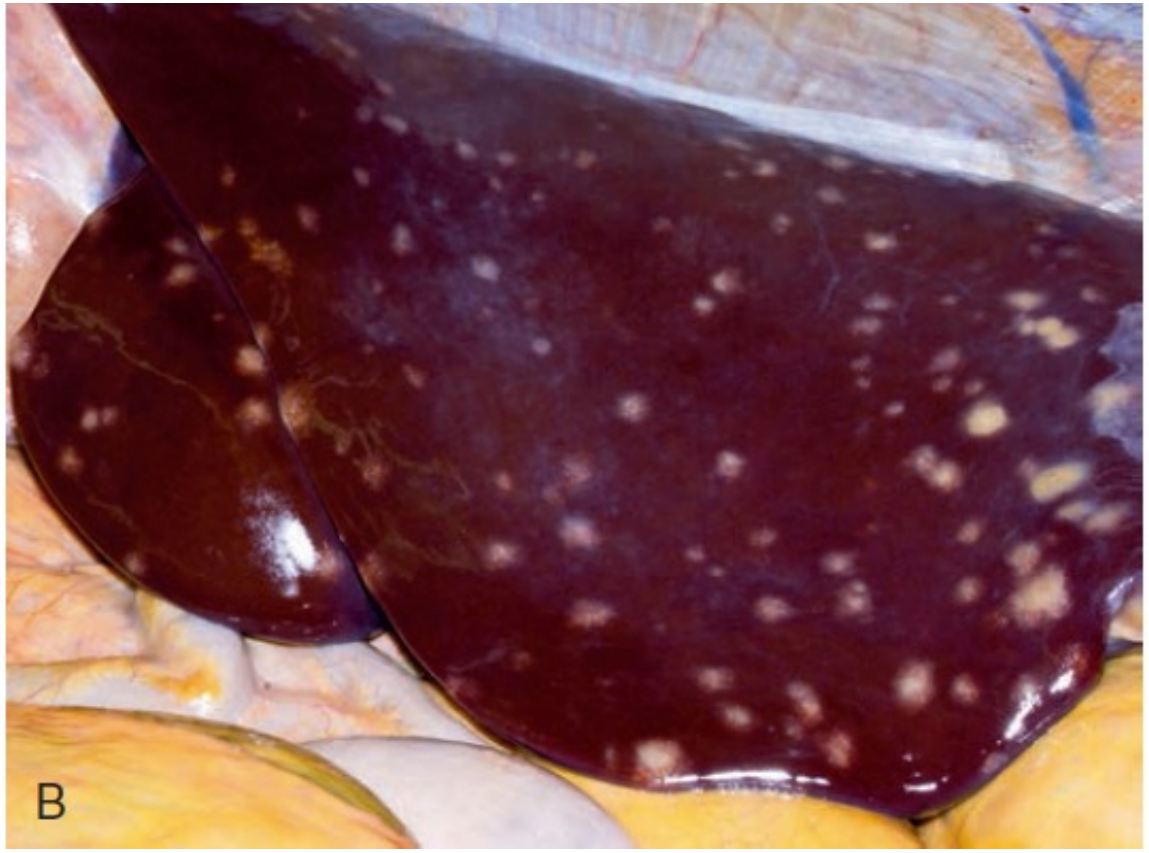
- Most notable clinical sign is therefore lymphoma (what kind? T or B cell?)
  - The clinical signs will depend on where the tumour develops:
    - Central and peripheral lymphadenopathy
    - Abomasum → abdominal pain, melena
    - Spinal cord → paresis or paralysis
    - Heart → arrythmias
    - Uterus → reproductive failure

BLV: lesions

- Lymphoma is a firm, white to tan mass that occurs in almost any organ



BLV: lesions





Lymphoma in the liver (left) and kidney (right)

Bovine leukemia virus (BLV): diagnosis and control

- Agar gel immunodiffusion (AGID) test forms basis for test & removal programs, international trade regulations, & CFIA's Canada Health Herd Accredited (CHAH)-EBL program.
  - CHAH-EBL certifies herds to be officially free from EBL (a requirement for export to European Union).

- AGID test detects persistent antibody to gp51 & p24 viral antigens:
  - Need 2 negative test results 12 weeks apart for animal to be considered BLV negative.
  - Herd test (for all animals above 6 months old) is done at 3-monthly intervals.
  - Many countries include conditions for Enzootic Bovine Leukosis (EBL) in their import health protocols
  - Only EBL-negative herds can export live bovines or beef to Europe
    - "The animals must originate from herds officially free of EBL".

Bovine leukemia virus (BLV): control

- No vaccine

Prevent the spread of EBL among your herd

Risk Factors	Preventative Measures
Any material able to transmit blood or infected lymphocytes (a type of blood cells carrying EBL) between animals	• Use individual, single-use needles and syringes during vaccination or therapeutic protocols • Use individual, single-use obstetrical sleeves • Use of disposable equipment (or at least cleaning, disinfection or sterilization of reusable materials and surgical instruments) in procedures such as dehorning, tattooing, implanting embryos, cauterizing, castration or ear-tagging • Use of electrical or gas burning devices rather than gouging equipment for dehorning
Colostrum and milk of an infected cow may carry EBL	• Feed calves with colostrum or whole milk from confirmed non-infected dams or use pasteurized colostrum or milk replacer • Use and maintain the milking machines adequately and according to manufacturer standards
Biting insects may spread EBL by mechanically transferring infected cells	• Eliminate or reduce insects, particularly in densely populated farm areas (e.g. milking areas, free-stalls, barns).



Small ruminant lentiviruses

SRLV: general

- Historically, Meadi Visna virus of sheep and caprine arthritis encephalitis virus of goats were considered unique, species-specific viruses
- It is now believed that they are a spectrum of SRLV, with some detected in certain species.
- Occur worldwide except in Australia, New Zealand & Iceland.
- Incubation period: up to 9 years.
- Virus replicates in macrophages & lymphocytes; does not induce neutralizing antibodies; results in persistent infection.



Major mode of transmission is ingestion of colostrum/milk

Inhalation of aerosols may also play a role



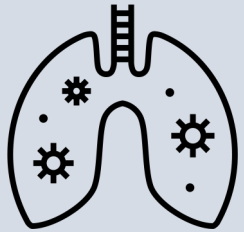
SRLV: pathogenesis

- Shared between all the SRLVS

- Infect monocytes and macrophages (not lymphocytes)
- Inflammation is common
- Immunodeficiency is NOT a feature

- What dictates the manifestation of different clinical syndromes is unclear.

Meadi Visna virus: clinical signs



”Meadi” or ovine progressive pneumonia

- Weight loss
- Exercise intolerance
- Dyspnea, nasal d/c, cough
- Markedly enlarged thoracic LNs
- Interstitial pneumonia



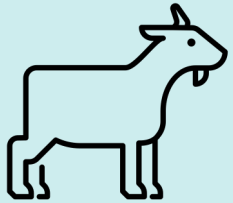
Encephalitis in adult sheep

- Pectoral limb weakness → Paresis
- Normothermic
- Good appetite
- Alert
- May last years

Relatively **rare**.

Caprine arthritis-encephalitis: clinical signs

- Generally, see one of 2 clinically presentations (not both in the same animal)



Chronic arthritis & mastitis in 2-9 year olds

- Insidious onset
- One or multiple joints
- Carpus often most severe (hock, stifle, shoulder, vertebral joints can also be affected)
- Progressive – fluid distension and eventually ankylosis



Encephalitis in kids 2-6 months old

- Paresis
- Normothermic
- Good appetite
- Alert
- Fatal

Relatively **rare**.

SRLV: diagnosis and control

- ELISA or AGID (antigen based tests)
  - Blood or milk

- Removal of offspring from infected mothers
- Test and cull