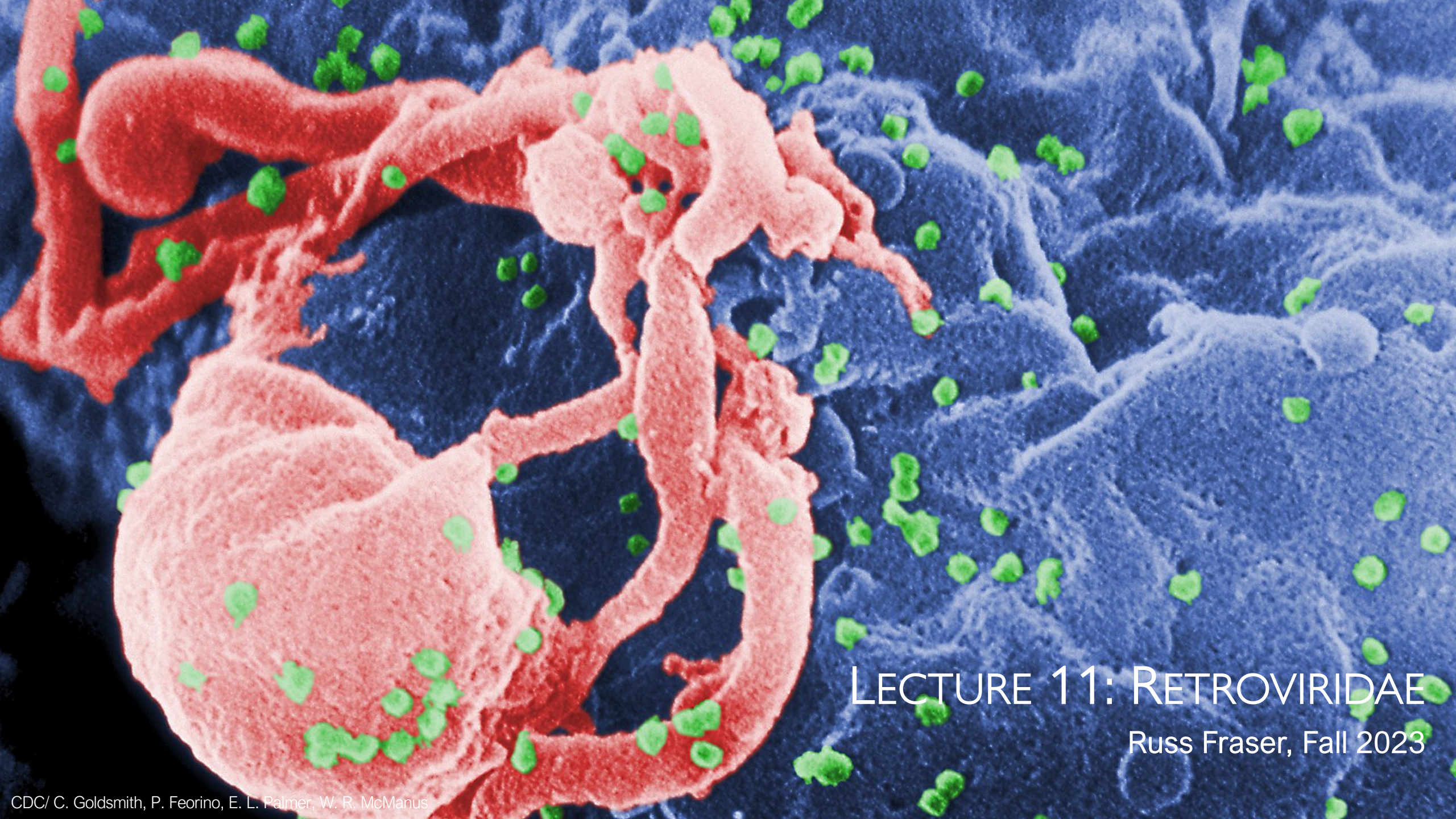


Lecture 11: Retroviridae

Russ Fraser, Fall 2023

CDC/ C. Goldsmith, P. Feorino, E. L. Palmer, W. R. McManus

Housekeeping

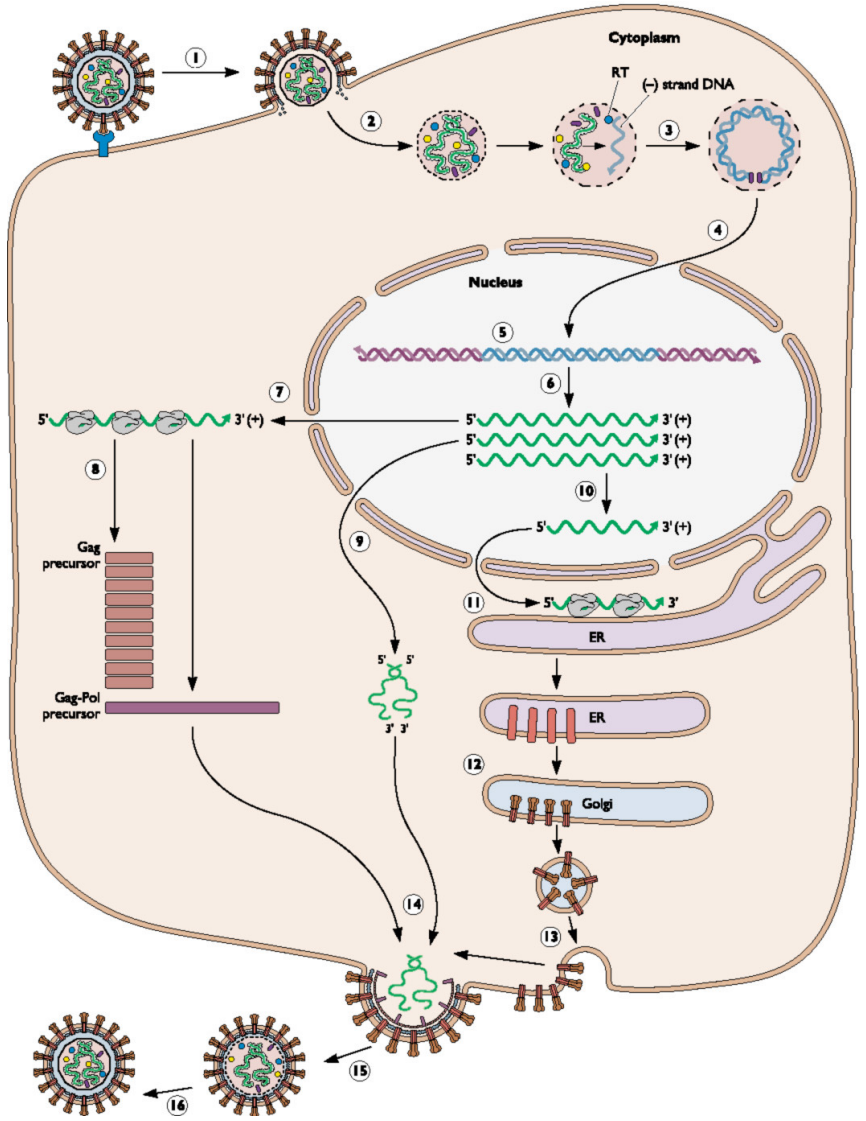
- Questions?
- Hooray for no Fiona!
- Thank you!

Retroviruses: general characteristics

Genome	• ssRNA (diploid)
Morphology	• Helical • Enveloped
Size	~100 nm
Features	• Carry a reverse transcriptase • Do not survive long in the environment (hours)
Oncogenic potential	• All the retroviruses we will discuss are oncogenic • Generally, retroviruses are either replication competent AND not rapidly tumorigenic OR • They are replication deficient AND rapidly tumorigenic

Retroviruses: general characteristics

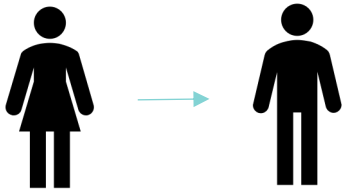
- Virus integrates into the host genome (required for replication)
- Once integrated it is called a provirus
- DNA provirus has its own powerful promoters (LTR) allowing for overexpression of its genes.
- Retrovirus replication is accompanied by high mutation rate (1:10,000 to 1:100,000 substitutions/replication cycle) because of:
  - No proof reading by reverse transcriptase (a mistake is carried unless it is lethal)
  - High rate of recombination with virus genes & with cellular genes.



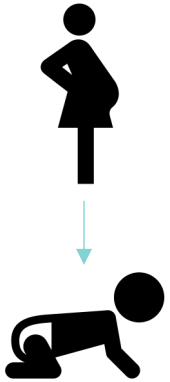
Retroviruses: general transmission

- Unique method to retroviruses: genetic
  - Form of vertical transmission
  - Remember: retroviruses insert their genomes into the host genome (provirus)
    - If that happens in a somatic cell, the virus is limited to the current host
    - If it happens in a germ cell (i.e. a sperm or ovum), the provirus can be transmitted to the next generation (and be present in all cells of the offspring)

Horizontal



Vertical



Retroviruses: general transmission

- Genetic transmission can give rise to “endogenous” retroviruses:
  - Retroviral DNA that has been incorporated into the host genome, generally over the course of many generations
  - Occurs in all organisms (human genome estimated to be ~5-8 % ERV!!!!!)
  - The majority have mutations and cannot produce new virions (but some may still be able to produce viral proteins)
  - Are under control by cellular regulatory genes (usually silent in infected animal – but can be activated or induced to synthesize virions);
  - Do not produce disease

Retroviruses: general characteristics

- Endogenous retroviruses are non-pathogenic

- All of the viruses we will talk about are exogenous retroviruses (typical viruses)

Retroviruses: transmission

Transmission of Animal Retroviruses

Transmission mode	Horizontal	Vertical		
		Congenital	Genetic	
Virus type	**Exogenous** retrovirus	**Exogenous** retrovirus	**Exogenous** retrovirus	All **Endogenous** retroviruses
Transmission routes	- **contact** (with body fluids) e.g., saliva in FeLV & FIV. - **aerosols** (inhalation), e.g., ALV in hatchery. - **mechanical vectors**, e.g., contaminated equipment in FeLV, FIV & EIAV; blood sucking insects in BLV & EIAV. - **milk during nursing**, e.g. in CAEV & BLV.	- ***in-utero***, i.e., transplacental infection in FeLV (100%), FIV, BLV (10%) & EIAV. - **via eggs** (locating in pancreas of embryos) in ALV. - **colostrum during nursing**, e.g. in CAEV. - **horizontal transmission in 1st few days of life** (up to 8 weeks in kittens)	- **provirus in ovum and/or sperm** results in provirus in all cells of embryo	- **provirus in ovum and/or sperm** results in provirus in all cells of embryo

Retroviruses: tumorigenesis

- Retroviruses may cause tumours either by:
  - Causing aberrant expression of cellular oncoproteins (c-onc)
  - Encoding viral oncoproteins (v-onc)
- In normal cells, oncoproteins are tightly regulated
- In neoplastic cells, they may be:
  - Overexpressed
  - Expressed in wrong cell
  - Expressed at wrong time
  - Abnormal oncoprotein (e.g., v-onc) is expressed, resulting in cell deregulation and cell transformation.

What's an oncoprotein?

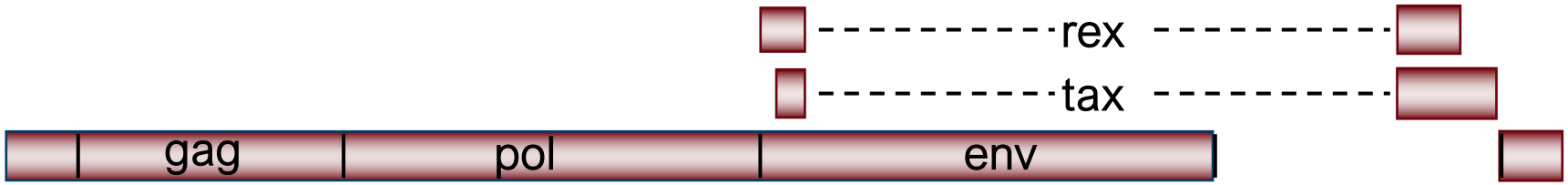
Retroviruses: tumorigenesis

Ways in which retroviruses cause tumors:



- Virus carries an oncoprotein.
- These cause rapidly developing tumors.
- In most cases, the oncogene has replaced an important structural protein, and these viruses cannot replicate.





Retroviruses: tumorigenesis

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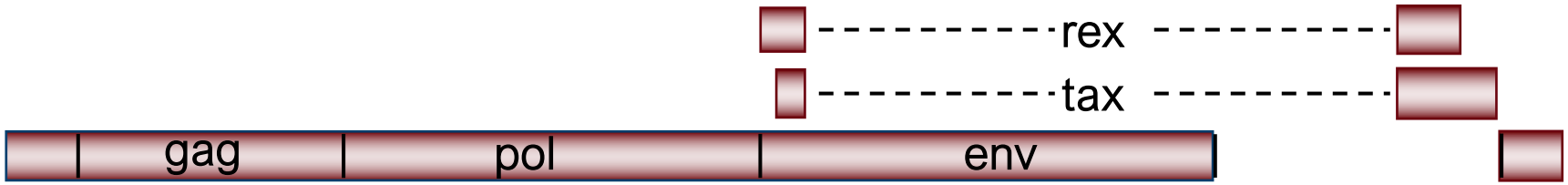
- In most cases, the oncogene has replaced an important structural protein, and these viruses cannot replicate.



- Virus does not have an oncoprotein.
- Tumours are caused by insertion in, or around, a cellular oncoprotein
- The increased local gene expression induced by the virus → ↑ c-onc production
- Tends to take weeks – months – years to produce tumours



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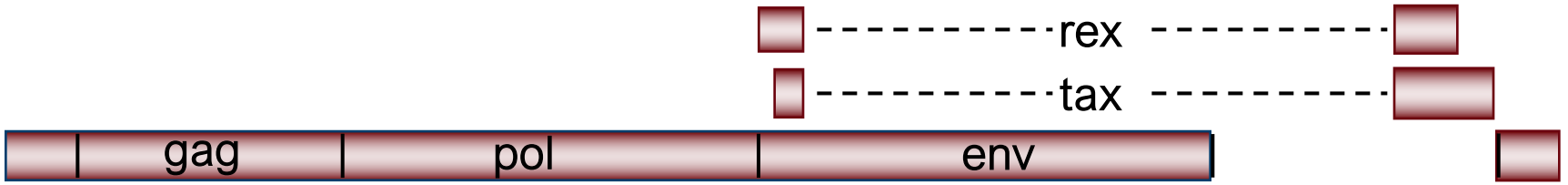
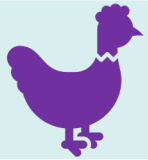
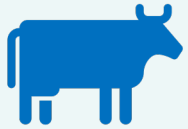
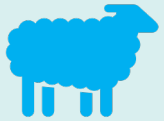
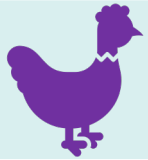


- Virus encodes a protein that regulates transcription (tax)
- Causes dysregulation/overexpression of cellular oncoproteins

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 

Retroviruses: important diseases

	Avian leukosis virus
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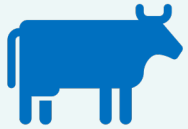
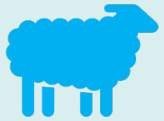
	Bovine leukemia virus
	Maedi/Visna virus
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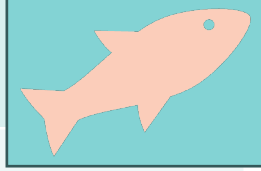

Atlantic Salmon swim bladder leiomyosarcoma virus





Avian leukosis virus

‘lymphoid leukosis’

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Avian leukosis virus: general

- Avian leukosis viruses are a large & complex group of viruses that cause tumors in chickens.

- Can be:
  - endogenous (in genome of every chicken as provirus) & nonpathogenic

  - exogenous & replication-competent (i.e., no v-oncogene), causing lymphoid leukosis, osteopetrosis & nephroblastoma only in congenitally infected birds with persistent viraemia >14 weeks old (3-20% incidence); or

  - exogenous & replication-defective (has v-oncogene), causing tumors depending on v-onc carried (see Table on page 65), in all infected birds <14 weeks old:
    - Erythroblastosis
    - Myeloblastosis
    - Myelocytomatosis
    - Hemangioma
    - Sarcomas

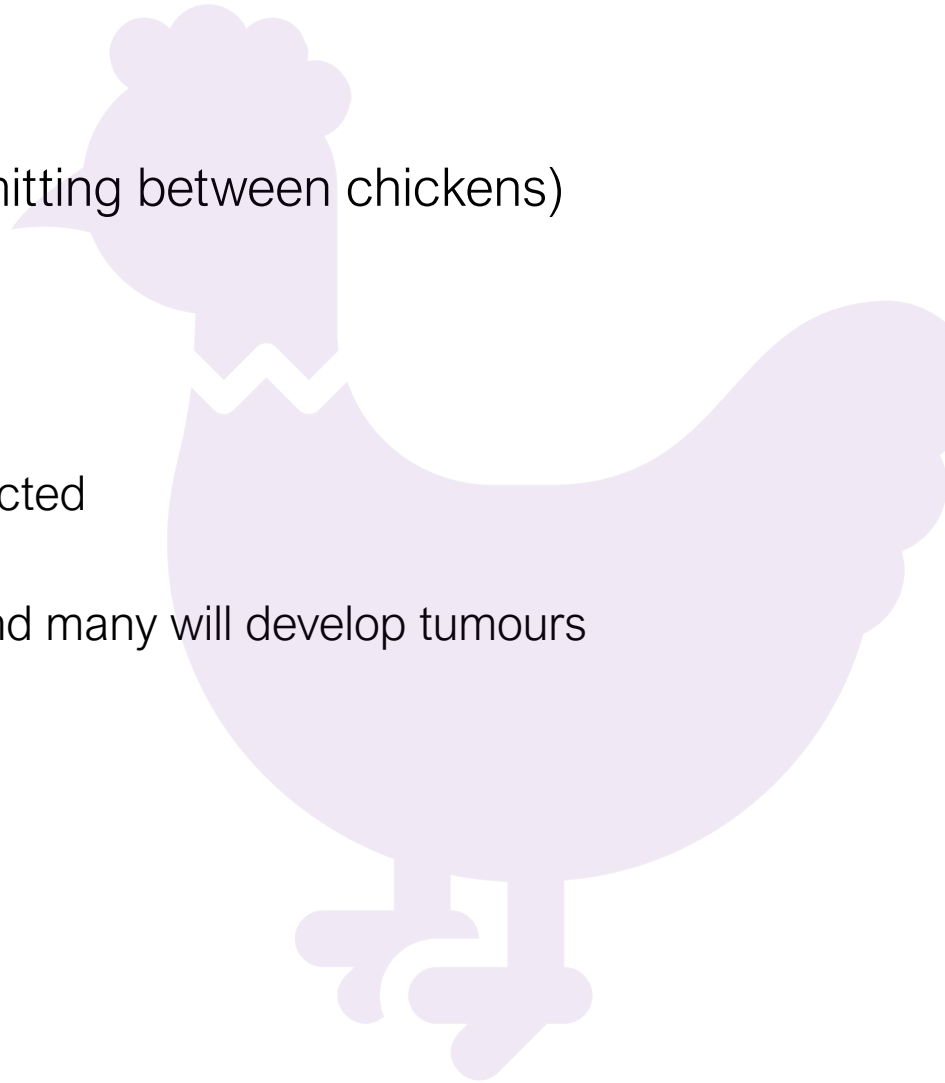
Avian leukosis virus

DISEASES PRODUCED BY AVIAN RETROVIRUSES (adapted from Veterinary Virology 4th edition, Table 14.4, page 253)

Type of virus	Disease	Rate of tumor development	Viral oncogene	Cell first affected	Type of lesion
Replication-competent (Avian Leukosis Viruses)	Lymphoid leukosis	Slow	-	Lymphoblast (B cell)	Lymphoid cell (IgM type) infiltrations of various organs
	Osteopetrosis	Slow	-	Osteoclast or osteoblast	Thickened long bones
	Renal tumors	Slow	-	Renal cells	Nephroblastoma, carcinoma
Replication-competent (Rous Sarcoma Virus)	Sarcoma	Very rapid	v-src	Various mesenchymal cells	Sarcomas
Replication-defective (Avian Erythroblastosis, Avian Myeloblastosis, Avian Myelocytomatosis Viruses)	Erythroblastosis	Rapid	v-erbB	Erythroblast	Anaemia
	Myeloblastosis	Rapid	v-myb	Myeloblast	Anaemia, leukemia
	Myelocytomatosis	Rapid	v-myc	Myelocyte	Sarcoma
	Hemangioma	Rapid	?	Capillary endothelium	Hemangioma
	Sarcomas	Rapid	v-fps, v-yes	Various mesenchymal cells	sarcoma, carcinoma

Avian leukosis virus: transmission

- Horizontal transmission:
  - Major mode of transmission
  - Saliva, feces, contaminated vaccines
  - Not particularly contagious (i.e. not very effective at transmitting between chickens)
  - Outcome of exposure depends on age
- Vertical transmission
  - Congenital transmission
    - Only 1-10% of embryos from an infected chicken will be infected
    - Minor mode of transmission, BUT
    - All chicks with congenital ALV will be persistently infected and many will develop tumours
  - Genetic transmission
    - Minor mode of transmission
    - Outcome the same as congenital

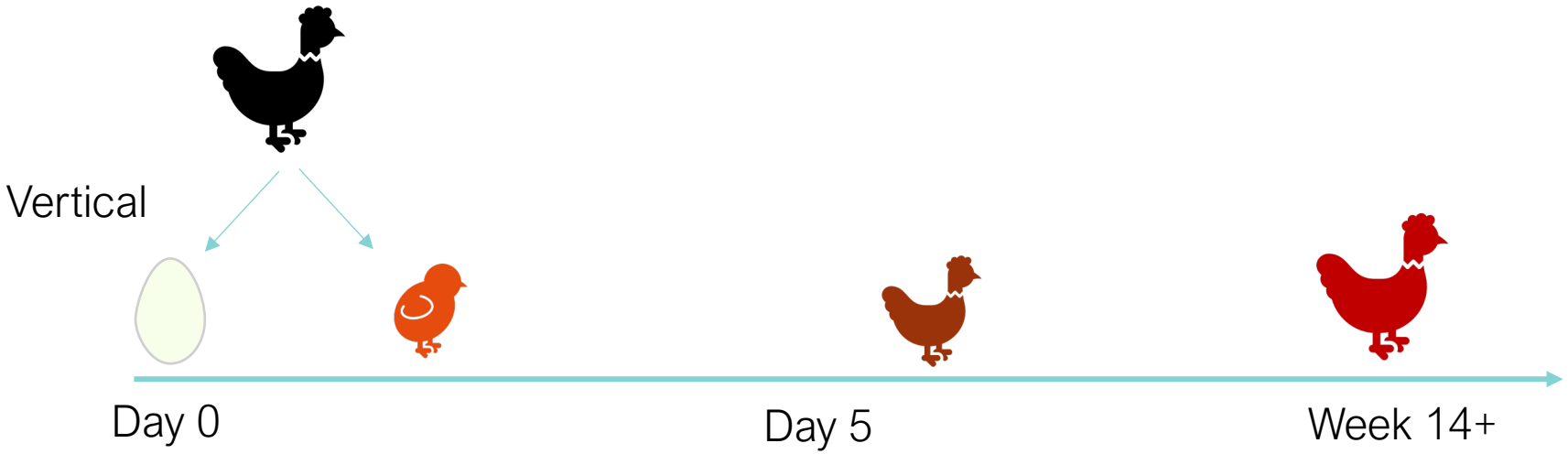


Avian leukosis virus: transmission

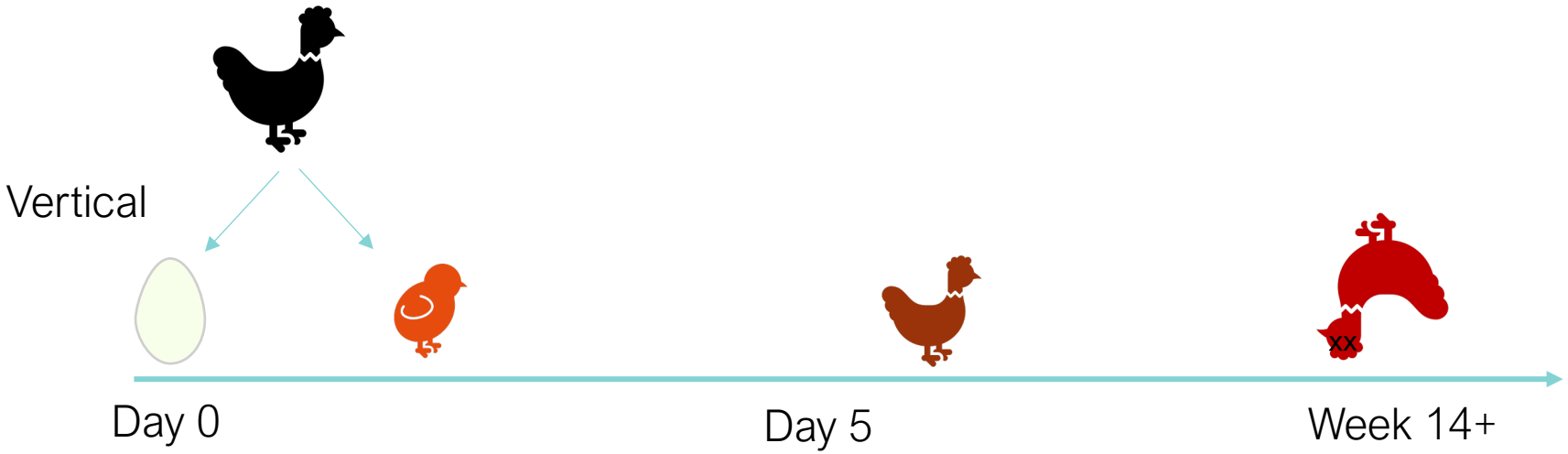
- Horizontal
  - Birds > 5 days old produce antibodies and clear the virus
  - Birds < 5 days old (including congenitally infected chicks) are susceptible, more likely to become immunotolerant and develop persistent infection
- Vertical
  - Congenital occurs via passage through the oviduct
  - Genetic



Avian leukosis virus: transmission

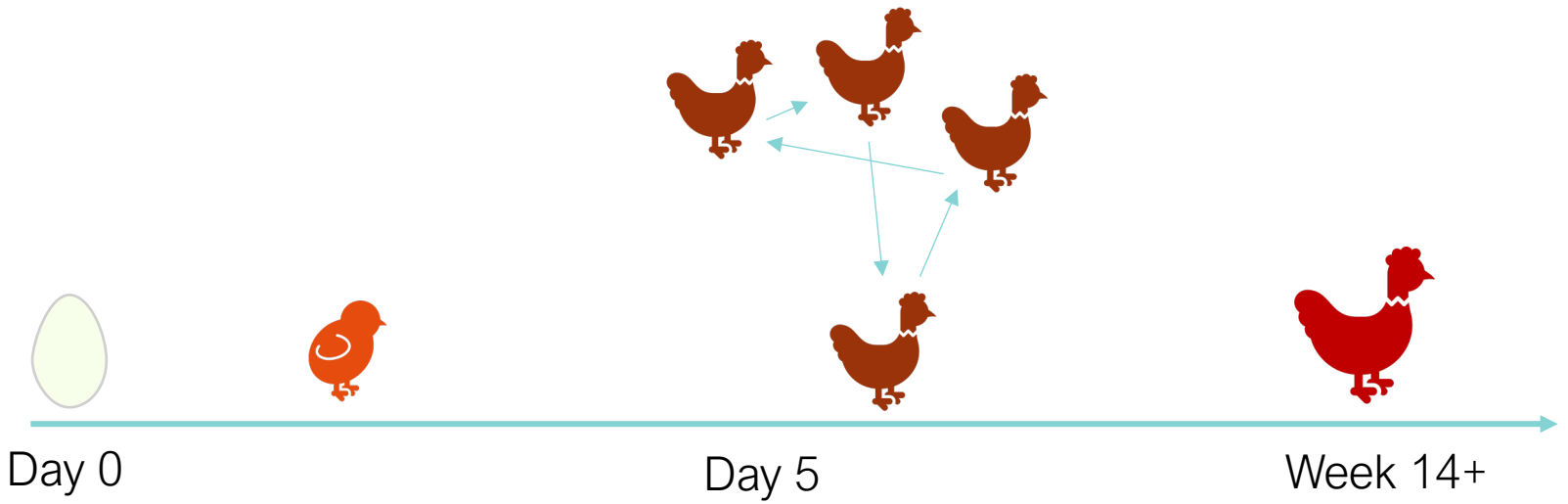


Avian leukosis virus: transmission

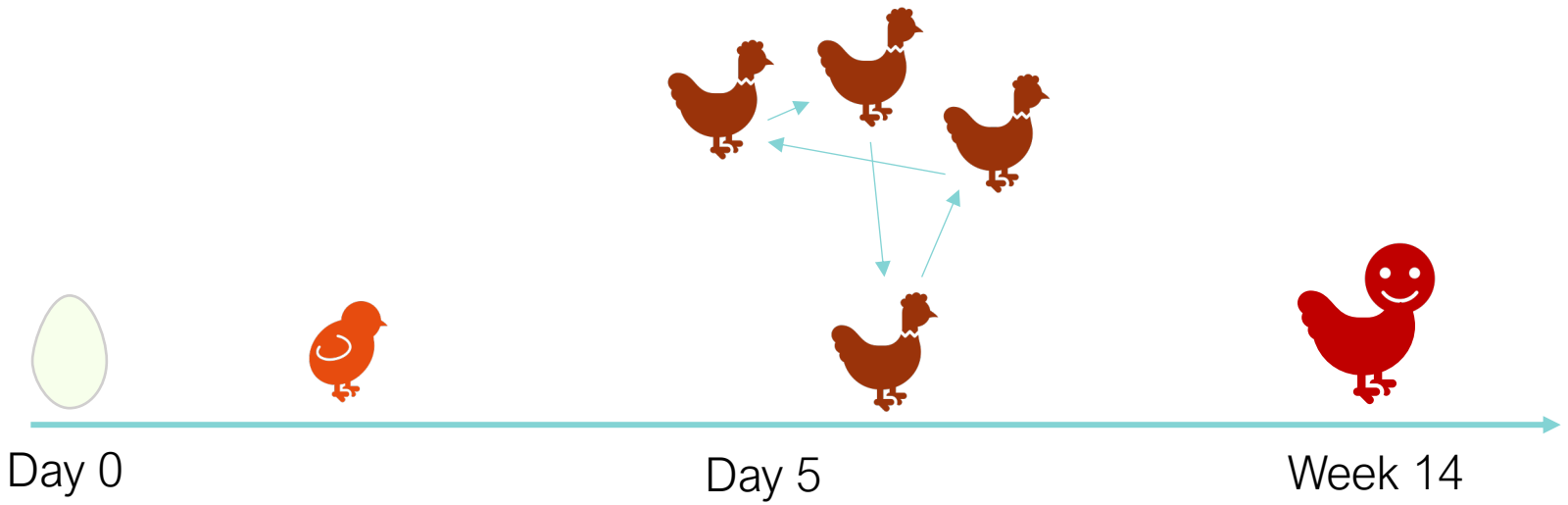


- Persistent infection
- Immunotolerant
- Sheds virus
- Will develop tumours

Avian leukosis virus: transmission



Avian leukosis virus: transmission



- Antibody response
- Transient viremia
- Clears virus
- Will <u>not</u> develop tumours

Transmission of Avian Leukosis Virus (pages 63-64)

by three distinct modes:

1. Horizontal transmission

- inefficient;
- viraemic birds excrete virus in saliva & faeces;
- in egg-transmitted virus, infected chicks shed virus in meconium at hatching.
- infection at >5-6 days old results only in transient viraemia & immunity.

2. Congenital transmission (& horizontal transmission at <5 days old) results in immunological tolerance, lifelong viraemia, & disease later in life (>14 weeks old).

3. Genetic transmission (provirus in germline;

if exogenous retrovirus – outcome as with congenital transmission;
if endogenous retrovirus – usually silent; no disease).

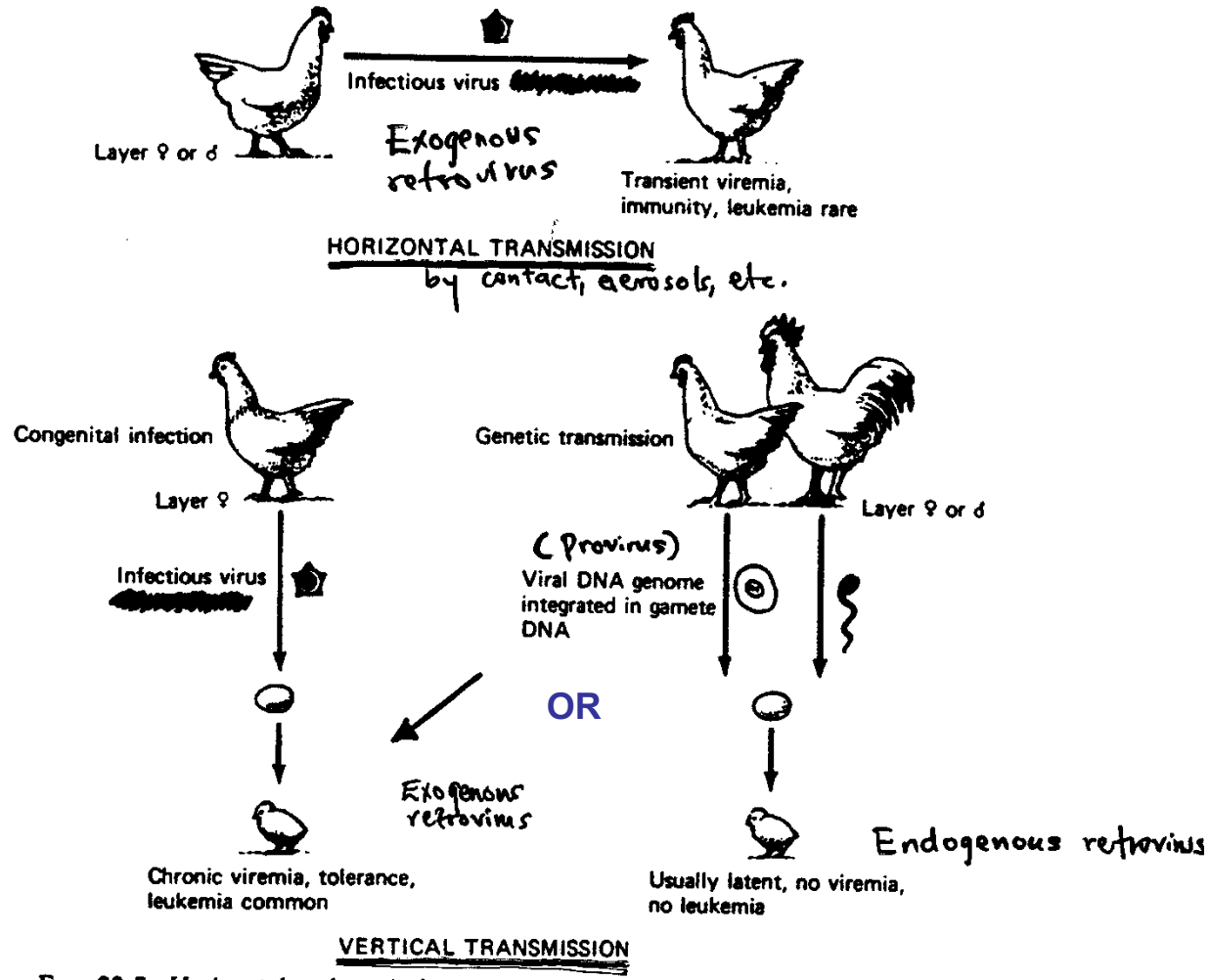


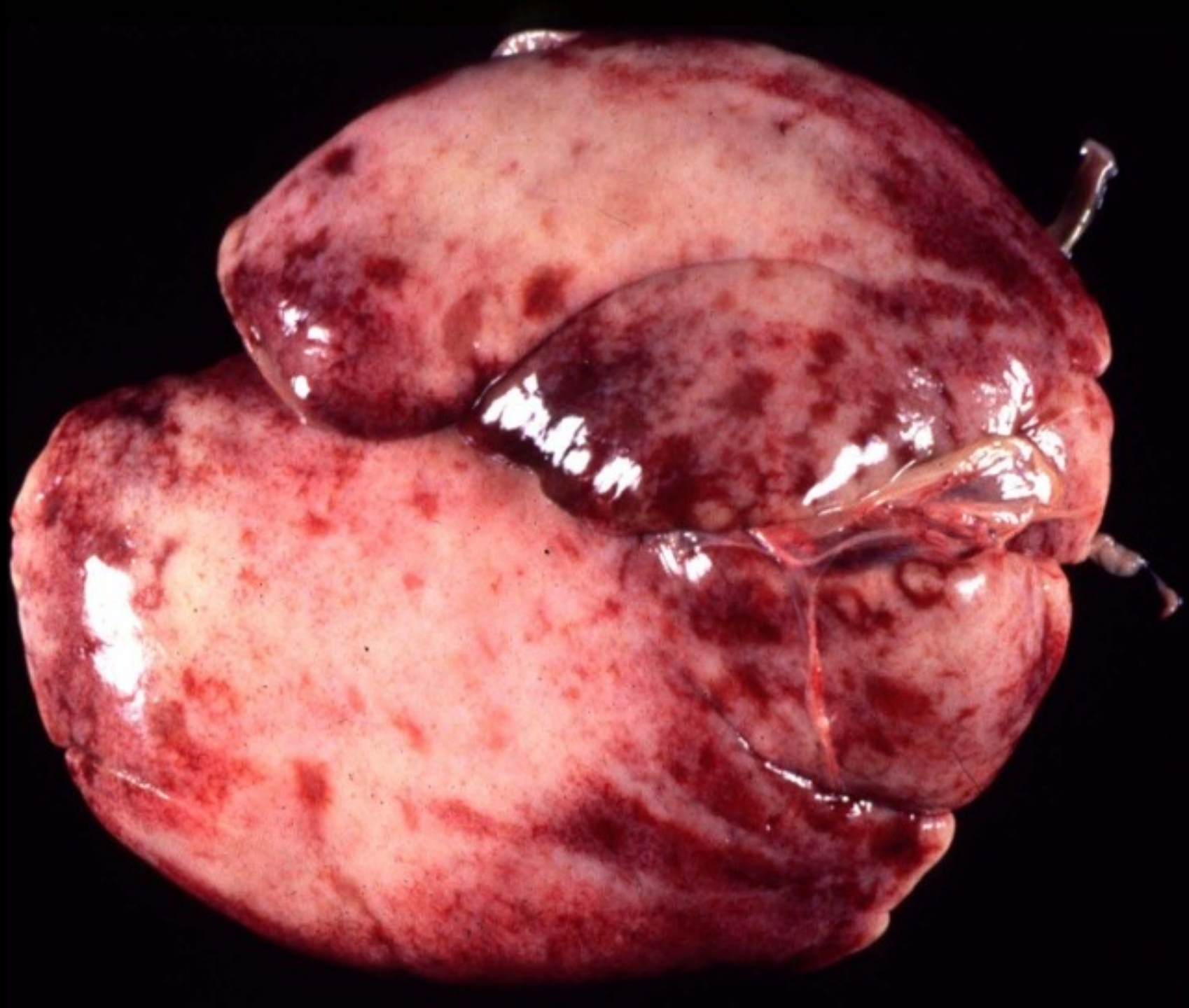
FIG. 33-5. *Horizontal and vertical transmission of avian leukosis viruses. [From R. A. Weiss, In "Virus Persistence" (B. W. J. Mahy, A. C. Minson, and G. K. Darby, eds.), 33rd Symposium Soc. Gen. Microbiol., p. 267. Cambridge Univ. Press, Cambridge, 1982.]*

Avian leukosis virus: clinical signs

- Lymphoid leukosis (visceral lymphomatosis, lymphoma) is the most common form of the disease.
- Occurs in chickens between 14-30 weeks old
- Clinical signs are nonspecific
    - Weakness
    - Dehydration
    - Diarrhea
    - Anorexia
    - Emaciation
- Lymphoid tumors (accumulations of B cells of originating from bursa of Fabricius)
    - Occur in liver, spleen, kidney & bursa.
- Liver lesion is indistinguishable from that in Marek's disease.

Avian leukosis virus: gross lesions

- Severely and diffusely enlarged, pale liver



Avian leukosis virus: gross lesions

- Multiple random white nodules in the liver, spleen, and heart



Avian leukosis: diagnosis

- DDx: Marek's disease (Gallid herpesvirus 2)

Criteria	ALV	Marek's Disease
Type of lymphoma	B cell	T cell
Bursa affected	Yes	No
Nerves affected	No	Yes
Age affected	> 14 weeks	> 4 weeks
Vaccine available	No	Yes

- Diagnosis:
  - Due to widespread presence of the virus in flocks, detection of the virus by conventional means is not useful
  - Gross and histological lesions are suggestive. IHC to confirm B cell lymphoma is helpful.

Avian leukosis: control

- No vaccine
- All-in, all-out
- Good hygiene, cleaning (virus is susceptible to many disinfectants and does not survive well in the environment)
- Genetic resistance has reduced incidence dramatically





Feline leukemia virus

FeLV, “feleuk”

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FeLV: general

- One of the most important viral diseases of cats

- Present worldwide
  - Prevalence has ↓ due to vaccination, testing and quarantine

- FeLV occurs in 4 subtypes: A, B, C & T (differ in envelope antigens and host cell spectrum)
  - Vaccine protects against all of them
  - Testing detects all of them

FeLV: transmission

- Host range: domestic and wild cats
  - Some large wild cats particularly sensitive

- Horizontal:
  - MAJOR mode of transmission
  - Prolonged, direct exposure
  - “Lover’s virus”: mutual grooming, transfer of saliva
  - … also biting.

- Vertical:
  - Pregnant queens → embryonic death or stillbirth
  - Lactating queens → usually 1-2 animals/litter become infected



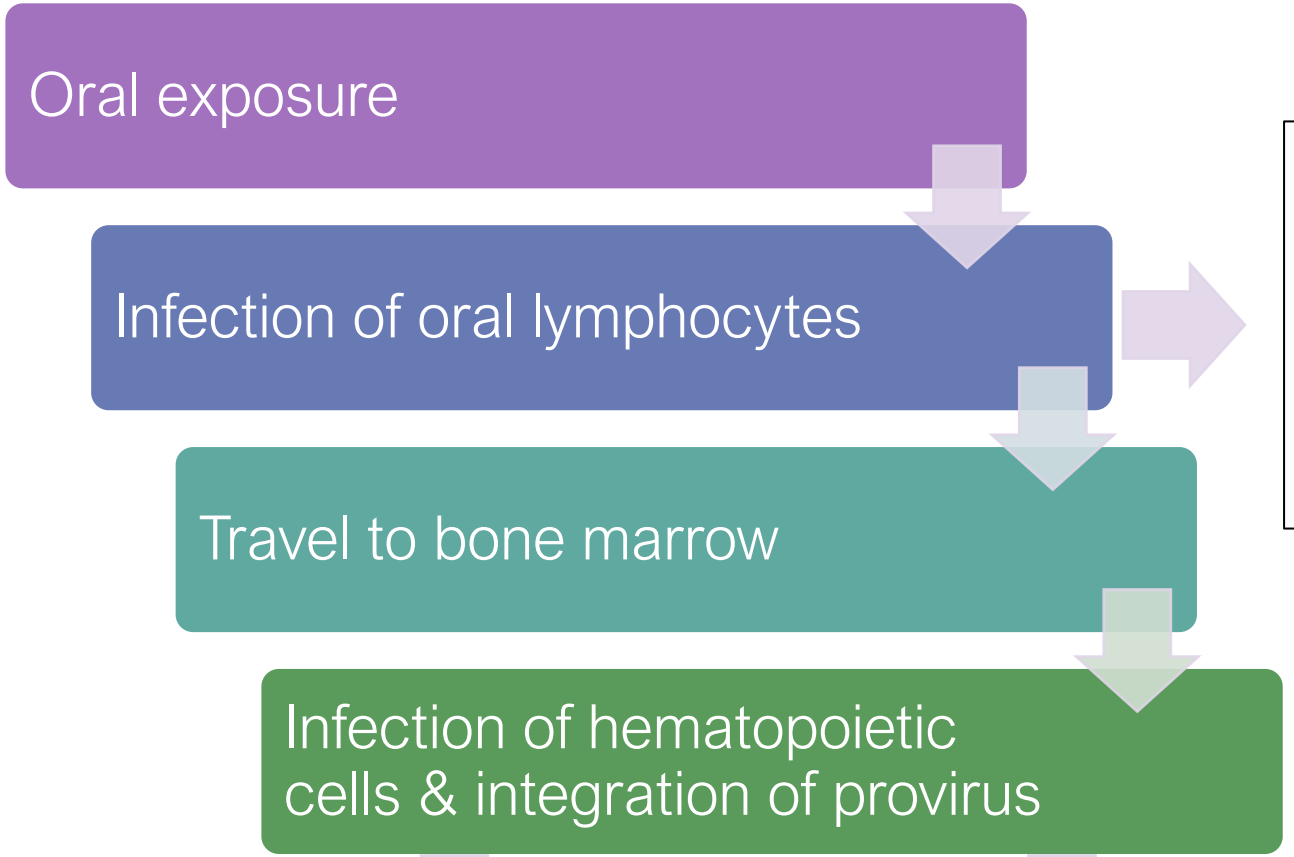
FeLV: transmission

- Young animals more susceptible than adults
  - ~15% of cats > 4 months become infected
  - (some studies show older animals can become infected with ↑ ↑ doses of virus)

- Poor hygiene, overcrowding are also risk factors



FeLV: pathogenesis



Abortive infection
Strong immune response or low infectious dose
No viremia, no provirus

Transient/regressive infection

- Viremia → successful immune response → prevents further viremia
- Cat ↓ ↓ ↓ risk of disease
- Provirus is present
- Antibody present
- Viremia is absent
- ~60 – 70 % of cats

- Rare possibility that in immunosuppressed cats provirus → reactivate → viremia

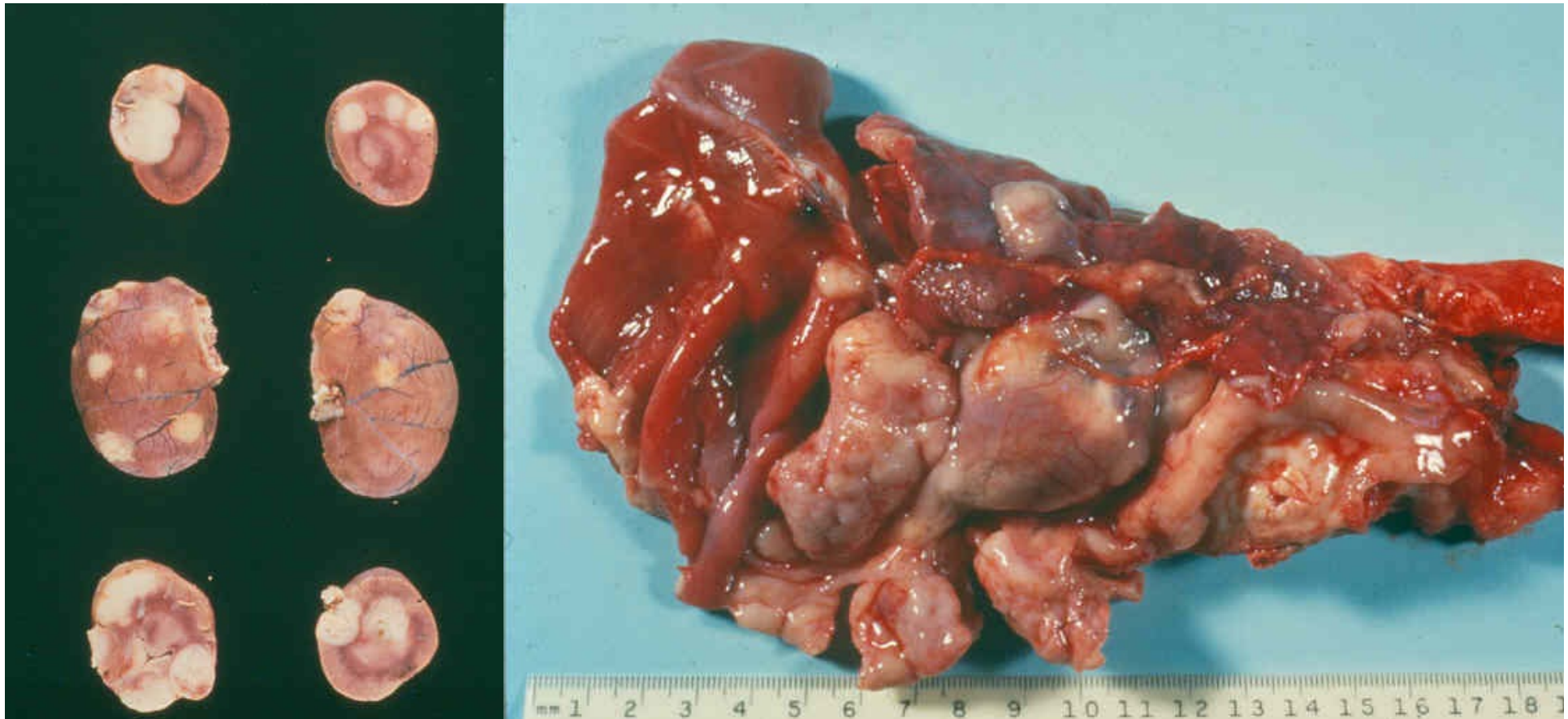
Persistent infection

- Immune response does NOT contain infection → viremia
- Replication in salivary glands and intestine
- Most will die of related illness in 2-3 years

Differentiating between transiently and persistently infected cats is very important
(see “Diagnosis” section)

FeLV: clinical signs

1. Lymphoma
2. Immunosuppression
3. Anemia



FeLV: clinical signs

1. Lymphoma
2. **Immunosuppression**
   - Persistently infected, viremic cats have a complex immunosuppression
   - Susceptible to secondary infections
   - Stomatitis, rhinitis, abscesses more common
3. Anemia





FeLV: clinical signs

1. Lymphoma
2. Immunosuppression
3. Anemia
   - Chronic, non-regenerative
   - ± other cytopenias
   - Clinically more common than lymphoma

FeLV: diagnosis (pages 70-72)

- Revolves around detection of virus or free p27 antigen (FeLV capsid protein)

- Sample selection:
  - blood, serum, plasma, saliva

- FeLV antigen ELISA: (e.g., SNAP® FeLV test kit detects p27)
  - Positive ELISA indicates viremia (and animal is potentially infectious)
  - **Does NOT tell you if the animals is in early stages of a regressive infection, or if the animal is persistently infected**
  - Animal with positive test result should be retested within 3-4 months.
    - If positive on repeat test, indicates persistent infection; if negative, indicates transient infection.
  - Specificity close to 100%

- PCR (on blood) can be used to confirm infection.

FeLV: diagnosis

- Discordant results are conflicting results of the two tests (e.g., ELISA positive BUT PCR negative)….. may be due to:
  - Testing in early phase of infection;
  - Presence of antigenemia without viremia
  - False +ve ELISA (due to faulty technique or cross reactive antigens).

In any of these cases, repeat test at 4-8 week intervals for at least 3 months.

FeLV: control

- Test and quarantine is the mainstay

- Vaccination
  - Several vaccines exist, with varying efficacies
  - Non-core
    - However, recommended for young kittens (lifestyle changes)
    - Based on your and your client's assessment of risk
    - Adults – every 2-3 years? (recs vary)