

Lecture 20: Flaviviridae

Russ Fraser, Fall 2023

Housekeeping

- Questions?

A cow dies develops peracute to acute severe bloody diarrhea. On physical exam you note the presence of moderate lymphadenopathy and oral mucosal ulcers. What do you tell the farmer regarding the remainder of their animals, based on your top differential diagnosis?

Model response
They are likely to be quarantined and then depopulated.
They should ensure they keep their herd separate from sheep.
Remaining animals will require supportive treatment but will likely survive.
This was an unusual presentation of a common disease: most remaining aimals will become subclinically infected.

Flaviviridae: general

Size	40-60 nm
Morphology	Enveloped
	• Icosahedral symmetry • Spherical
Genome	Linear, ssRNA, ~ 11 kb
Site of Replication	• Cytoplasm
Properties	• *Flavivirus* are arboviruses • *Pestivirus* are NOT arboviruses • Infectious RNA • ”Flavi” = yellow

Flaviviridae: general

- *Flavivirus*
  - West Nile virus
  - Japanese encephalitis virus
  - Louping ill virus (U.K.)
  - (Dengue, Yellow fever)
- *Pestivirus*
  - Bovine viral diarrhea virus
  - Border disease virus
  - Classical swine fever virus
- Clinical relevance of *Hepacivirus* and *Pegiviruses* is uncertain



1999: A mortality event at the Bronx zoo was the start of an outbreak.

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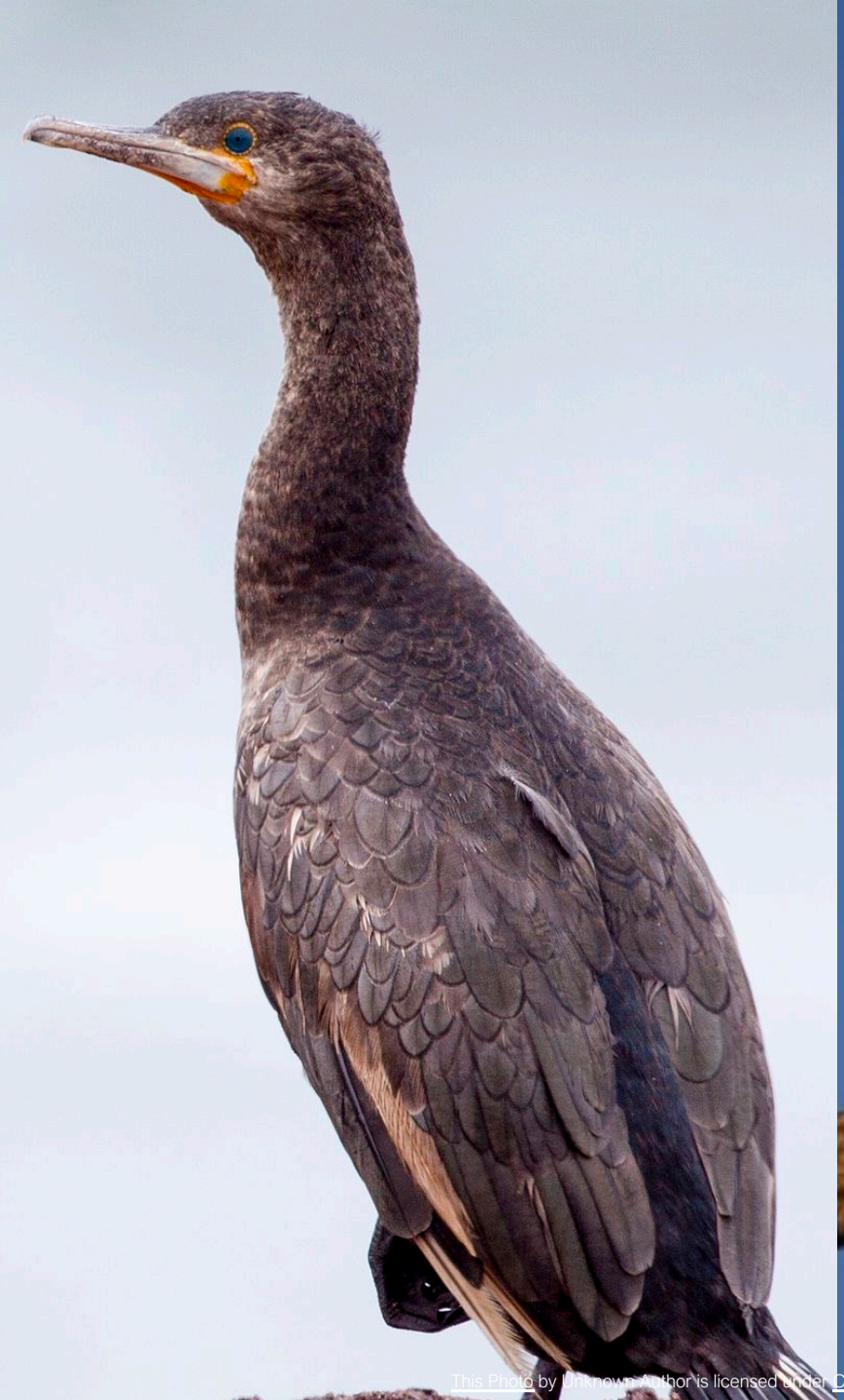
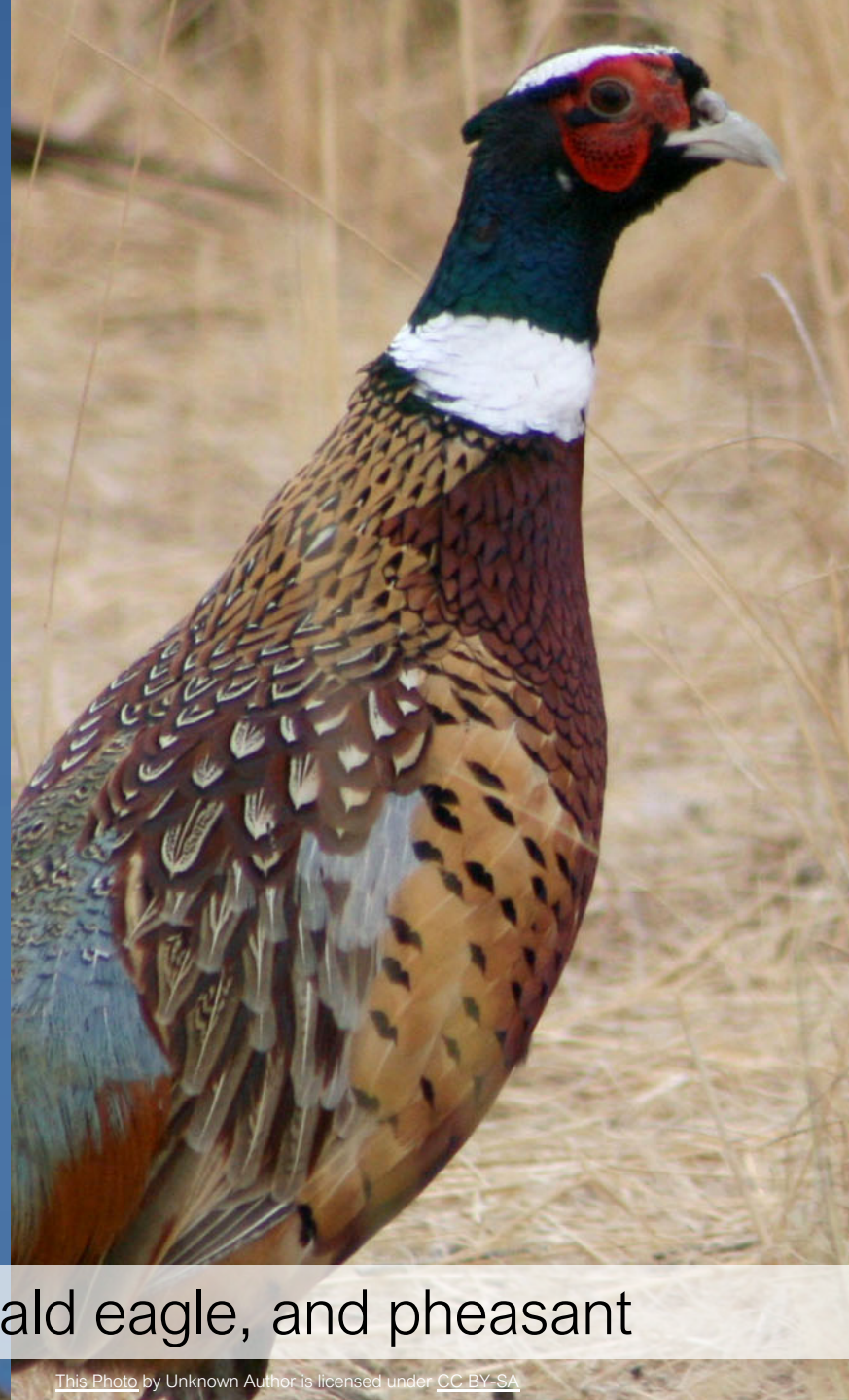
Over the course of ~~Labour~~ Labor day weekend, 3 flamingos died.







Along with a cormorant, bald eagle, and pheasant

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Dr. T MacNamara, veterinary pathologist at the Bronx zoo, linked the recent appearance of numerous dead crows to the dead birds in their collection.



Meanwhile, doctors in NYC were noticing an increase in cases of encephalitis.



West Nile Virus

"WNV"

WNV: general

- Worldwide distribution
  - Incursion into N.A. in 1999 has resulted in endemic disease
- Host range is wide:
  - A large variety of species have had documented infections, but **wild birds, horses, and humans** are most important.
  - **Zoonotic**
  - In humans, **most infections (80%) are asymptomatic**, but can result in fatal encephalitis (1% of cases)
  - In horses, exact %age of asymptomatic animals is unknown, but mortality is 40% in clinically affected animals
- It is an arbovirus
  - Vector is primarily the mosquito (*Culex spp.*)
  - Incidence is seasonal

Numbers correct as of January 9, 2019

Select year: 2018

Province	Examined	Tested	Positive	Negative
British Columbia	149	29	3	26
Alberta	56	7	1	6
Saskatchewan	335	20	5	14
Manitoba	46	46	37	9
Ontario	236	97	41	56
Québec	605	105	73	32
New Brunswick	53	4	3	1
Nova Scotia	120	1	1	0
Prince Edward Island	151	11	5	6
Newfoundland and Labrador	23	0	0	0
Yukon	1	0	0	0
Northwest Territories	0	0	0	0
Nunavut	4	0	0	0
TOTAL	**1779**	**320**	**169**	**150**

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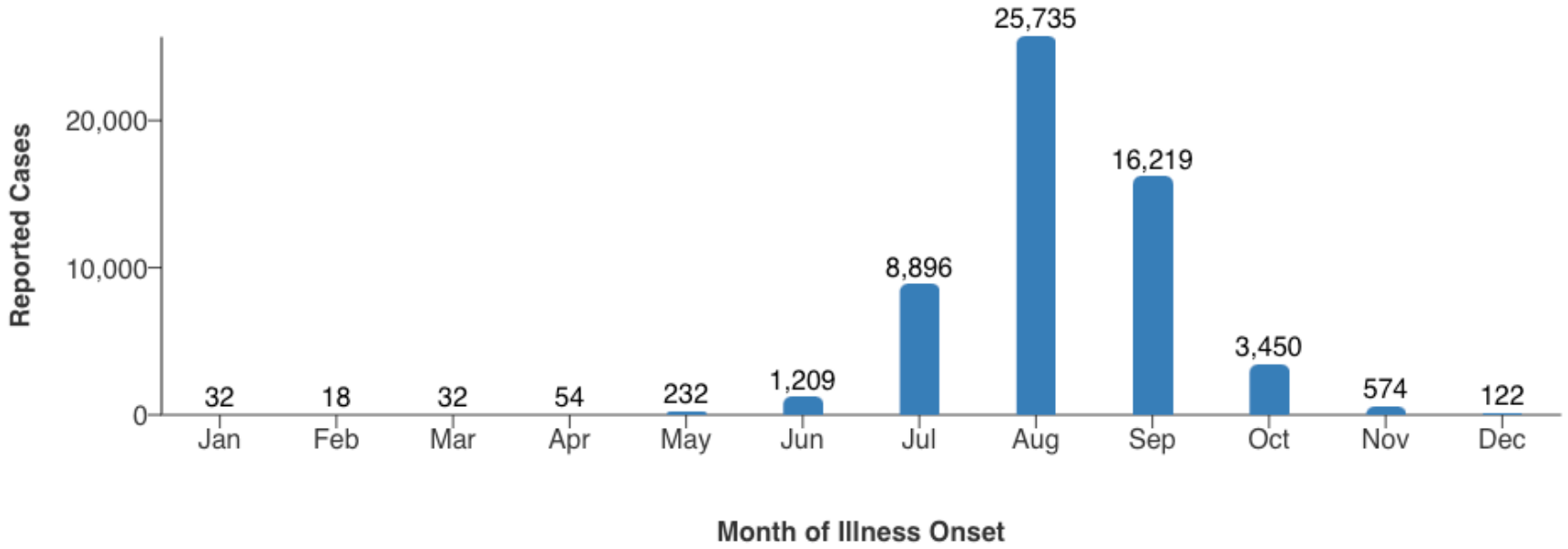
WNV: general

Human Disease Cases	Hospitalizations	Deaths
56,575	**25,777**	**2,776**
Cases from year(s) and type of case selected above	*Hospitalizations from year(s) and type of case selected above*	*Deaths from year(s) and type of case selected above*

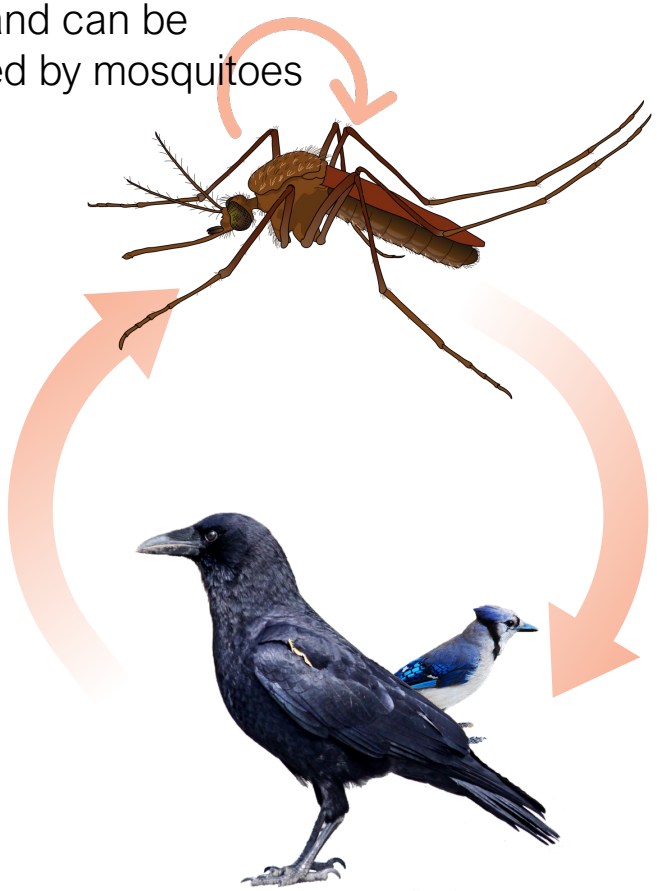
West Nile virus human disease cases reported by month of illness onset, 1999-2022, All disease cases





WNV: transmission

Virus replicates in and can be vertically transmitted by mosquitoes



Wild birds are the reservoir, and some (crows) are extremely susceptible to the disease

Dead-end hosts, such as horses and humans, are sporadically infected. They are not considered a source of the virus.



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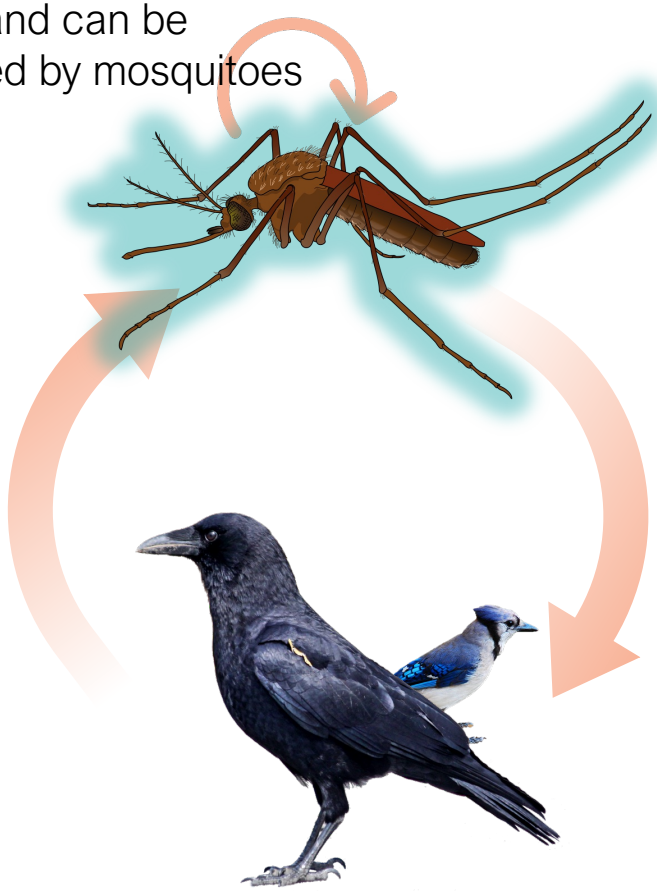

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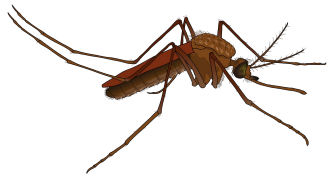
WNV: pathogenesis

Pathogenesis is incompletely understood.



Bite (injection) by a mosquito



First round of replication:
Keratinocytes, fibroblasts, dendritic cells

↓

Viremia
Very low levels of virus

↓

Entry into the CNS

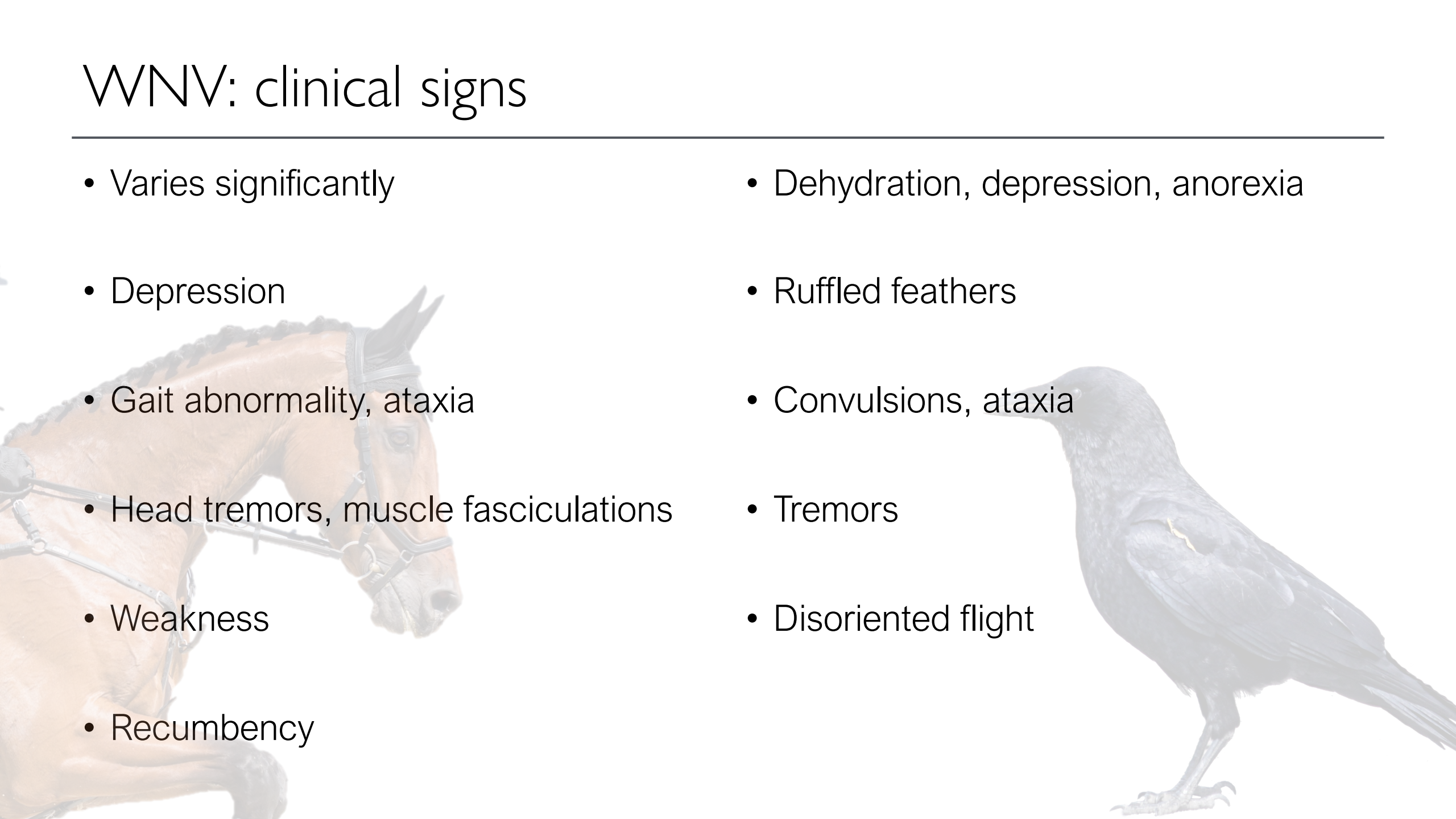
↓

Encephalomyelitis
Non-suppurative

Migration through neurons?

WNV: clinical signs

- Varies significantly
- Depression
- Gait abnormality, ataxia
- Head tremors, muscle fasciculations
- Weakness
- Recumbency
- Dehydration, depression, anorexia
- Ruffled feathers
- Convulsions, ataxia
- Tremors
- Disoriented flight



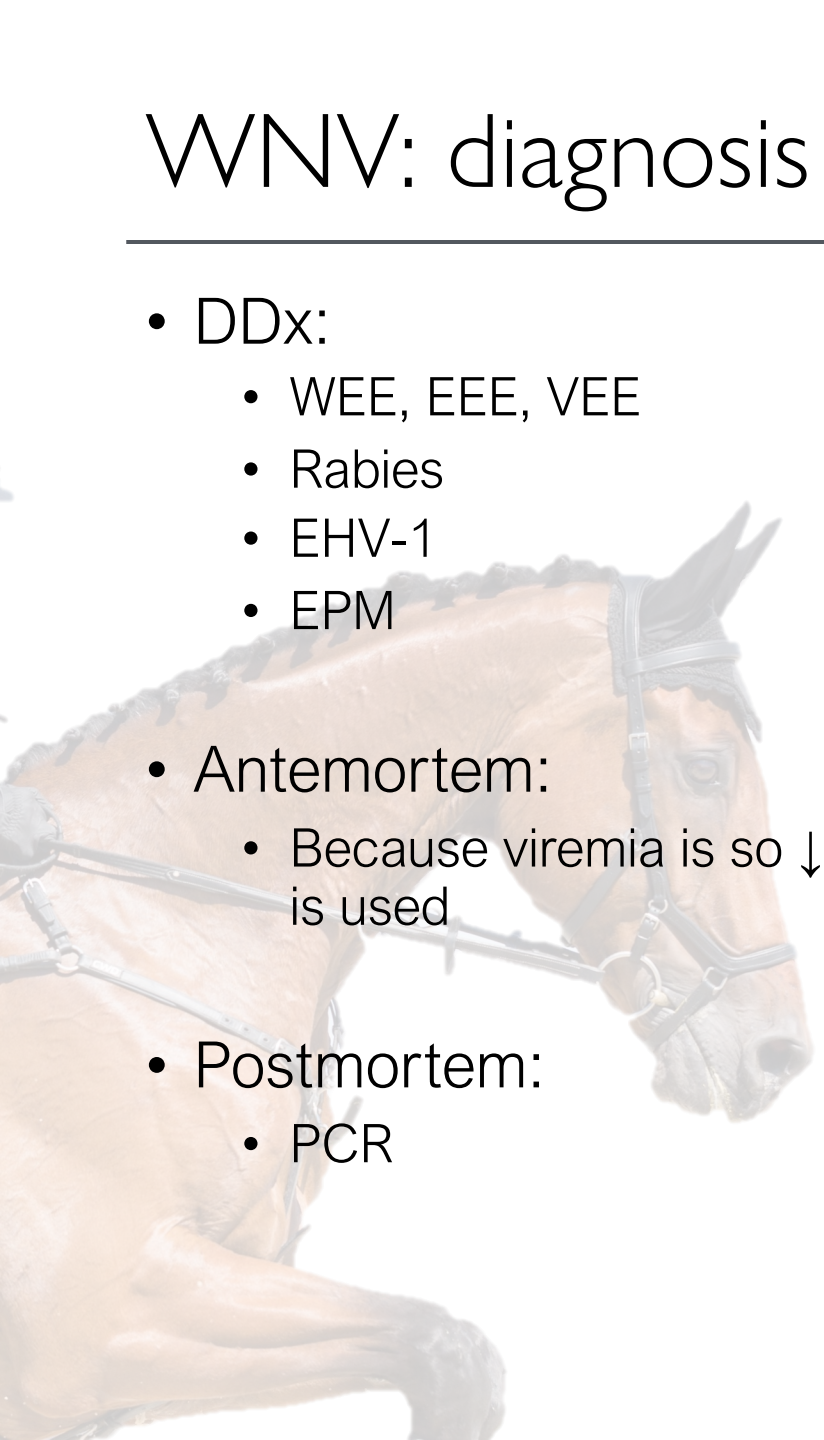
WNV: gross lesions

- None

- Wide-spread tissue necrosis with hemorrhage
  - Heart
  - Brain
  - Skeletal muscle
  - Lungs
  - Spleen
  - Liver
  - Kidney

WNV: diagnosis

- DDx:
  - WEE, EEE, VEE
  - Rabies
  - EHV-1
  - EPM
- Antemortem:
  - Because viremia is so ↓, paired serology is used
- Postmortem:
  - PCR



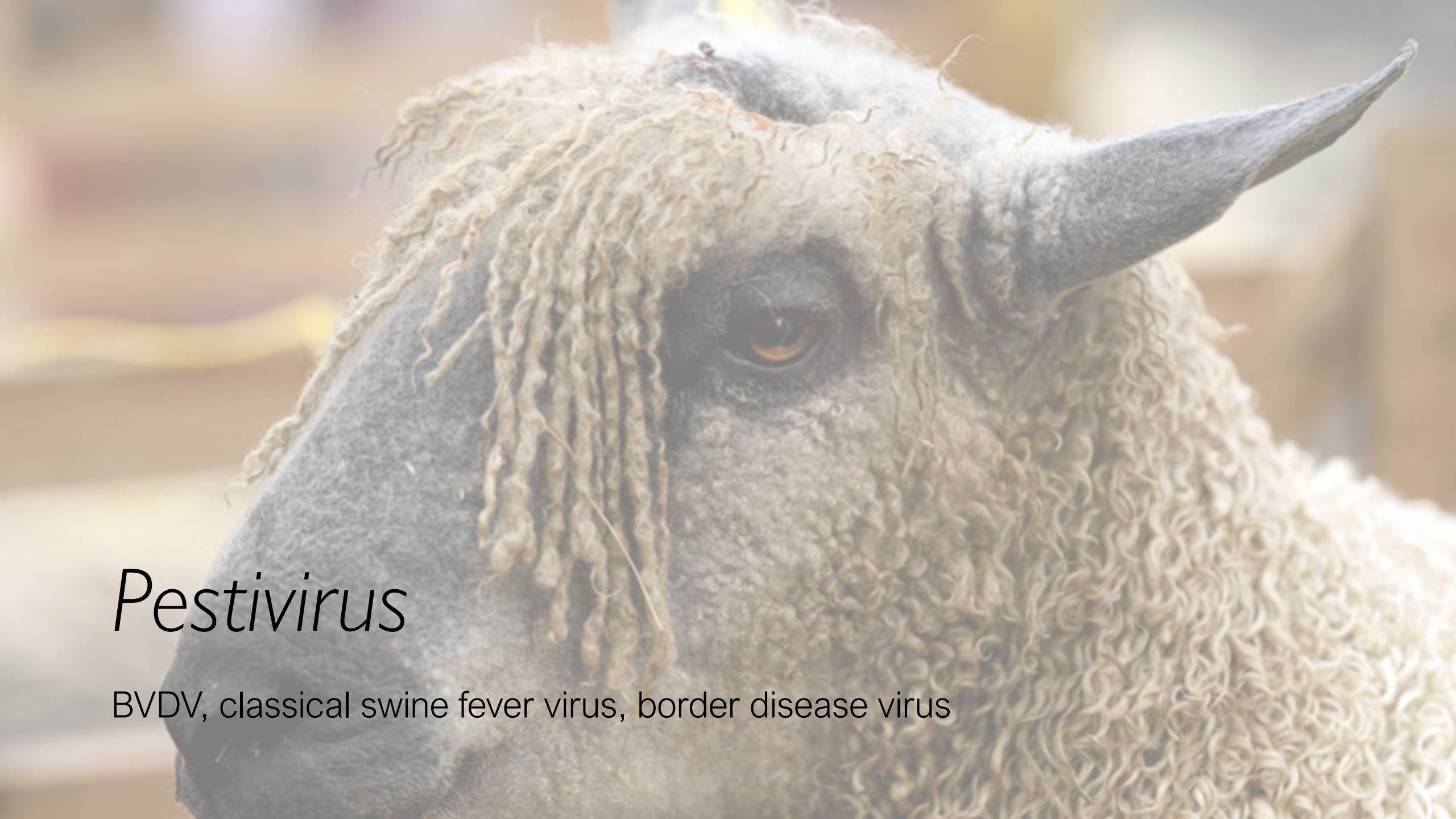
- DDx:
  - Newcastle disease virus
  - AI
  - Marek's disease
- Disease is peracute in crows especially – PM testing by PCR

WNV: control

- Vaccine is available for horses, and is considered a **core** vaccine in N.A.
  - Guidelines are provided by the AAEP

- Control of the vector: good mosquito control helps
  - Clean gutters, avoid standing water, fans in stalls, avoid nighttime lights, etc.

- Monitoring wildlife (esp crows) can serve as an indicator of spread of the disease.



Pestivirus

BVDV, classical swine fever virus, border disease virus

Pestivirus: general

- Pestiviruses have an overlapping host spectrum
  - Classical swine fever virus can infect cattle
  - Bovine viral diarrhea virus can infect sheep & goats.
  - Border disease virus can infect small ruminants, cattle (read more about this virus in your notes)

- Pestiviruses cause generalized infections with
  - Immunosuppression
  - Respiratory symptoms
  - Reproductive symptoms
  - Gastroenteritis
  - ± Neurological symptoms

- Persistently infected animals are critical to the maintenance of the disease



Bovine viral diarrhea virus

BVDV

BVDV: general

- Complex virus with different strains, ***biotypes*** and ***genotypes***.

Cytopathic (CP) can cause vacuolation and death of cultured cells

Non-cytopathic (NCP) has little effect on cultured cells.

Genotype 1 (BVDV-1)

Strain: BVDV-1a (e.g.)

Genotype 2 (BVDV-2)

Strain: BVDV-2b (e.g.)

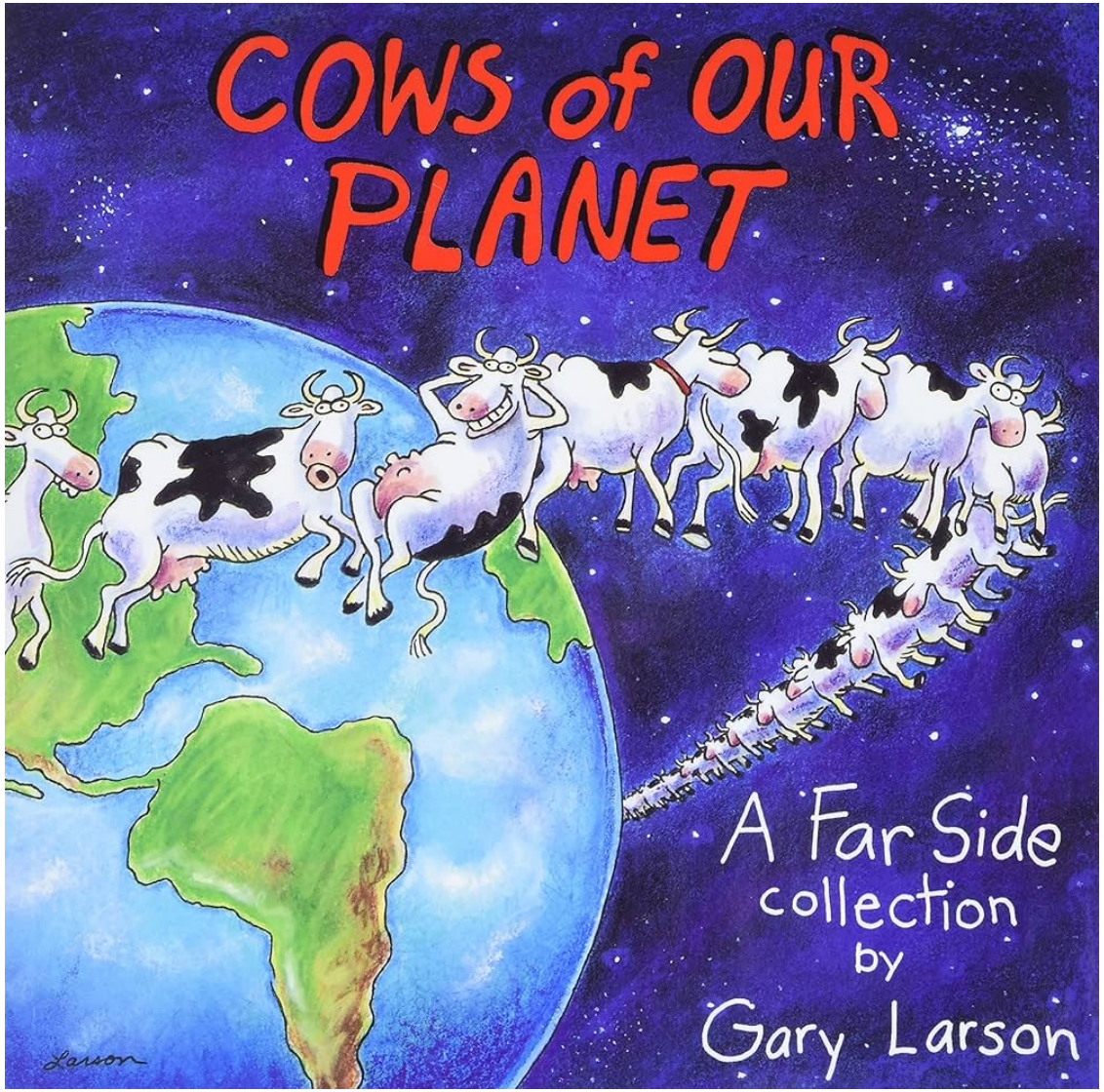
Neither of these correlate definitively with pathogenicity

ONLY NCP CAN LEAD TO A PERSISTANT INFECTION

NCP BVDV are dominant in the cattle population.

BDVD: general

- Widespread globally
- Mostly thought of as a disease of **cattle**, but can infect a **wide** range of animals, including pigs, sheep, and goats.
- Importance is primarily in economic loss, rather than morbidity or mortality.



BVDV: transmission

- Both horizontal and vertical transmission occur.
  - Direct contact (usually inhaled or ingested)
- Virtually all secretions from infected animals contain virus:
  - Nasal secretions, feces, urine, semen, milk, saliva, tears, uterine fluids.



Persistently infected animals are the most important drivers of BVDV transmission

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- Both horizontal and vertical transmission occur.
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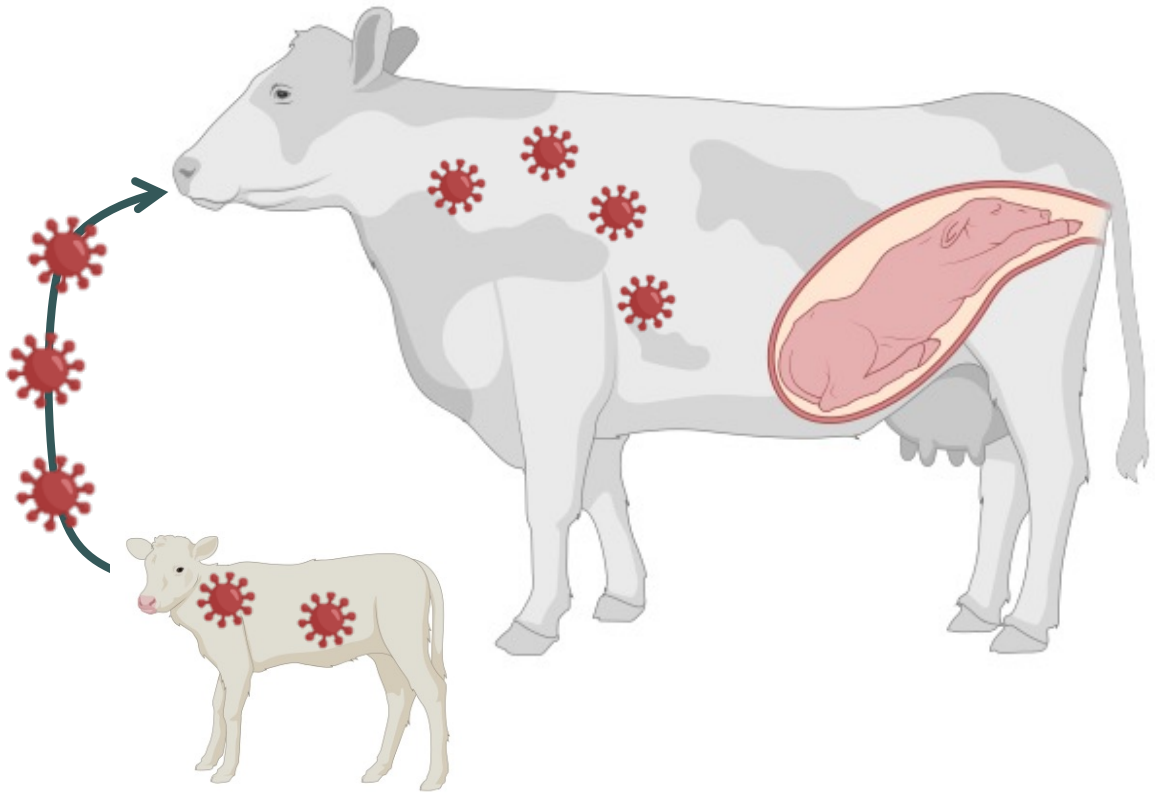
<u>A persistently infected animal sheds large amounts of the virus continuously and is immunotolerant to the virus.</u>

In other words, there is no antibody or other immune response to BVDV.

BVDV: transmission

Horizontal transmission of BVDV to a pregnant cow



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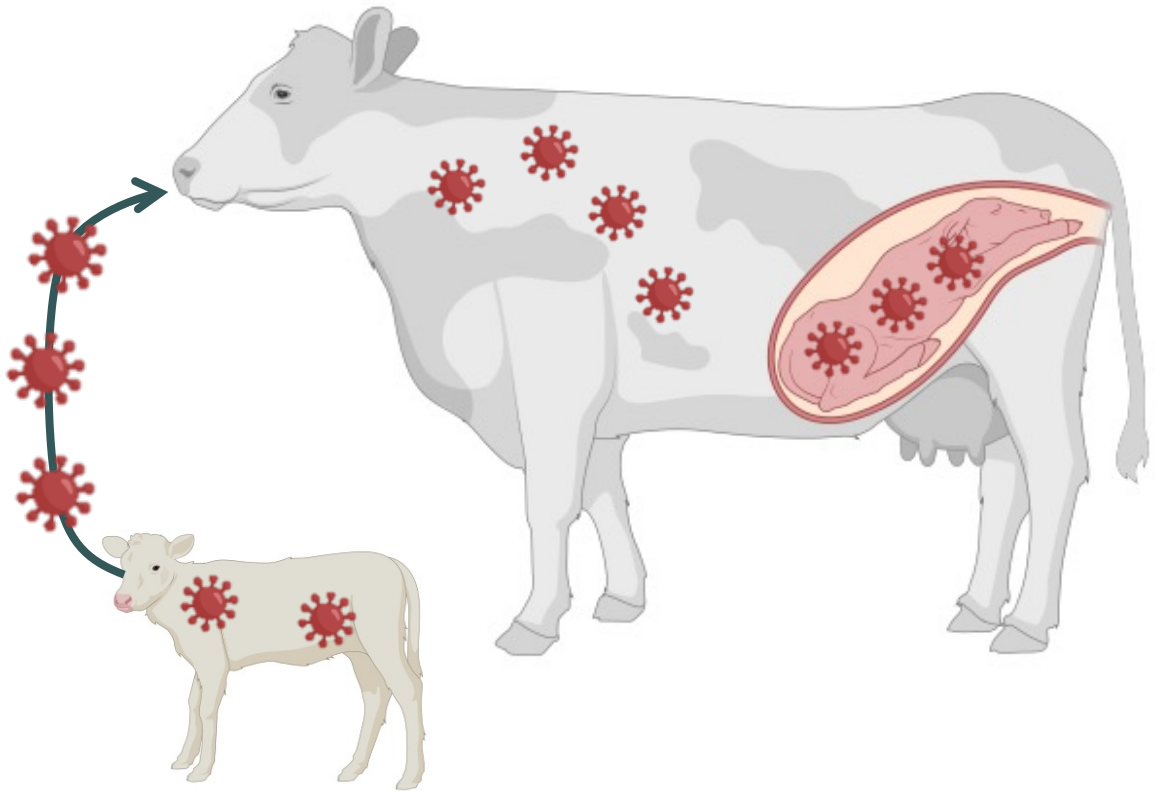

Horizontal transmission of BVDV to a pregnant cow

↓

Infection of cow **and** fetus

↓

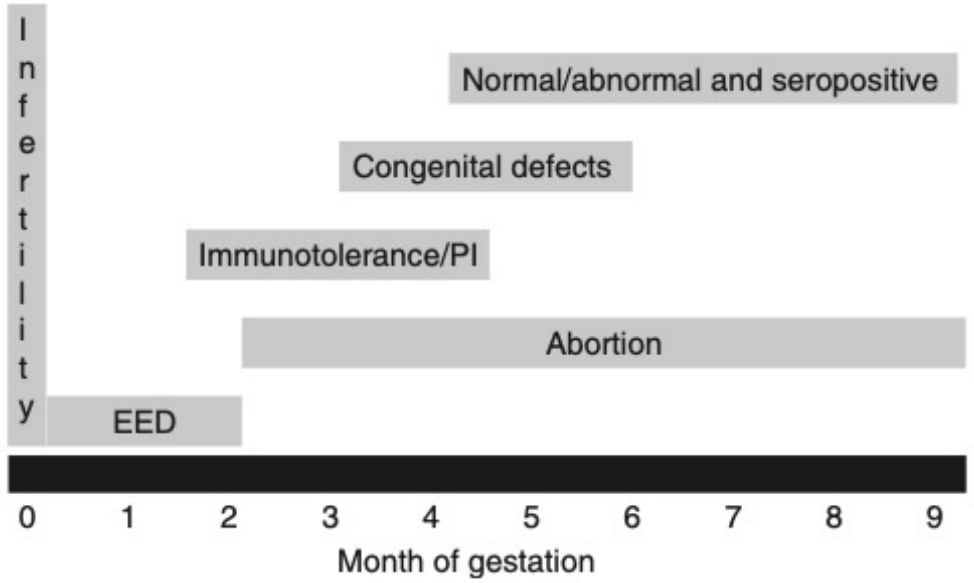

FIG. 32.92 Potential reproductive outcome after infection with bovine virus diarrhea virus during different stages of gestation. *EED*, early embryonic death; *PI*, persistent infection.

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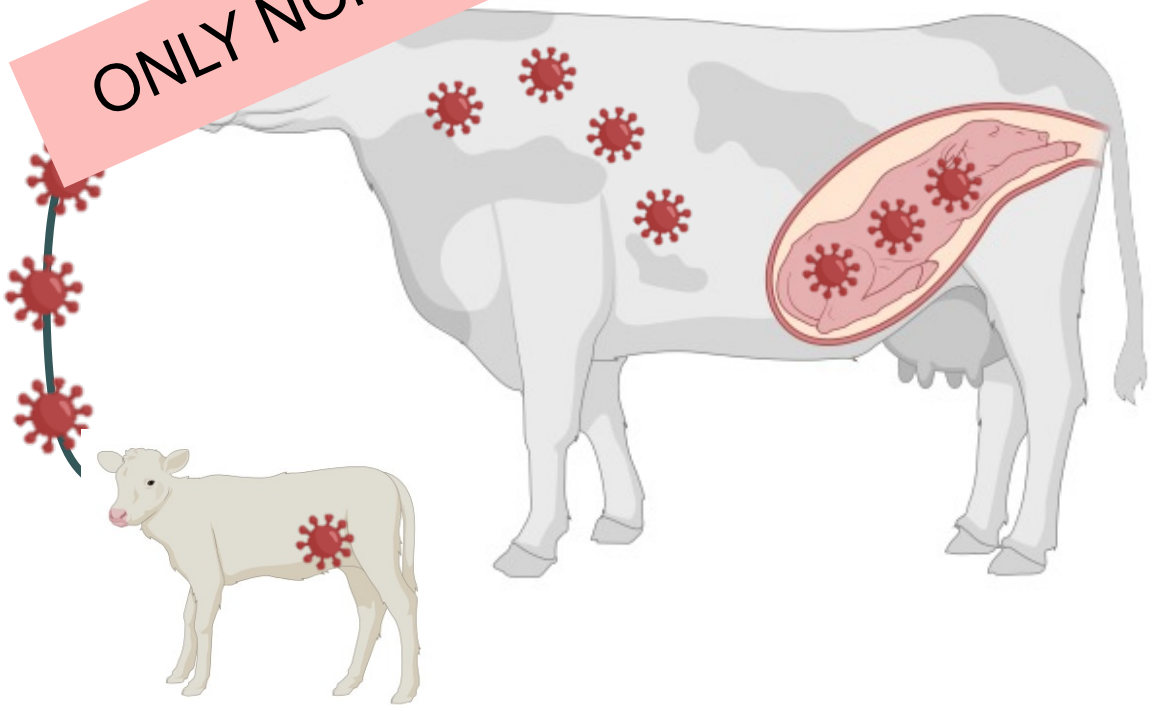

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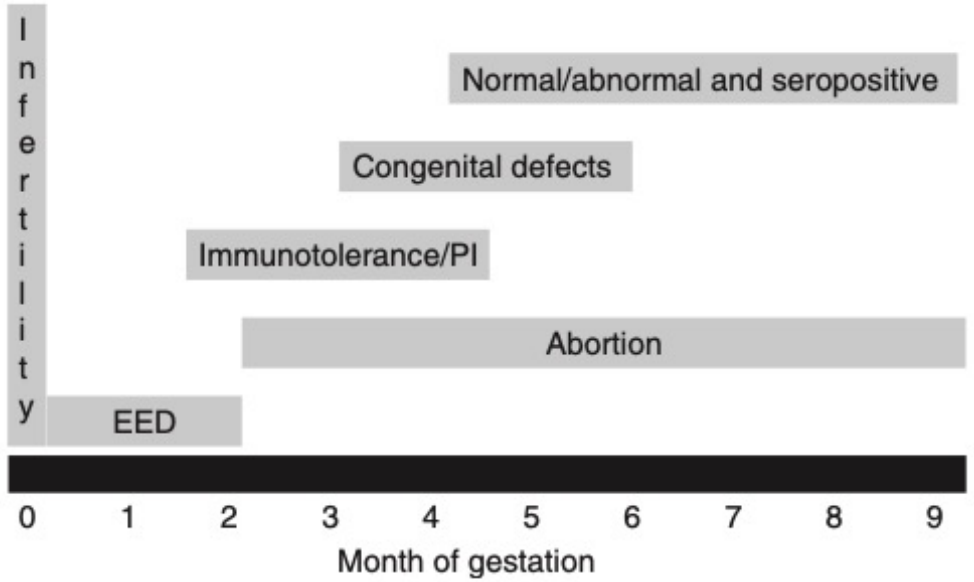
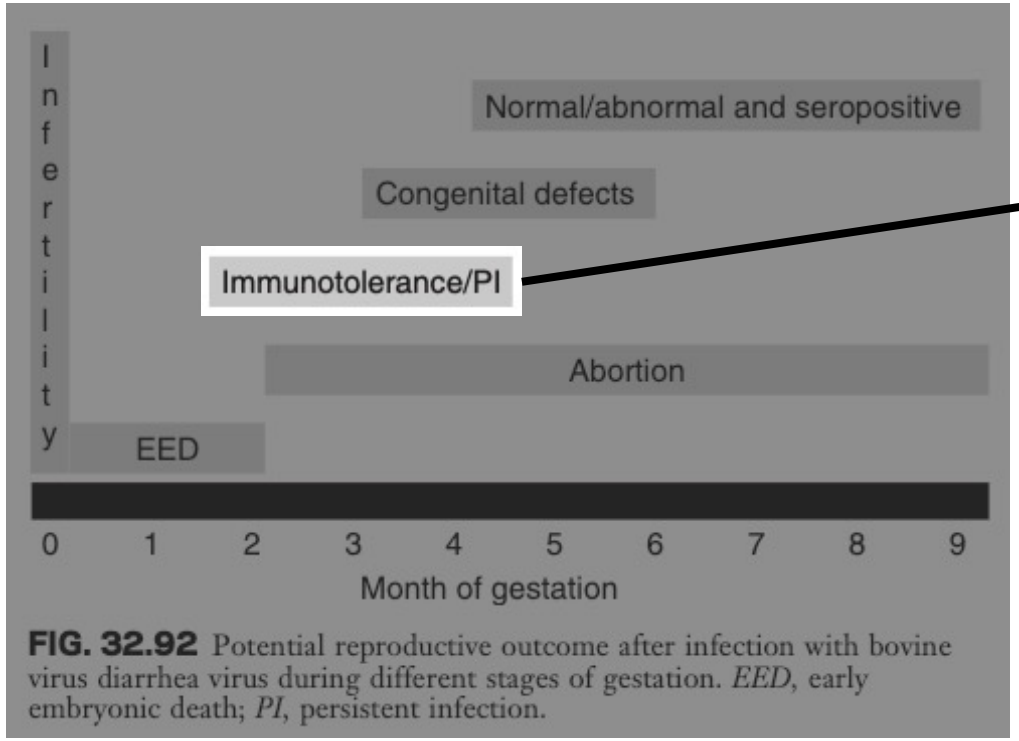


FIG. 32.92 Potential reproductive outcome after infection with bovine virus diarrhea virus during different stages of gestation. *EED*, early embryonic death; *PI*, persistent infection.

BVDV: transmission

Immunotolerance is to the ***infecting strain*** of BVDV.



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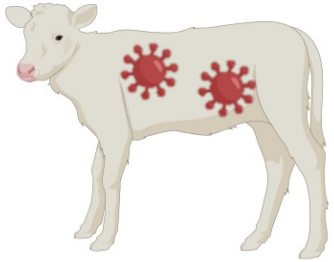
<u>Example</u>:

Calf is infected with NCP BVDV-1a *in utero* and is a PI.



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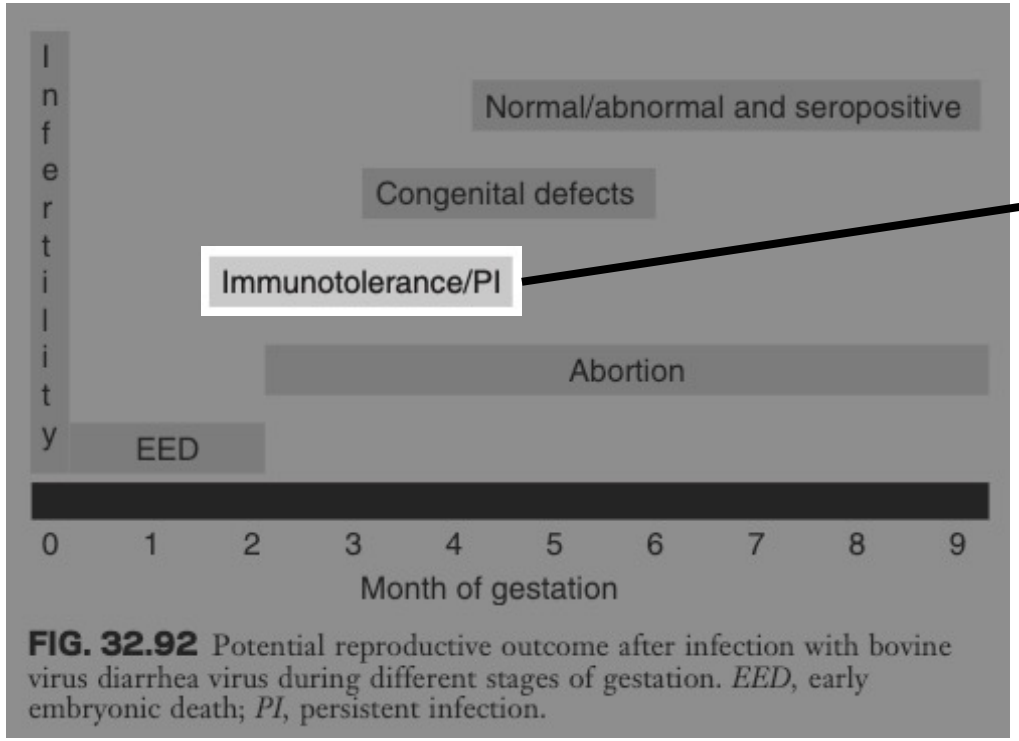

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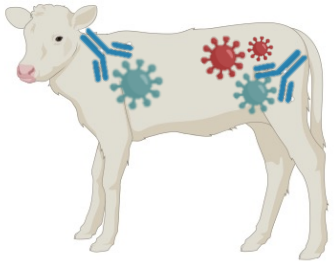
<u>Example</u>:

Calf is infected with NCP BVDV-1a *in utero* and is a PI.

If calf then becomes exposed to, and infected with, NCP BVDV-1d, it can mount an immune response against NCVP BVDV-1d.



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- Virtually all secretions from infected animals contain virus:
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Persistently infected animals are the most important drivers of BVDV transmission

Acutely infected cattle have a more limited role in horizontal transmission.

BVDV: pathogenesis

Initial replication in nasal mucosa and tonsils

↓

Primary viremia

↓

Infection of and replication in GI, respiratory tract, spleen, thymus, lymph nodes, lymphocytes

↓

± clinical signs

± leukopenia

± more widespread infection, depending on virulence of strain

BVDV: clinical syndromes

- MOST immunocompetent animals will be subclinically affected by BVDV
  - Subclinical infection of pregnant animals can still have the effects discussed earlier.
- Acute BVD
  - Classical/clinical BVD
  - Respiratory
  - Reproductive
- Severe, acute BVD
- Hemorrhagic BVD
- Mucosal Disease
  - Only occurs in Pis
  - Clinically very similar to severe acute BVD



BVDV: acute BCDV infection

- Disease most likely to manifest in naïve, 6 - 24 month old cattle.
  - Maternal Abs are likely protective
  - Can be seen in younger calves if there has been FPT
- When present, common signs include:
  - Depression, fever, anorexia
  - Oculonasal discharge
  - Oral erosions and ulcers
  - Diarrhea
  - ↓ milk production
- Immunosuppression may occur in either symptomatic or asymptomatic animals
  - ↑ secondary infections

BVDV: acute BVDV infection and respiratory disease

- BVDV is often detected in cases of BRDC
- Exact mechanism is unclear: primary infection vs. immunosuppression
- Direct damage to URT → bacterial colonization
- Synergistic effects with BoHV-1 and BRSV; *M. hemolytica* and *Mycoplasm bovis*



BVDV: acute BVDV infection and reproductive disease

- Economic damage caused by infection of pregnant cattle is significant.
- Acute BVDV infection can → ↓ semen quality
- Can cause fertilization failure
- In pregnant animals, can cause embryonic death and abortion
- Outcome of fetal infection is dependent on gestational age
- PI – see earlier
- Congenital defects – especially <u>cerebellar hypoplasia</u>

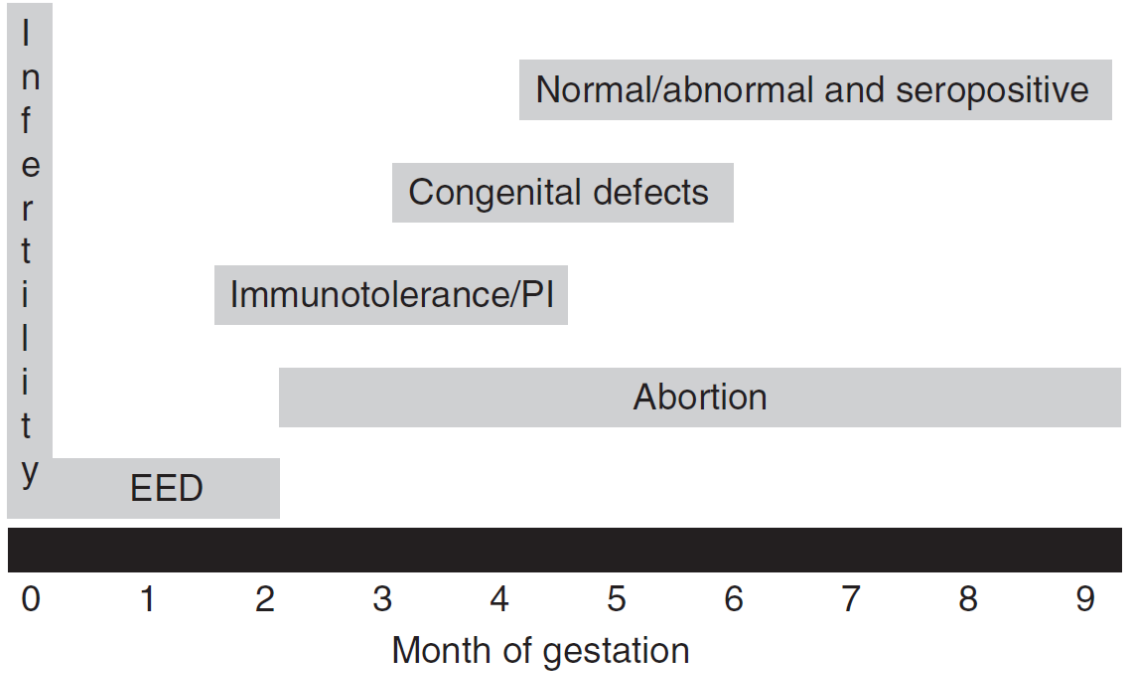


FIG. 32-91 Potential reproductive outcome after infection with BVDV during different stages of gestation. *BVDV,* Bovine virus diarrhea virus; *EED,* early embryonic death; *PI,* persistently infected.

BVDV: severe, acute BVD

- *Usually* associated with BVDV-2.
- Clinical progression is usually acute to peracute
- Signs are severe and include:
  - Anorexia, fever
  - Severe, often bloody, diarrhea
  - Oral ulcers are frequent
  - ↓ milk production
- Mortality is ↑ ↑ compared to acute BVD.



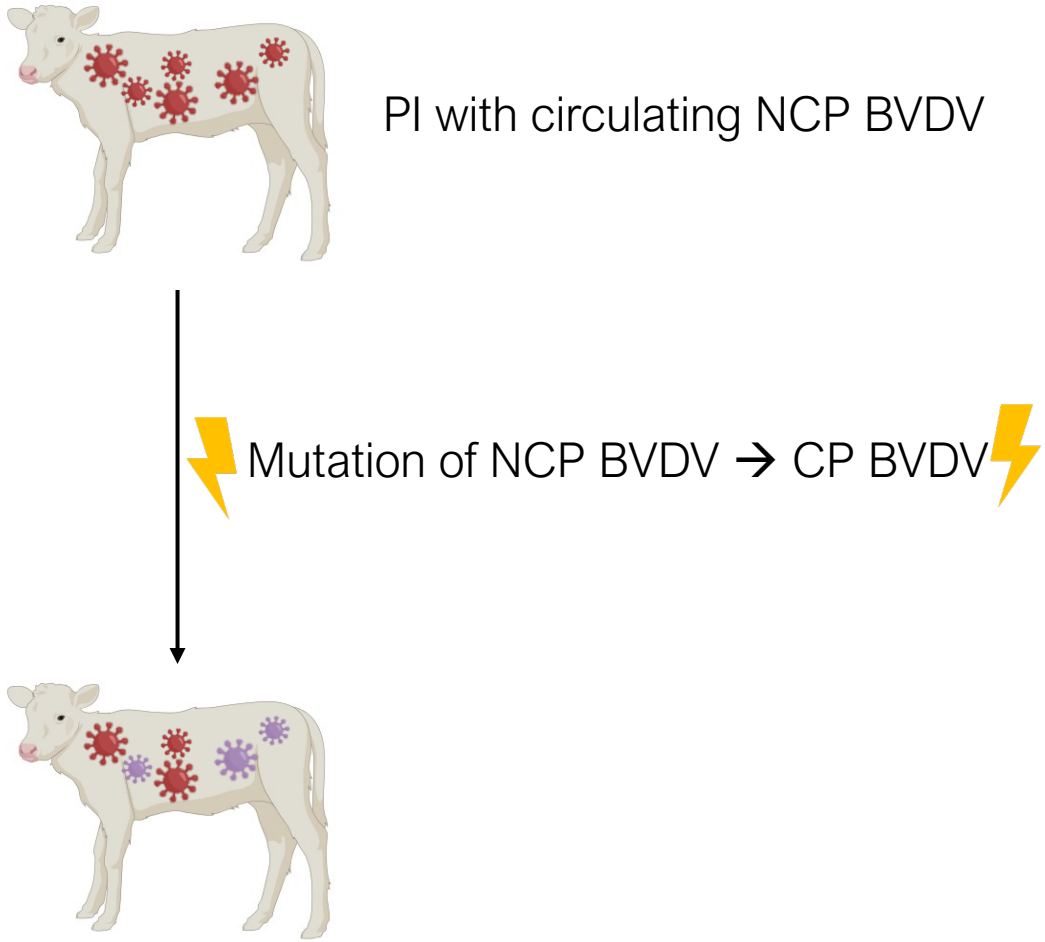
BVDV: hemorrhagic BVDV

- Thrombocytopenia and leukopenia
  - Infection of BM
- Clinical signs relate to ↓ platelets:
  - Bloody diarrhea
  - Petechia
  - Ecchymoses
  - Hyphema
- Strong (experimental) association with BVDV-2



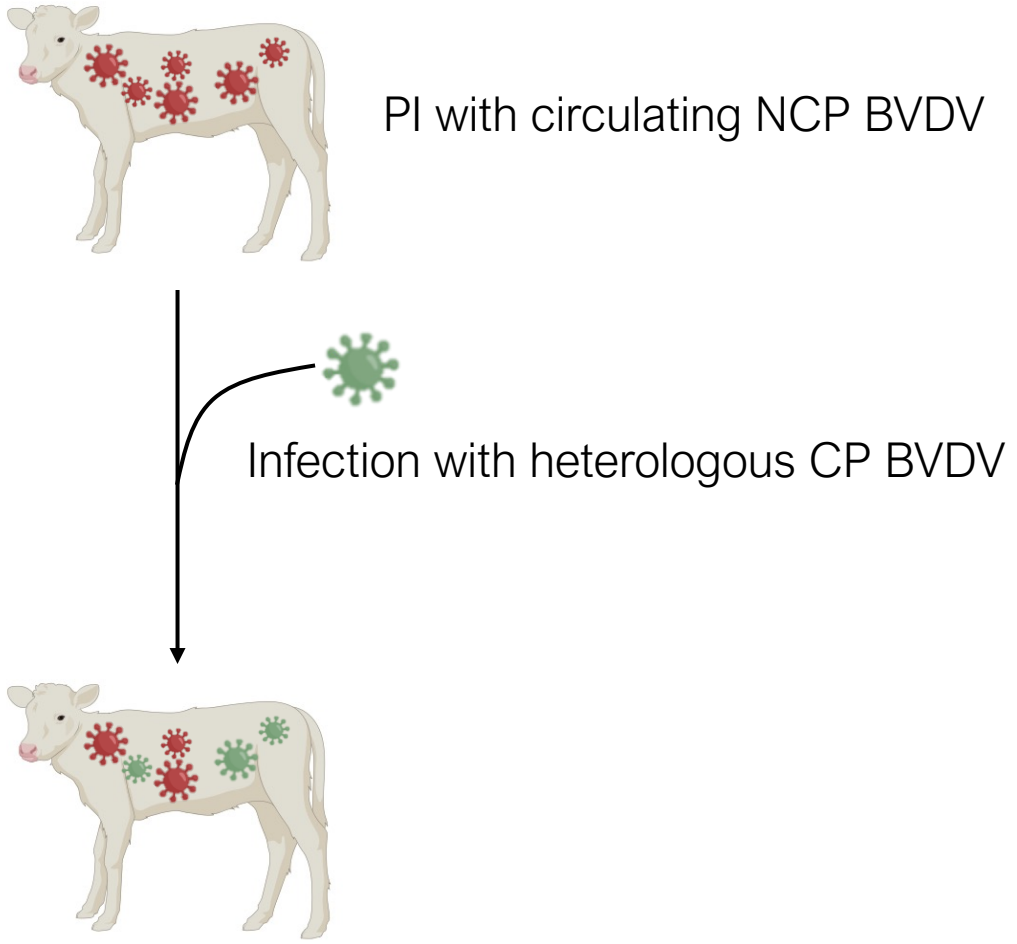
BVDV: mucosal disease

- Only affects PIs
- Occurs when immunotolerant PIs are coinfected with a CP BVDV.
  - Since PIs are uncommon (~ < 1 %), this disease is uncommon.
- Is severe and 100 % fatal
- Is caused by mutation of the circulating NCP BVDV → CP
- Clinical signs are very similar to severe acute BVDV:
  - Severe diarrhea
  - Marked oral ulcers
  - Mucopurulent nasal d/c



BVDV: chronic mucosal disease

- Only affects PIs
- Occurs when immunotolerant PIs are coinfected with a CP BVDV.
  - Since PIs are uncommon (~ < 1 %), this disease is uncommon.
- Much less common than classical mucosal disease.
- Clinical signs include:
  - Anorexia and weight loss
  - Diarrhea
  - Alopecia
  - Oral ulcers
  - Lameness
- Recovery can rarely occur (only from CP strain)



BVDV: gross lesions

- Many are non-specific
- Depends to some degree on presence of secondary infections
- Often most pronounced in the GI tracts
  - Ulcers
  - Hemorrhage in the case of hemorrhagic form



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BVDV: diagnosis

- PCR is most often the method of choice.
- Identification of PIs is critical to controlling BVDV.
  - Recall: they have a constant, high level of viremia.
  - **Serum** or **buffy coat (whole blood)** will always be positive and is more or less diagnostic.
  - If the animal is very valuable, may be worth segregating and testing again in 3-4 weeks to ensure it is not a transient infection.
  - In really young animals, some concern that maternal Abs may ↓ viremia – some recommend using ear notches instead.
- Pooled testing can be useful for approaching herd-level issues
  - Bulk milk tank, serum, whole blood, skin.

BVDV: control

- Control revolves around preventing the addition of, finding, and removing PIs.
- Vaccination is available.
  - limits disease rather than infection.
  - Helps reduce PIs.
- Closed herd
- If open, quarantine and test.
  - Including pregnant cattle (“trojan dams”!)



Classical swine fever

‘Hog cholera’

Classical swine fever virus

- Economically important disease of pigs
- Reportable
- Aka ‘hog cholera’
- Similar properties to other Pestiviruses:
  - Presence of PI animals is important
  - Highly contagious
  - Various strains of differing pathogenicity circulate
  - BUT: no cytopathic form → no mucosal disease like syndrome
- Introduction of infected pigs is major cause of outbreaks
  - Fomites also contribute to spread
  - Once within a herd, virus spreads horizontally
  - Contaminated pork and pork products also play a role

CSFV: pathogenesis

Oronasal infection

↓

Primary replication in tonsil

↓

Viremia

↓

Liver, kidney, lungs, vascular endothelium, and leukocytes

↓

DIC, splenomegaly + infarctions, lymphoid depletion

Necrosis and hemorrhage in kidneys, LNs

CSFV: clinical signs

- Acute (↑ pathogenicity)
  - Vomiting, diarrhea
  - Secondary infections (e.g. pneumonia)
  - Dermal hyperemia
  - Severe leukopenia
  - Acute death in young swine
  - Mortality ↑↑ (close to 100% in naïve herds)
  - Non-specific neurological signs
- Chronic (↓ pathogenicity)
  - Anorexia, depression, fever, leukopenia, sometimes recurrent
  - Reproductive failure may be the only sign
  - May produce PIs → die within a year



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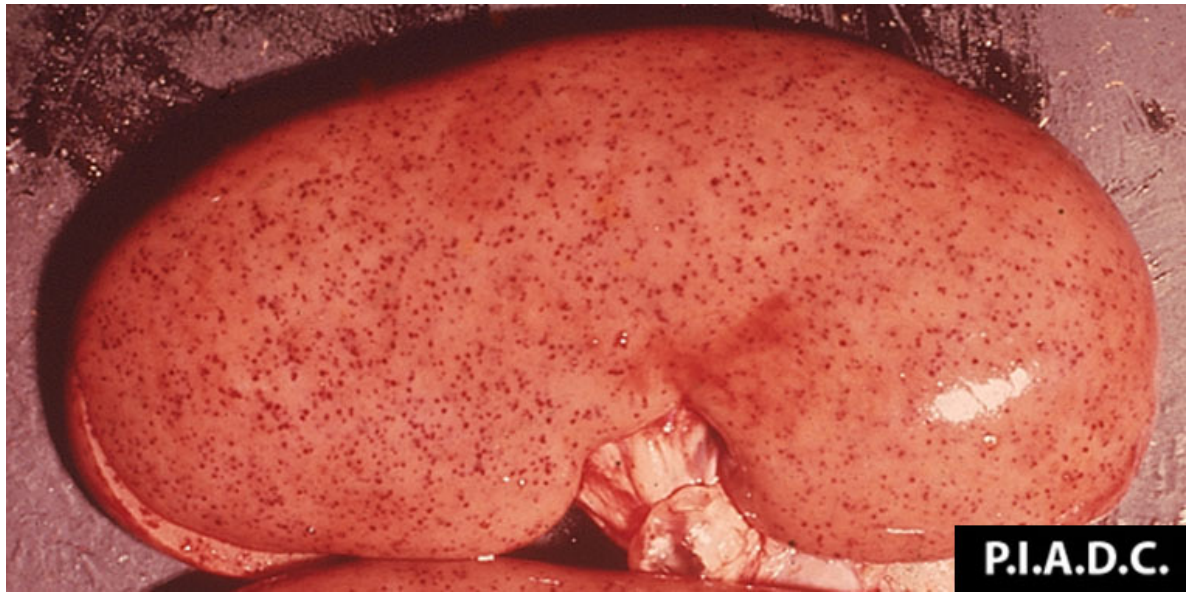
  - Reproductive failure may be the only sign
  - May produce PIs → die within a year



CSFV: gross lesions

- Multifocal necrosis and hemorrhage
  - Kidneys
  - Lymph nodes
- Splenic infarcts
- Colonic ‘button’ ulcers



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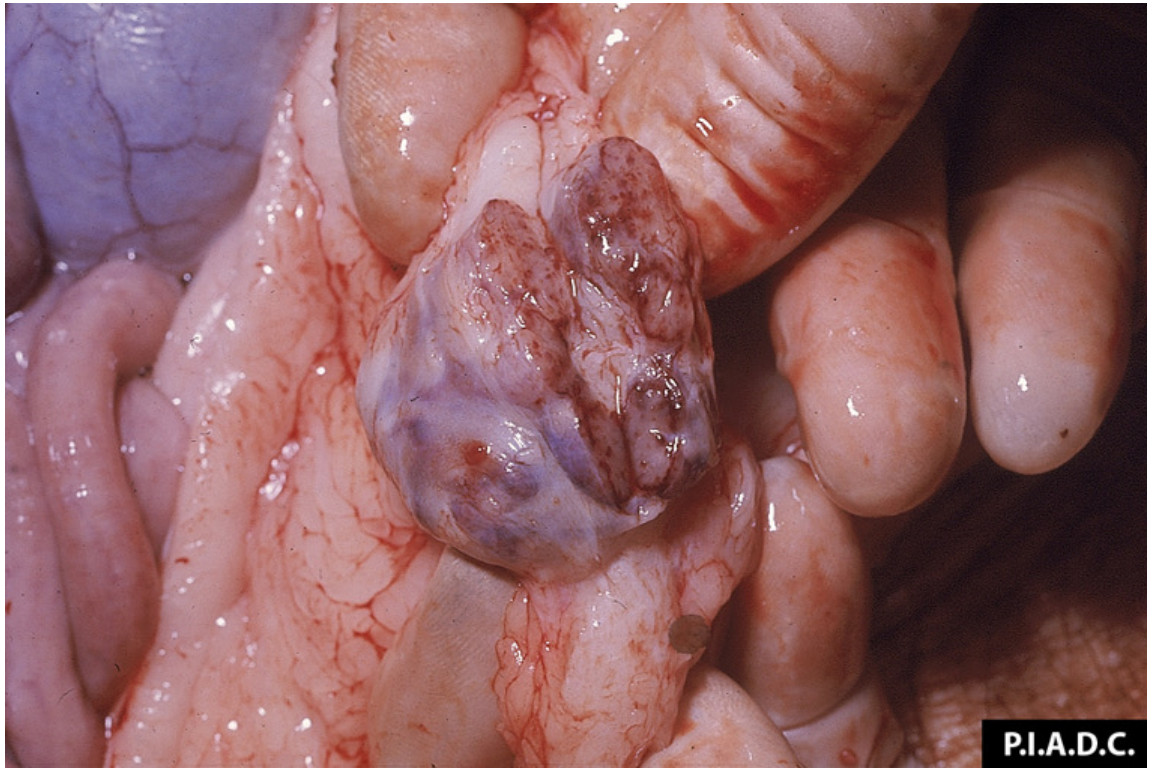

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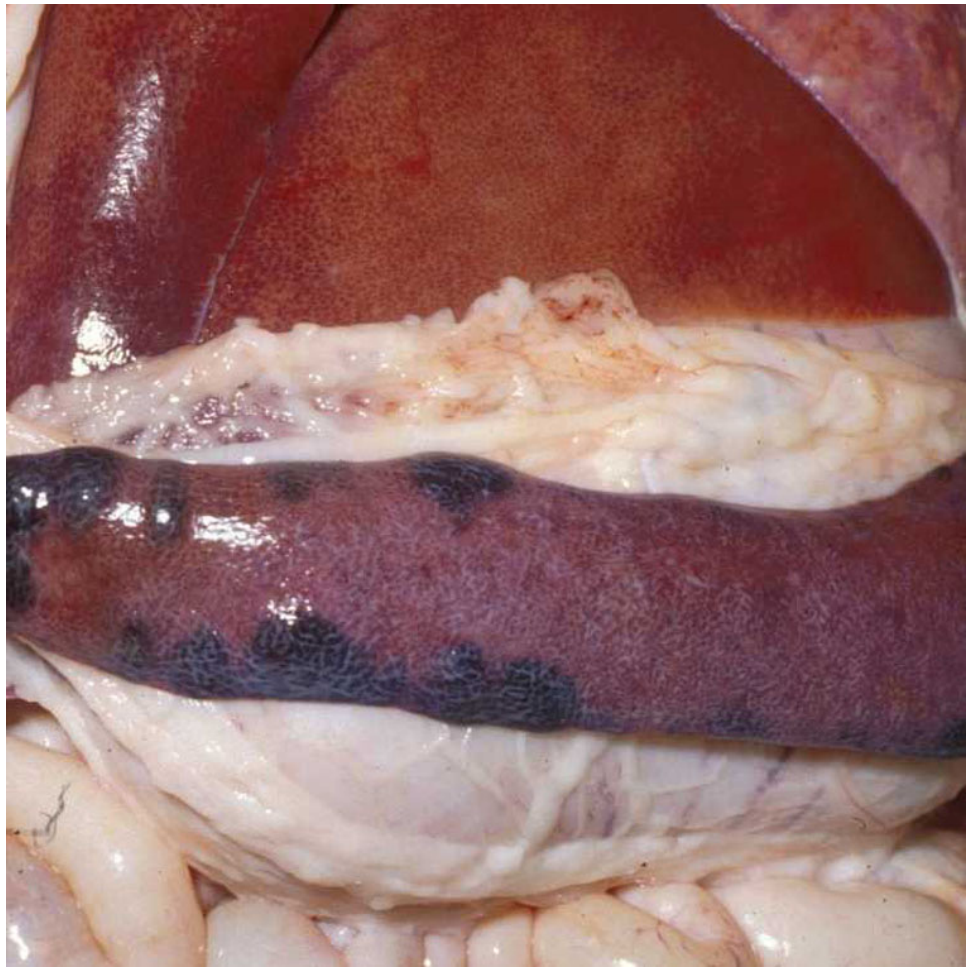

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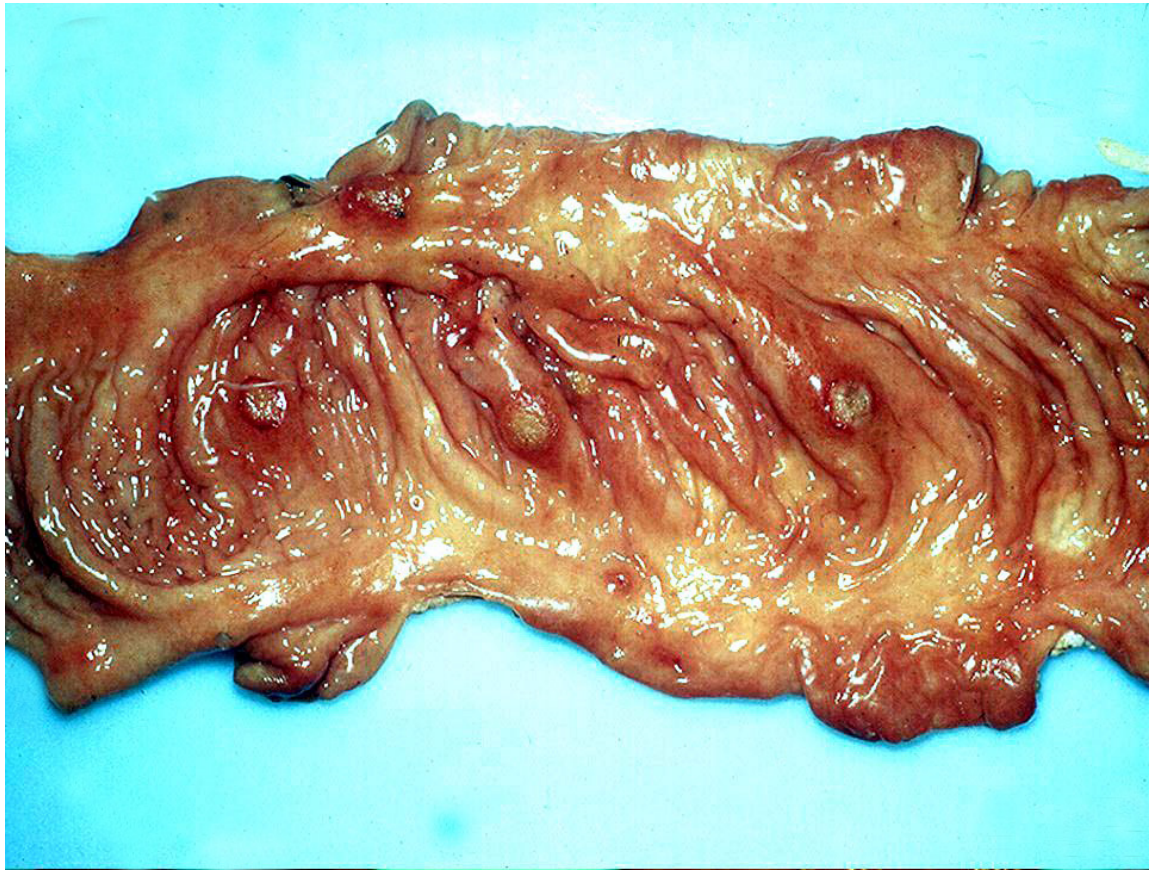

CSFV: diagnosis

- What is your major differential diagnosis?

- Diagnosis:
  - PCR
  - LN, tonsil, spleen, kidney, blood

- Control:
  - Not present in N.A. – control is through slaughter, quarantine, movement restrictions, etc.
  - Vaccines are available (but not used here, since the disease is foreign)