Practical aspects in the diagnosis of PCV2 associated diseases

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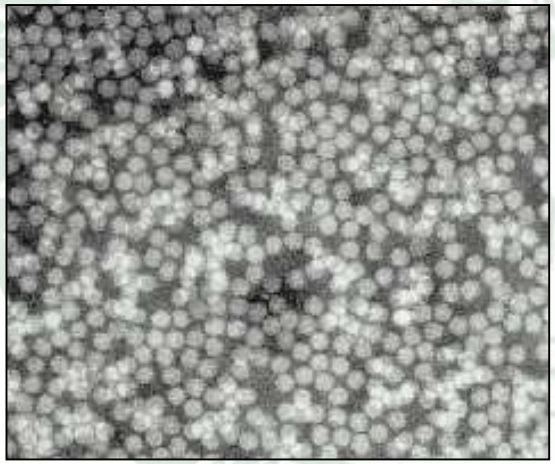
Porcine Circovirus Type 2

- Small size (~1800 bp)
- Non-enveloped
- Single-stranded DNA
- Circular genome
- Family *Circoviridae*
- Genus *Circovirus*
- Genotypes: 8 (a-h). PCV2d > PCV2b > PCV2a



(Nawagitgul et al., 2000)

Porcine Circovirus Associated Diseases (PCVAD)

- PCV2 systemic disease PCV2
- PCV2 reproductive disease
- PCV2 lung disease
- PCV2 enteric disease
- Porcine dermatitis and nephropathy syndrome (PDNS)

PCV2 systemic disease

(Postweaning Multisystemic Wasting Syndrome, PMWS)

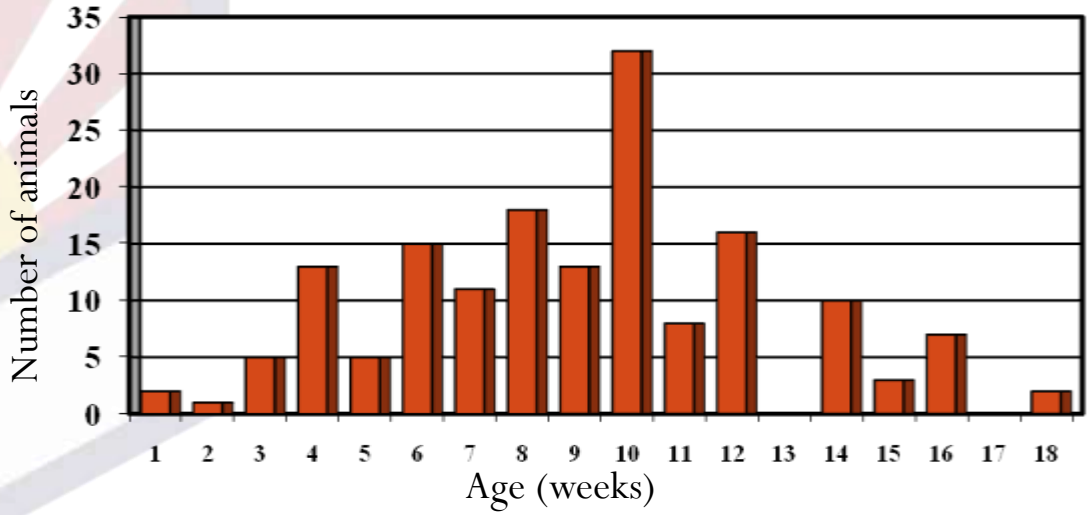
Case definition (Sorden, 2000)

- Clinical signs
  - Growth retardation and wasting, enlargement of inguinal lymph nodes, dyspnea, jaundice, pallor of the skin, diarrhea, gastric ulcers
- Characteristic histopathological lesions
  - Lymphocyte depletion with histiocytes and giant multinucleate cells infiltration
- Moderate to high amounts of PCV2 within the lesions
  - **Antigen:** Immunohistochemistry (IHC)
  - **Nucleic acid:** *In situ* hybridization (ISH)

PCV2 systemic disease

(Postweaning Multisystemic Wasting Syndrome, PMWS)

- **Clinical signs**
  - PCV2 is ubiquitous (most are subclinical infections)
  - Endemic/epidemic presentation
  - Morbidity 4-30% (occasionally 50-60%)
  - Mortality 4-20%
  - 2-4 months of age




Growth retardation and wasting

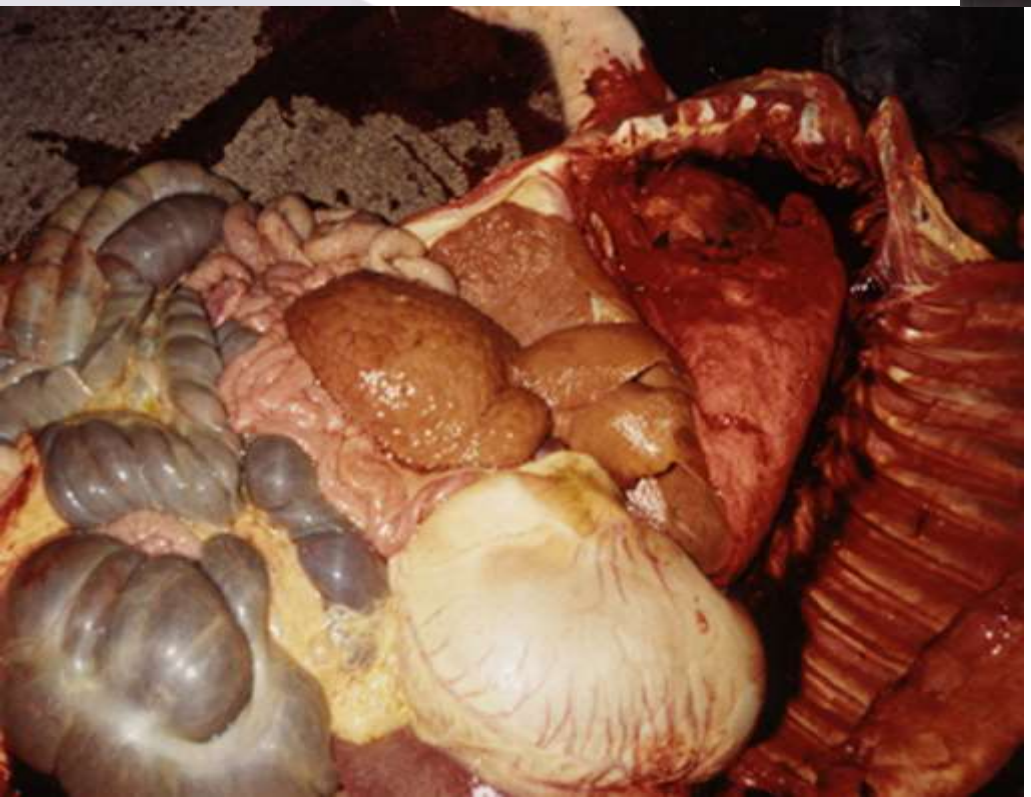
Growth retardation and wasting

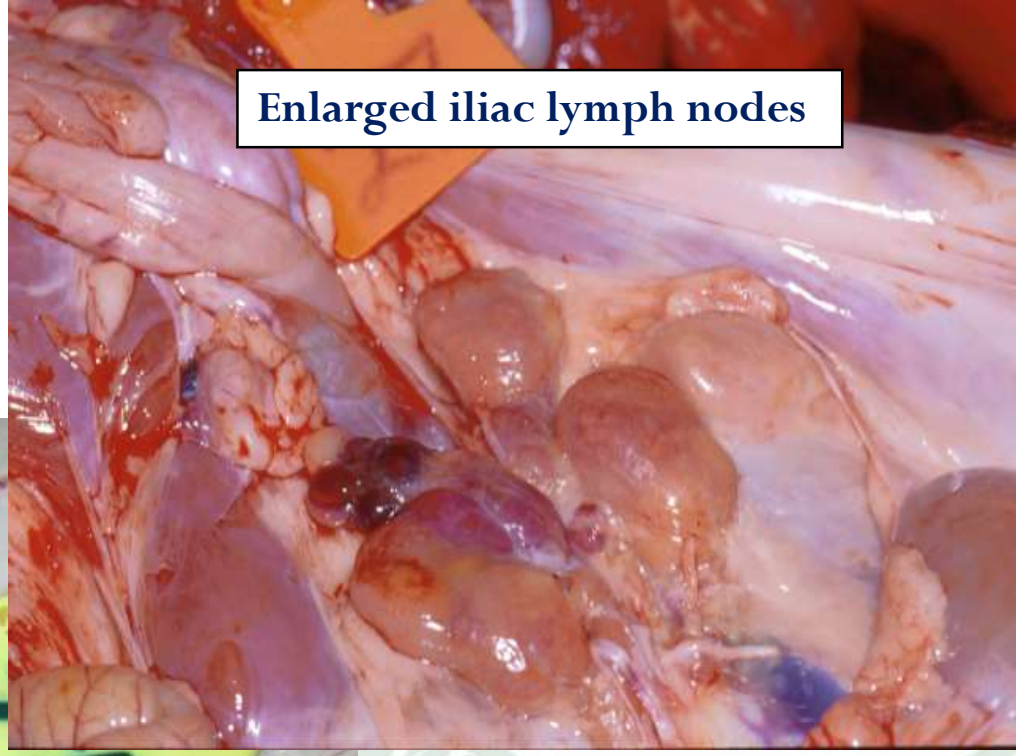
Growth retardation and wasting

Growth retardation and wasting

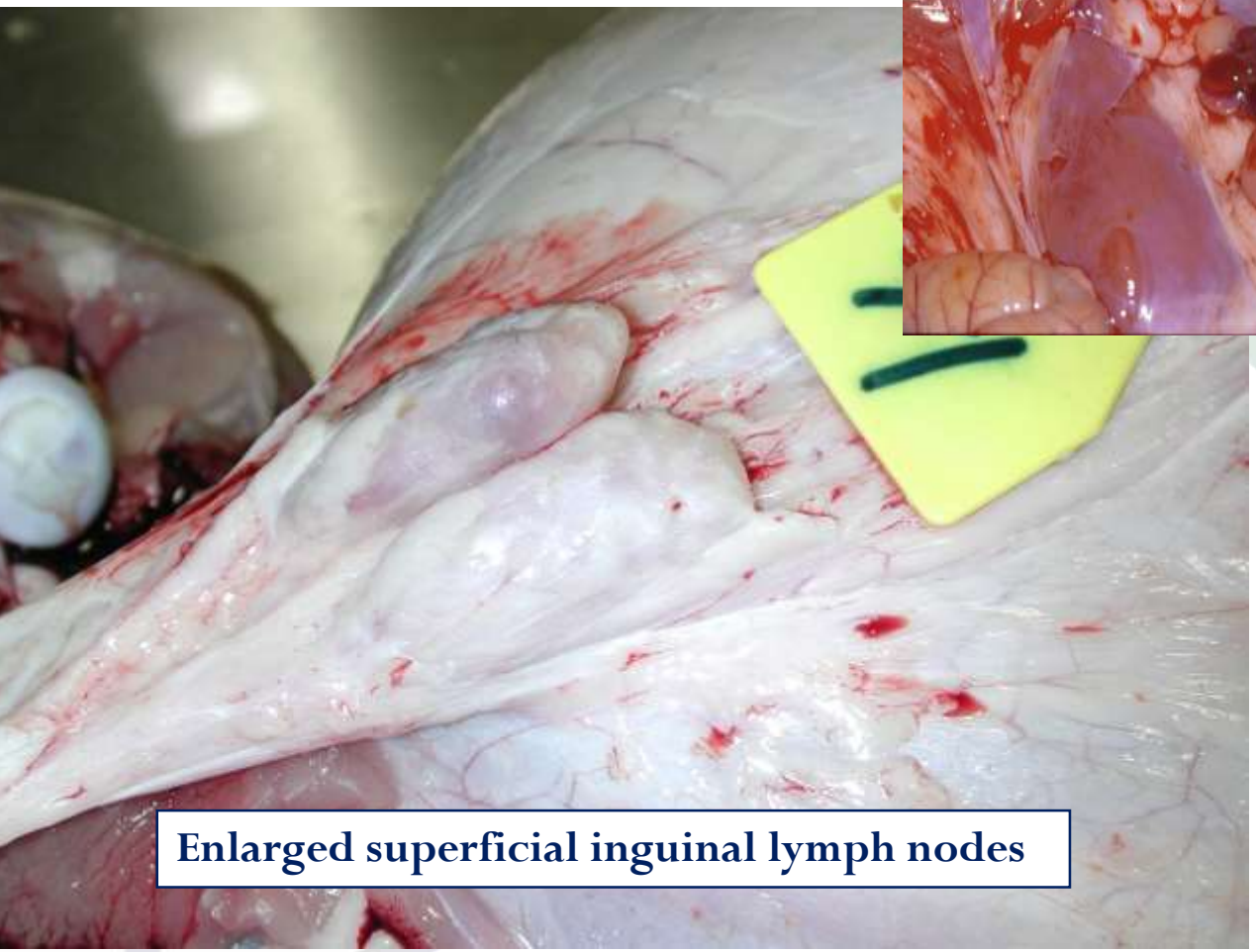
Jaundice

Jaundice



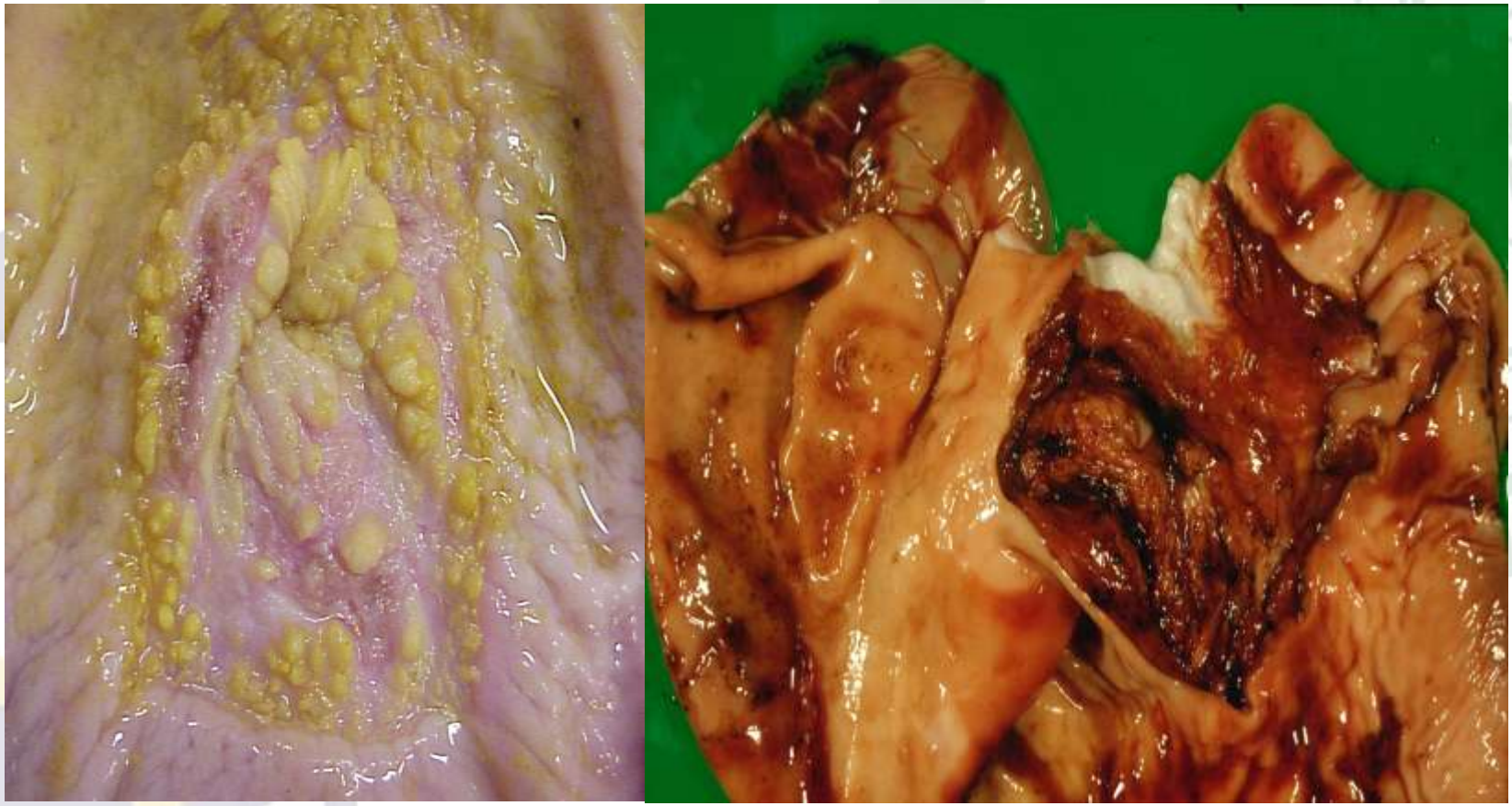


Enlarged iliac lymph nodes



Enlarged superficial inguinal lymph nodes

Enlarged superficial inguinal lymph node



Gastric ulcers

PCV2 systemic disease

(Postweaning Multisystemic Wasting Syndrome, PMWS)

Case definition (Sorden, 2000)

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  - Growth retardation and wasting, enlargement of lymph nodes, dyspnea, jaundice, pallor of the skin, diarrhea, gastric ulcers
- Characteristic histopathological lesions
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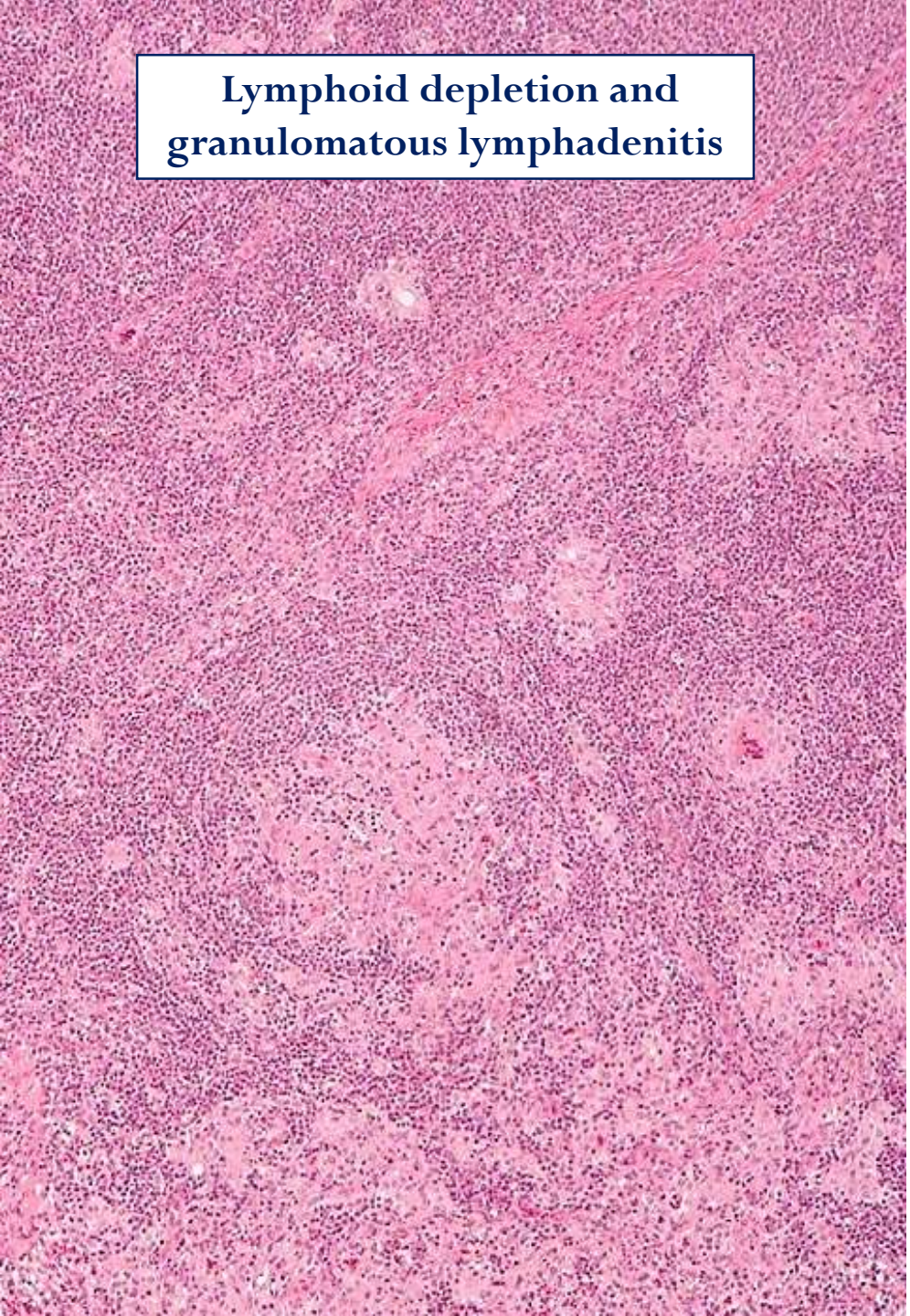
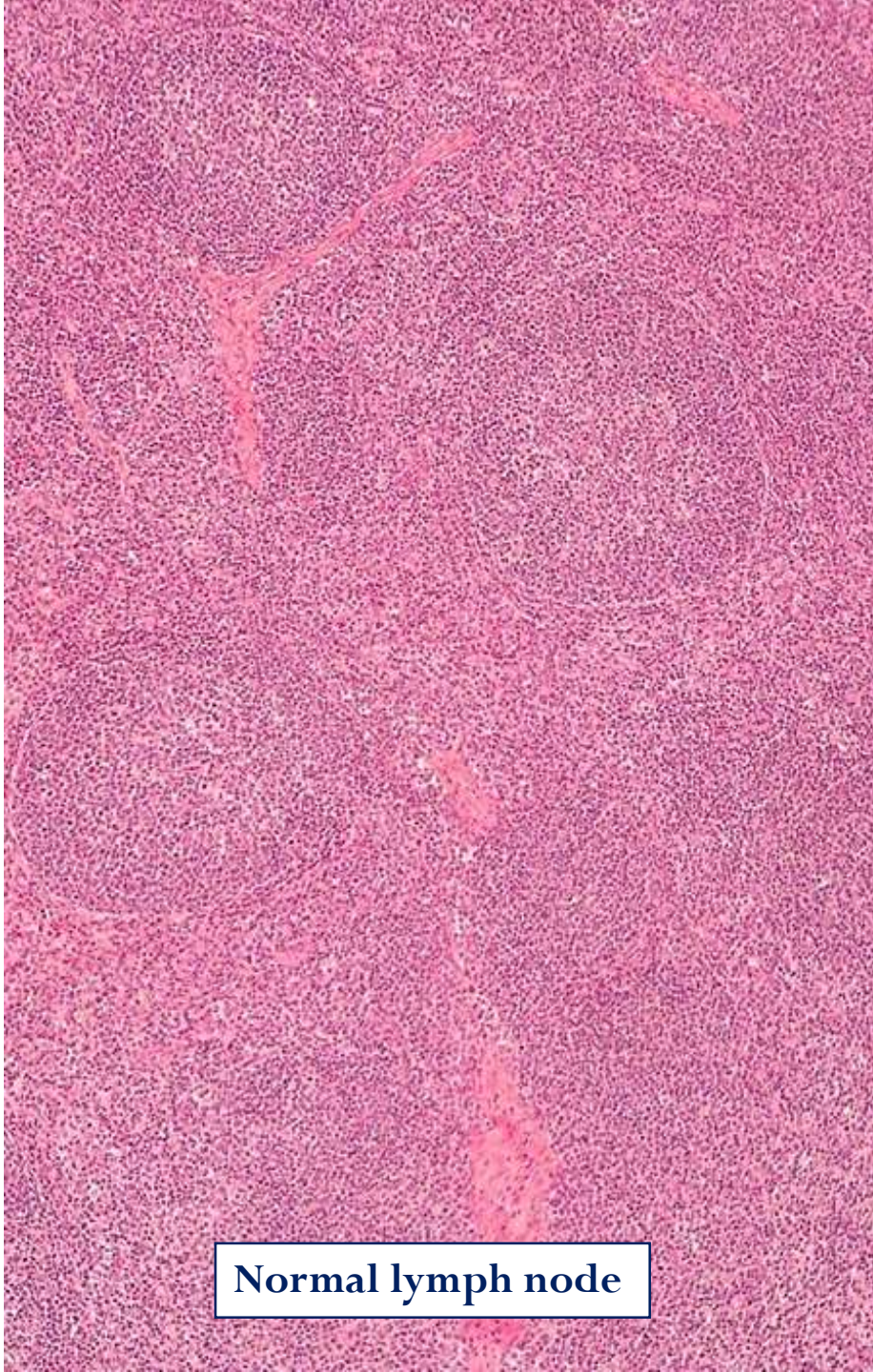
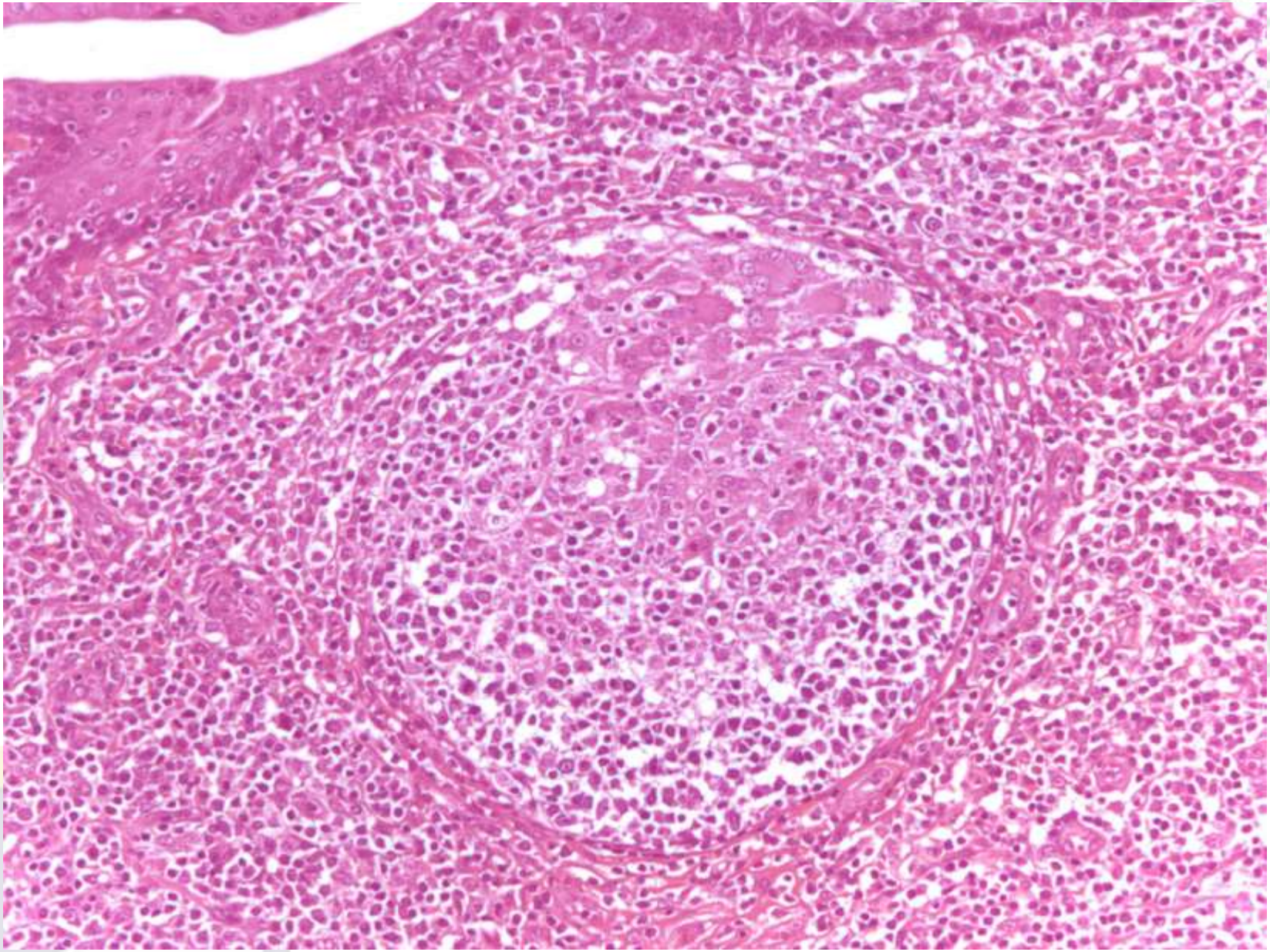
- Moderate to high amounts of PCV2whitin the lesions
  - **Antigen:** Immunohistochemistry (IHC)
  - **Nucleic acid:** *In situ* hybridization (ISH)

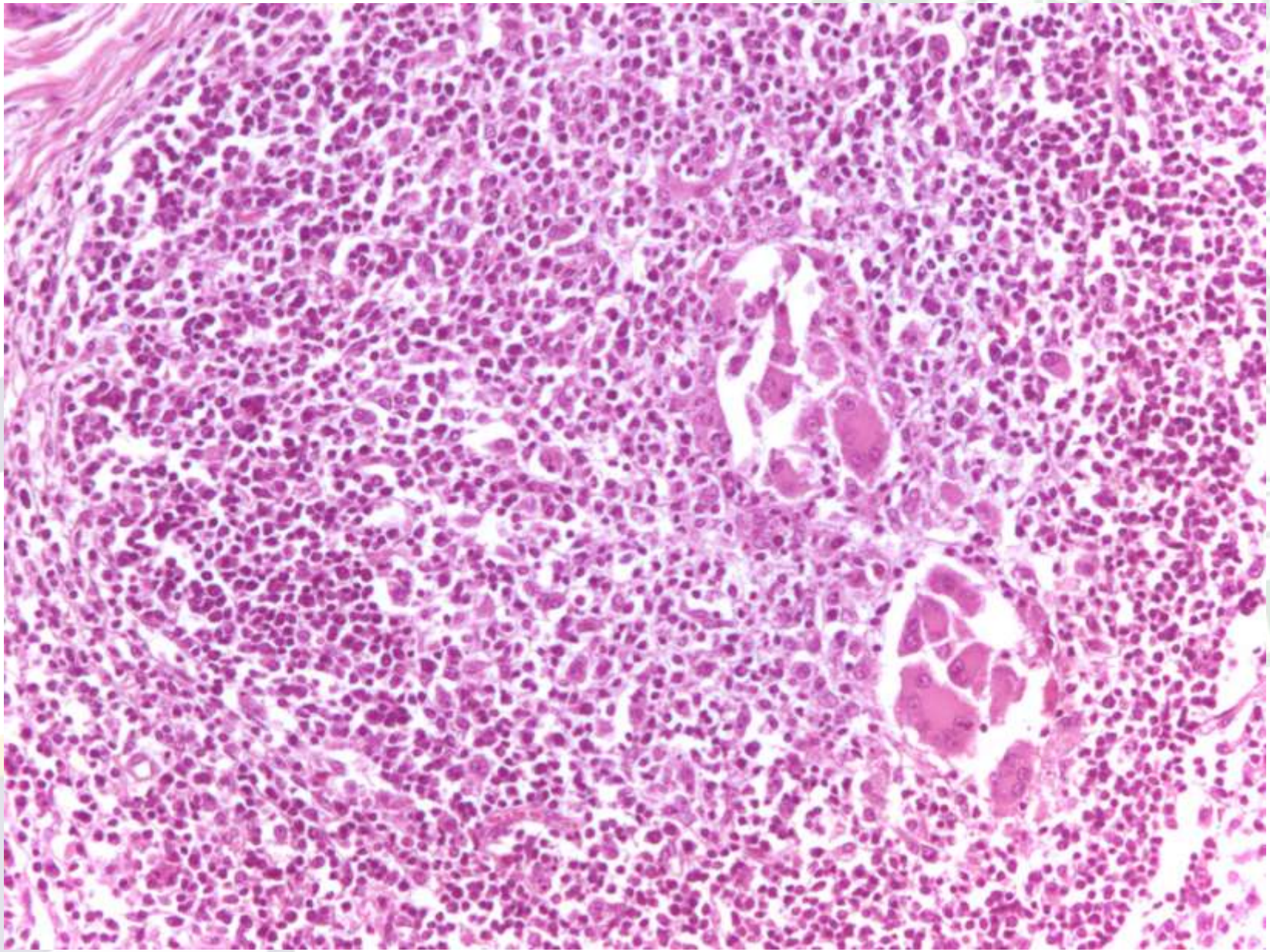

Lymphoid depletion and
granulomatous lymphadenitis


Normal lymph node

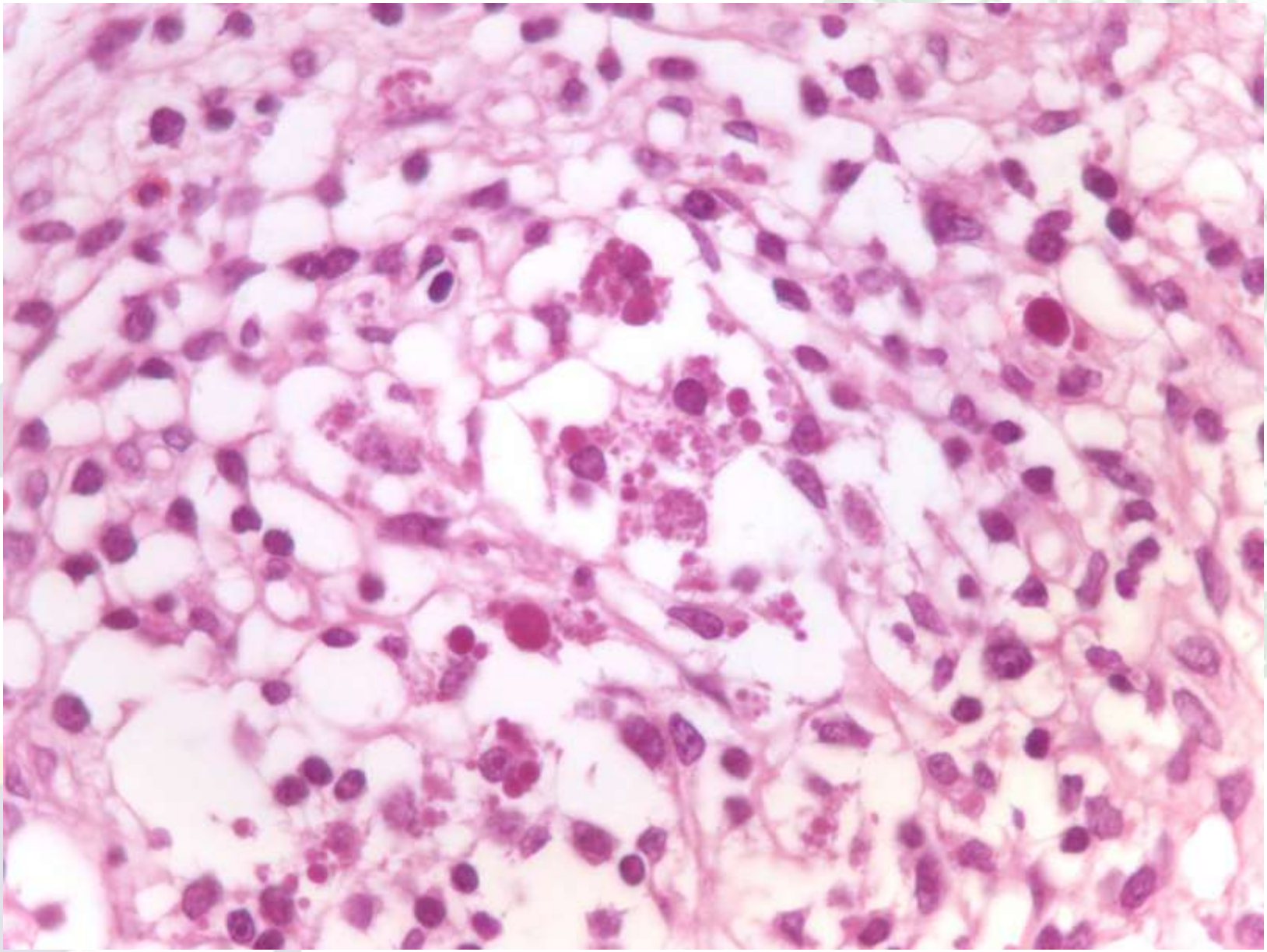

Tonsil with severe lymphoid depletion
Normal tonsil



Lymphoid depletion and infiltration of histiocytes



Presence of giant multinucleate cells



Cytoplasmic viral inclusion bodies

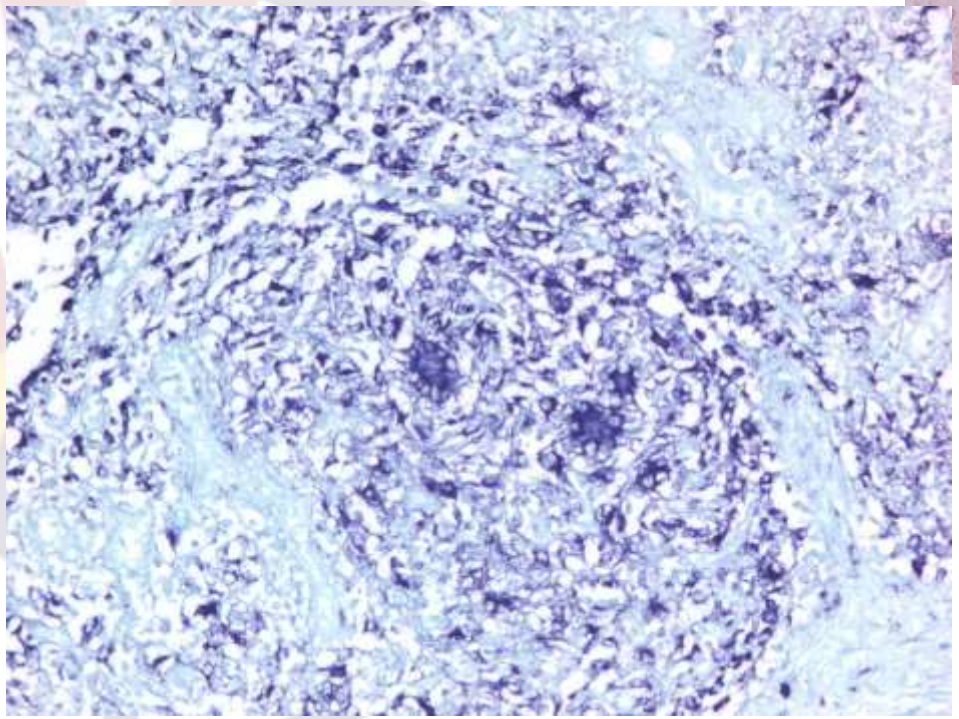
PCV2 systemic disease

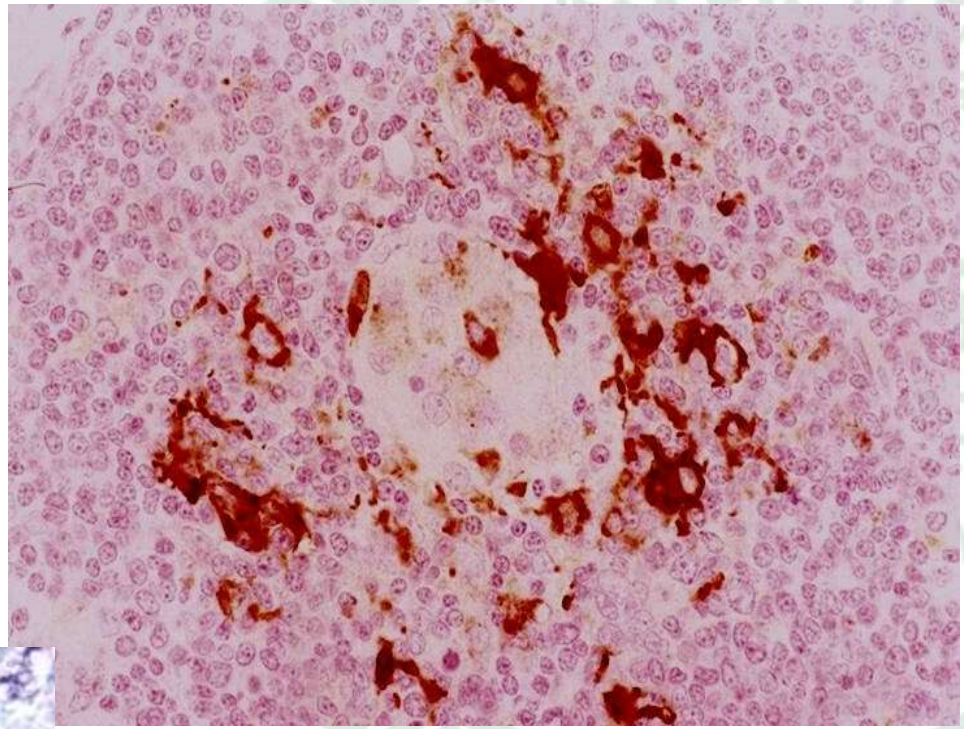
(Postweaning Multisystemic Wasting Syndrome, PMWS)

Case definition (Sorden, 2000)

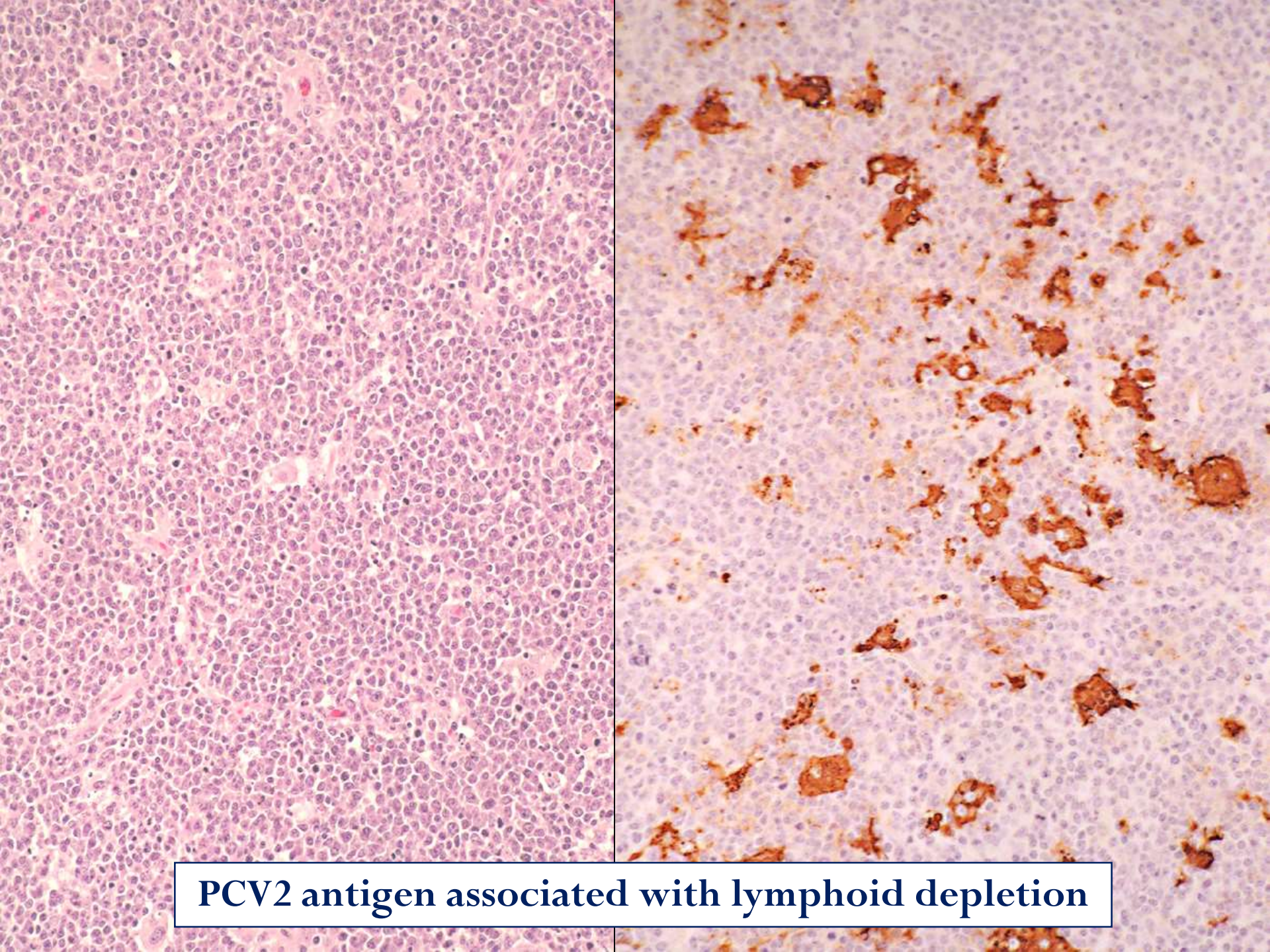
- Clinical signs
  - Growth retardation and wasting, enlargement of lymph nodes, dyspnea, jaundice, pallor of the skin, diarrhea, gastric ulcers
- Characteristic histopathological lesions
  - Lymphocyte depletion with histiocytes and giant multinucleate cells infiltration
- Moderate to high amounts of PCV2whitin the lesions
  - **Antigen:** Immunohistochemistry (IHC)
  - **Nucleic acid:** *In situ* hybridization (ISH)

In situ hybridization (nucleic acid)



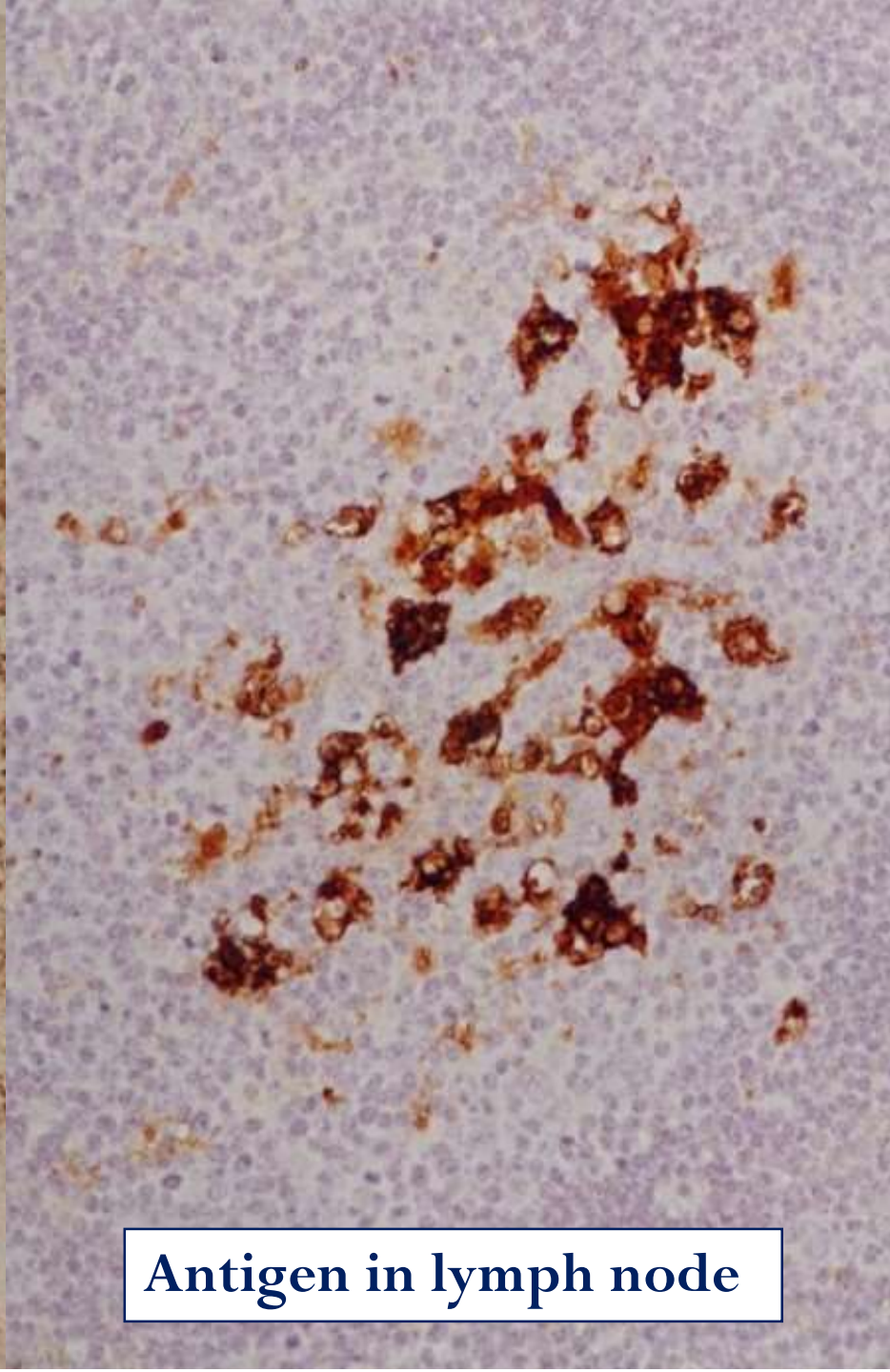


Immunohistochemistry (antigen)



PCV2 antigen associated with lymphoid depletion

Antigen in tonsil



Antigen in lymph node

PCV2 systemic disease

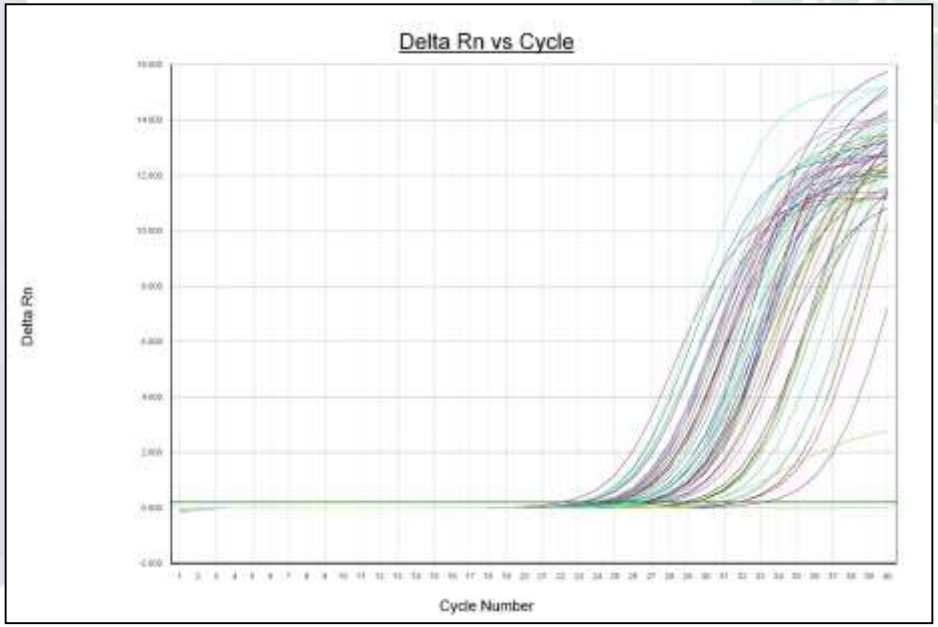
(Postweaning Multisystemic Wasting Syndrome, PMWS)

- Clinical signs are not diagnostic
  - Many things cause wasting…..
- Gross lesions are not diagnostic
  - Many things cause lymph nodes enlargement….
- PCV2 infection ≠ PCV2 systemic disease
  - Healthy pigs with histologically normal lymphoid tissues may have low numbers of PCV2-positive cells in lymphoid follicles
  - PCV2-free herds apparently do not exist

PCV2 systemic disease

(Postweaning Multisystemic Wasting Syndrome, PMWS)

- Use of other diagnostic techniques
  - High correlation between quantity of PCV2 and severity of microscopic lesions
  - PCV2 quantification techniques as qPCR could be potentially used for the diagnosis



PCV2 systemic disease

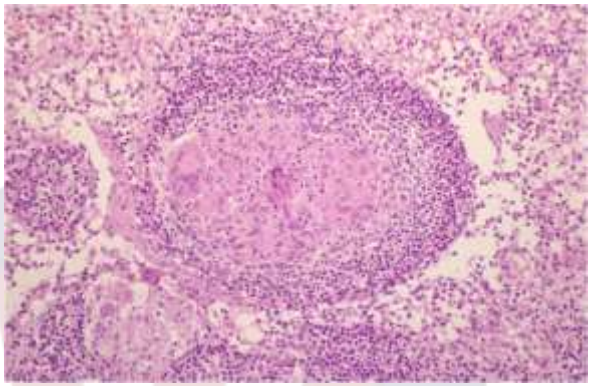
(Postweaning Multisystemic Wasting Syndrome, PMWS)

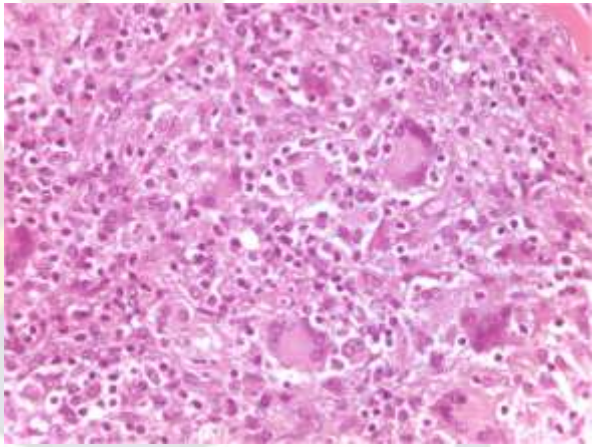
- Use of other diagnostic techniques: **qPCR**
  - Serum
    - Negative/not relevant: $<10^2$
    - Low or doubtful: $10^2 - 10^4$
    - Subclinical: $10^4 - 10^8$
    - High possibility of clinical involvement: $>10^8$
  - Tissues
    - Subclinical: $<10^9$
    - High possibility of clinical involvement: $\geq 10^9$

PCV2 systemic disease

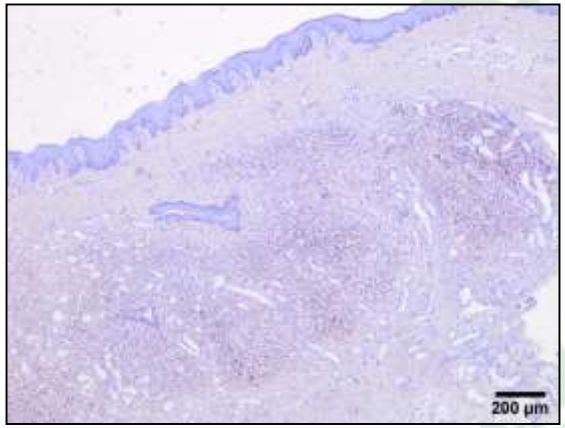
(Postweaning Multisystemic Wasting Syndrome, PMWS)

qPCR: 10^{11} (tissue pool)

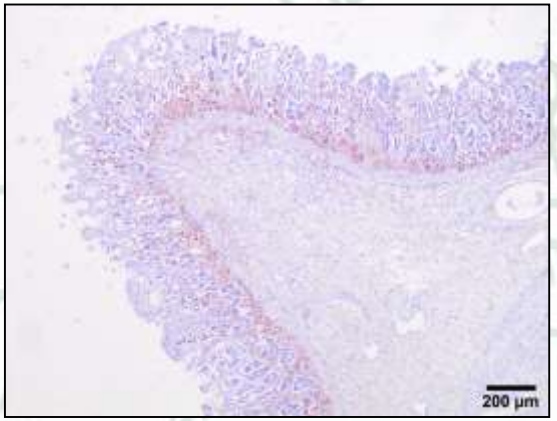




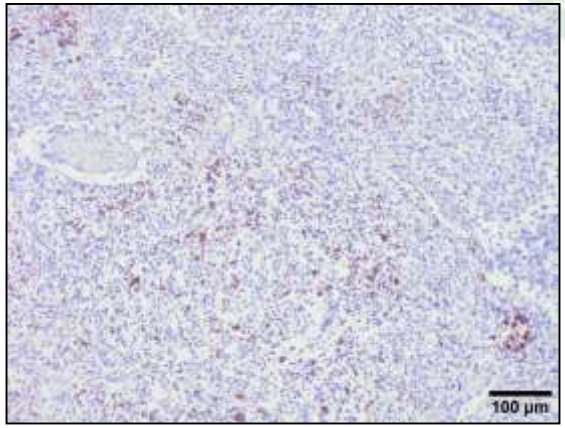
Lymphoid tissues



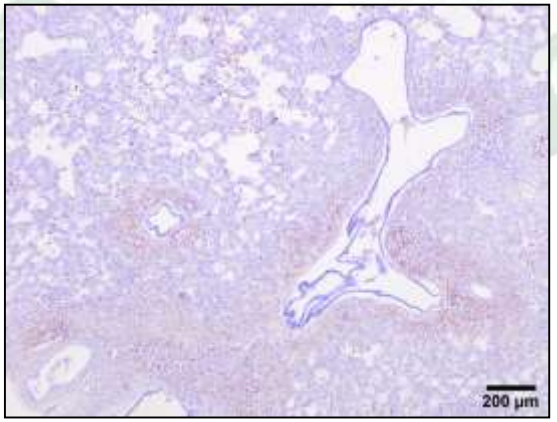
Tonsil



Intestine



Lymph node

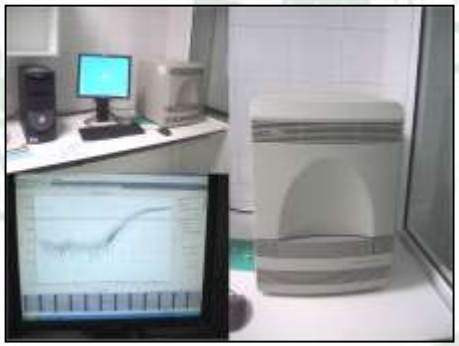


Lung

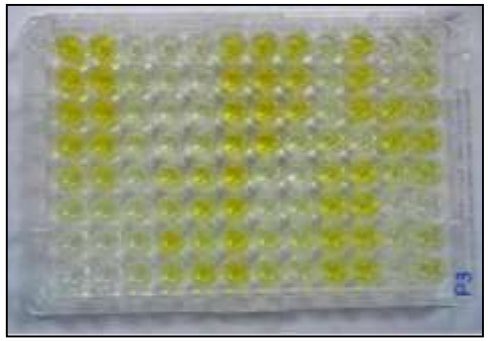
PCV2 systemic disease

(Postweaning Multisystemic Wasting Syndrome, PMWS)

- Use of other diagnostic techniques
  - **qPCR** should be used on a **population basis**
    - Not for individual diagnosis
    - Serum samples
    - Positive results without clinical disease



  - Use of **serology problematic**
    - Seroconversion patterns similar in affected and non-affected farms

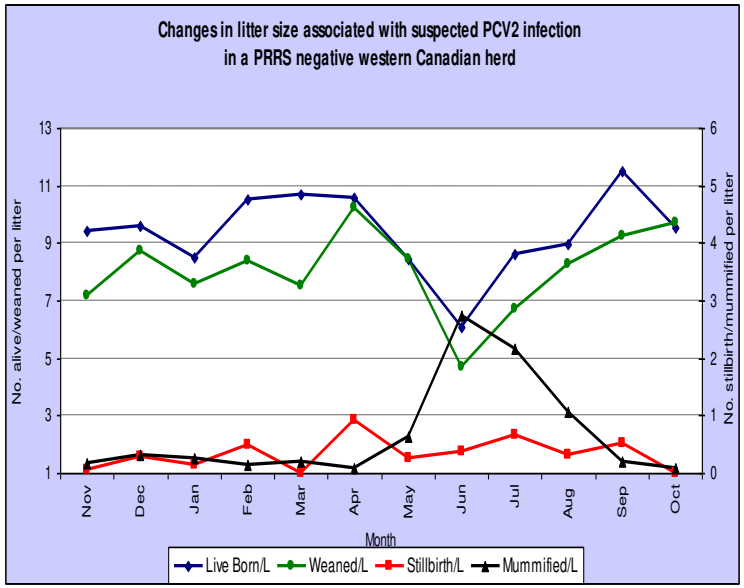


Porcine Circovirus Associated Diseases (PCVAD)

- PCV2 systemic disease PCV2
- PCV2 reproductive disease
- PCV2 lung disease
- PCV2 enteric disease
- Porcine dermatitis and nephropathy syndrome (PDNS)

PCV2 reproductive disease

- Late-term abortions and stillbirths
- Mummifications
- Increased number of piglet deaths during lactation
- Infection at early gestation: return to estrus
  - Seroconversion and/or PCV2 positivity
- Discrepancies regarding the real number of cases in the field



PCV2 reproductive disease

- Fetal lesions
  - Hepatic enlargement and congestion
  - Cardiac hypertrophy with areas of discoloration
  - Ascites
  - Hydrothorax
  - Hydropericardium

PCV2 reproductive disease

Diagnosis (Segalés, 2012)

- Late-term abortions and stillbirths, sometimes with evident hypertrophy of the fetal heart
- Presence of heart microscopic lesions characterized by extensive fibrosing and/or necrotizing myocarditis
- Presence of high amounts of PCV2 in myocardial lesions and other fetal tissues

PCV2-associated abortions

PCV2-associated fetal heart hypertrophy

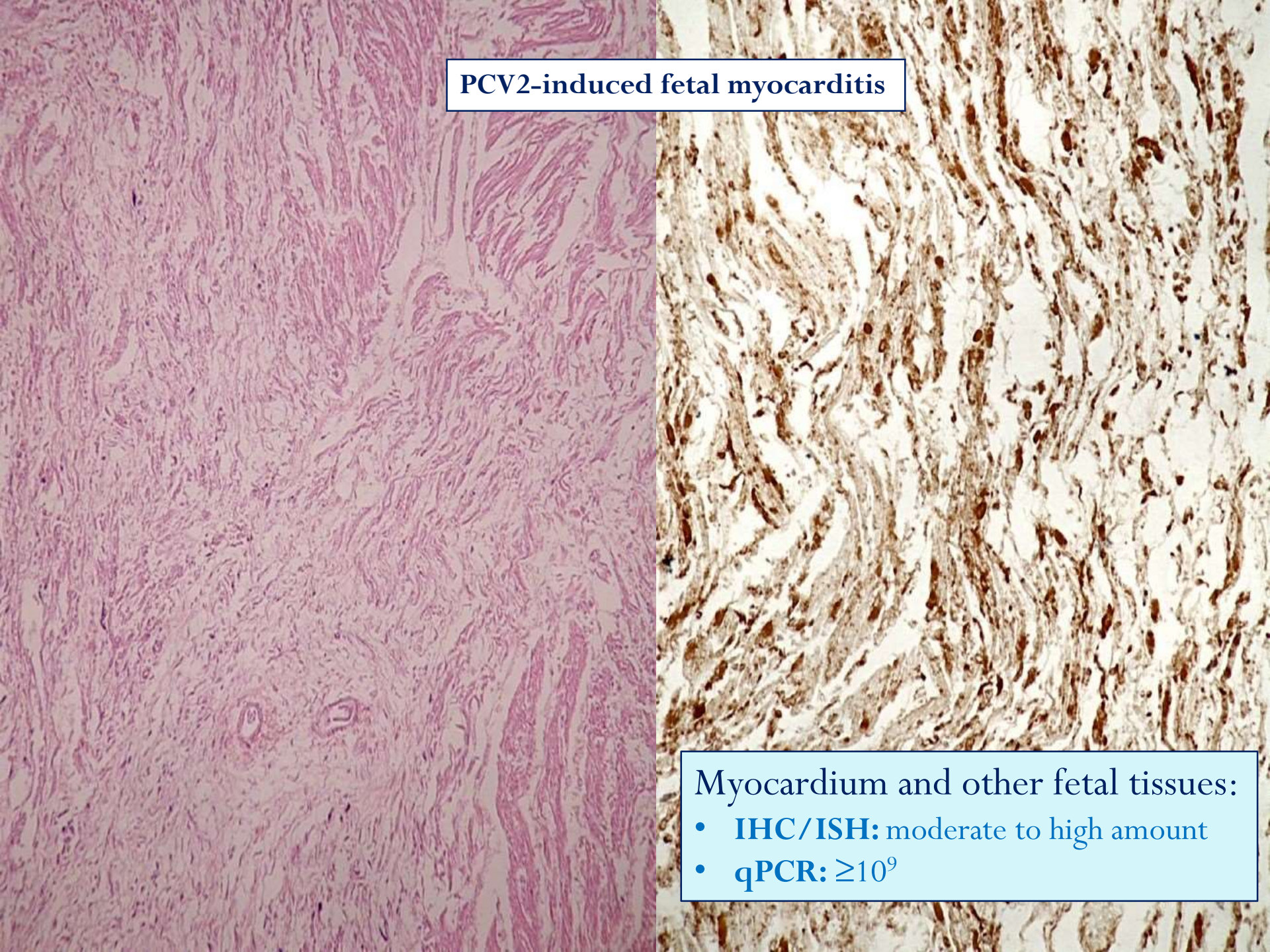
PCV2 reproductive disease

Diagnosis (Segalés, 2012)

- Late-term abortions and stillbirths, sometimes with evident hypertrophy of the fetal heart

- Presence of heart microscopic lesions characterized by extensive fibrosing and/or necrotizing myocarditis

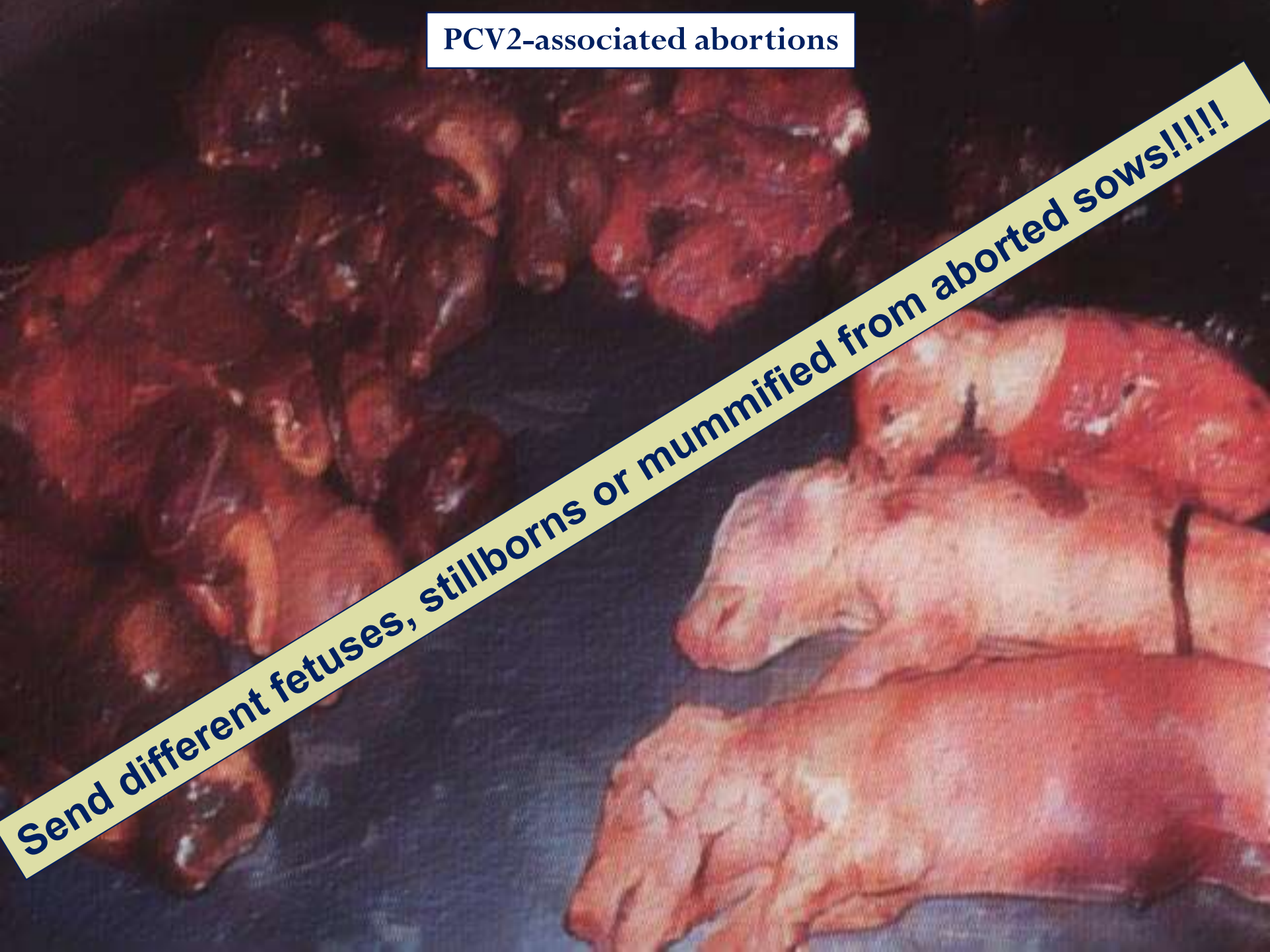
- Presence of high amounts of PCV2 in myocardial lesions and other fetal tissues



PCV2-induced fetal myocarditis

Myocardium and other fetal tissues:

- **IHC/ISH:** moderate to high amount
- **qPCR:** $\geq 10^9$


PCV2-associated abortions
Send different fetuses, stillborns or mummified from aborted sows!!!!!

PCV2 reproductive disease

- Differential diagnosis
  - Clinically indistinguishable from other diseases causing late-term abortions and stillbirths
    - PRRSV, *Leptospira* spp.

  - Presence of mummies of different sizes
    - Differentiate from PPV infection

Porcine Circovirus Associated Diseases (PCVAD)

- PCV2 systemic disease PCV2
- PCV2 reproductive disease
- PCV2 lung disease
- PCV2 enteric disease
- Porcine dermatitis and nephropathy syndrome (PDNS)

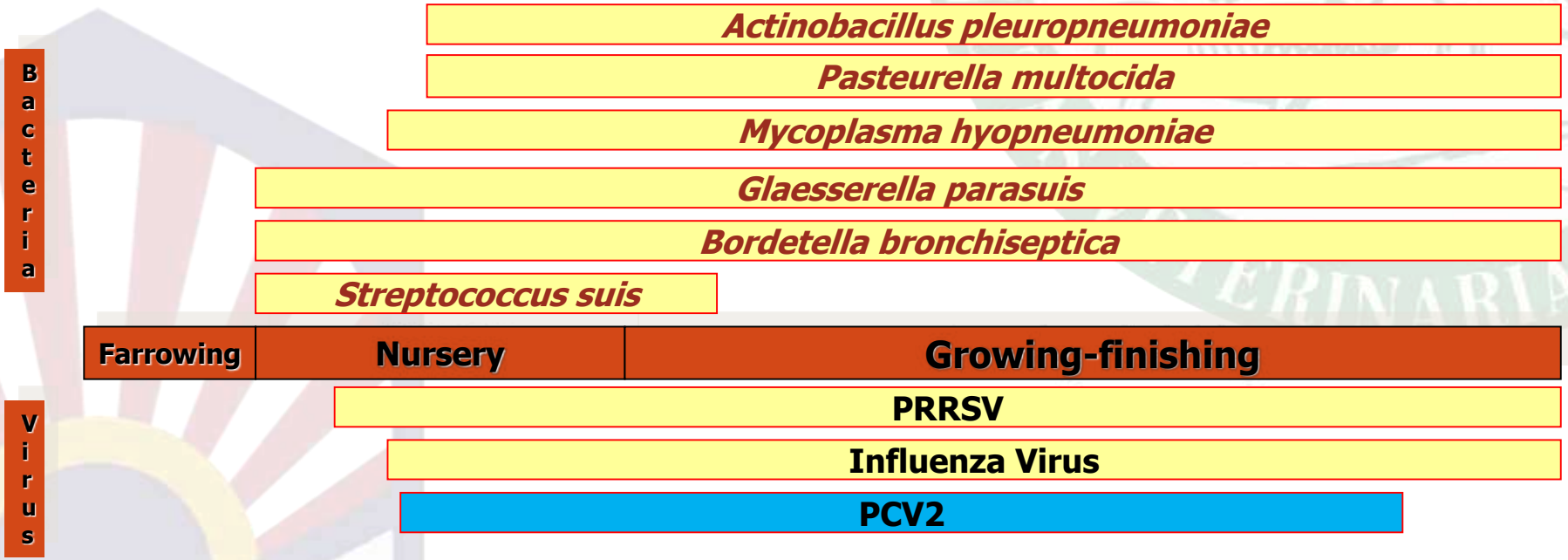
PCV2 lung disease

Main pathogens involved in the Porcine Respiratory Disease Complex (PRDC)

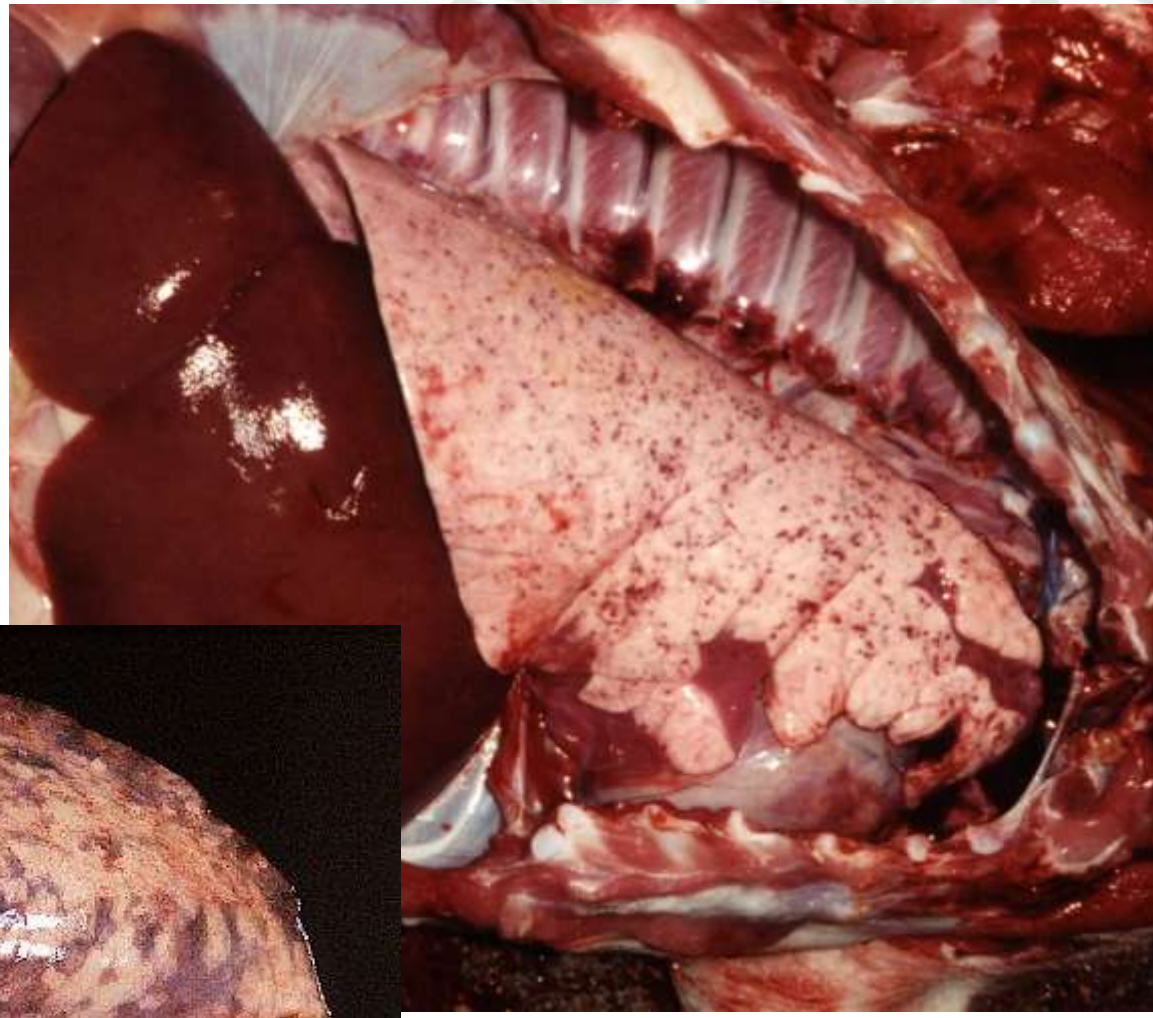
VIRUS	MYCOPLASMA	BACTERIA
PRRSV	***M. hyopneumoniae***	***A. pleuropneumoniae***
Influenza	*M. hyorhinis*	***B. bronchiseptica***
PCV2		*P. multocida*
Coronavirus		*G. parasuis*
		S. suis

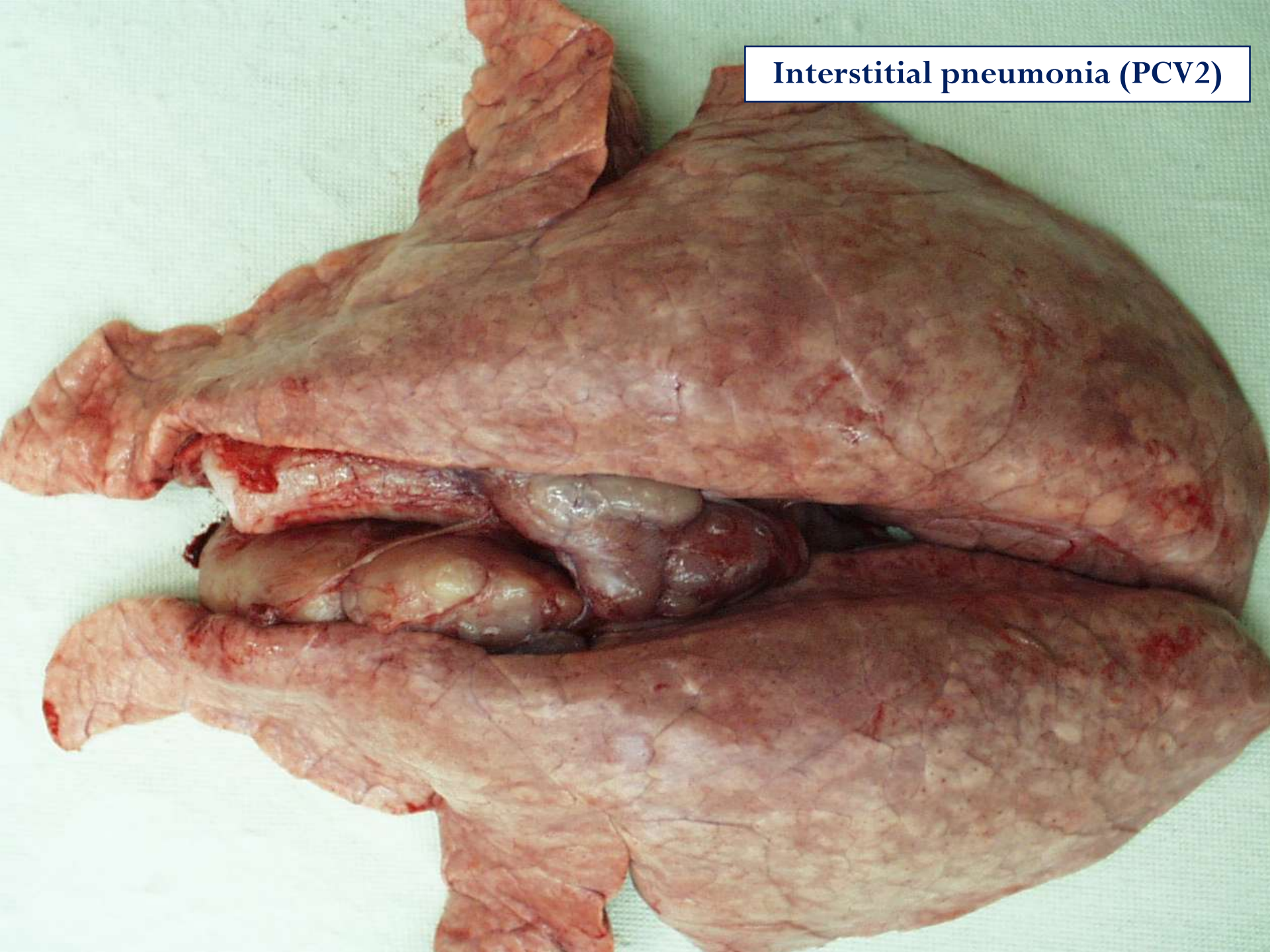
PCV2 lung disease

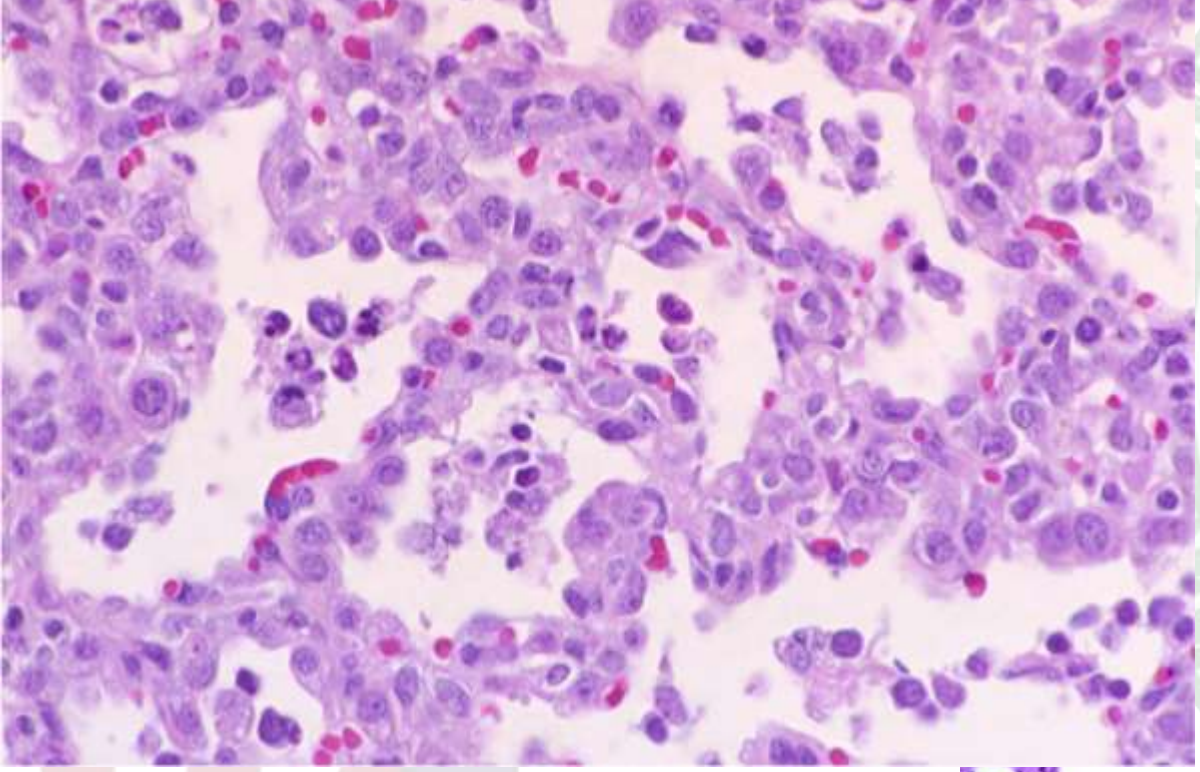
Most probable time of infection of the main pathogens involved in the Porcine Respiratory Disease Complex

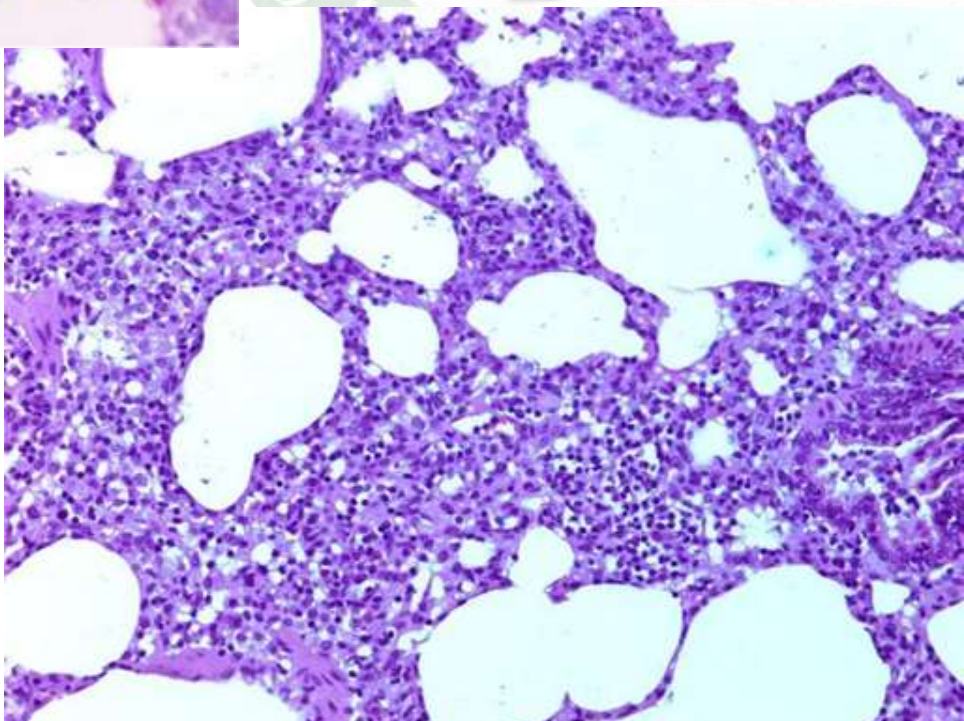


Interstitial pneumonia (PCV2)




Interstitial pneumonia (PCV2)

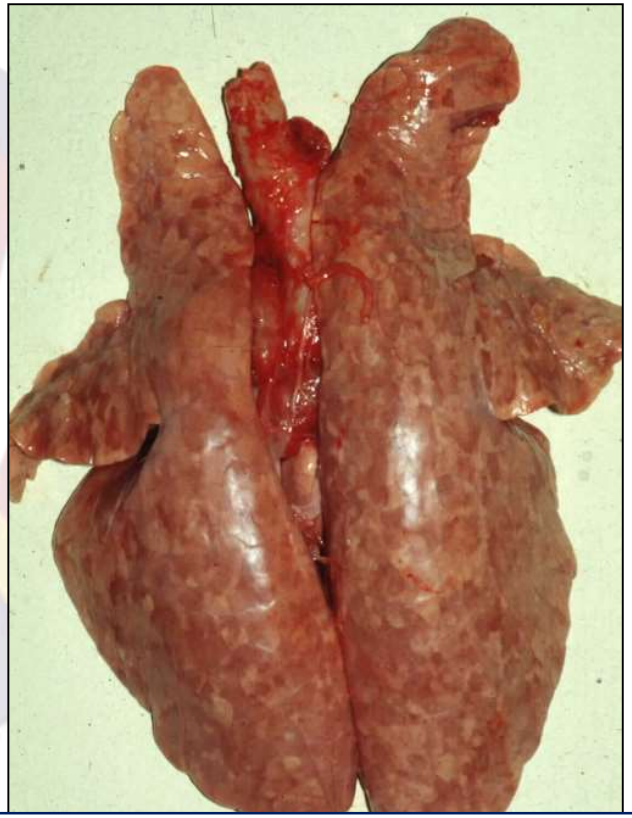




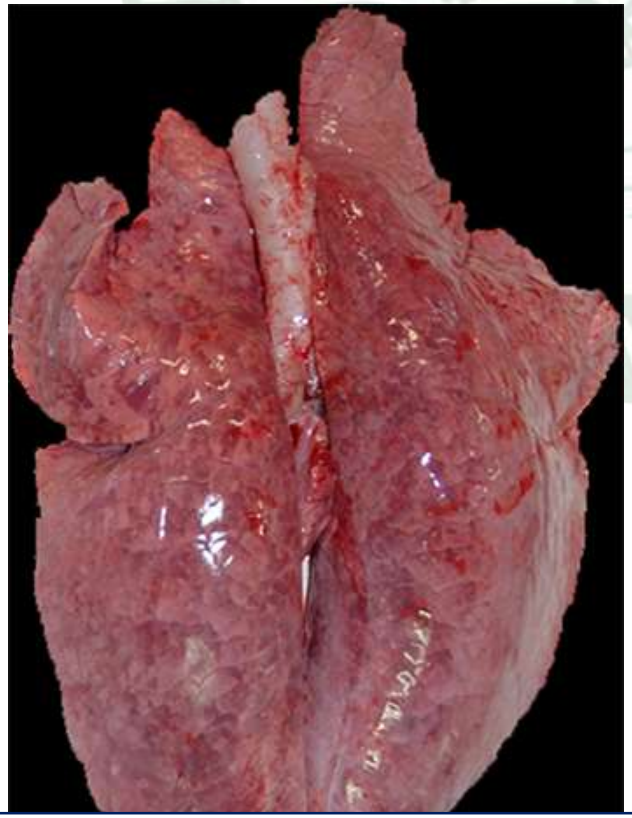
Interstitial pneumonia (PCV2)

PCV2 lung disease

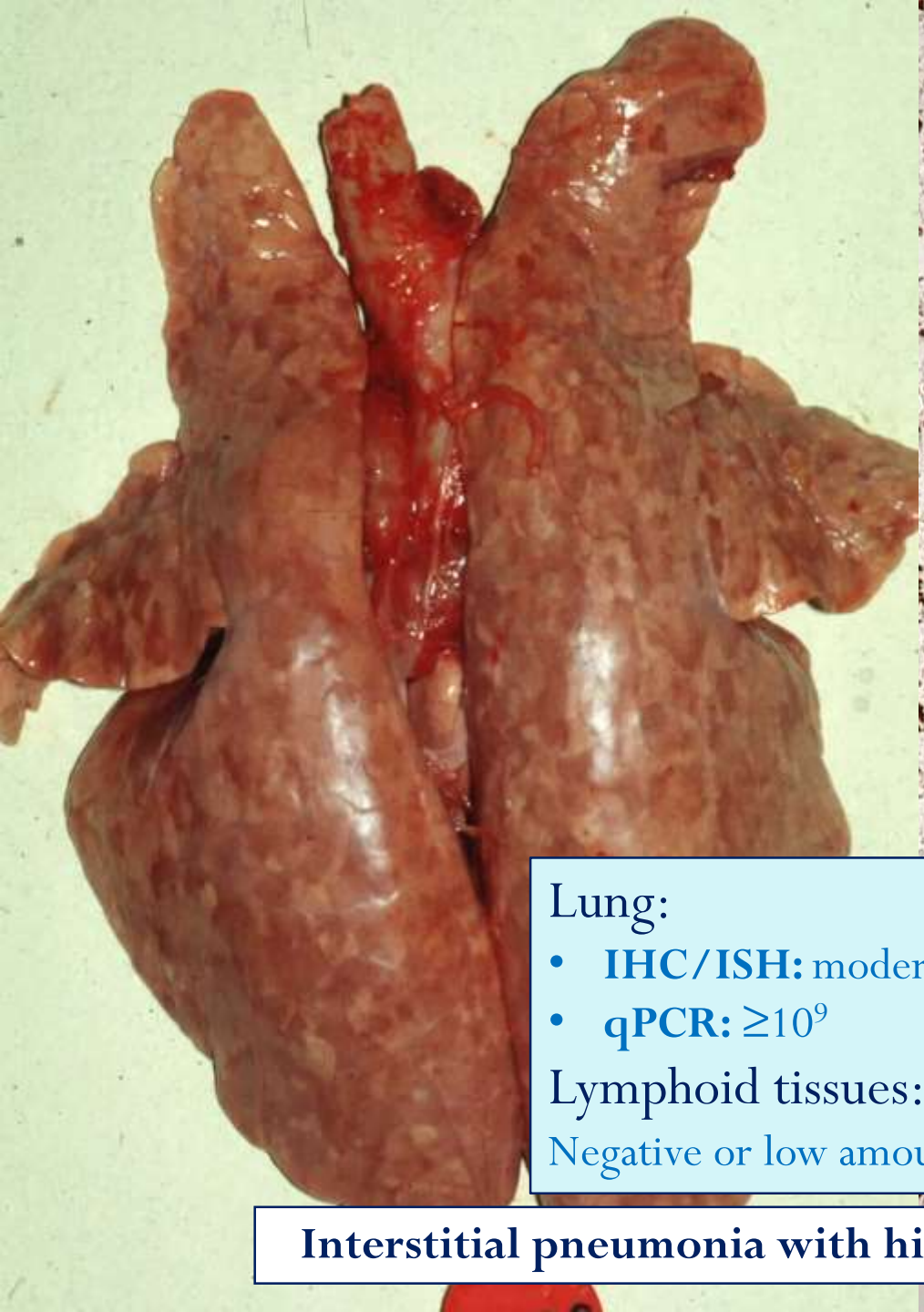
- Differential diagnosis
  - Interstitial pneumonia caused by PRRSV

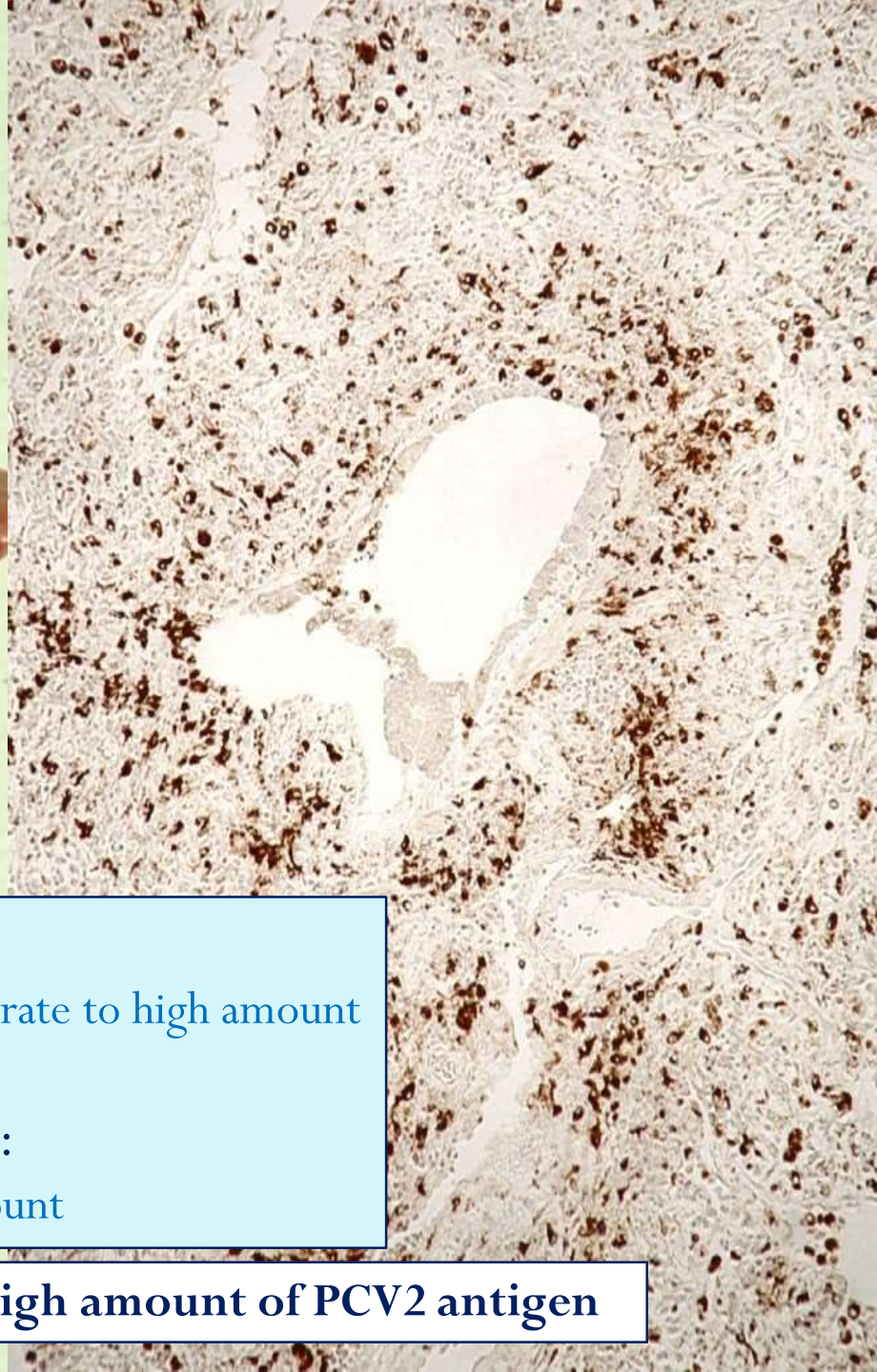


Interstitial pneumonia (PCV2)



Interstitial pneumonia (PRRSV)





Lung:

- **IHC/ISH:** moderate to high amount
- **qPCR:** $\geq 10^9$

Lymphoid tissues:

Negative or low amount

Interstitial pneumonia with high amount of PCV2 antigen

PCV2 lung disease

- Clinical signs
  - 8-26 weeks of age
  - Growth retardation and wasting
  - Fever
  - Coughing
  - Dyspnea
  - Rough hair coat

PCV2 lung disease

- Differential diagnosis

PCV2-SD or PRDC?

PCV2 lung disease

	PCV2-SD	PRDC
Wasting	+	+
Respiratory tract lesions	+/-	+
Systemic lymphoid lesions	+	-
Jaundice/liver lesions	+/-	-
Paleness/gastric ulcers	+/-	+/-
Kidney lesions	+/-	-
Other pathogens in lesioned tissue	+/-	+

Must submit other tissues with lungs to confirm PCV2-SD or PCV2-LD diagnosis

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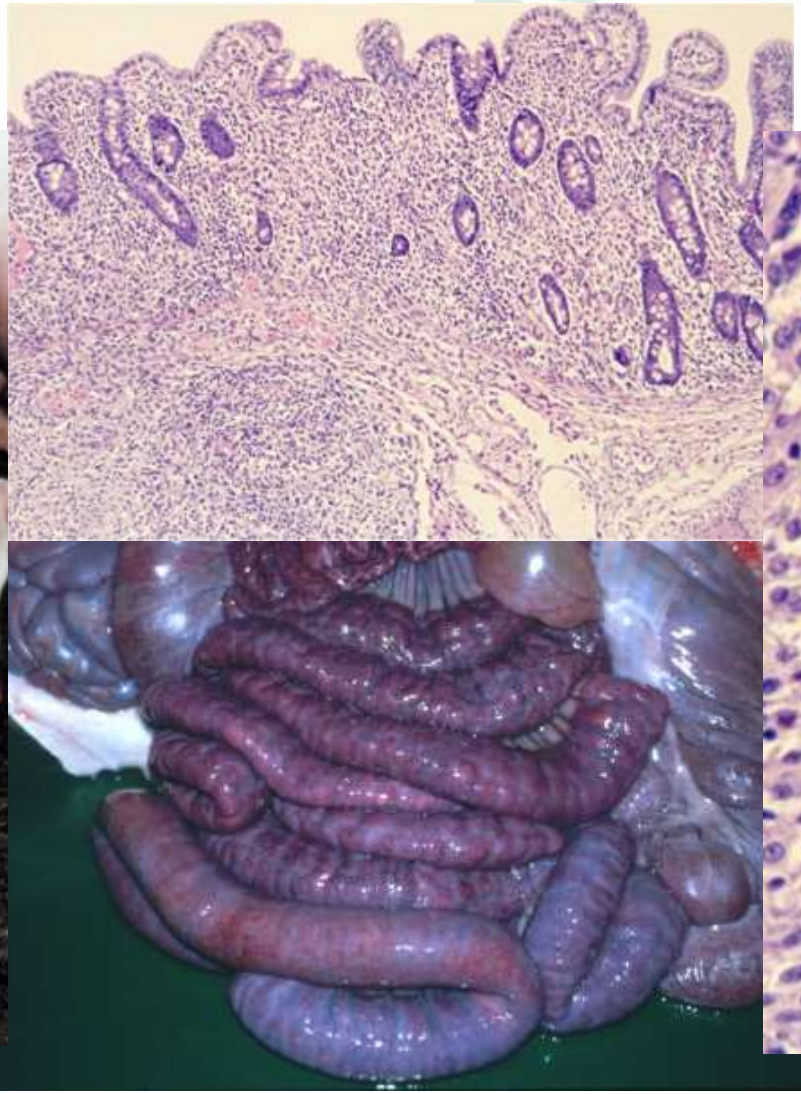
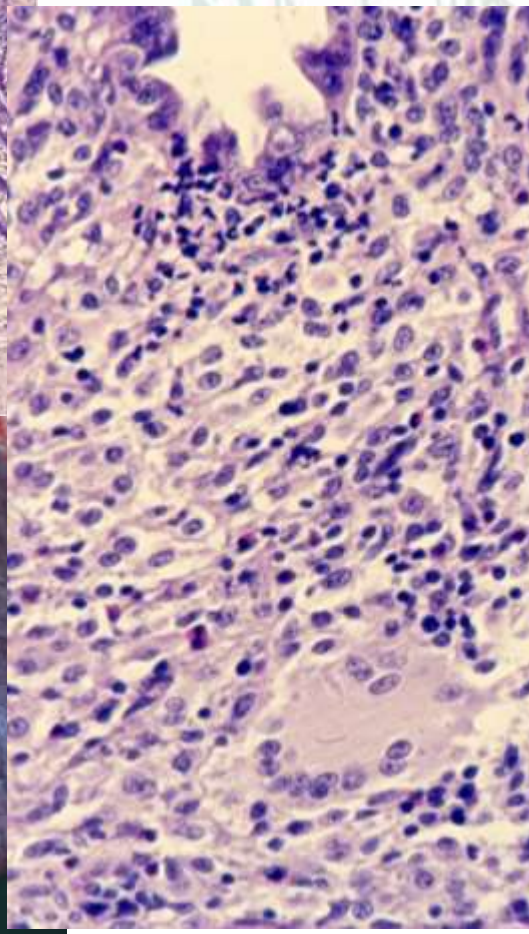
PCV2 enteric disease

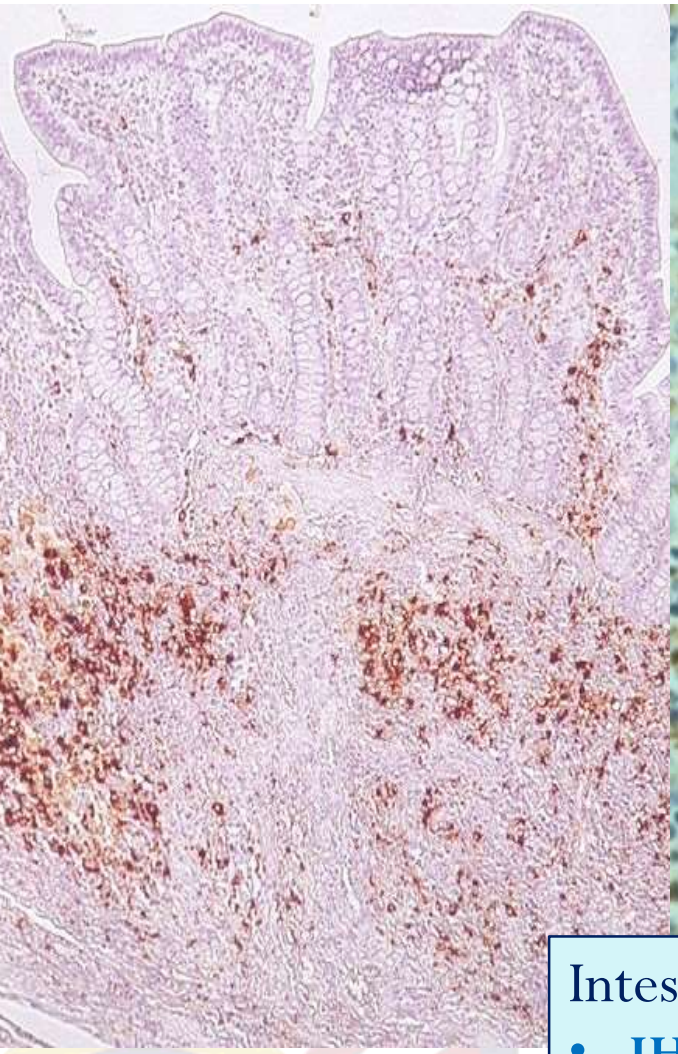
- Diarrhea in growing-finishing pigs
- Ileum and first sections of colon
- Thickened mucosa and enlarged mesenteric lymph nodes
- Granulomatous enteritis with viral antigen/genome
- Lymphocyte depletion with granulomatous inflammation in Peyer´s patches
  - Not in other lymphoid tissues!!!

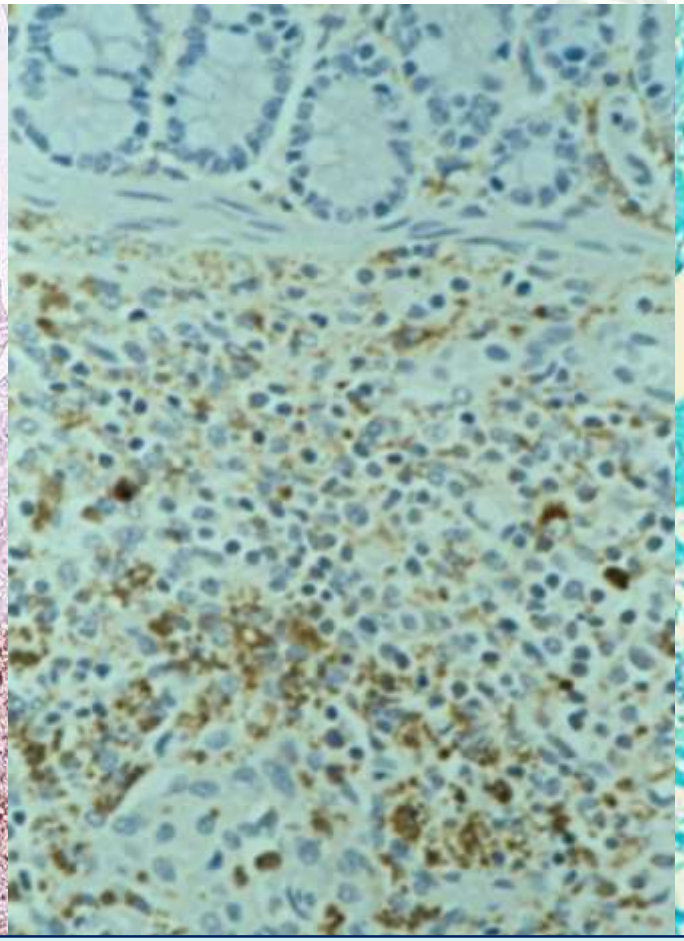
PCV2 enteric disease

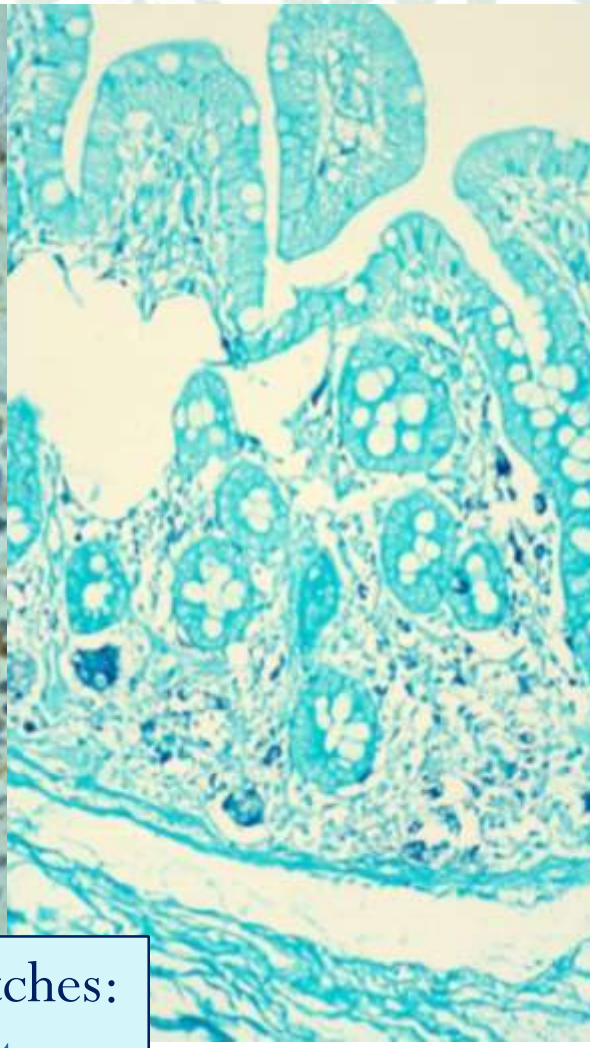




Granulomatus enteritis (PCV2)







Intestinal mucosa and Peyer´s patches:

- **IHC/ISH:** moderate to high amount
- **qPCR:** $\geq 10^9$

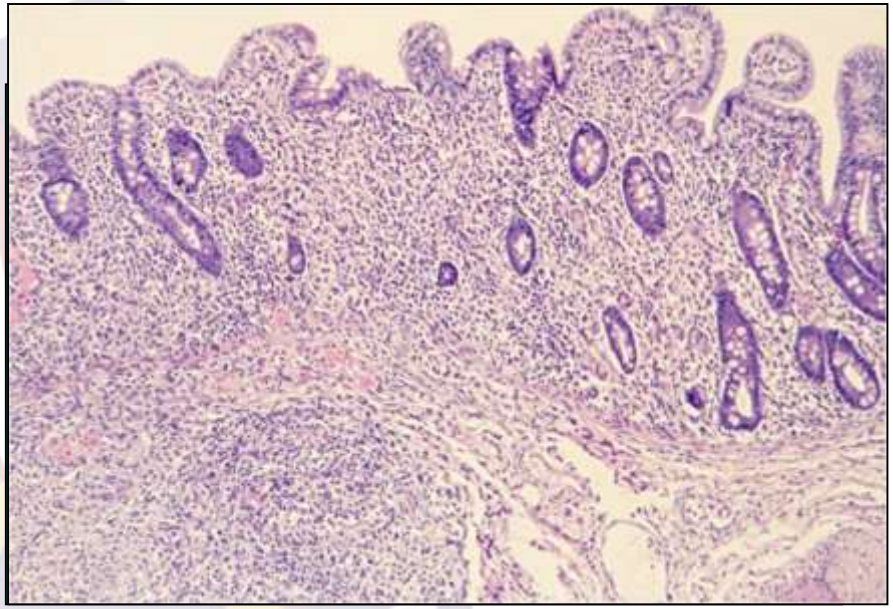
Lymphoid tissues:

Negative or low amount

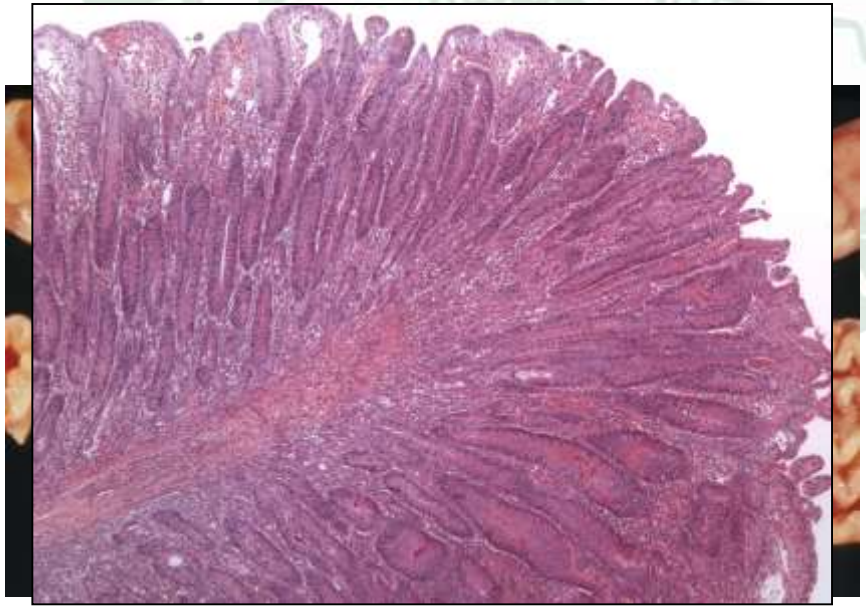
Granulomatous enteritis with abundant PCV2 antigen/genome

PCV2 enteric disease

- Differential diagnosis
  - Intestinal adenomatosis caused by *Lawsonia intracellularis*



Granulomatous enteritis (PCV2)



Intestinal adenomatosis (*L. intracellularis*)

Porcine Circovirus Associated Diseases (PCVAD)

- PCV2 systemic disease PCV2
- PCV2 reproductive disease
- PCV2 lung disease
- PCV2 enteric disease
- Porcine dermatitis and nephropathy syndrome (PDNS)

Porcine dermatitis and nephropathy syndrome (PDNS)

- Dermatitis
  - Irregular, red-to-purple macules and papules
  - Hind limbs and perineal area
  - Subcutaneous hemorrhages and edema
  - Recovered animals: scars
- Nephropathy
  - Enlarged kidneys (bilateral)
  - Small cortical petechiae
  - Renal pelvis edema
- Other lesions (enlarged lymph nodes and occasionally, spleen infarcts)
- Nursery, growing and adult pigs
- Prevalence <1%, sometimes higher
- Mortality ≈ 100% in older than 3 months and 50% in youngers

Porcine dermatitis and nephropathy syndrome (PDNS)

Diagnosis

- Hemorrhagic and necrotizing skin lesions (hind limbs and perineal area), and/or swollen and pale kidneys with generalized cortical petechiae
- Systemic necrotizing vasculitis and necrotizing fibrinous glomerulonephritis

Hemorrhagic and necrotizing skin lesions (PDNS)

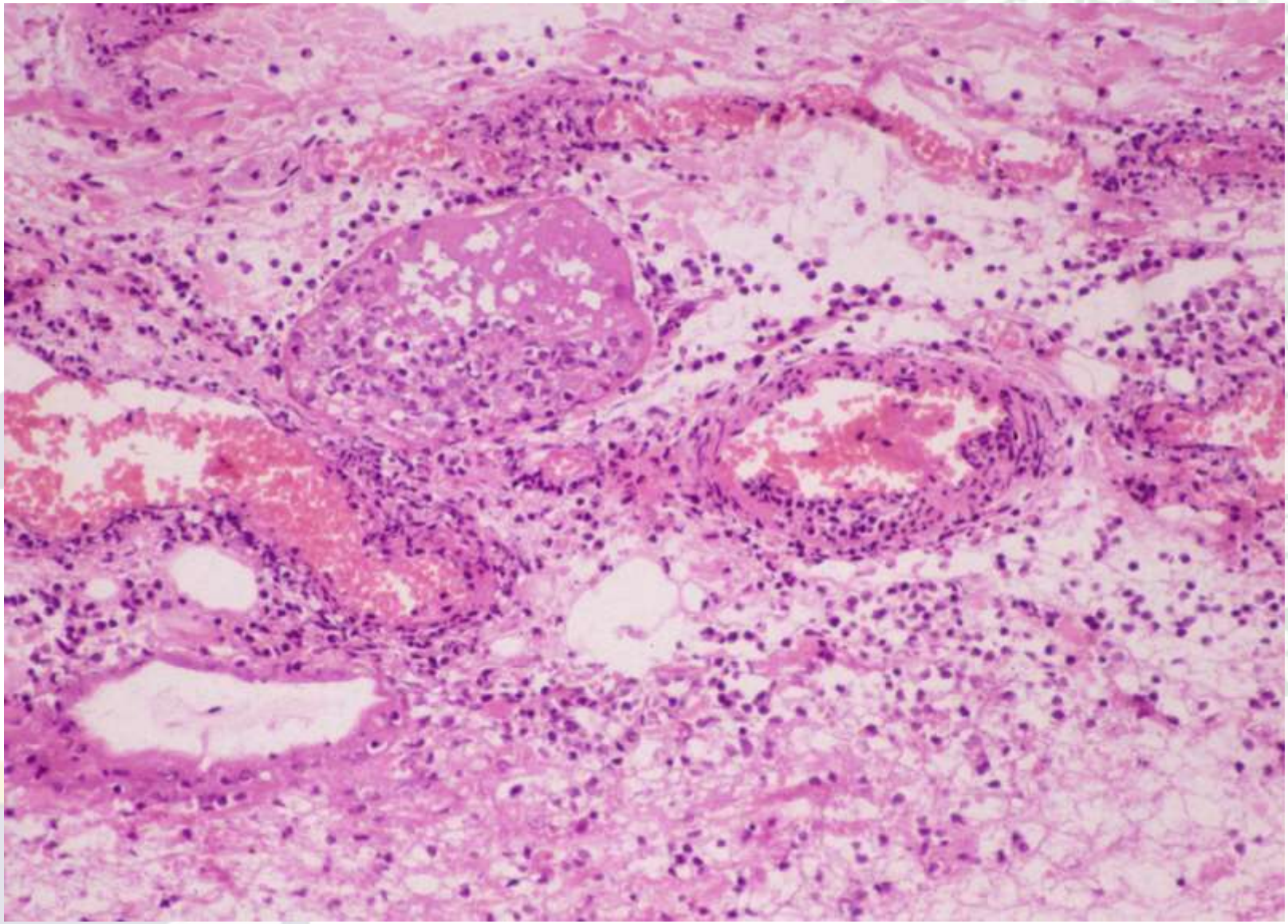
Hemorrhagic and necrotizing skin lesions (PDNS)

Cortical petechiae in kidney (PDNS)

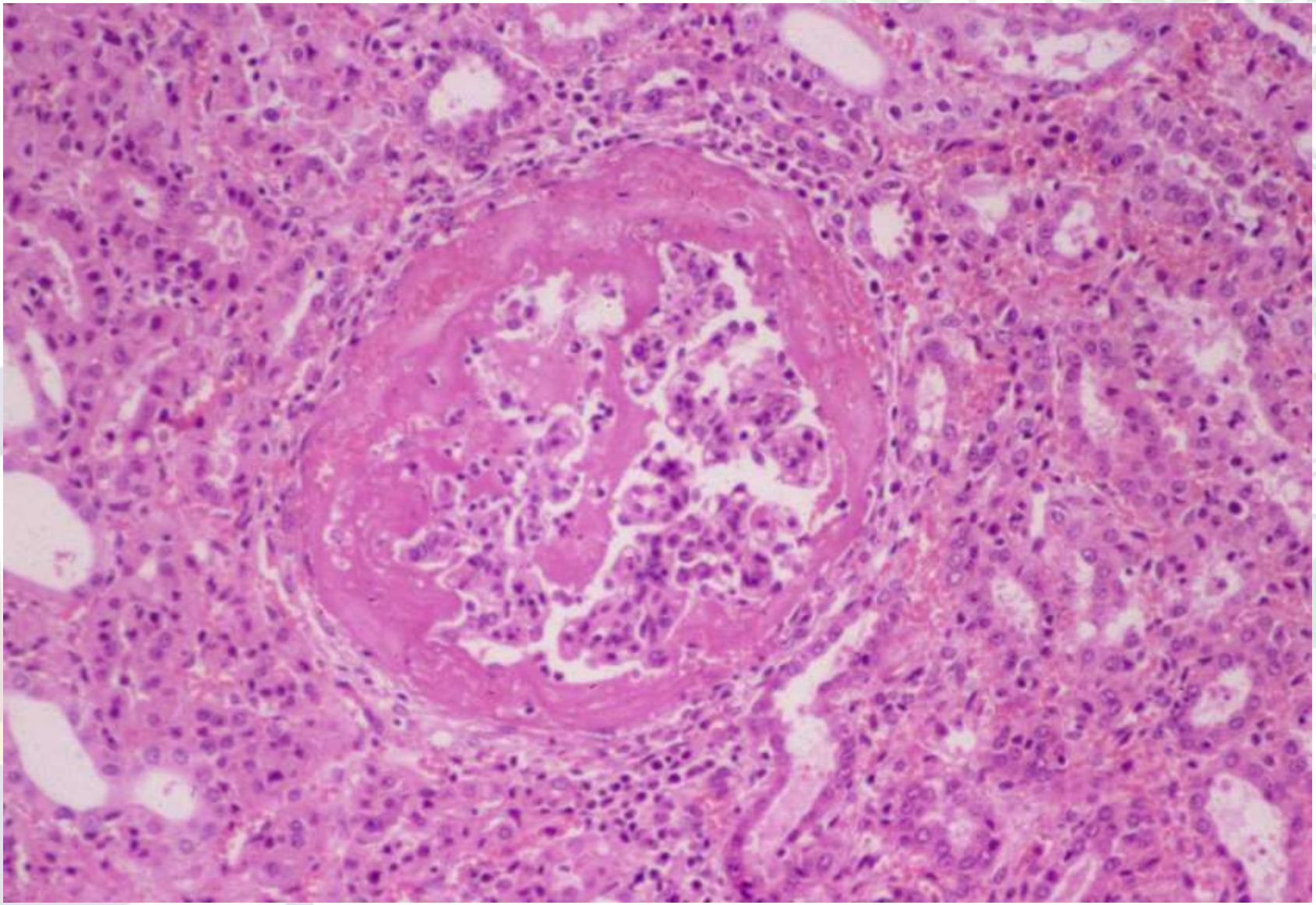
Porcine dermatitis and nephropathy syndrome (PDNS)

Diagnosis

- Hemorrhagic and necrotizing skin lesions (hind limbs and perineal area), and/or swollen and pale kidneys with generalized cortical petechiae
- Systemic necrotizing vasculitis and necrotizing fibrinous glomerulonephritis



Necrotizing vasculitis (PDNS)



Necrotizing fibrinous glomerulonephritis (PDNS)

Porcine dermatitis and nephropathy syndrome (PDNS)

Diagnosis

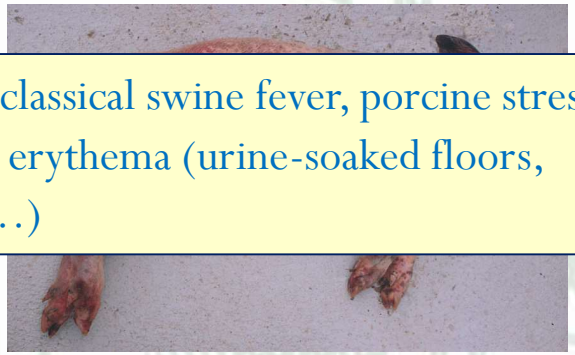
- Hemorrhagic and necrotizing ski… (hind limbs and perineal area), a… wollen and pale kidneys with generali… ical petechiae
- Systemic ne… g vasculitis and necrotizing fibrino… erulonephritis

PCV2 detection is not a diagnostic requirement

PCV2 reproductive disease

- Differential diagnosis

Other diseases: classical swine fever, porcine stress syndrome, transit erythema (urine-soaked floors, chemical burns, …)



Pityriasis rosea (unknown cause)

Septicemia

Erysipelas

African swine fever

PCV2 reproductive disease

- Differential diagnosis

PDNS

Septicemia

African swine fever

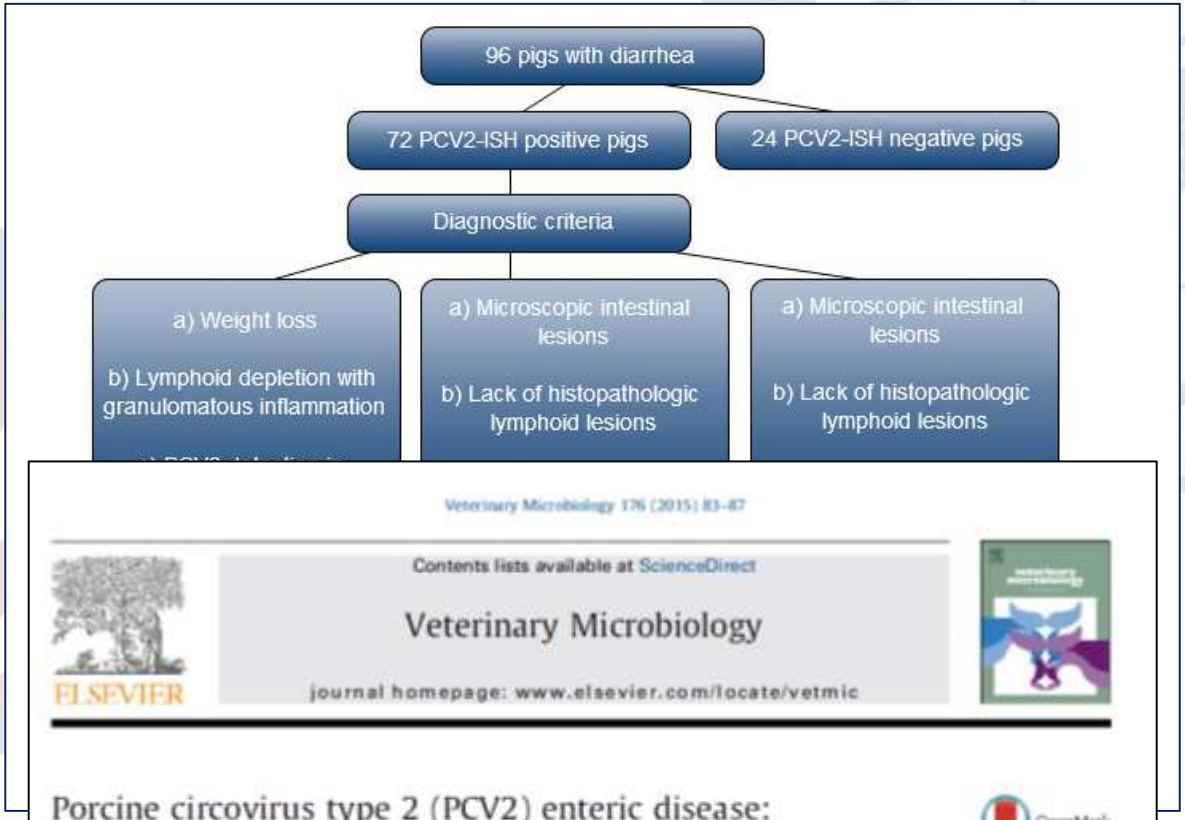
Classical swine fever

PCV2 subclinical infections

- Most common manifestation
- No evident clinical signs
- Decreased average daily gain
- Lymphoid tissues
  - None or slight lymphocyte depletion with granulomatous inflammation
  - None or low amount of PCV2 (IHC/ISH)
    - **qPCR:** $<10^9$ (10^4-10^8 in serum)
- Vaccination improves productive parameters (field evidences)
- Decreased vaccine efficacy (experimental conditions)

PCV2 associated diseases diagnosis

- Example of diagnostic criteria for PCV2 infected pigs



Laboratory techniques offered in diagnostic laboratories in Spain:
- ISH
- qPCR
- Sequencing

New circoviruses

PCV3 and PCV4

- **PCV3** (USA, 2015/2016) by metagenomic analisys
  - Associated with:
    - Reproductive disease (abortions, stillbirths, weak-borns and momifications)
    - PDNS
    - Myocarditis (fetuses and weak-born neonatal piglets)
    - Encephalitis (weak-born neonatal piglets)
    - Systemic periarteritis (weaned pigs)
  - Detected in sick and healthy animals
  - Circulating in the pig population in an endemic way
  - Prevalence of associated diseases unknown

New circoviruses

PCV3 and PCV4

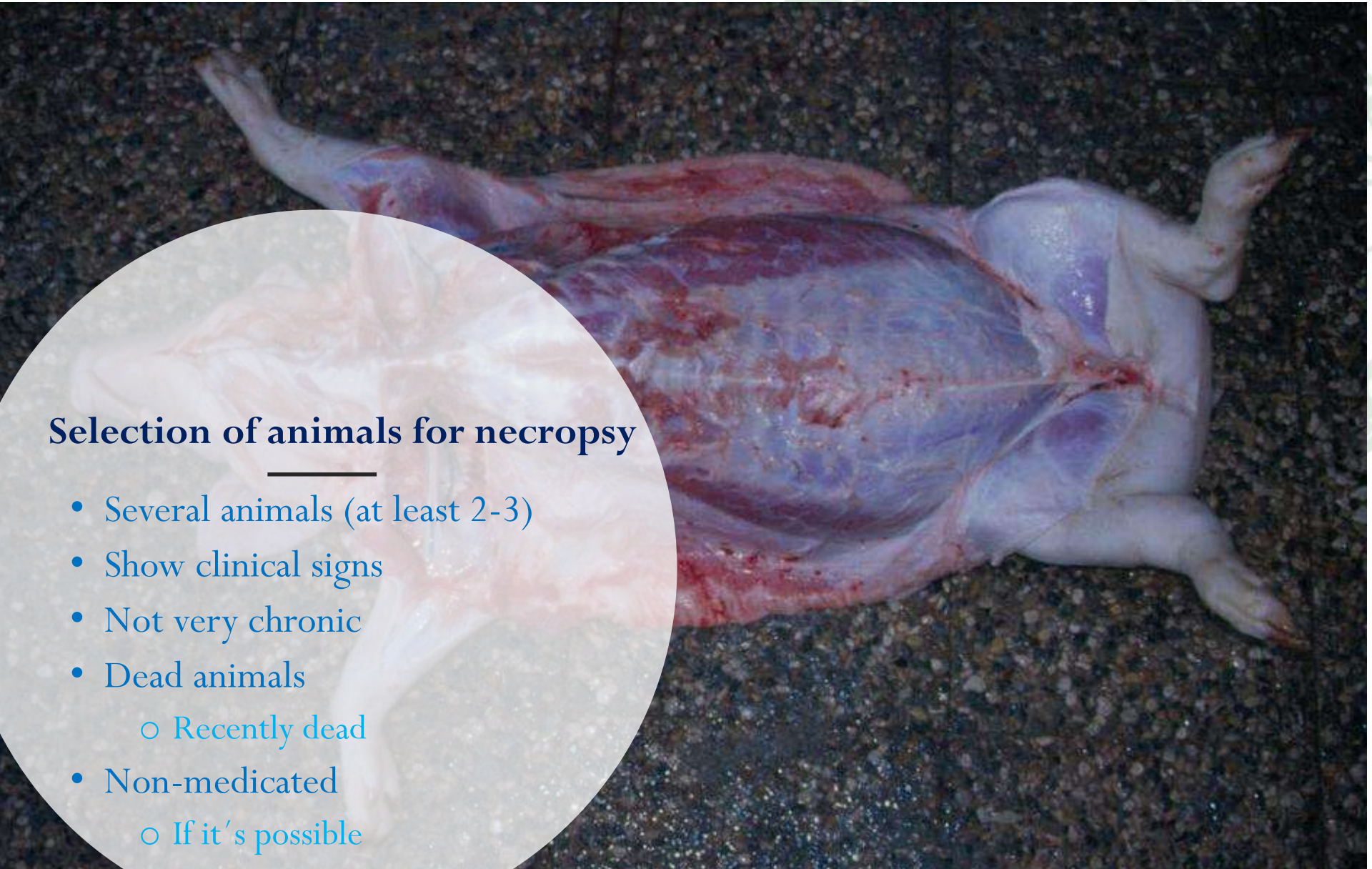
- **PCV4** (China, 2019)
  - Respiratory signs
  - Enteric signs
  - PDNS

- Clinical significance and pathogenesis needs further investigation

Sampling

- Selection of animals for necropsy
- Histopathology
- PCR
- Serology
- Bacteriology

Sampling



Selection of animals for necropsy

- Several animals (at least 2-3)
- Show clinical signs
- Not very chronic
- Dead animals
  - Recently dead
- Non-medicated
  - If it's possible

Sampling

- Selection of animals for necropsy
- Histopathology
- PCR
- Serology
- Bacteriology

Sampling

Lung
- Several lobes

Hollow organs
- 5-10 cm
- Open longitudinally

Histopathology

- Take samples as soon as possible
- Samples with lesioned and non-lesioned tissue
- **No freezing**
- Fixation: immersion in 10% buffered formalin
  - 1 part formalin and 9 tap water
- Tissue:formalin: 1:10
- No refrigeration
- Plastic can with hermetic closure and proper identification
- Thickness maximum 1 cm

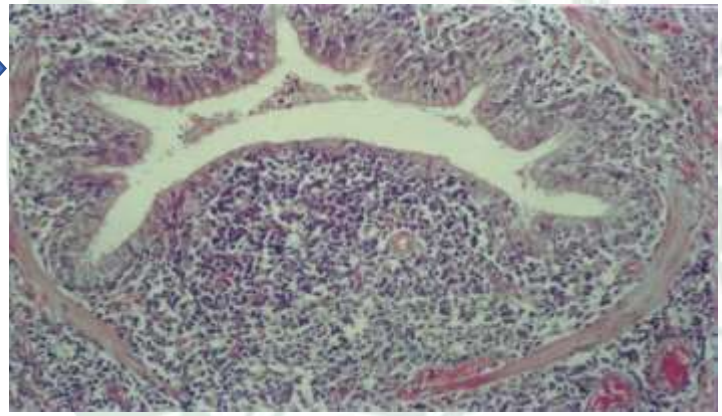
Sampling

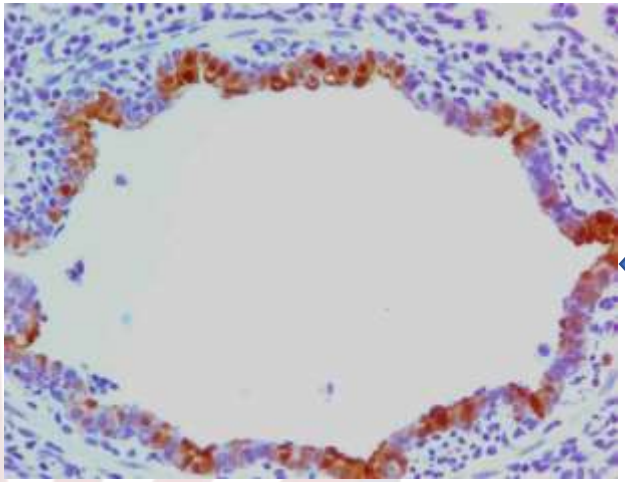
- Histopathology

Sampling

- Histopathology

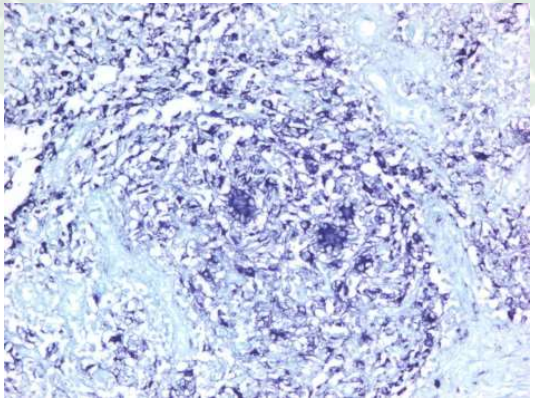
Lesions

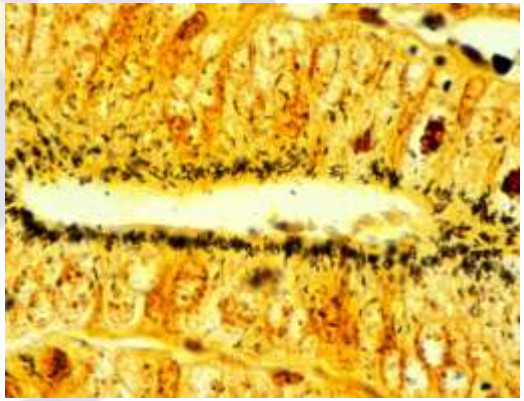




Immunohistochemistry

In situ hibridization



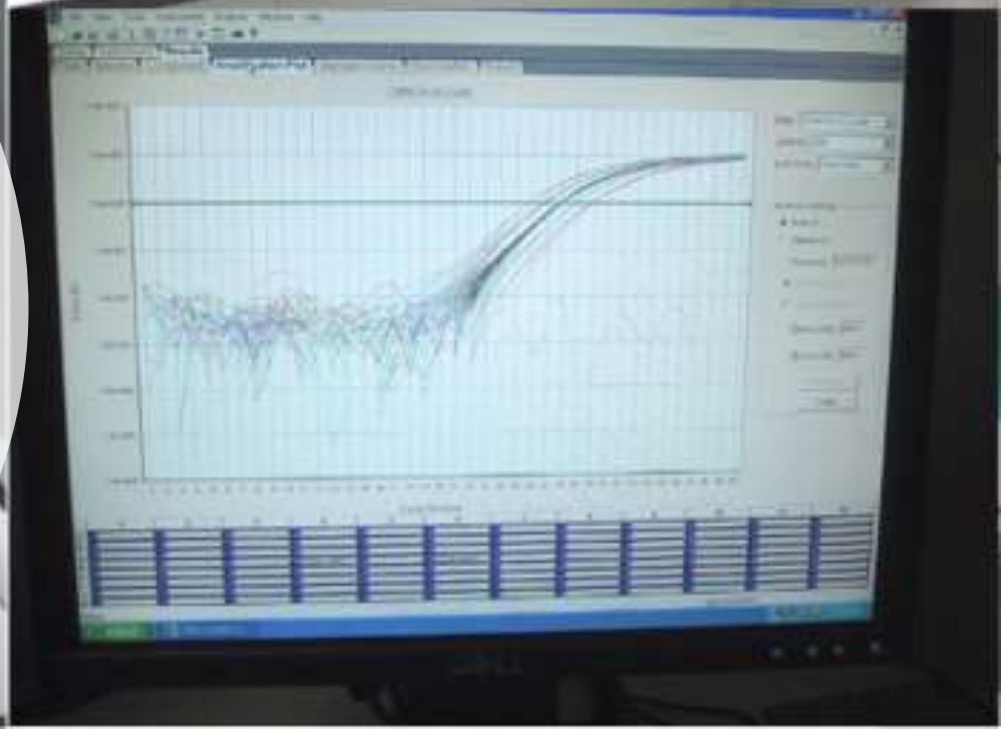


Special stains: Warthin-Starry, PAS, grocott,…

Sampling

- Selection of animals for necropsy
- Histopathology
- PCR
- Serology
- Bacteriology

Sampling



PCR

- Sterile plastic containers or bags
- Proper identification
- Suitable for freezing
  - Tissue
  - Nasal, tracheal, bronchial swabs
  - Bronchoalveolar lavages
  - Oral fluids
  - Feces,…….

Sampling

- Selection of animals for necropsy
- Histopathology
- PCR
- Serology
- Bacteriology

Sampling

Serology

- Proper identification
- Better send serum (plastic tubes)
- Serum suitable for freezing
- Do not freeze collection tube with clot

Sampling

- Selection of animals for necropsy
- Histopathology
- PCR
- Serology
- Bacteriology

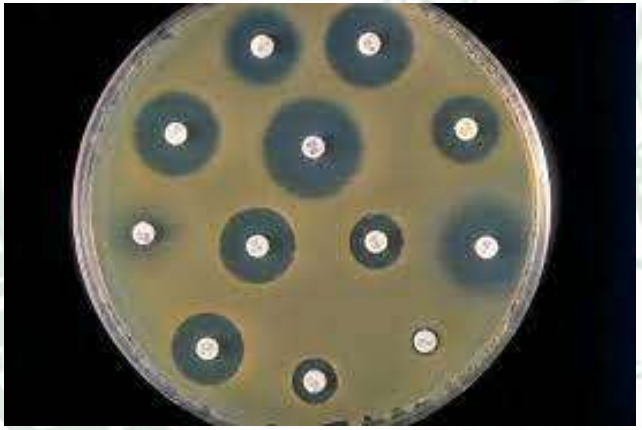
Sampling

Bacteriology

- Sterile plastic cans or bags
- Proper identification
- Tissue, swabs (growth medium)
- Hollow organs with tied margins
- Do not mix intestine samples with others in the same container
- Refrigeration

Sampling

- Bacteriology
  - Bacterial isolation
  - Antibiogram
    - Sensitivity/Resistance
  - Minimum inhibitory concentration (MIC)
    - Minimum concentration that inhibits bacterial growth
  - Autogenous vaccines

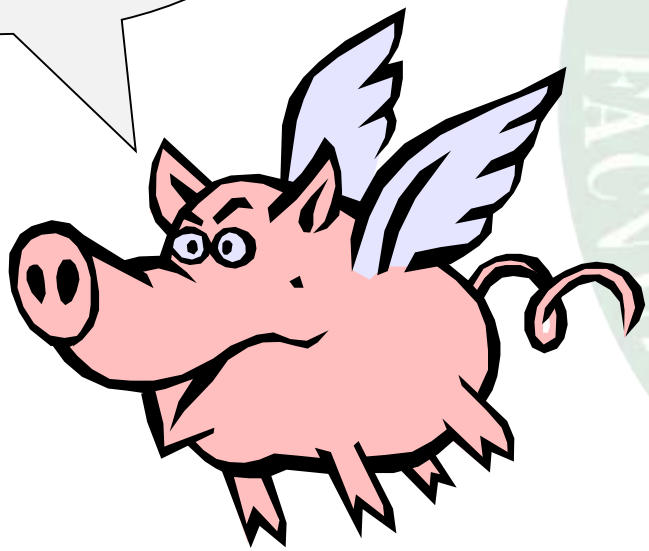


Sampling

- Selection of animals for necropsy
- Histopathology
- PCR
- Serology
- Bacteriology

IMPORTANT!!! When submitting different samples for several diagnostic approaches use independent containers to avoid cross-contamination among them that may invalidate the results obtained

¡¡THANK YOU FOR YOUR ATTENTION!!



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