



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

**“L’istopatologia in diagnostica suina:
quando una metodica diventa discriminante”**

**Quando una metodica
diventa discriminante?**

**L’esempio del PCV2:
stesso agente eziologico
ma malattie diverse**

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PCV2 and (PCVD or PCVAD)

Table 1

Summary of historical events related with knowledge on porcine circoviruses.

Year	Event	Reference ^a
1962	Earliest retrospective evidence of porcine circovirus type 2 (PCV2) detection by PCR	Jacobsen et al., 2009
1974	The subsequently named porcine circovirus type 1 (PCV1) was initially discovered as a potential picornavirus-like agent in permanent pig kidney cell lines	Tischer et al., 1974
1982	First description of porcine circovirus (nowadays PCV1) as a very small porcine virus with circular single-stranded DNA	Tischer et al., 1982
1985	Earliest retrospective evidence of PCV2-systemic disease (PCV2-SD)	Jacobsen et al., 2009
1996	First description of a new, sporadic disease named postweaning multisystemic wasting syndrome (referred in this review as PCV2-SD) – initial cases from 1991 to 1994. Association with a porcine circovirus-like agent (subsequently identified as PCV2)	Clark, 1996; Harding, 1996
1997	First descriptions of PCV2-SD as severe disease outbreaks in Europe	LeCann et al., 1997; Segalés et al., 1997
1998	First isolation and characterization of PCV2 isolates	Allan et al., 1998; Ellis et al., 1998
1998	First sequences of complete PCV2 genome available	Hamel et al., 1998; Meehan et al., 1998; Morozov et al., 1998
1997–99	First descriptions of PCV2-SD as severe disease outbreaks in Asia	Choi et al., 2000; Sato et al., 2000
1999	First experimental reproduction of PCV2-SD by means of PCV2 and porcine parvovirus co-inoculation	Allan et al., 1999
2004	First PCV2 vaccine available in France and Germany; inactivated, adjuvanted product temporarily licensed for its use in sows	Segalés et al., 2005
2004–05	First descriptions of PCV2-SD as severe disease outbreaks in North-America	Carman et al., 2006
2006	First year of availability of PCV2 vaccines in North-America; products licensed for using in piglets (USA and Canada) and sows (Canada)	Opriessnig et al., 2007
2007	First year of general availability of PCV2 vaccines all over the world	Chae, 2012
2008	Formal proposal of nomenclature and definition of PCV2 genotypes by a EU consortium on PCVD	Segalés et al., 2008

^a Reference in which the event was described or quoted.

PCV2 and (PCVD or PCVAD)

Malattie

Infezioni subcliniche

PMWS

PDNS

Reproductive failure

PRDC

- PNP
- APE (Acute pulmonary edema)

Necrotizing lymphadenitis

Granulomatous enteritis

PCVD - Europa

PCV2-SI

PCV2-systemic disease (SD)

PCV2-PDNS

PCV2-reproductive disease (RD)

PCV2-lung disease (LD)

Necrotizing lymphadenitis

PCV2 enteric diseases (ED)

PCVAD - America

PCV2-SI

- Necrotizing lymphadenitis?

PCV2A-systemic infection

PCV2A-PDNS

PCV2A-reproductive failure

PCV2A-respiratory disease

Necrotizing lymphadenitis

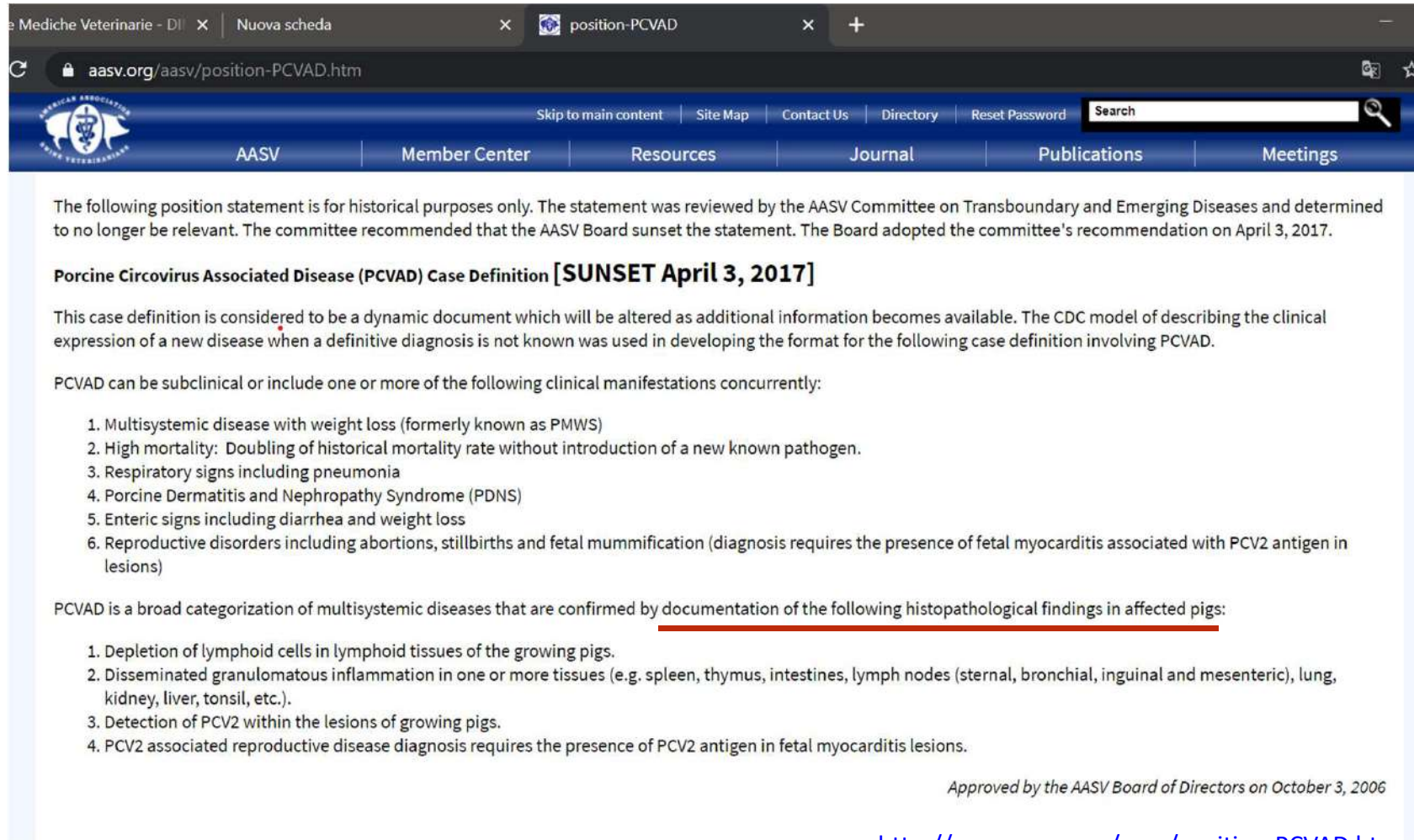
PCV2A-enteritis

Segales, Virus Res., 2012

Oppriessnig et al., J Vet Diagn Invest, 2007

<http://www.aasp.org/aasv/position-PCVAD.htm>

PCV2 e istologia



The screenshot shows a web browser window with the URL aasv.org/aasv/position-PCVAD.htm. The page header includes the AASV logo and navigation links: AASV, Member Center, Resources, Journal, Publications, and Meetings. A search bar is also present. The main content area contains the following text:

The following position statement is for historical purposes only. The statement was reviewed by the AASV Committee on Transboundary and Emerging Diseases and determined to no longer be relevant. The committee recommended that the AASV Board sunset the statement. The Board adopted the committee's recommendation on April 3, 2017.

Porcine Circovirus Associated Disease (PCVAD) Case Definition [SUNSET April 3, 2017]

This case definition is considered to be a dynamic document which will be altered as additional information becomes available. The CDC model of describing the clinical expression of a new disease when a definitive diagnosis is not known was used in developing the format for the following case definition involving PCVAD.

PCVAD can be subclinical or include one or more of the following clinical manifestations concurrently:

1. Multisystemic disease with weight loss (formerly known as PMWS)
2. High mortality: Doubling of historical mortality rate without introduction of a new known pathogen.
3. Respiratory signs including pneumonia
4. Porcine Dermatitis and Nephropathy Syndrome (PDNS)
5. Enteric signs including diarrhea and weight loss
6. Reproductive disorders including abortions, stillbirths and fetal mummification (diagnosis requires the presence of fetal myocarditis associated with PCV2 antigen in lesions)

PCVAD is a broad categorization of multisystemic diseases that are confirmed by documentation of the following histopathological findings in affected pigs:


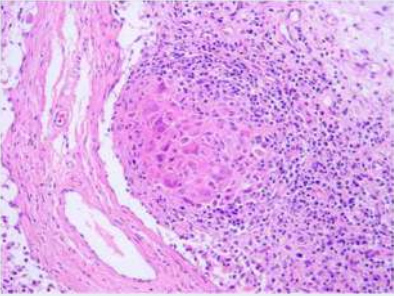
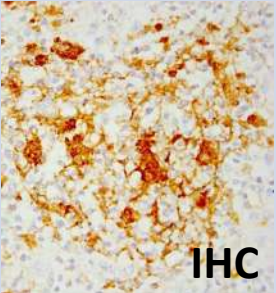
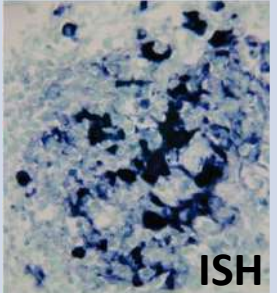
1. Depletion of lymphoid cells in lymphoid tissues of the growing pigs.
2. Disseminated granulomatous inflammation in one or more tissues (e.g. spleen, thymus, intestines, lymph nodes (sternal, bronchial, inguinal and mesenteric), lung, kidney, liver, tonsil, etc.).
3. Detection of PCV2 within the lesions of growing pigs.
4. PCV2 associated reproductive disease diagnosis requires the presence of PCV2 antigen in fetal myocarditis lesions.

Approved by the AASV Board of Directors on October 3, 2006

<http://www.aasv.org/aasv/position-PCVAD.htm>

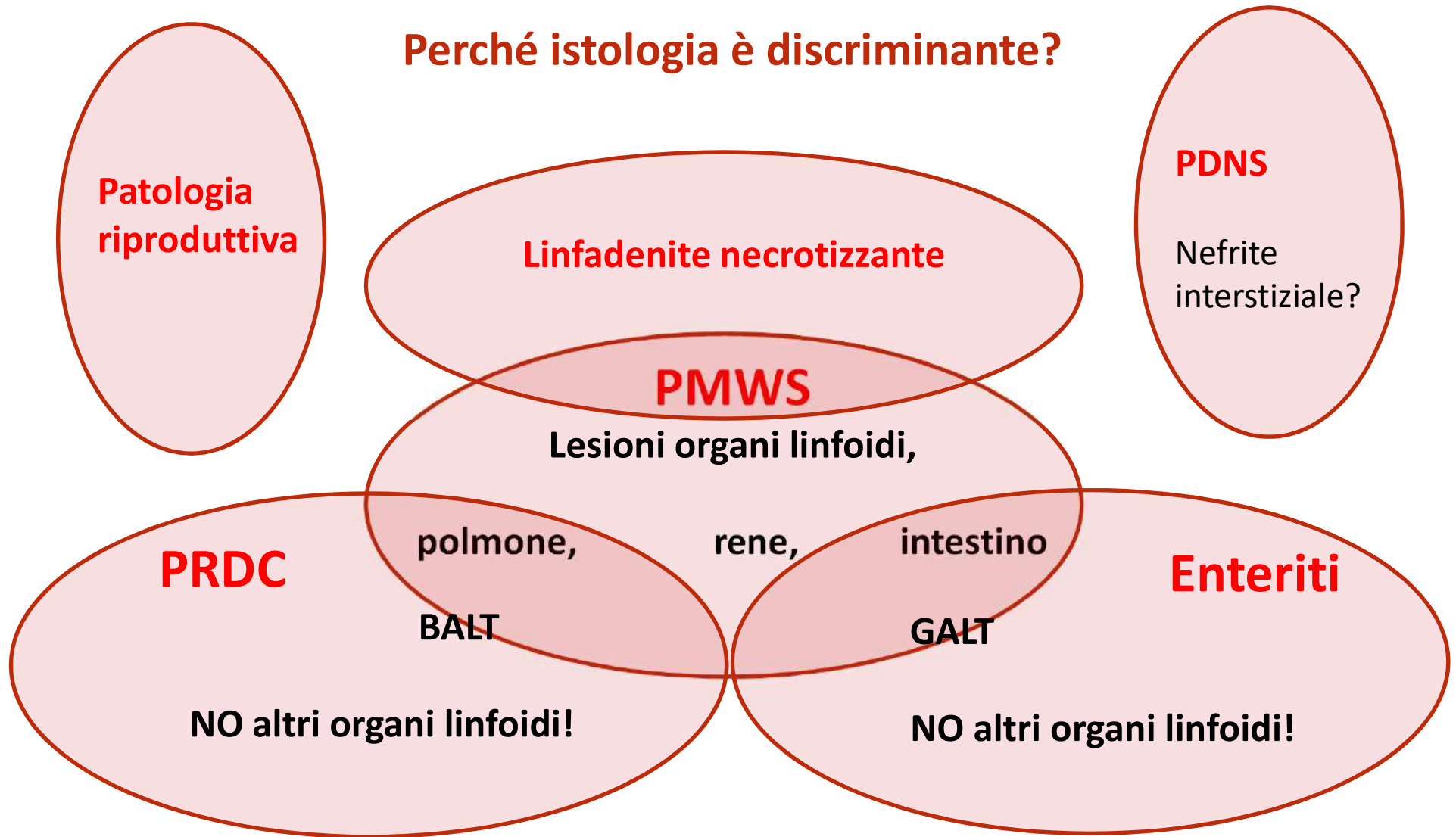


Iter diagnostico nelle PCVD/PCVAD

step		... PMWS	
1	Sintomatologia	Deperimento, perdita peso, difficoltà respiratorie	
2	Lesione istologica	Deplezione linfocitaria e/o istiociti centrofollicolari	
3	Colocalizzazione lesione-PCV2	Ag dei capsomeri (IHC) o DNA virale (ISH) nelle lesioni	 



Perché istologia è discriminante?



Perché istologia è discriminante?

Permette di escludere infezioni subcliniche =
Presenza virus ma assenza di lesioni

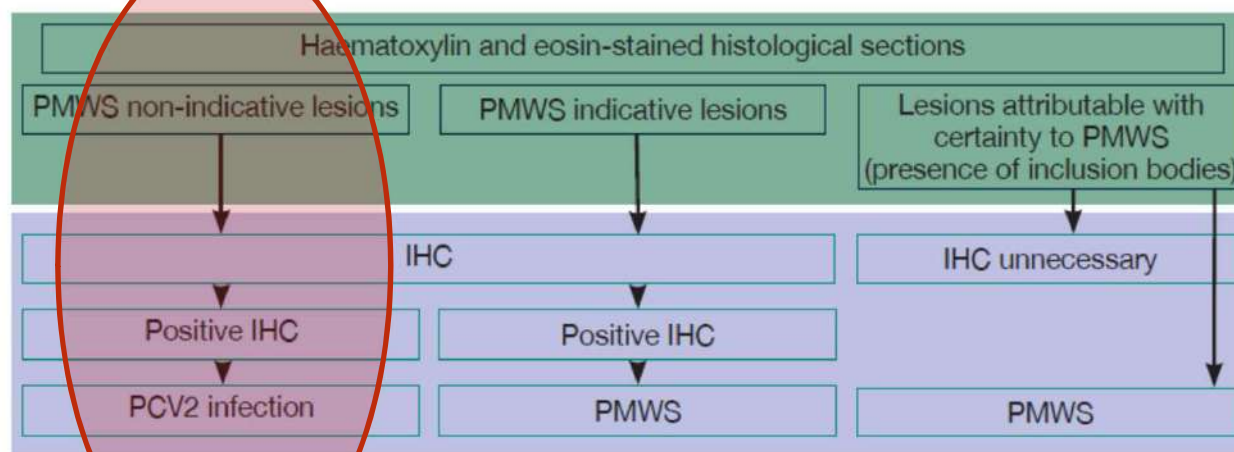
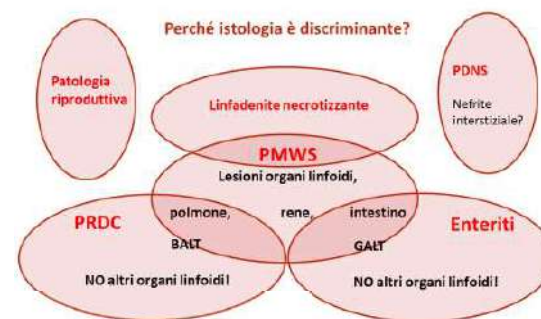


FIG 1: Diagnostic algorithm for the diagnosis of postweaning multisystemic wasting syndrome (PMWS). IHC Immunohistochemistry, PCV-2 Porcine circovirus type 2

Application of a protocol for the diagnosis of postweaning multisystemic wasting syndrome in Italy

- 61 aziende
- 217 suini

G. Sarli, F. Ostanello, F. Morandi, L. Fusaro, M. Gnudi, B. Bacci, A. Nigrelli, L. Alborali, M. Dottori, F. Vezzoli, G. Barigazzi, L. Fiorentini, V. Sala, G. Leotti, F. Joisel Vet. Rec. 2009



Perché istologia è discriminante?

TABLE 2: Numbers of farms and single pigs with the three types of diagnosis: postweaning multisystemic wasting syndrome (PMWS), porcine circovirus type 2 (PCV-2) infection (non-PMWS but PCV-2 positive [PCV-2+], or neither (non-PMWS and PCV-2 negative [PCV-2-])

Numbers of farms	Numbers of pigs that were			Total number of pigs
	Non-PMWS and PCV-2-	Non-PMWS and PCV-2+	PMWS	
Non-PMWS and PCV-2-	13	44	0	44
Non-PMWS and PCV-2+	3	7	6	13
PMWS	45	45	109	160
Total	61	96	109	217

Sarli et al., Vet. Rec. 2009



Quindi

L'approccio diagnostico alle
PCVD/PCVAD

- Deve considerare gli organi coinvolti
- Deve potere dimostrare la presenza di lesioni «specifiche»
- Deve potere dimostrare la presenza di PCV2 nelle lesioni «specifiche»

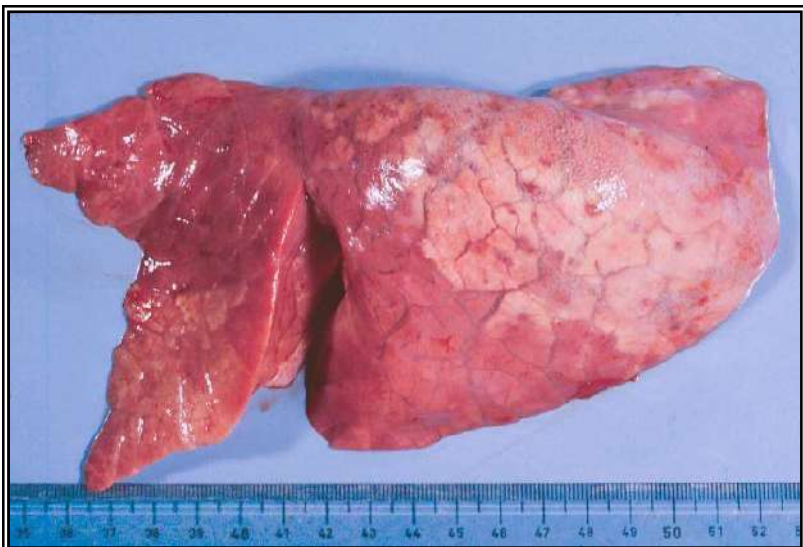
Per una corretta
identificazione della
malattia

Per una corretta
attribuzione
eziologica

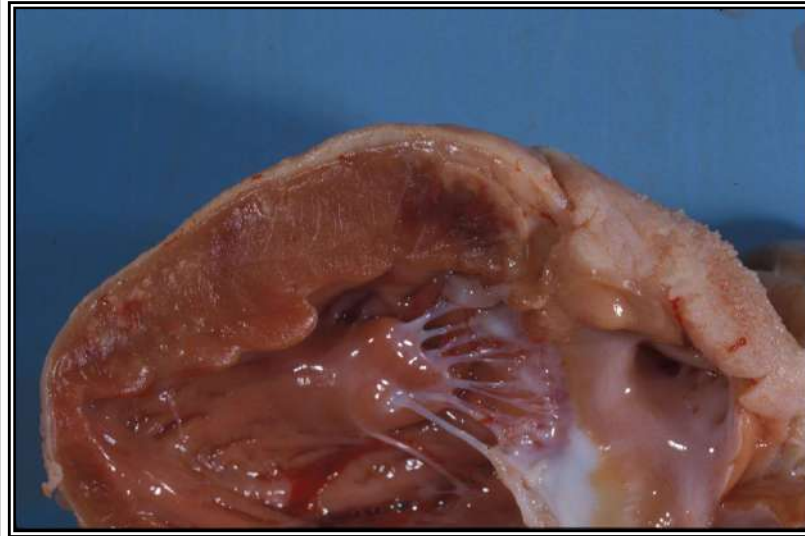


PCVD - PCVAD

Quadro macroscopico indicativo



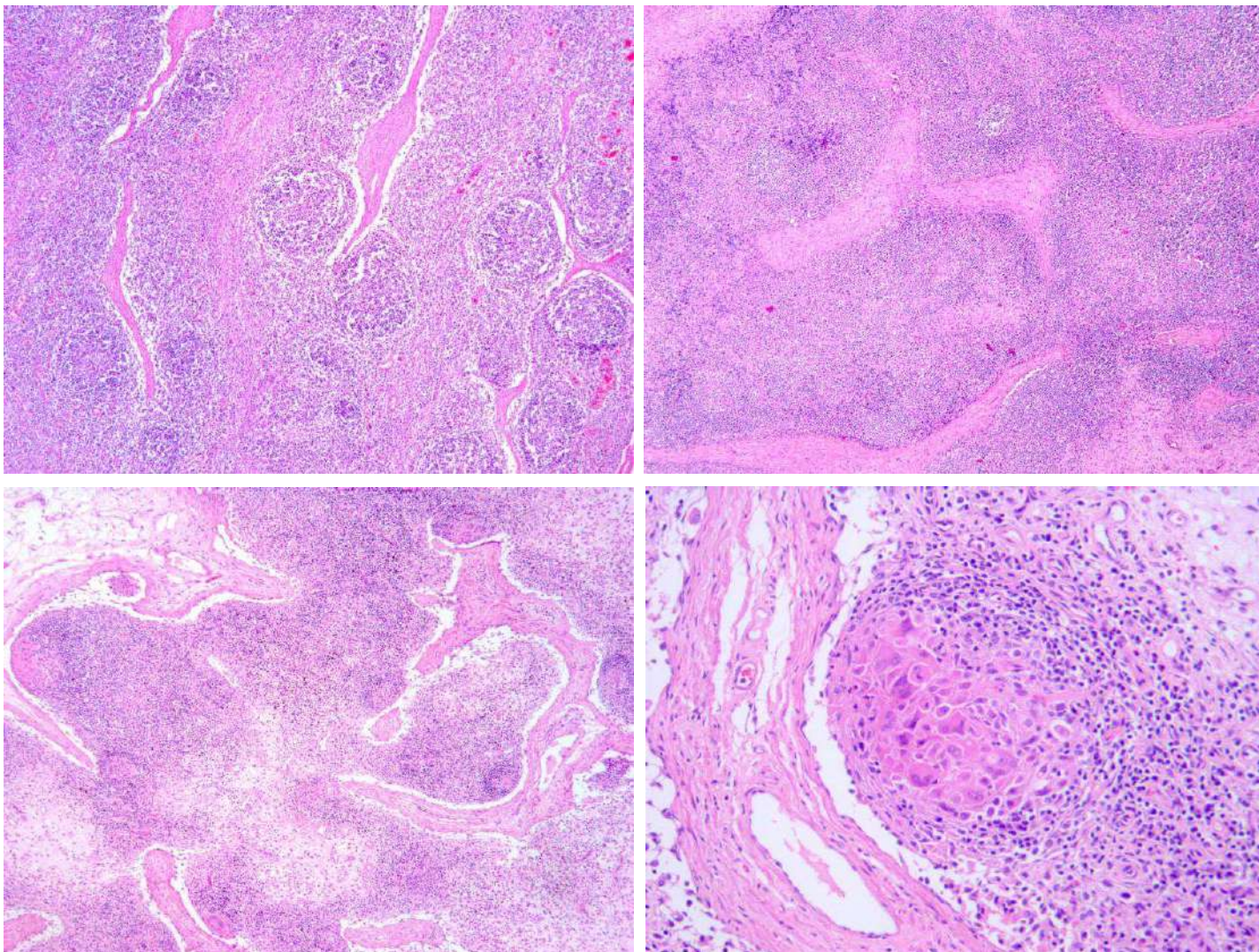
PCVD - PCVAD
Quadro macroscopico indicativo



Tessuti linfoidei

Quadro microscopico indispensabile per la diagnosi

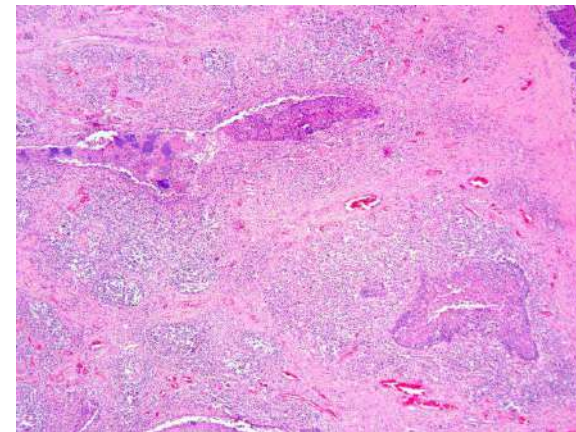
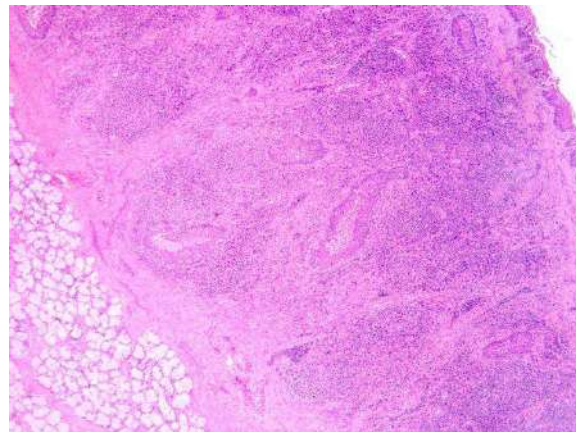
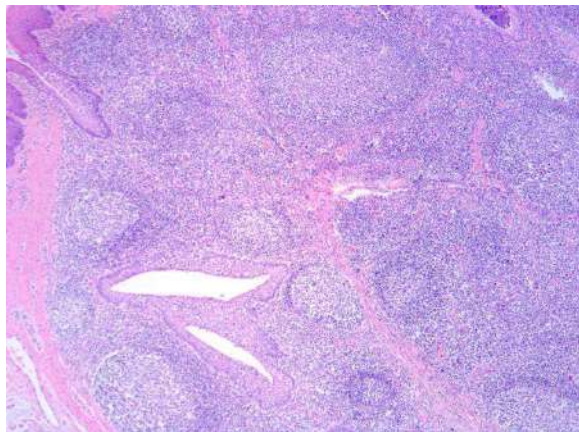
LINFONODI



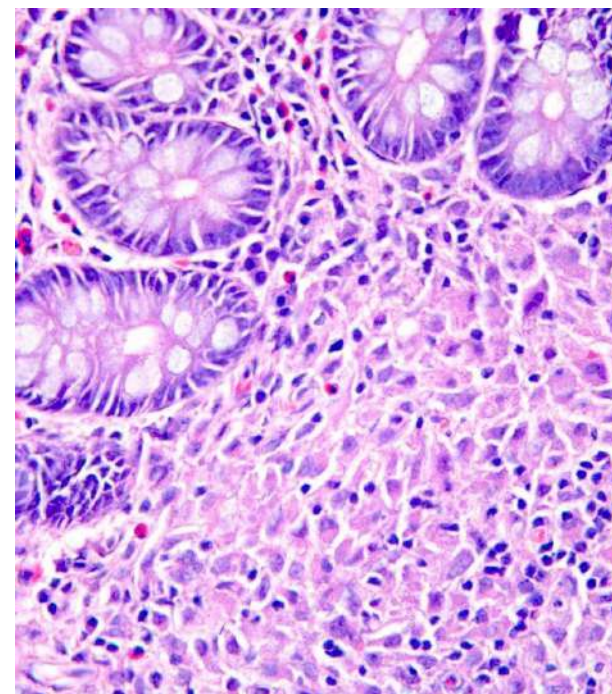
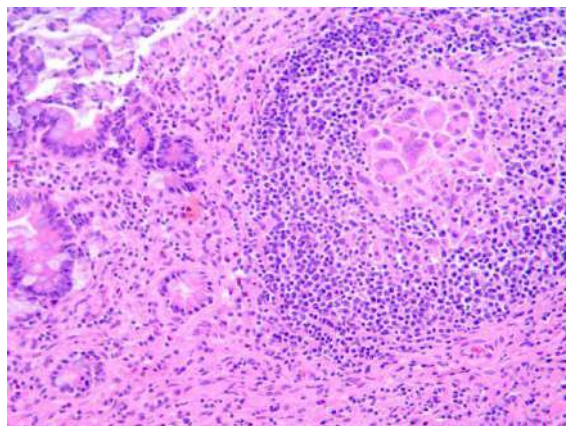
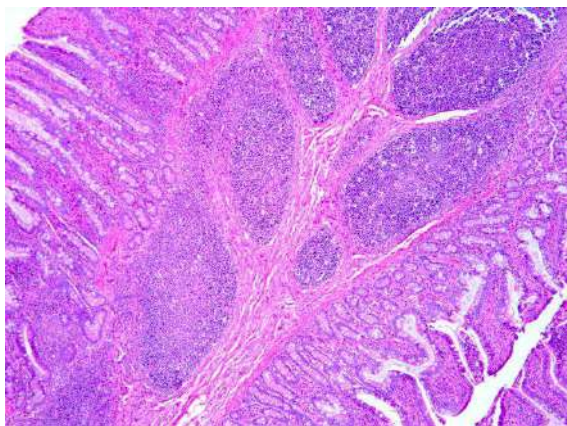
Tessuti linfoidei

Quadro microscopico indispensabile per la diagnosi

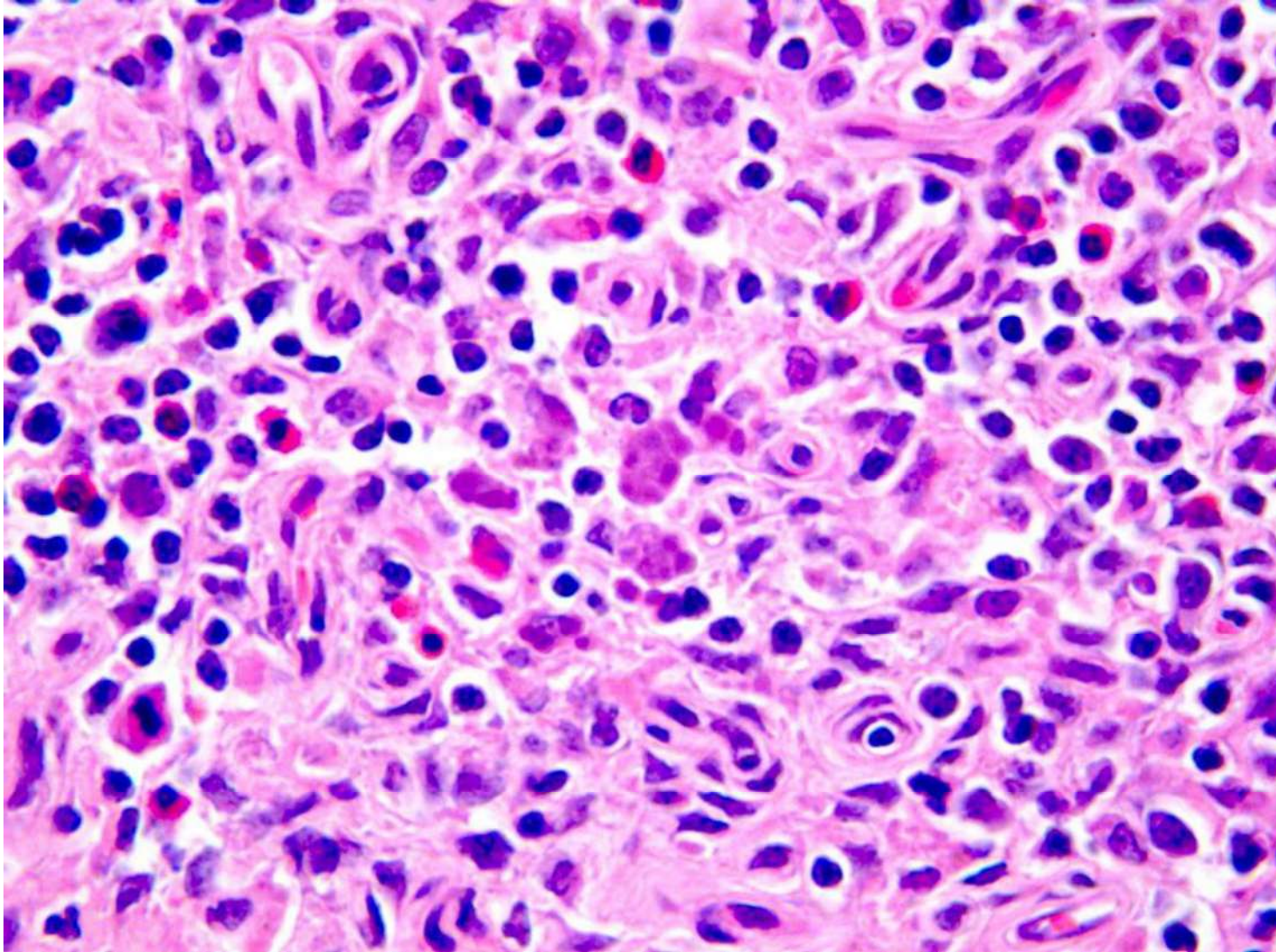
TONSILLA



ILEO

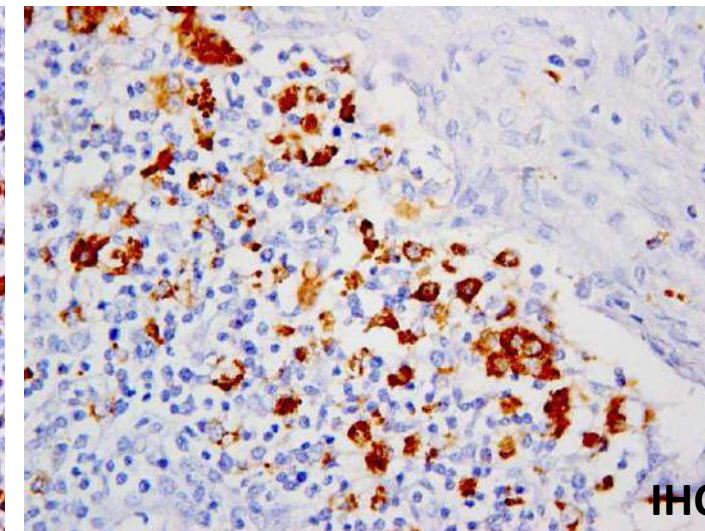
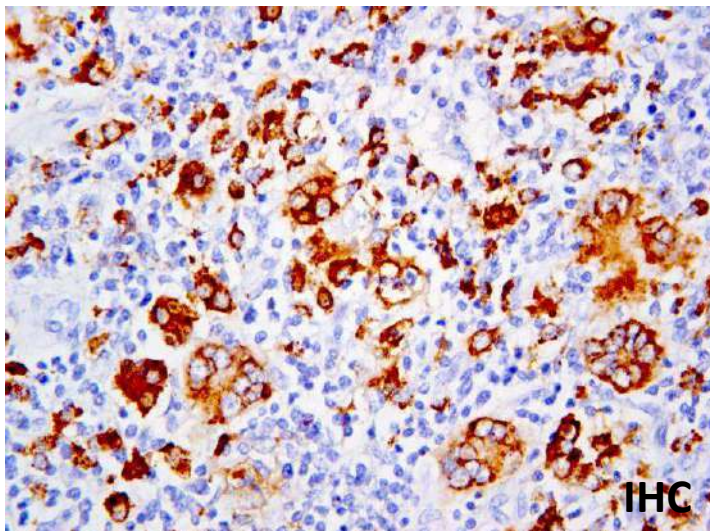
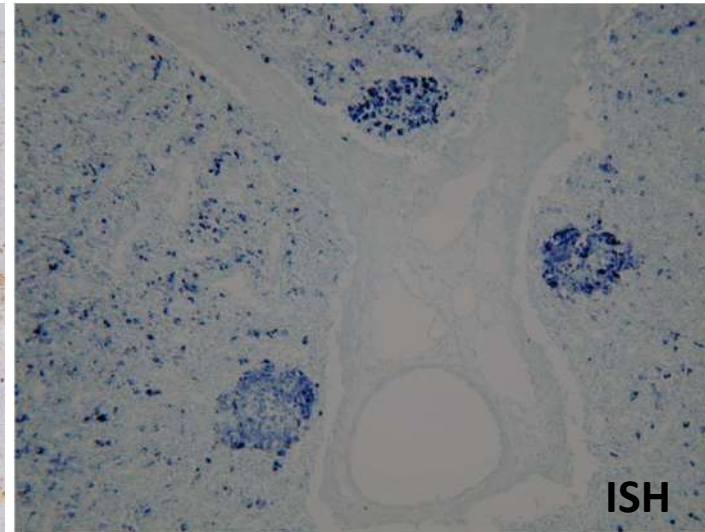
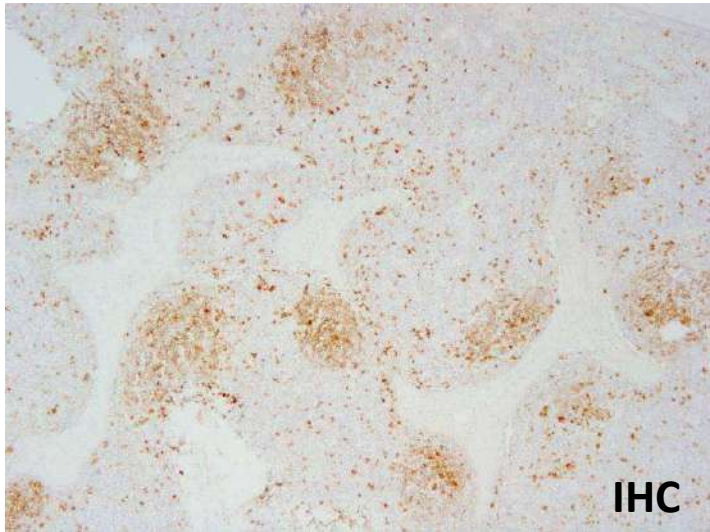


Corpi inclusi: se li vedo ho la diagnosi eziologica!



Tessuti linfoidei

Istologia: la prova del 9 IHC o ISH



Conclusione

Lesioni specifiche

- Deplezione linfocitaria
- Istiociti e cellule giganti centrollicolari

Lesioni specifiche positive per PCV2

Distribuite a diverse stazioni linfoidi (Lfn, tonsille, milza) ed eventualmente associate a infiammazione da «*interstiziale a granulomatosa*» in diversi parenchimi

=

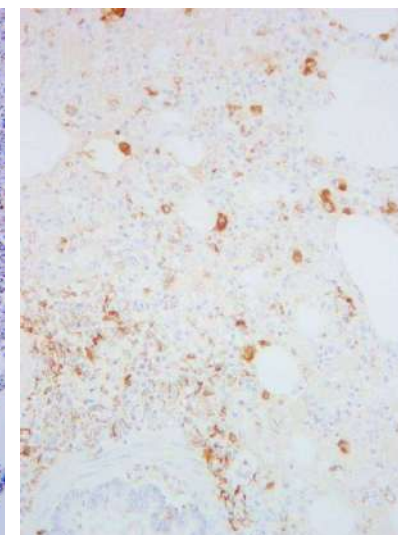
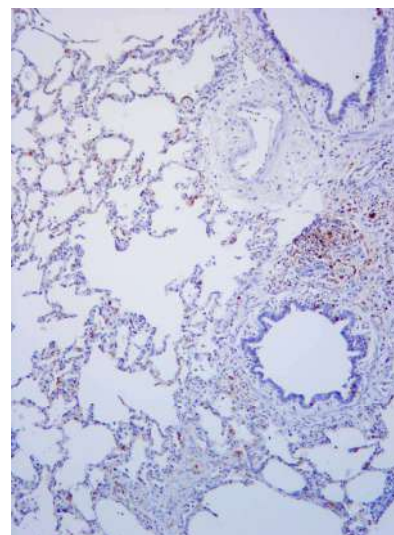
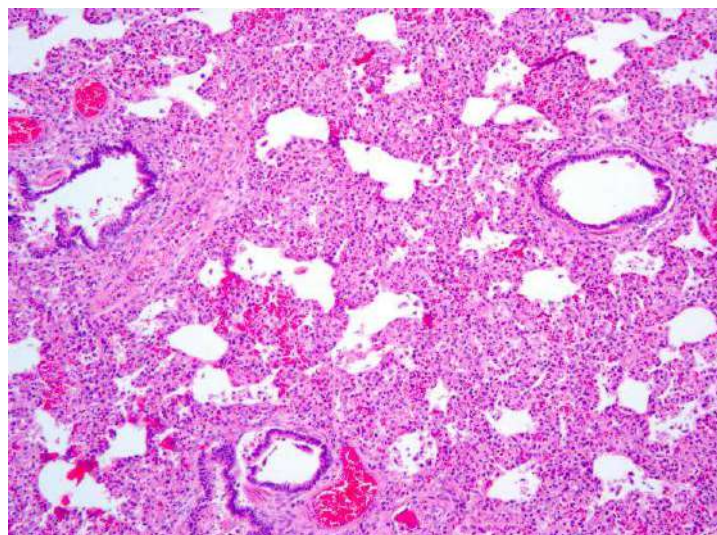
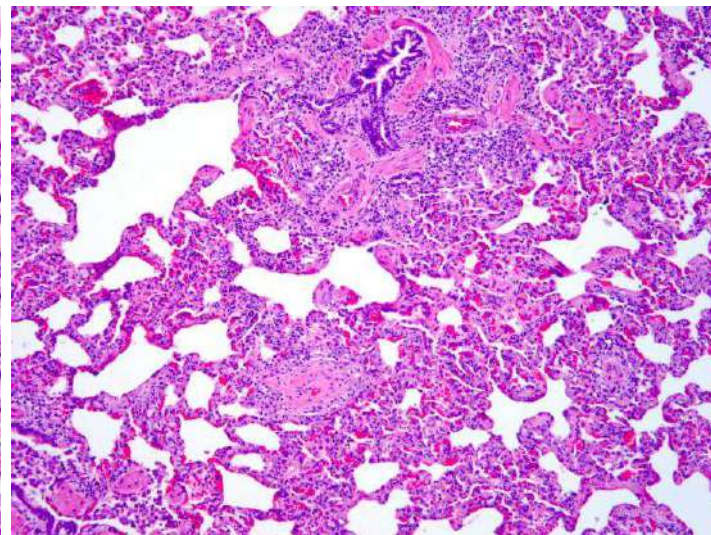
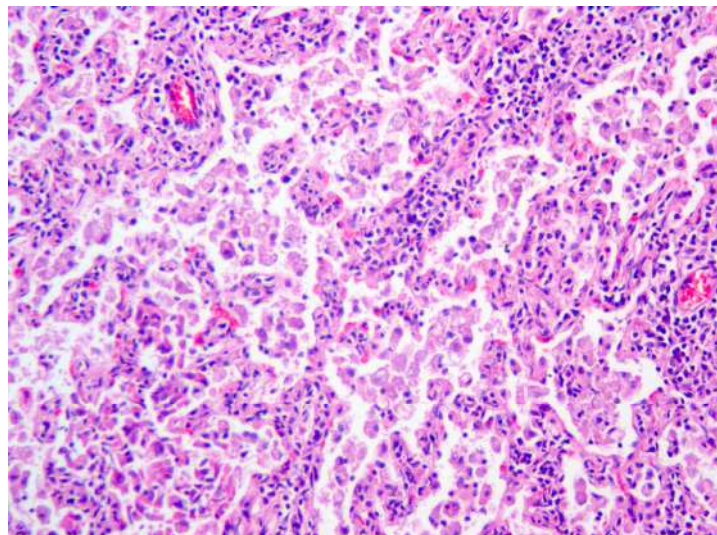
PMWS o PCV2-SD o PCV2A-SI



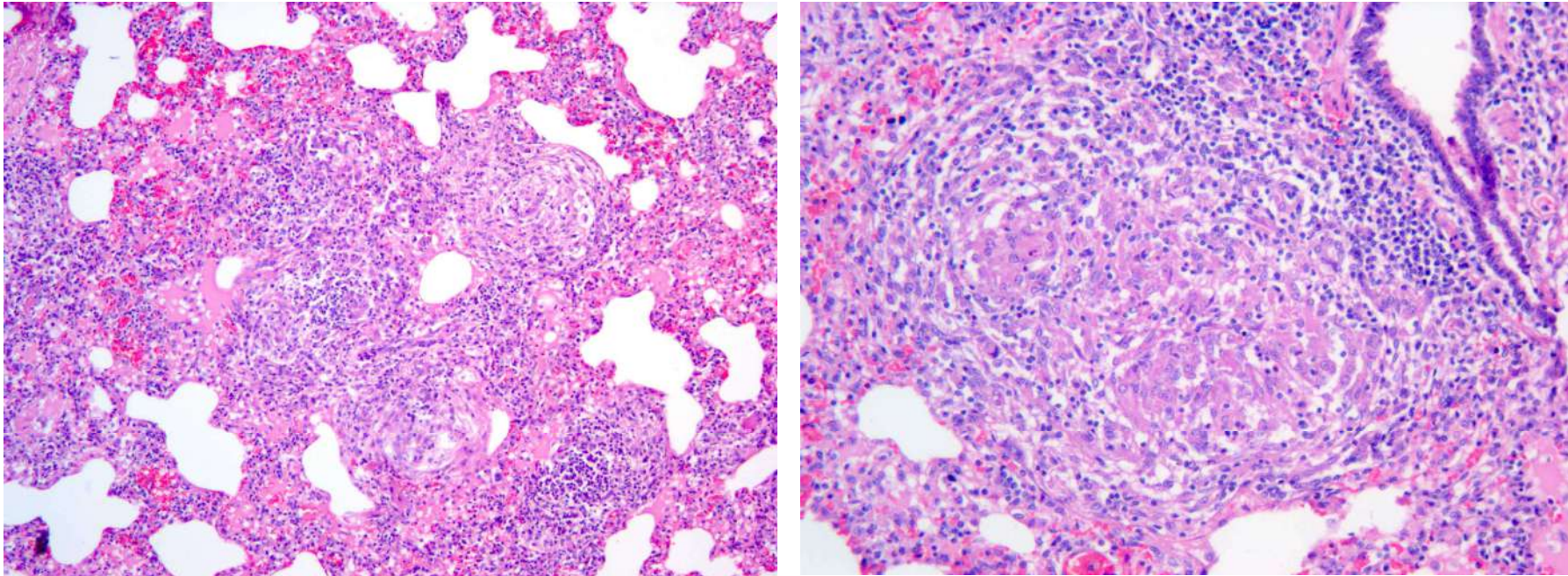
Polmone: PMWS o PRDC?

Istologia indispensabile per la diagnosi

Polmonite interstiziale



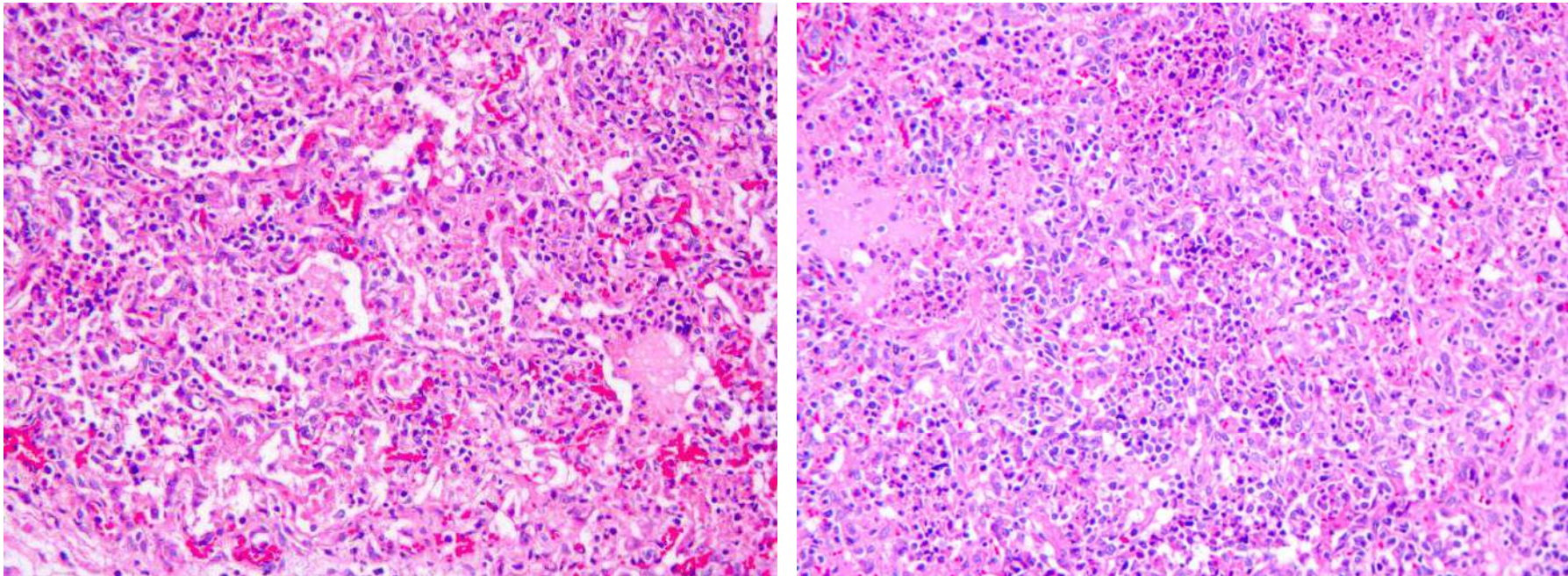
Polmone: PMWS o PRDC?
Istologia indispensabile per la diagnosi



**Polmonite interstiziale ma anche granulomatosa
con granulomi centrati sul BALT**



Polmone: PMWS o PRDC?
Istologia indispensabile per la diagnosi



**Forme essudative non legate al PCV2 ma a
complicazioni batteriche**



Conclusione

Lesioni specifiche

- Polmonite da interstiziale a granulomatosa

Lesioni specifiche positive per PCV2



Distribuite anche a diverse stazioni linfoidi (Lfn, tonsille, milza) ed eventualmente associate a infiammazione da «*interstiziale a granulomatosa*» in diversi parenchimi

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**PMWS o PCV2-SD
o PCV2A-SI**



Se limitate a polmone e BALT

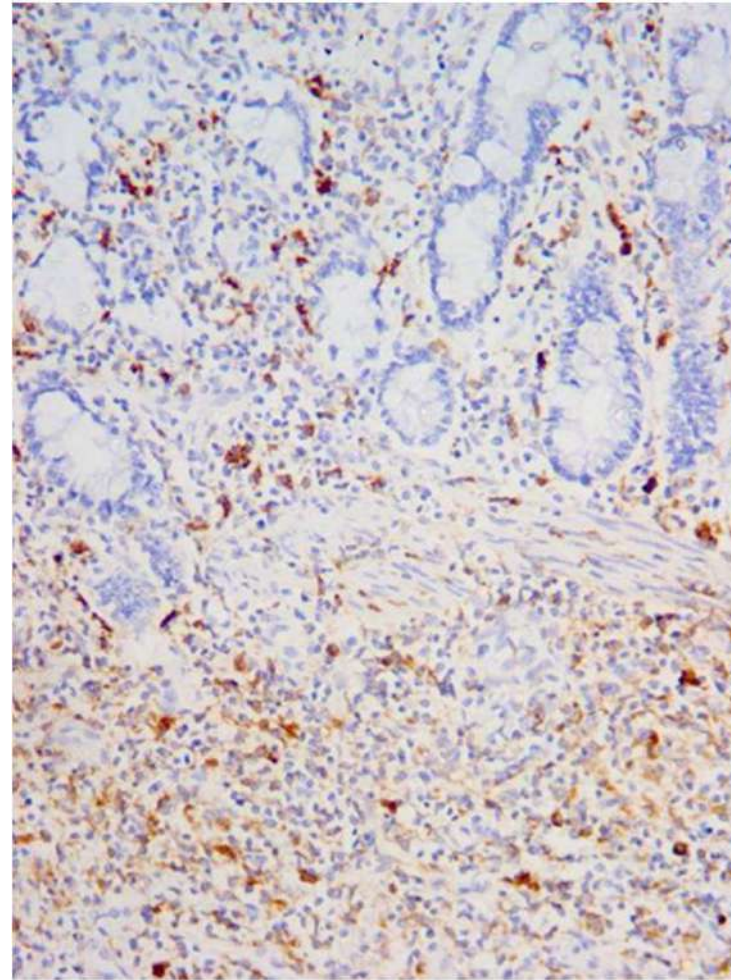
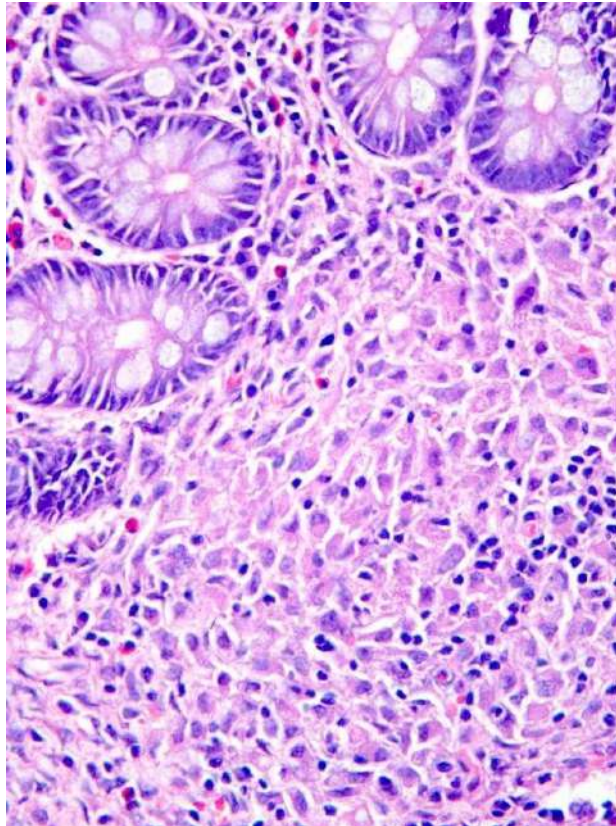
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**PRDC o PCV2-LD o
PCV2A-respiratory
disease**



Intestino

Quadro microscopico indispensabile per la diagnosi



Intestino

Attenzione: dd con enterite da Lawsonia o coinfezioni PCV2-Li

Patologia spontanea

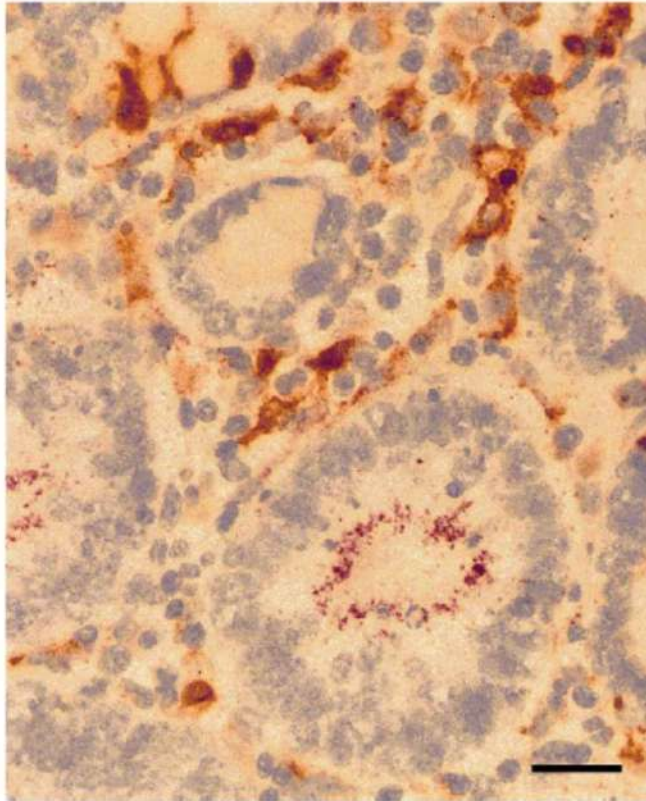


Fig. 4. Double immunolabelling for PCV2 antigen (brown) and *L. intracellularis* antigen (red) in ileum from a pig with a dual infection. *L. intracellularis* is seen within the crypt epithelium whereas PCV2 is demonstrated within macrophages in the adjacent lamina propria. IHC. Bar, 25 μ m.

Jansen et al., JCP, 2006

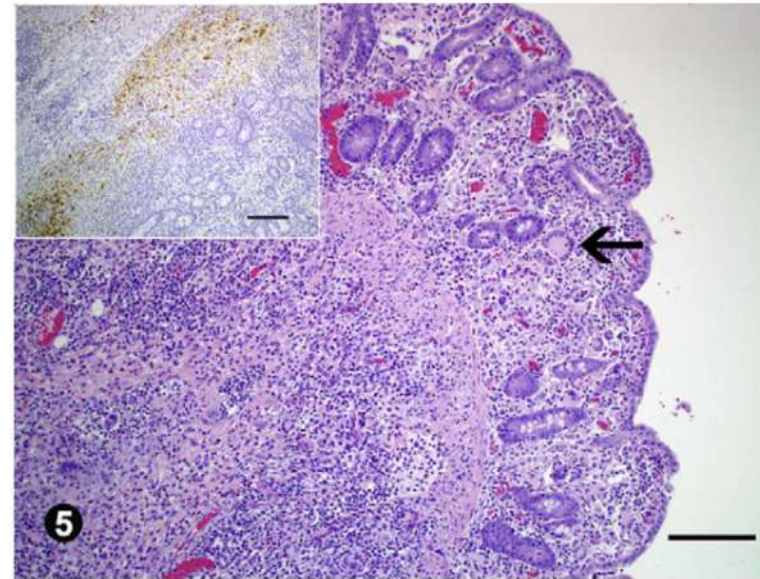


Fig. 5. Ileum of a pig inoculated with PCV2 28 days previously. There is marked depletion of Peyer's patches with occasional multinucleated giant cells. Villi are blunted and fused with histiocytic inflammation within the lamina propria. Multinucleated giant cells are also present within the lamina propria (arrow). HE. Bar, 200 μ m. Inset: PCV2 IHC. There is marked labelling of Peyer's patches. Bar, 200 μ m.

Oppriessnig et al., JCP, 2011

Patologia sperimentale



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Conclusione

Lesioni specifiche

- Enterite da interstiziale a granulomatosa

Lesioni specifiche positive per PCV2



Distribuite anche a diverse stazioni linfoidi (Lfn, tonsille, milza) ed eventualmente associate a infiammazione da «*interstiziale a granulomatosa*» in diversi parenchimi

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**PMWS o PCV2-SD
o PCV2A-SI**



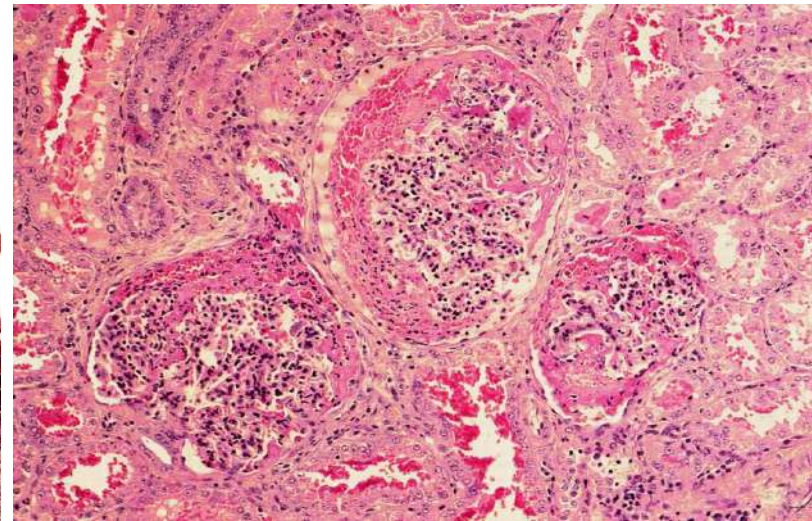
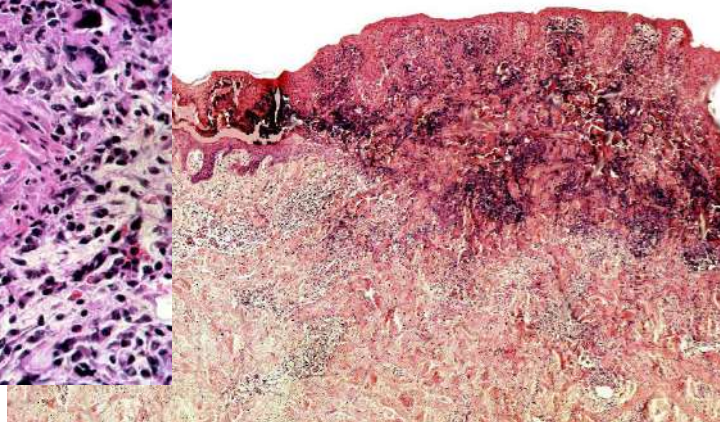
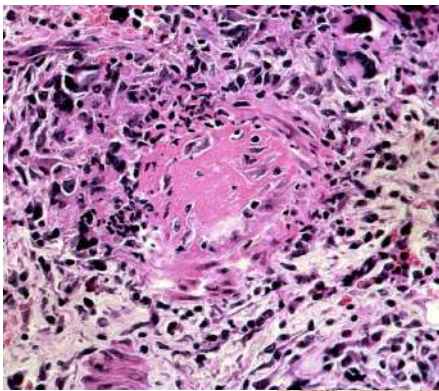
Se limitate a intestino e GALT

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**PCV2-ED o PCV2A-
enteritis**



Lesioni renali: PDNS (associazione non stretta!)



Marcato Patologia Sistematica Veterinaria



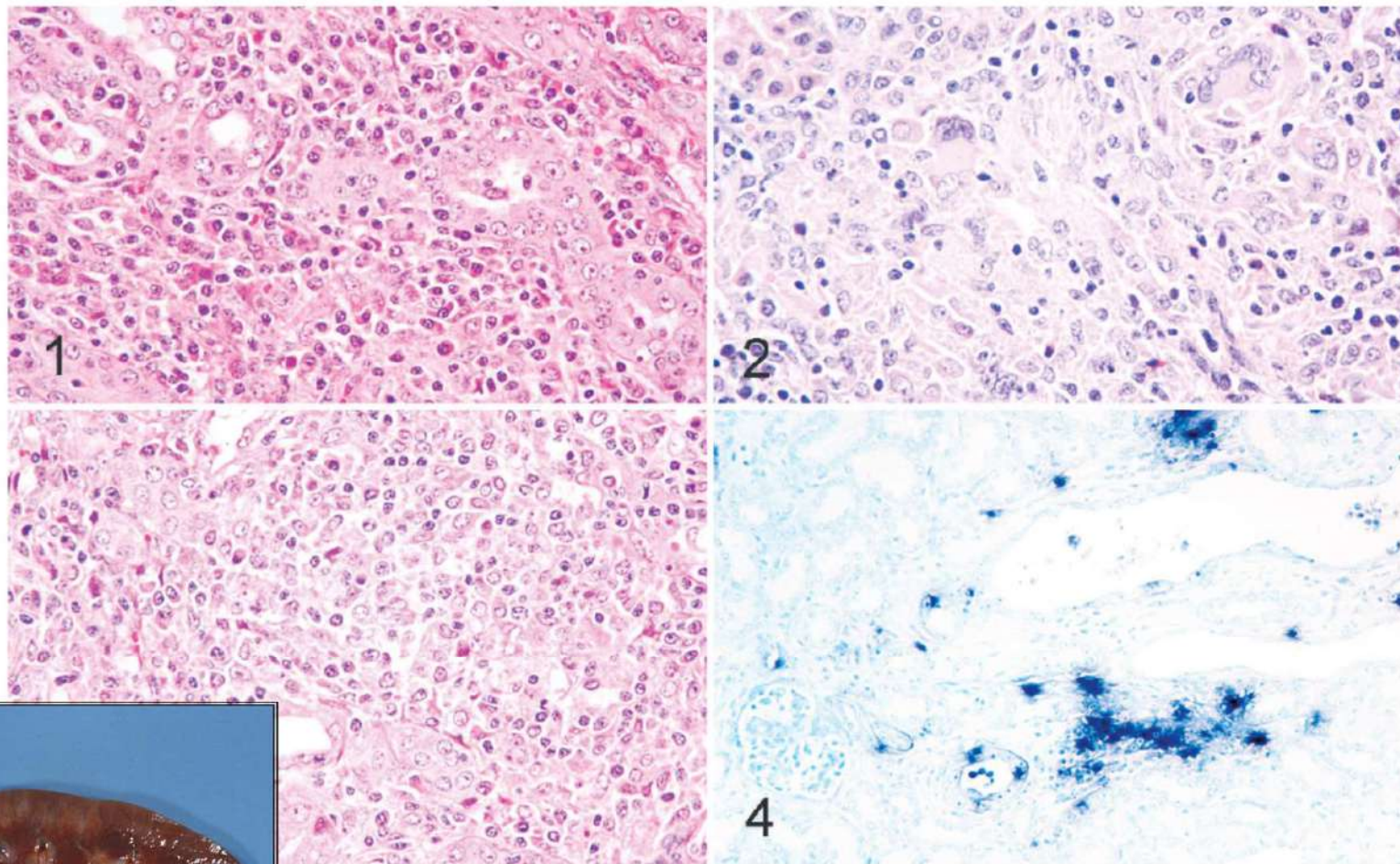
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Ricorda il rene nella PMWS - PCV2-SD – PCV2A-SI

14

Sarli, Mandrioli, Panarese, Brunetti, Segalés, Domínguez, and Marcato

Vet Pathol 45:1, 2008



Patologia riproduttiva

Due contesti:

Abortions or mummifications	<ol style="list-style-type: none">1. Reproductive failure at late gestation2. Fibrous to necrotizing myocarditis of fetuses3. Moderate to high amount of PCV2 in heart <p>The use of real time quantitative PCR on fetal tissues might be more sensitive to detect PCV2-RD</p>
Regular return-to-estrus	<ol style="list-style-type: none">1. Regular return-to-estrus2. PCV2 sero-conversion following the return-to-estrus and/or PCV2 PCR positivity around return-to-estrus occurrence

Segales, Virus Res., 2012



Patologia riproduttiva: aborti e organo target il cuore

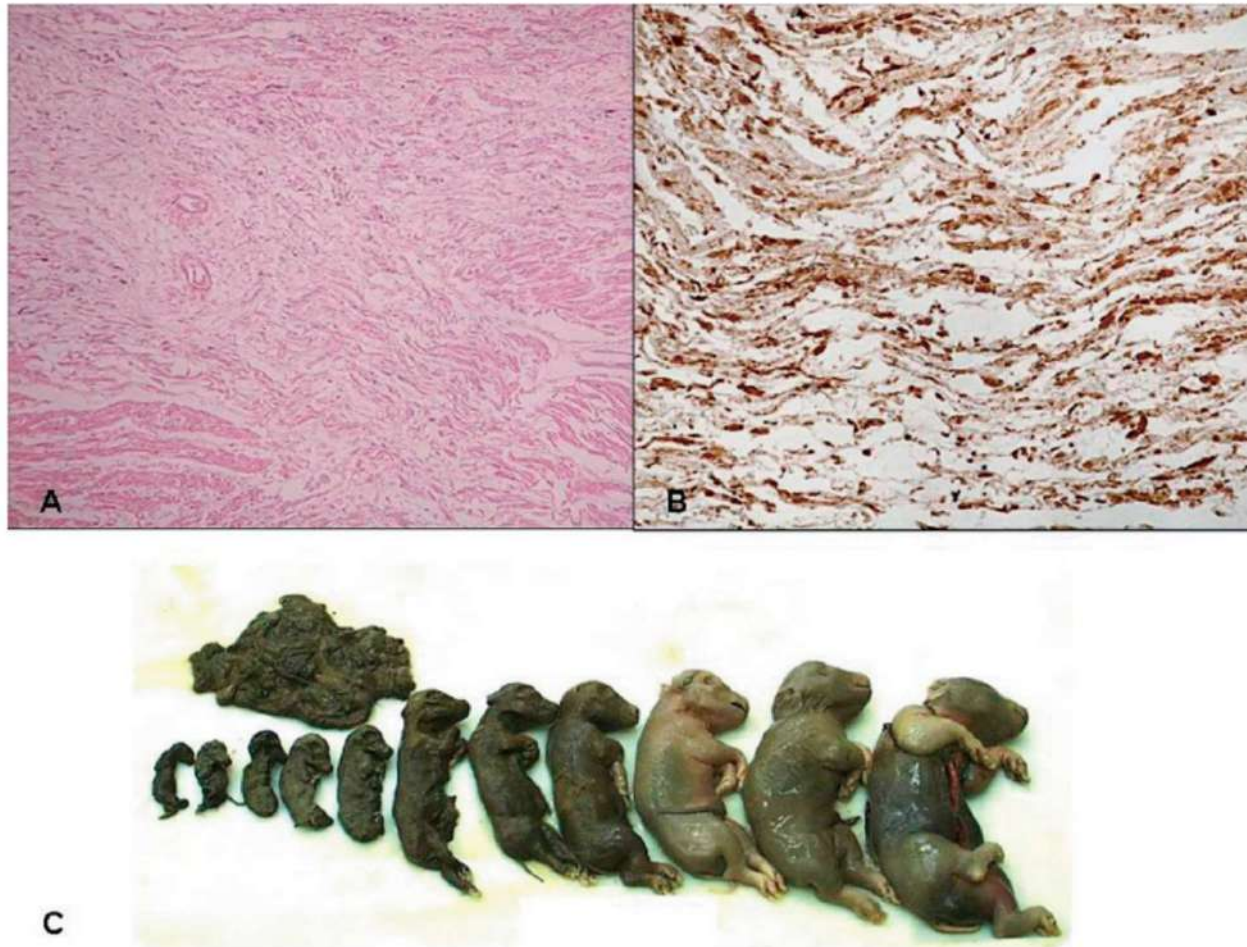
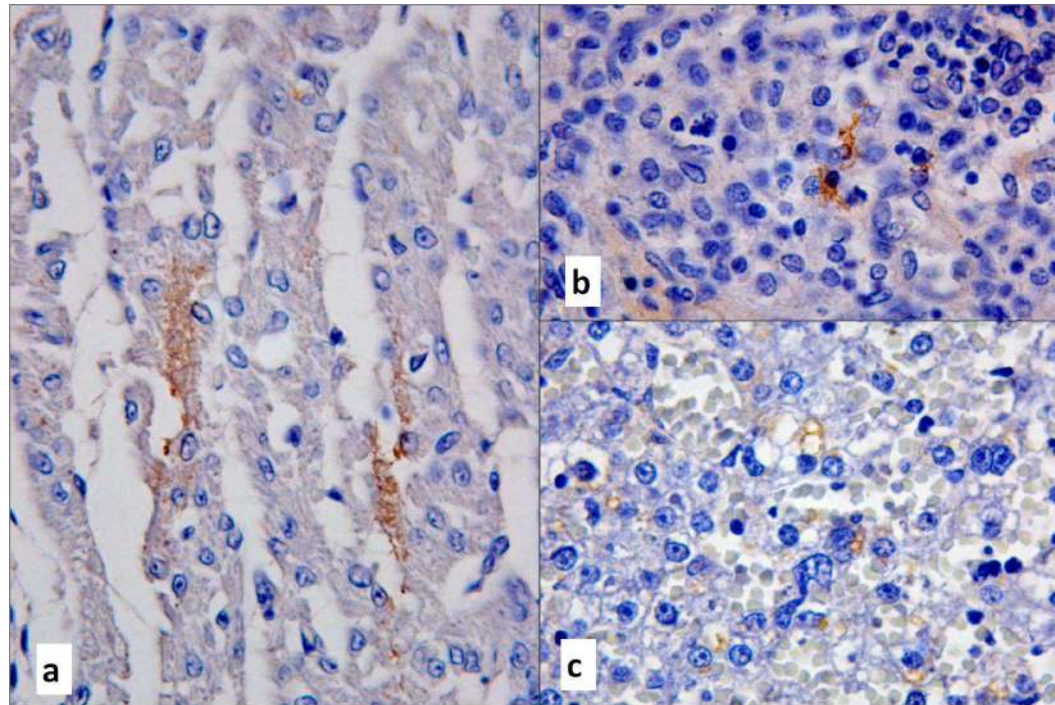


Figure 8. Porcine circovirus-associated reproductive failure. A, Myocardium, fetus. Separation of myocytes by edema and low numbers of inflammatory cells. Hematoxylin and eosin. B, PCV2 antigen (brown staining) within the cytoplasm of myocytes, immunohistochemistry. Streptavidin-biotin peroxidase complex method, hematoxylin counterstain. C, Affected litter showing fetuses at different stages of mummification and maceration.

Oppriessnig et al., J Vet Diagn Invest, 2007



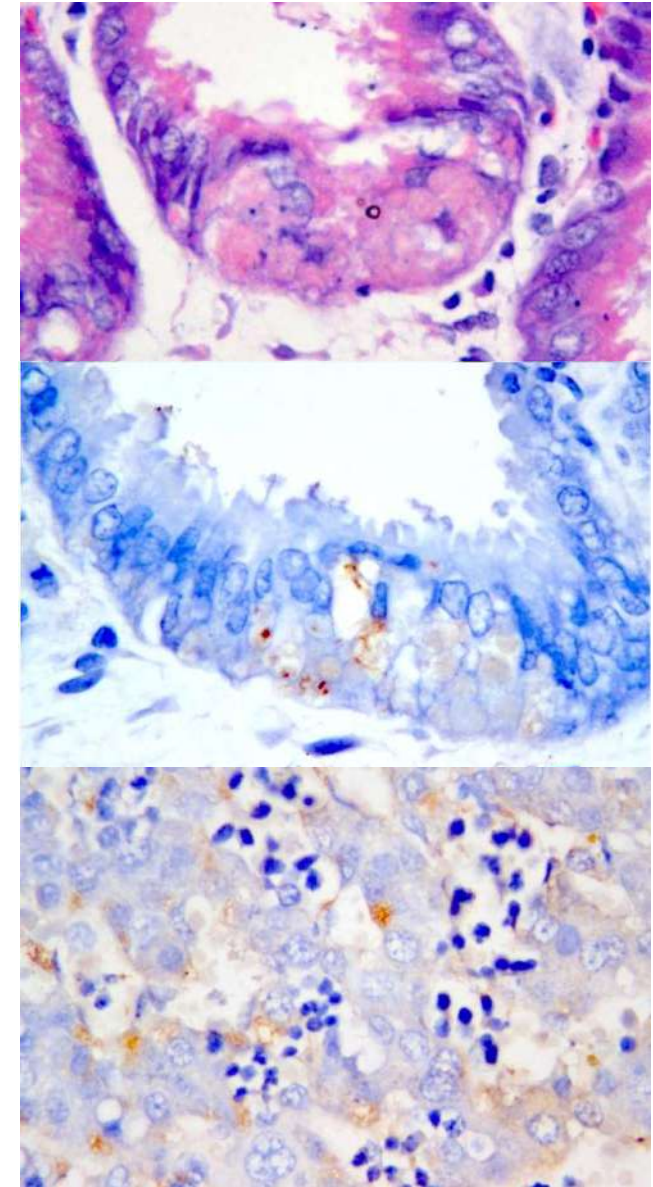
Patologia riproduttiva

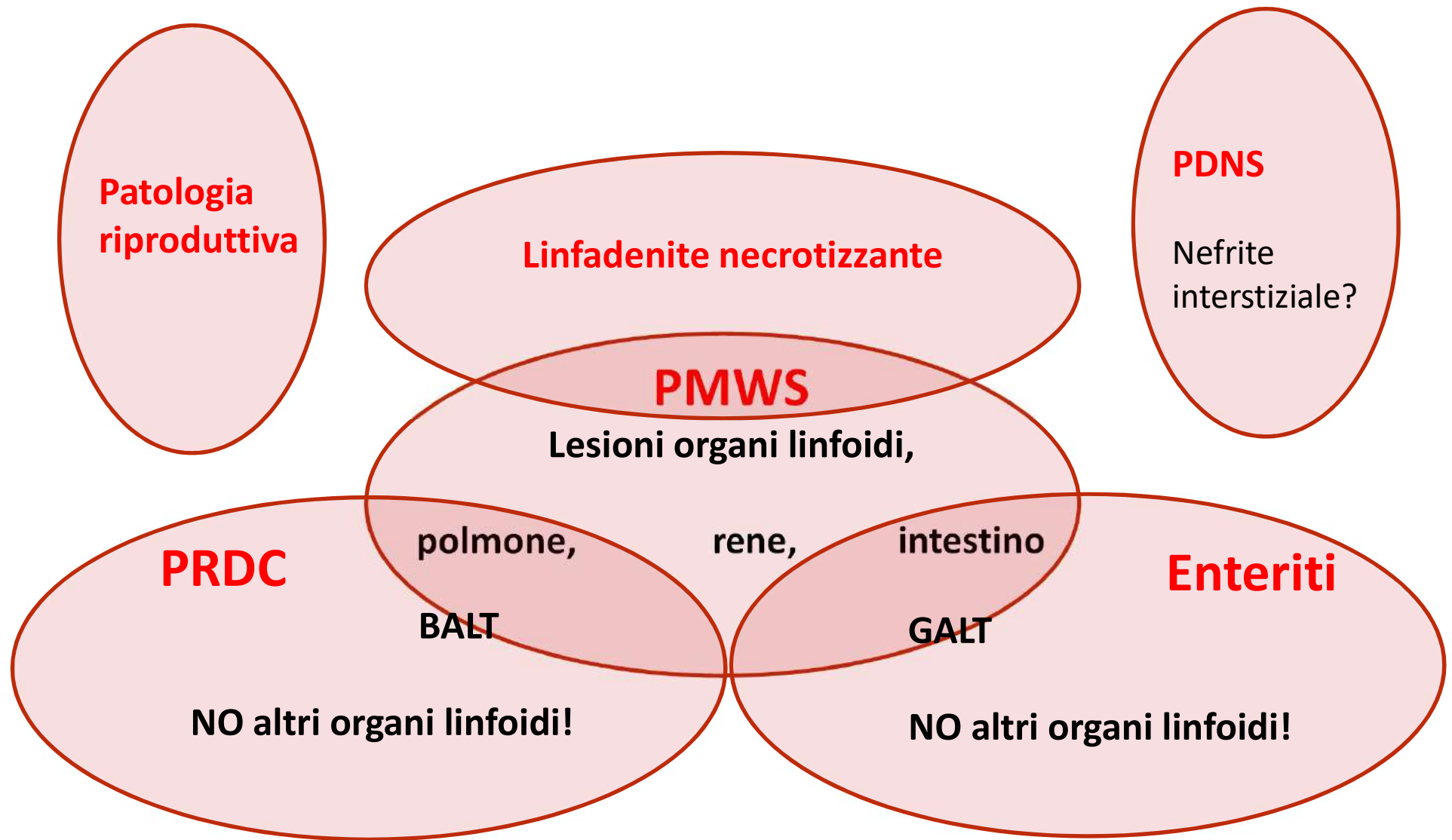


*PRELIMINARY RESULTS OF A DIAGNOSTIC PROTOCOL
FOR THE ASSESSMENT OF PCV2 INVOLVEMENT IN SWINE
REPRODUCTIVE FAILURE*

MORANDI F.¹, PANARESE S.¹, MASERATI A.², GRANITO G.², DOTTORI M.³,
BONILAURI P.³, LUPPI A.³, LELLI D.⁴, LEOTTI G.⁵,
BIANCHI M.⁵, BRUNETTI B.¹, FERRARA D.¹, BIANCO C.¹, VILA T.⁶,
JOISEL F.⁶, OSTANELLO F.¹, SARLI, G.¹

- 2 aziende
- 11 nidiati
- 91 suinetti di cui 25 PCR+ per PCV2
- Solo 3 positivi IHC! Protocollo attuale?





L'istologia è discriminante poiché permette di individuare la malattia che la sola positività in PCR per PCV2 non sempre è in grado di rilevare



Cosa campionare e cosa aspettarsi dall'istologia

Le malattie da PCV2 prevedono più una diagnosi sul gruppo che sul singolo

Per istologia

- 3-5 animali
- **Linfonodi, polmone, tonsilla, ileo, milza** altri organi parenchimatosi in cui si vedono lesioni



Forniscono nell'insieme un quadro capace di discriminare fra le diverse PCV(A)D in base alla tipologia di organi coinvolti

- Concentrare il campionamento in fx della sintomatologia
 - Polmone per sindromi respiratorie
 - Intestino per sindromi enteriche
- Nella PDNS non aspettarsi certezza eziologica
- Nella patologia riproduttiva l'istologia ha limiti





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