Immune System Histology Boehringer-Ingelheim

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Poultry Immunology and Immunosuppressive Diseases Training

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Ploufragen, France

Overview

Primary lymphoid tissues

- Bursa of Fabricius and thymus

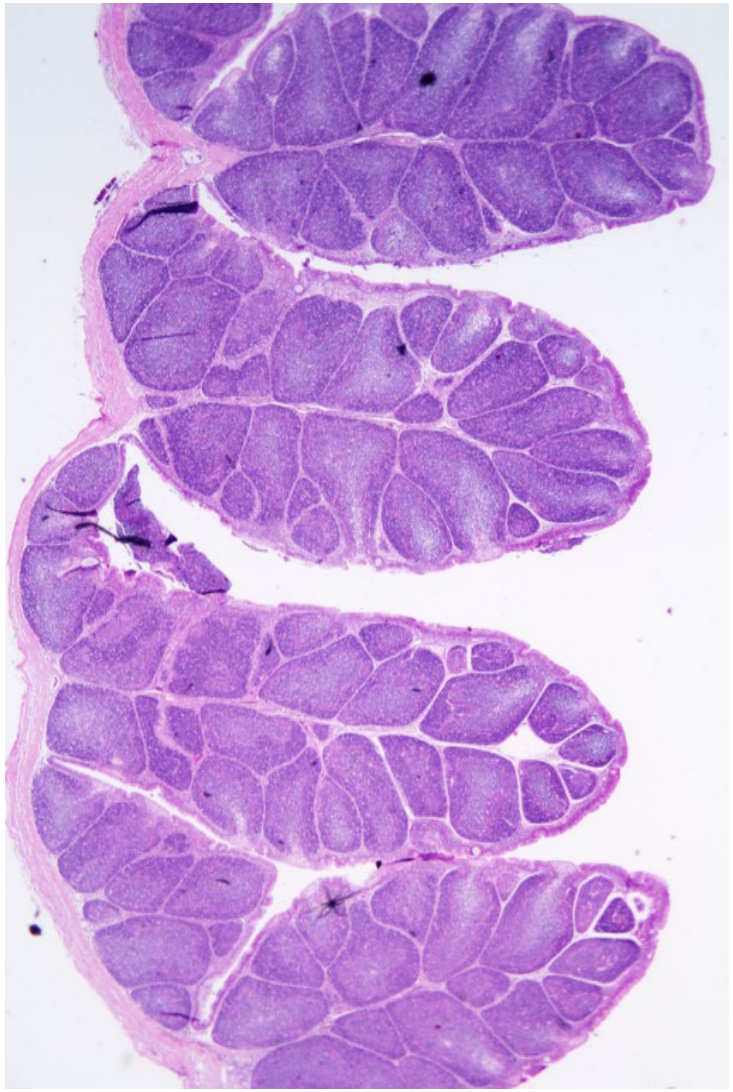
Secondary lymphoid organs

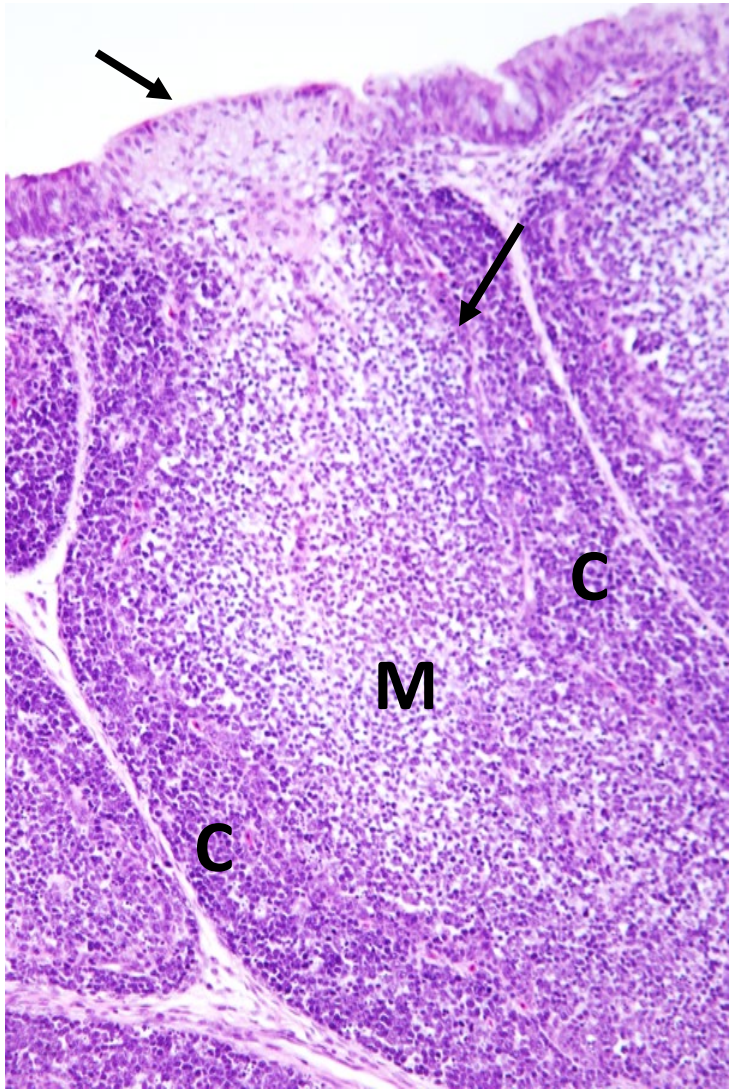
- Spleen
- Conjunctival (CALT), nasal (NALT) and bronchial (BALT)
- Gut (GALT), proximal and distal digestive tract, Peyer's patches and cecal tonsil
- Urinary, skin

Disease progression in selective immunosuppressive diseases

- Necrosis, apoptosis
- Atrophy, depletion
- Hyperplasia
- Inflammation
- Neoplasia

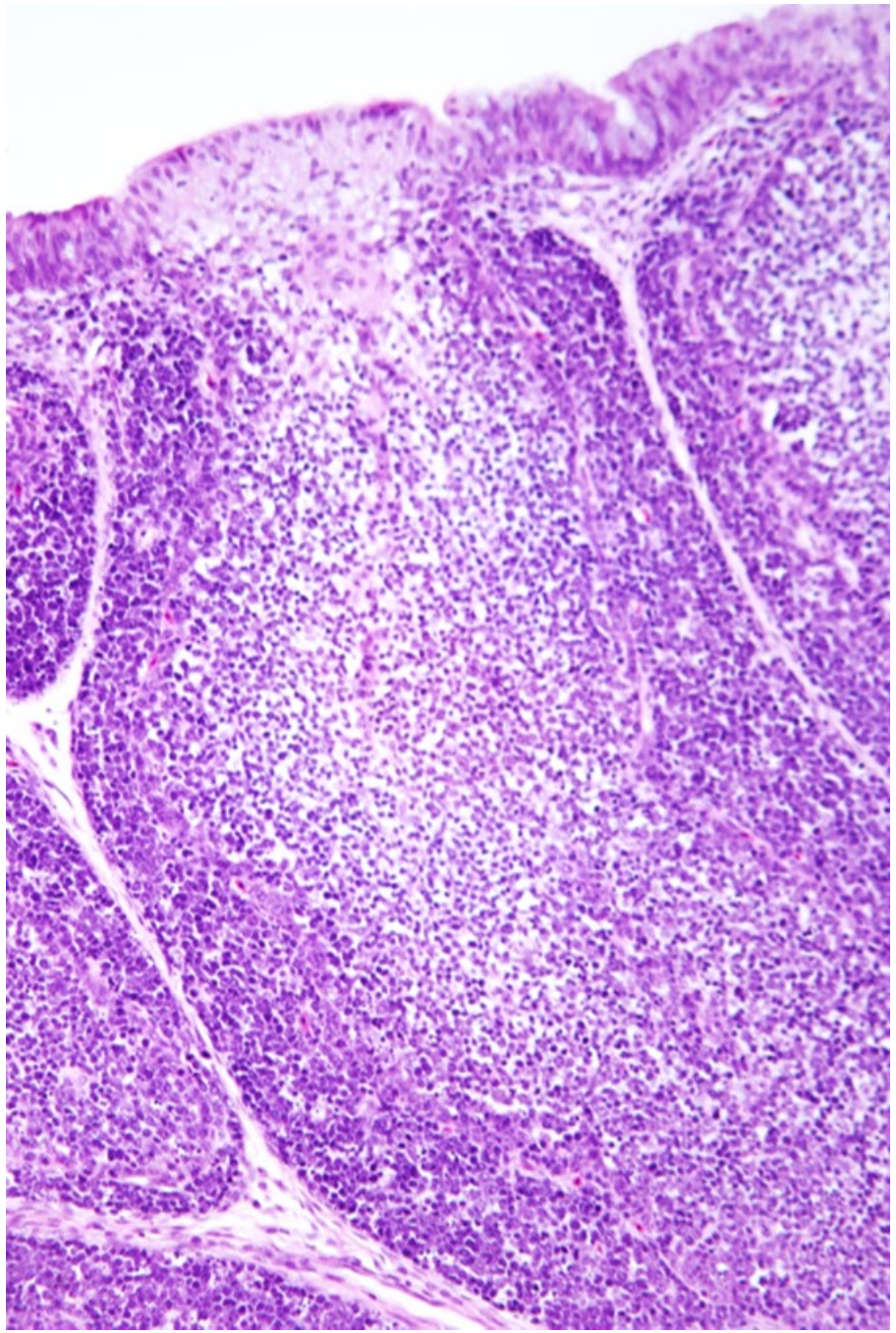
Normal bursa of Fabricius



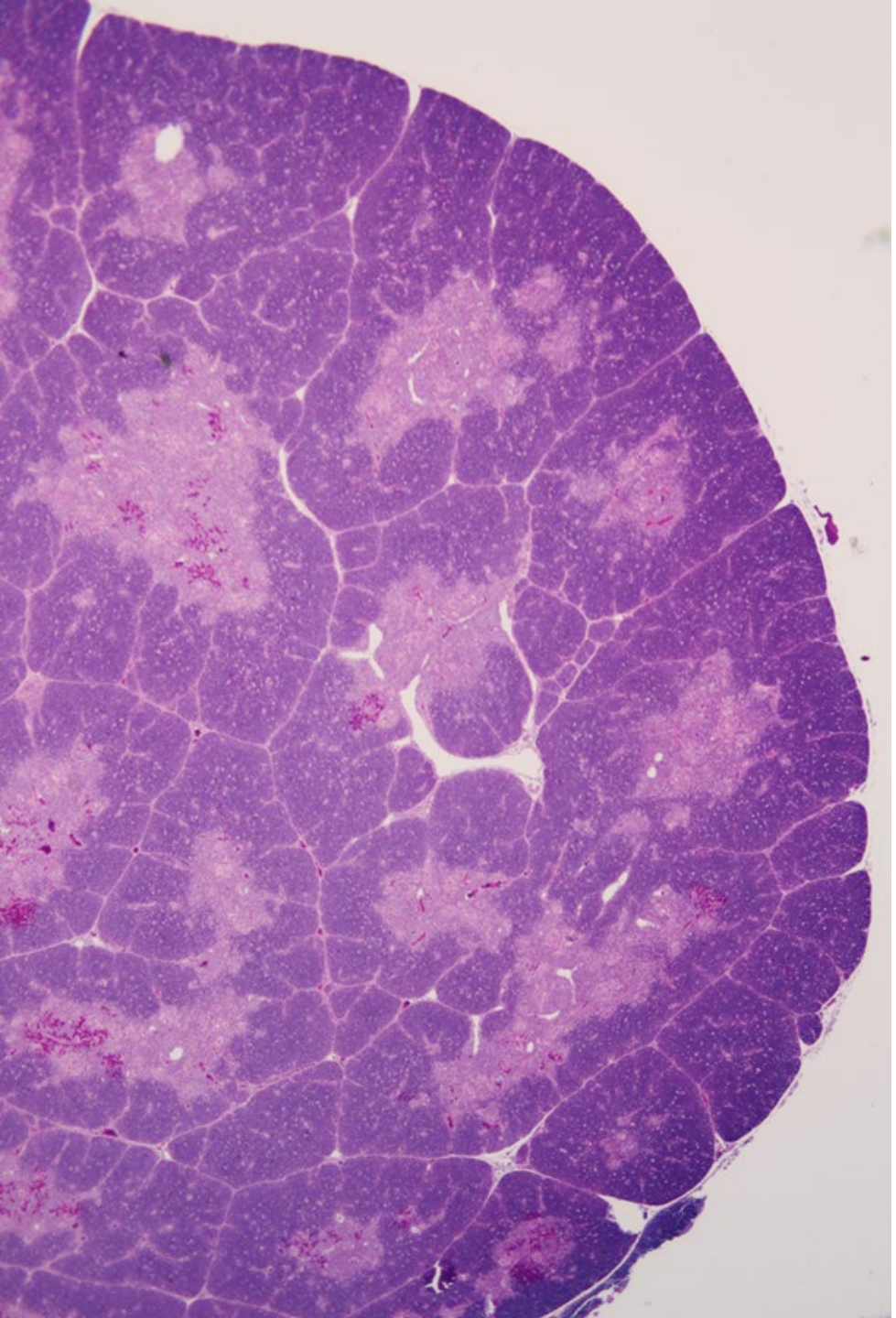


Bursa and B Lymphocytes

- The embryonic bursa is populated by precursor embryonic cells from yolk sac and liver
- B Lymphocytes develop in medulla and migrate to cortex
- Most are destroyed by MHC selection
- Migrate to blood stream and populate secondary tissues
- Medulla: epithelial cells, lymphocytes and lymphoblasts
- CM junction: capillary network and undifferentiated epithelium
- Cortex: B lymphocytes, plasma cells, and macrophages
- Perifollicular tissue: neuroendocrine dendritic cells
- Each follicle has 2-500,000 cells

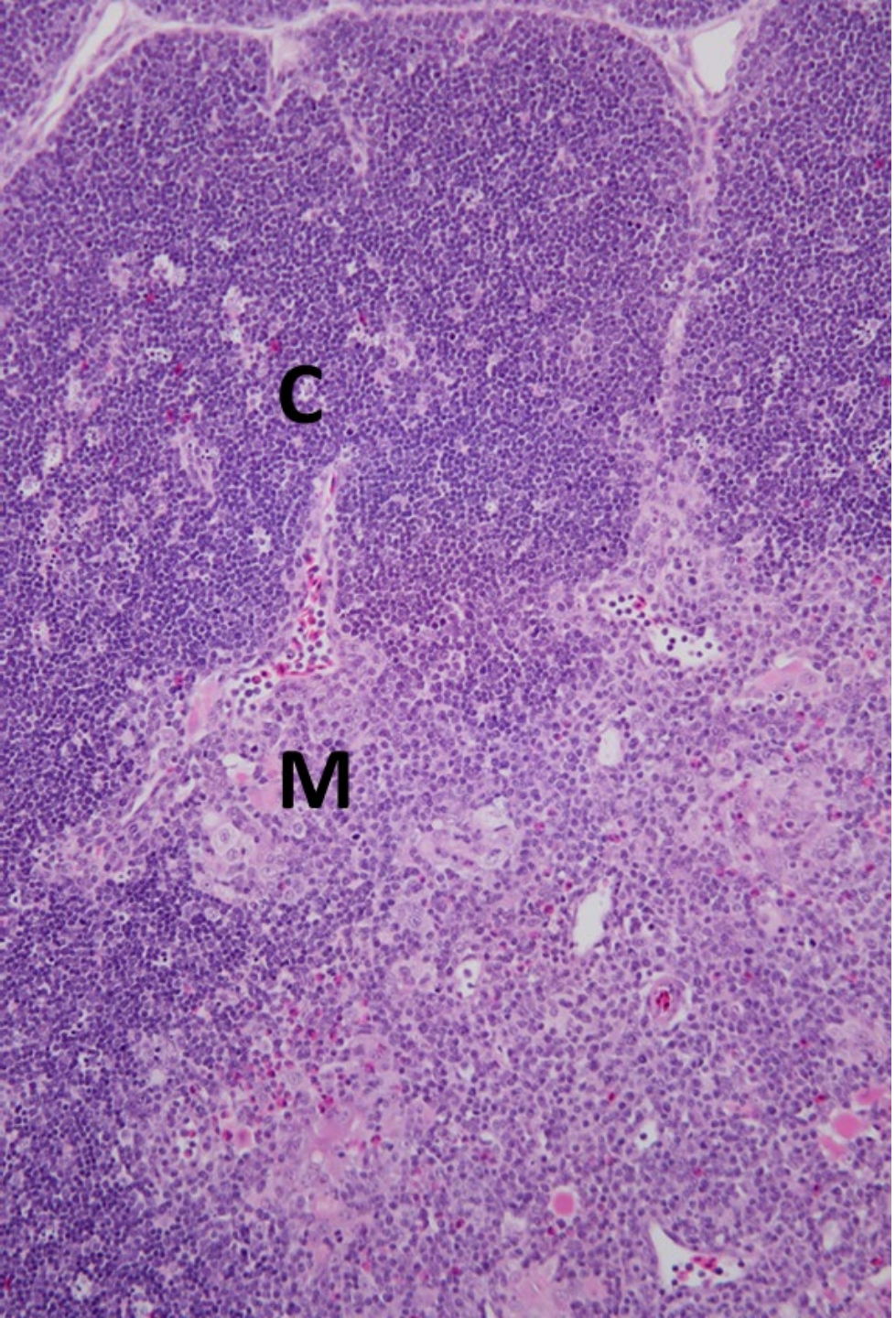


Sci Rep. 2018; 8: 16905.
Published online 2018 Nov 15. doi: 10.1038/s41598-018-34897-4; Kwang Hyun Ko, et al.



Thymus

- Lymphoepithelial organ
  - Lymphoid population from progenitor cells from yolk sac
  - Stromal cells from branchial arches and pharyngeal clefts
- Epithelial cells in cortex (ectoderm origin) and medulla (endoderm)
  - Hassall's corpuscles
  - Contribute to microclimate for T-lymphocyte development at all ages
- Other cells
  - Reticular cells, macrophages, heterophils and myoid cells (smooth muscle-like)
- Subcapsular zone: entry of progenitor from bone marrow and circulation



Thymus

- T-cells mature while traversing from cortex to medulla
- Recognize self Major Histocompatibility Complex (MHC) antigens
  - Mature by positive selection
  - Cells that do not recognize self-MHC are removed by apoptosis
- Cells that recognized both self-MHC and self-nonMHC antigens are removed by negative selection
- 95% of developing T lymphocytes are destroyed in development
- Mature T lymphocytes depart through venules at cortico-medullary junction
  - Distribute to secondary lymph organs: spleen, Peyer's patches, cecal tonsil
- Delicate balance of mature T Lymphocyte production, development, and supply to secondary organs – easily disrupted

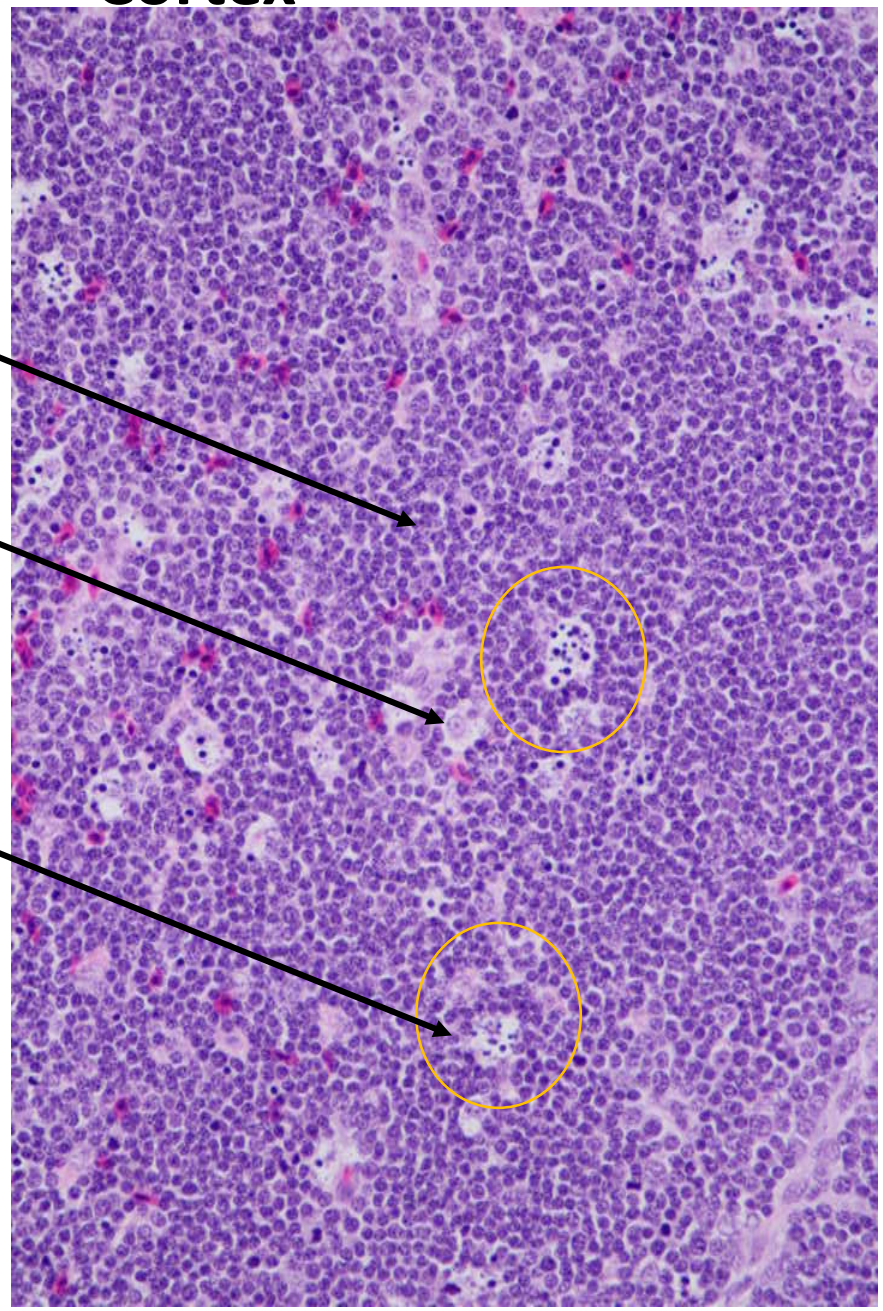
Thymus

Developing T lymphocytes

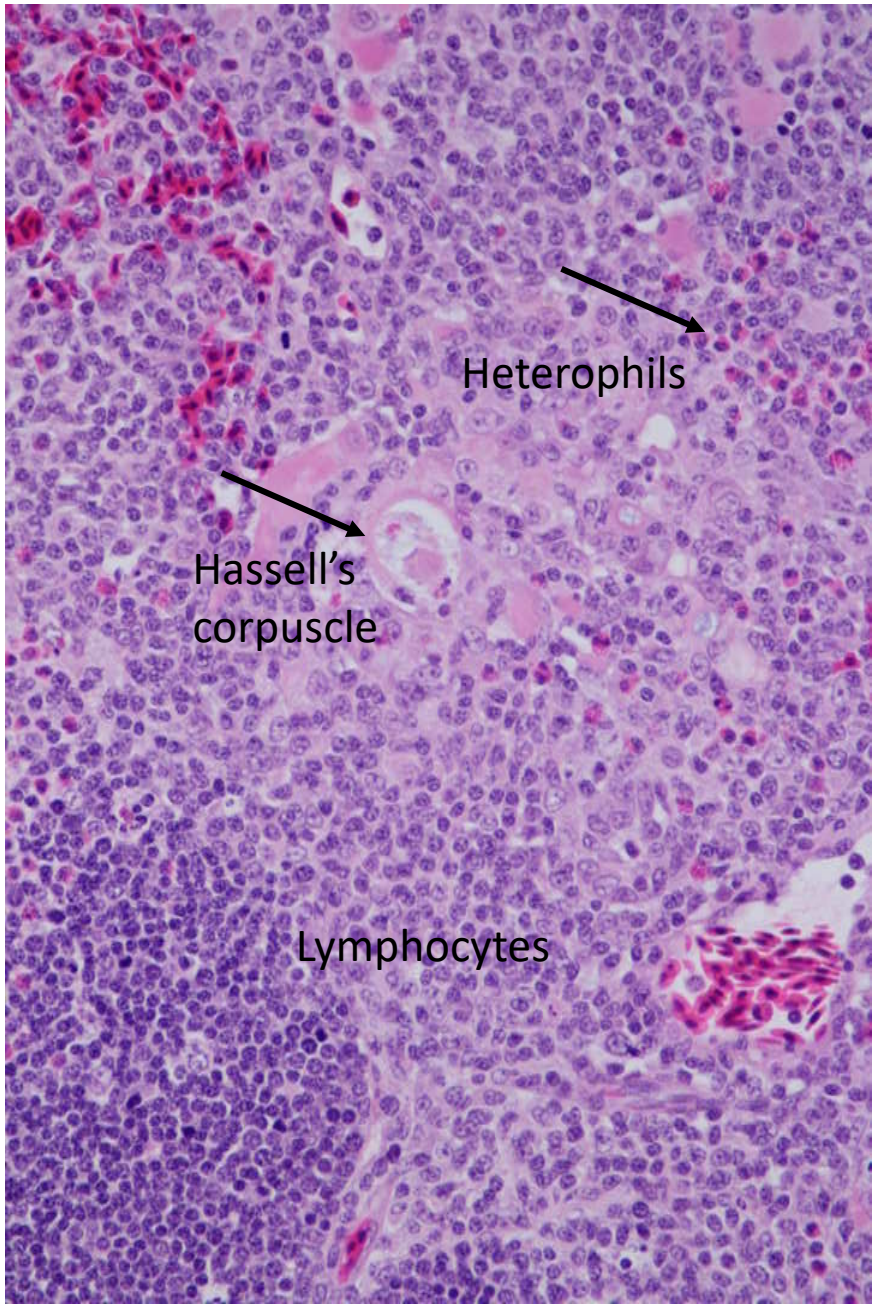
Cortical epithelial cells

Apoptosis: programmed cell death

Cortex



Medulla



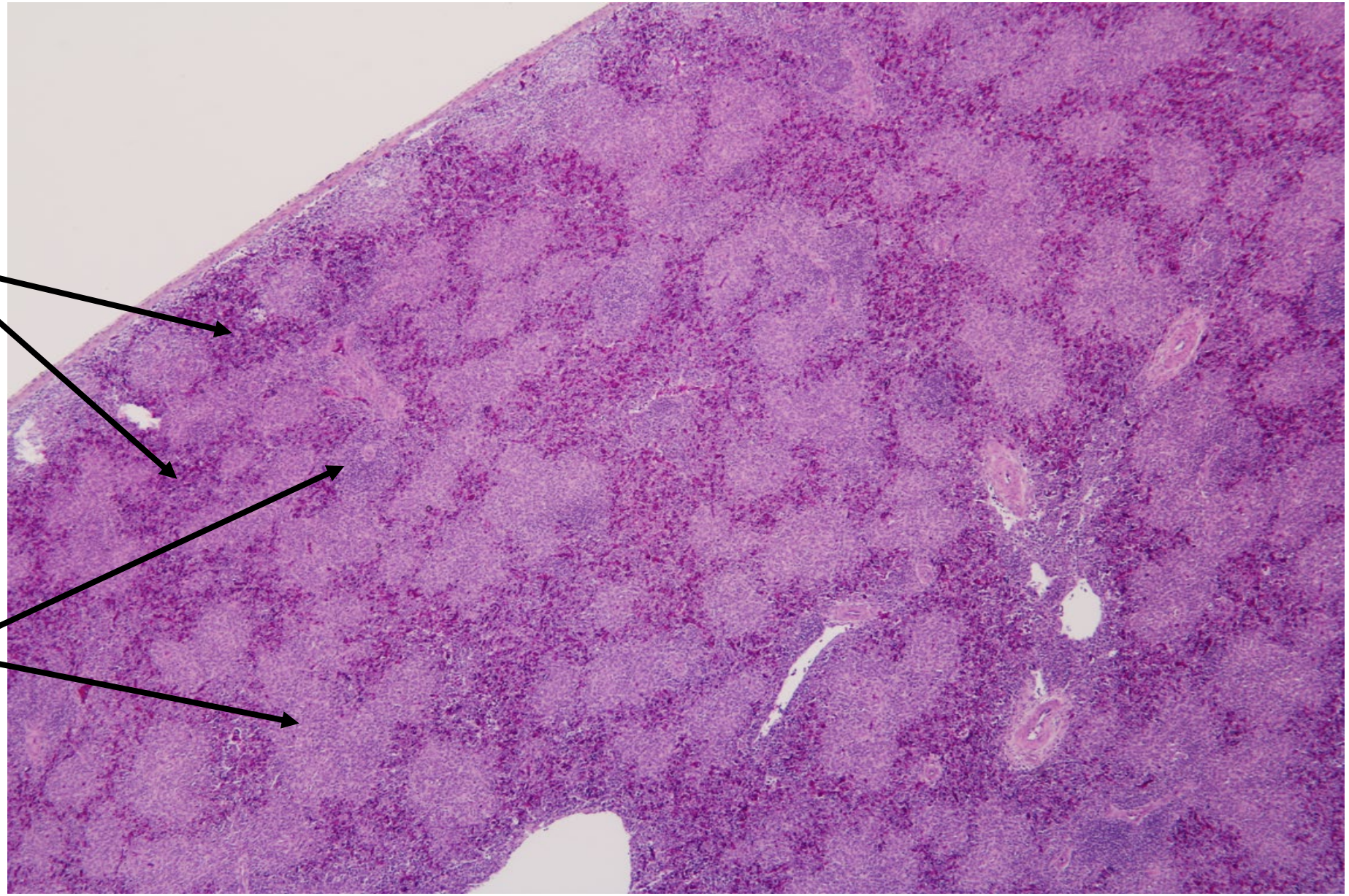
Spleen

Three anatomic systems	Red pulp	White pulp
• Monocyte-macrophage system • Lymphopoietic syste • Vascular system	• Removal of foreign material (microorganisms) and senescent erythrocytes • Storage of mature erythrocytes • Hematopoiesis, if stimulated	• Immunologic response • B lymphocyte and plasma cells produce antibody and memory lymphocytes

Spleen

Red pulp - blood storage and removal of senescent erythrocytes and pathogens

White pulp – antigen detection, neutralization, and processing by humoral and cell-mediated immune responses.



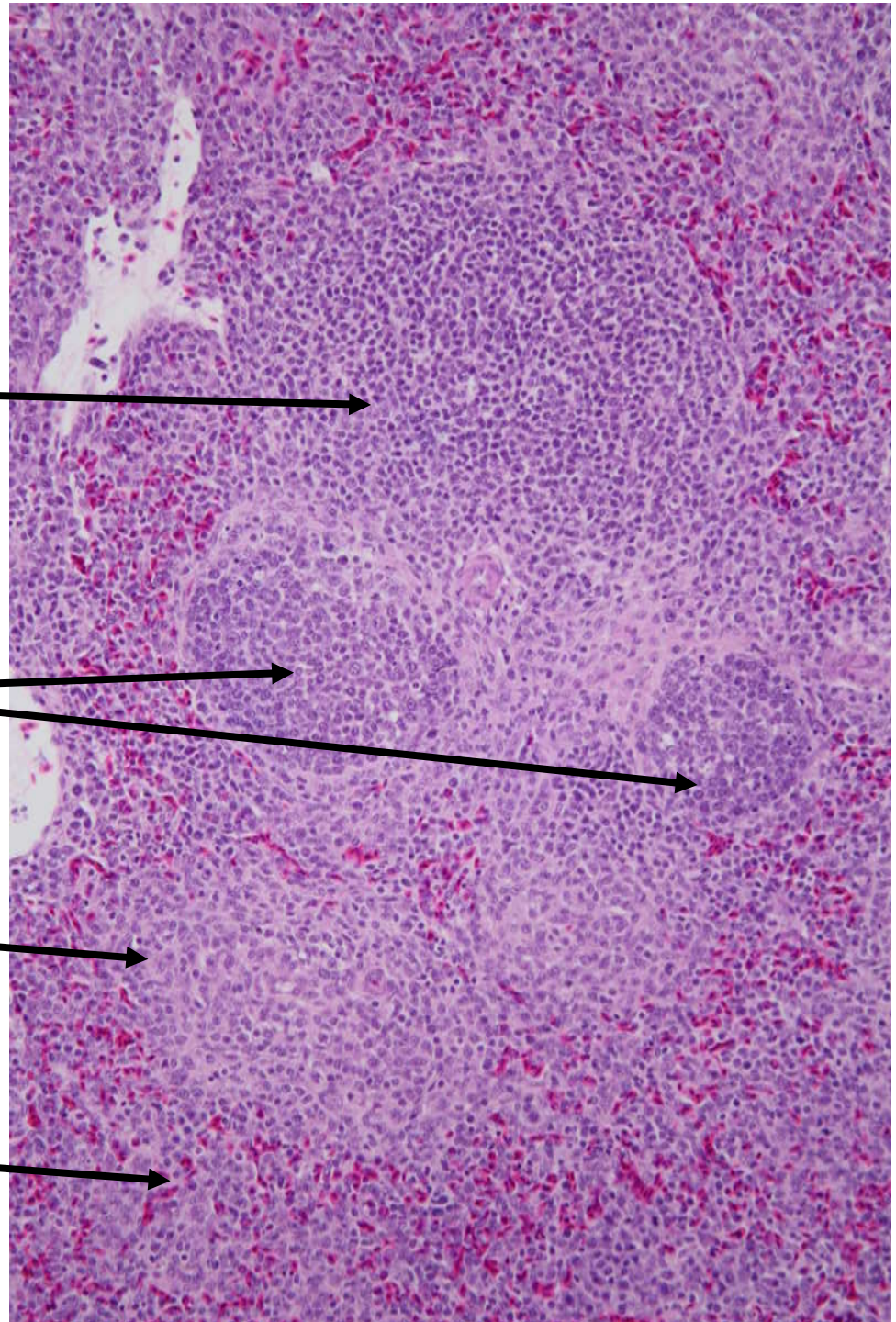
Spleen

Periarteriolar lymphoid sheath (PALS), rich in T lymphocytes that are seeded by the thymus

Germinal centers composed of B Lymphocytes, seeded by the bursa, and plasma cells

Ellipsoid containing lymphocytes and reticular cells

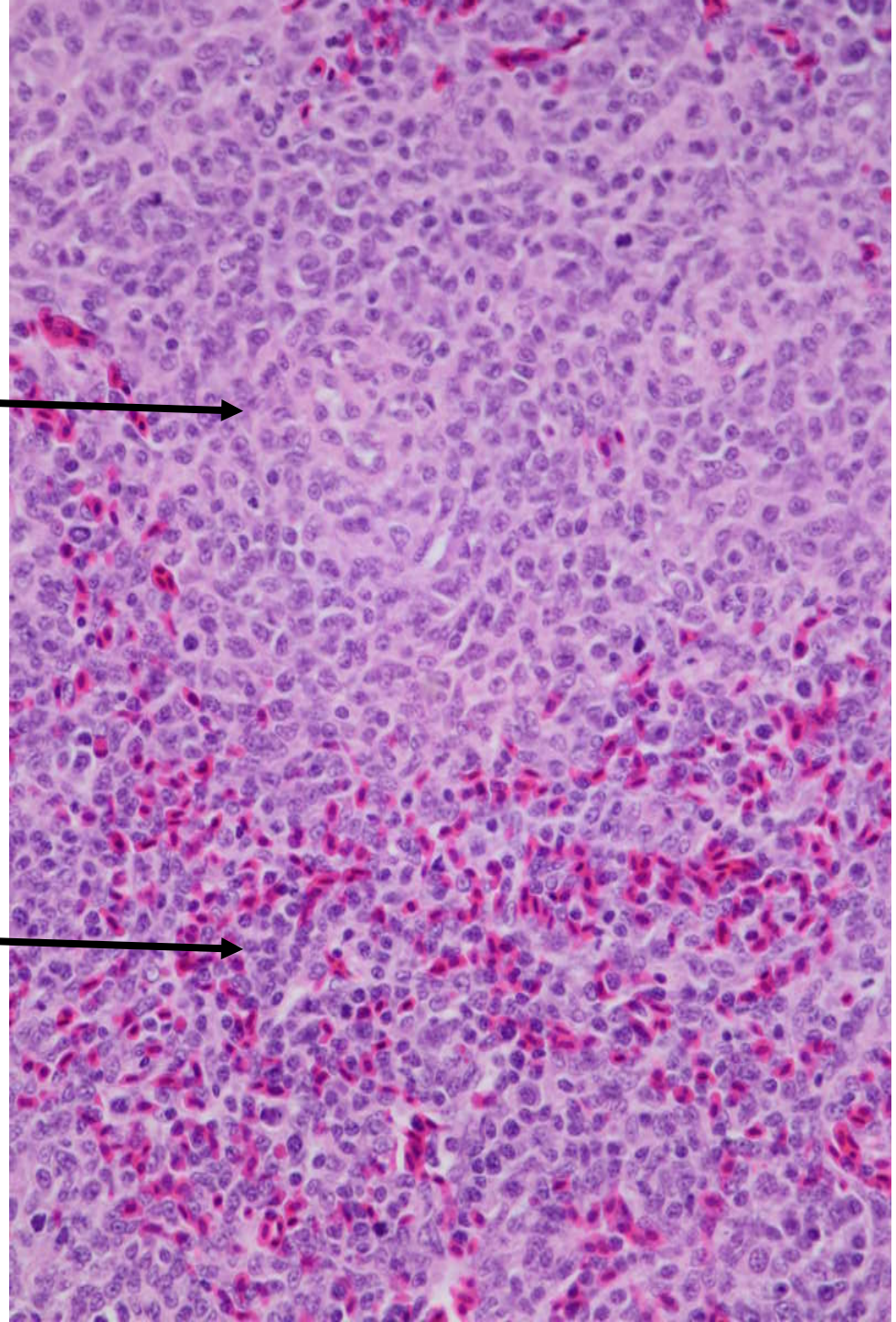
Red pulp

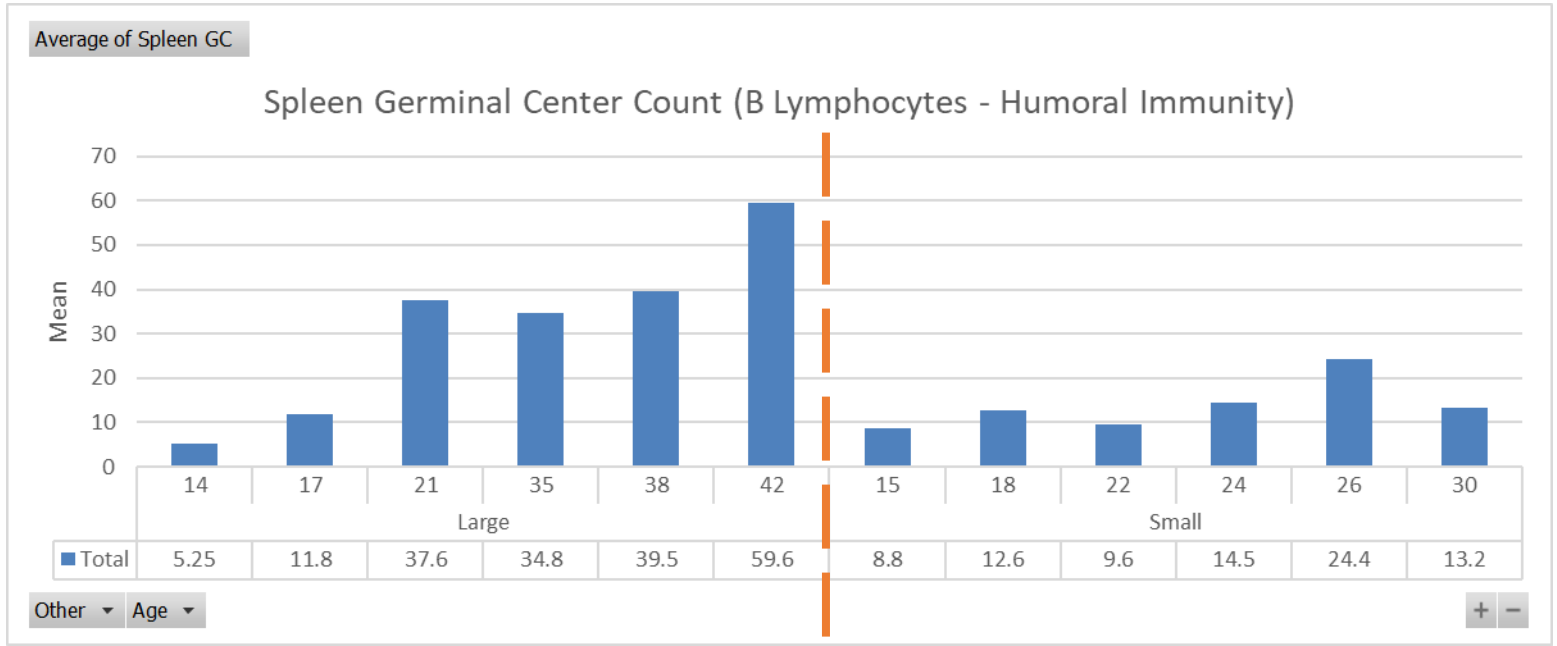


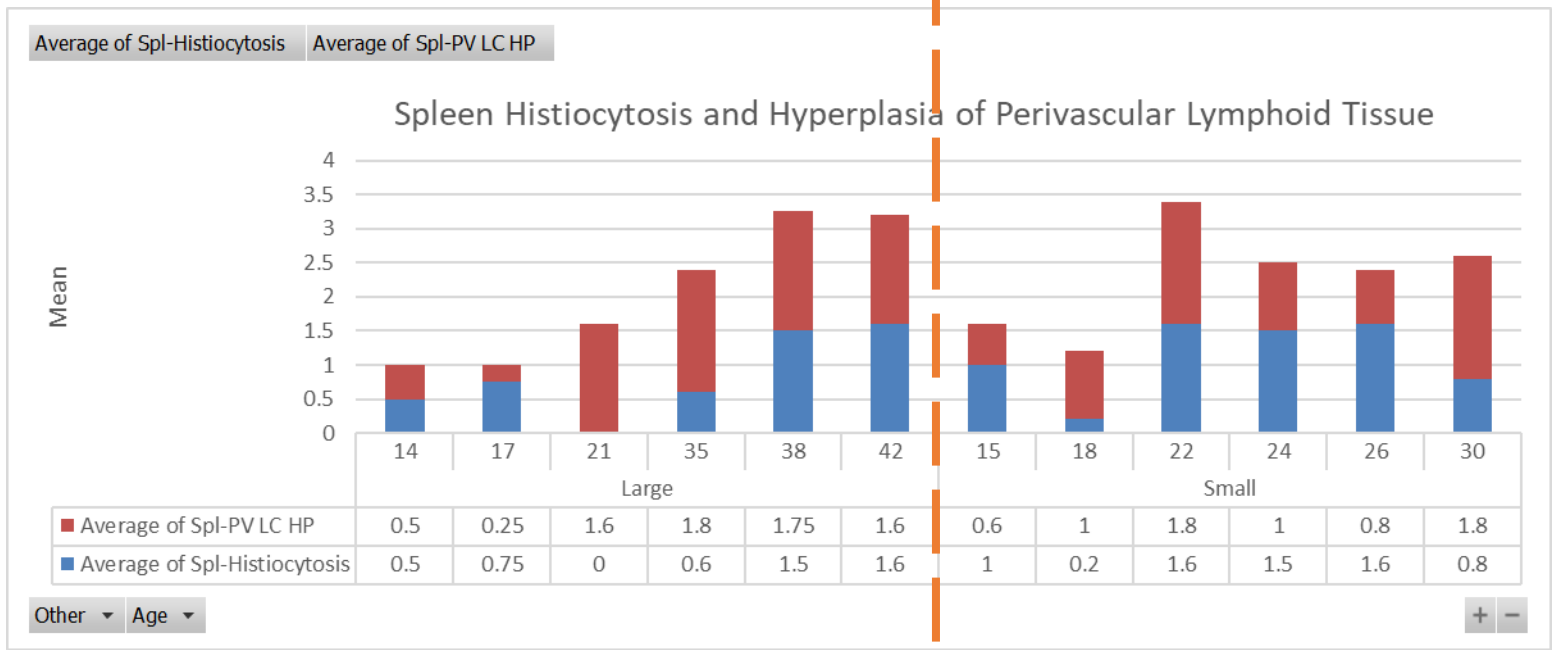
Spleen

Ellipsoid (white pulp) – trapping and phagocytosis of blood-born foreign antigens and presentation of antigens to T lymphocytes, leading to activation of T and B lymphocytes, and antibody production.

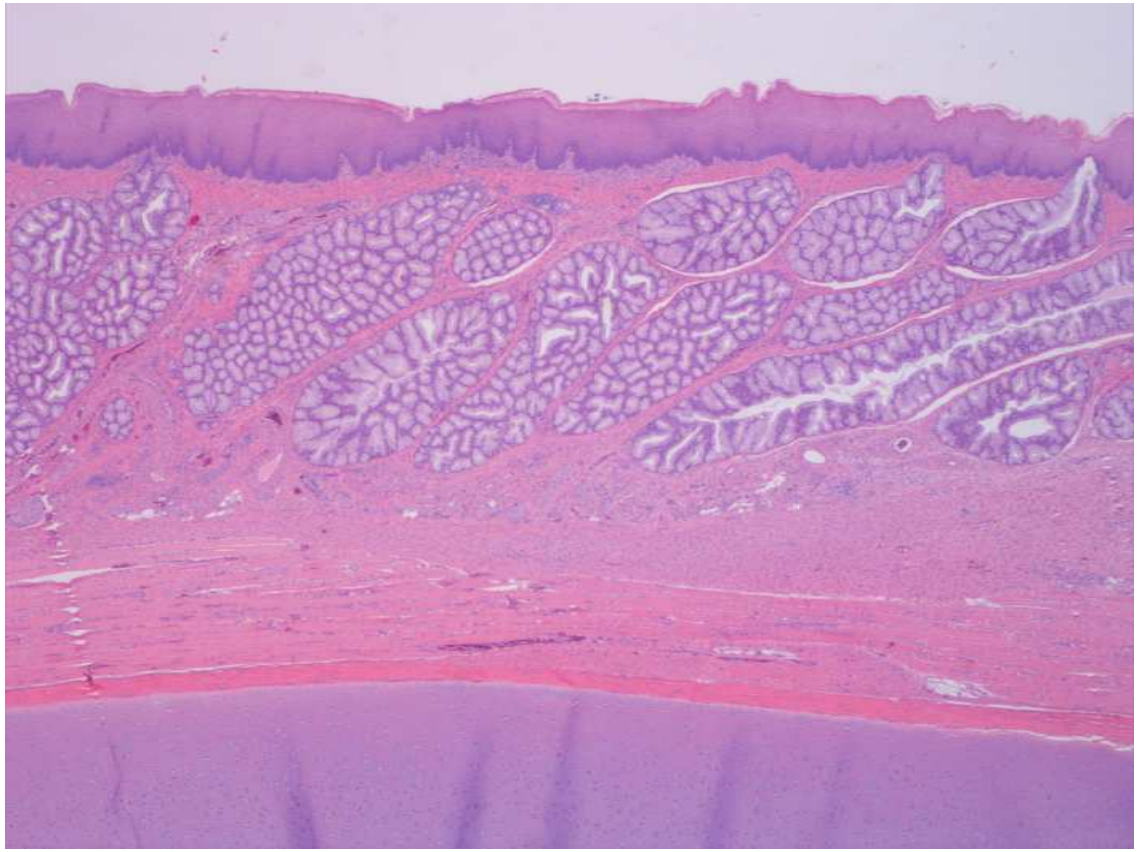
Red pulp – endothelial lined sinuses with macrophages that removed pathogens and damaged cells. Part of monocyte-macrophage system. The red pulp also stores blood.

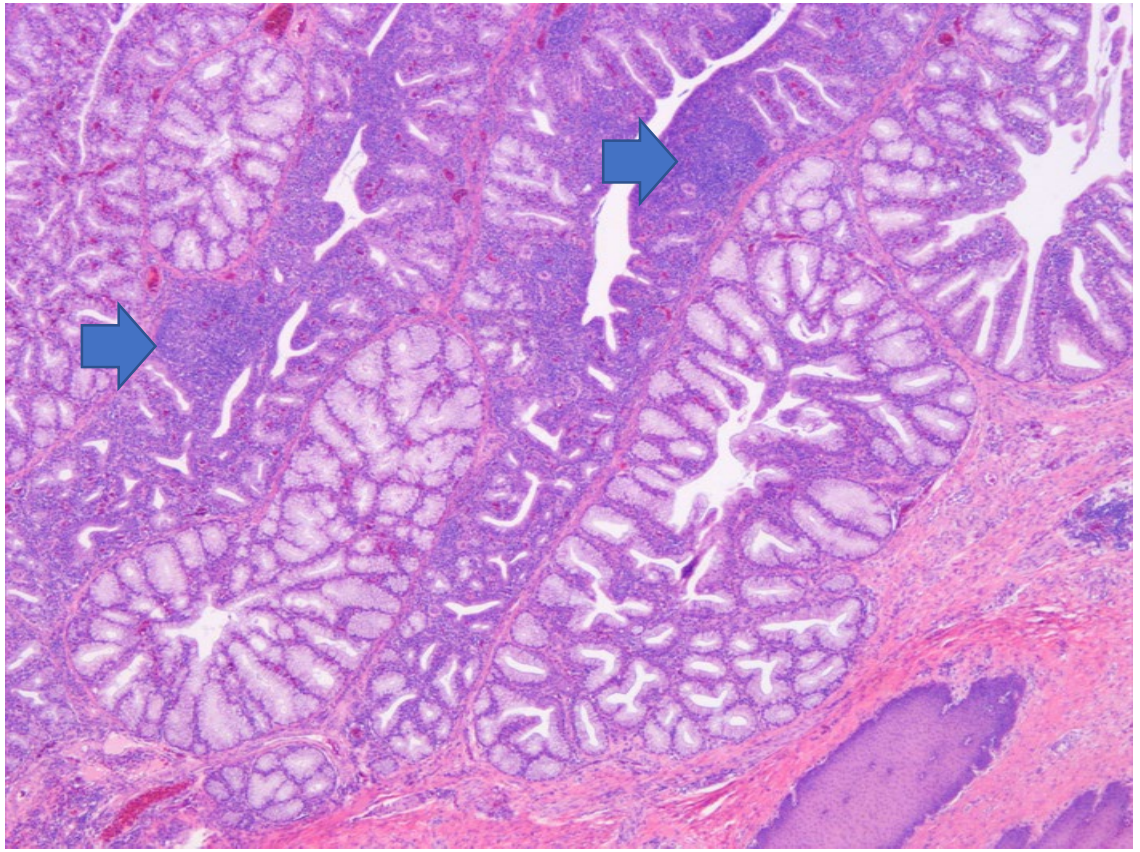



Average of Spleen GC
Spleen Germinal Center Count (B Lymphocytes - Humoral Immunity)
Mean
70
60
50
40
30
20
10
0
14 17 21 35 38 42 15 18 22 24 26 30
Large
Small
Total 5.25 11.8 37.6 34.8 39.5 59.6 8.8 12.6 9.6 14.5 24.4 13.2
Other
Age


Average of Spl-Histiocytosis
Average of Spl-PV LC HP
Spleen Histiocytosis and Hyperplasia of Perivascular Lymphoid Tissue
Mean
4
3.5
3
2.5
2
1.5
1
0.5
0
14 17 21 35 38 42 15 18 22 24 26 30
Large
Small
Average of Spl-PV LC HP 0.5 0.25 1.6 1.8 1.75 1.6 0.6 1 1.8 1 0.8 1.8
Average of Spl-Histiocytosis 0.5 0.75 0 0.6 1.5 1.6 1 0.2 1.6 1.5 1.6 0.8
Other
Age

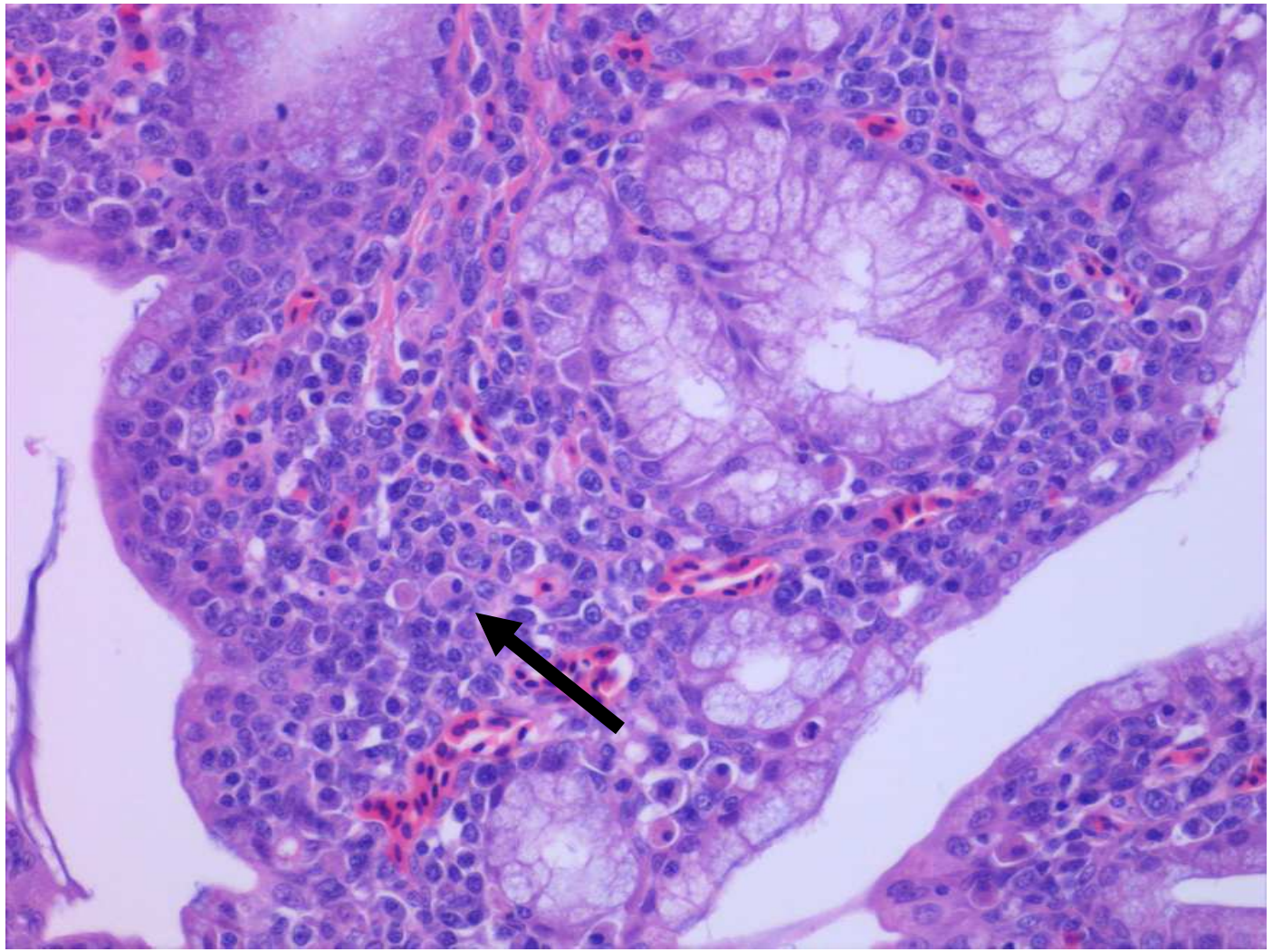
Normal Tongue: Salivary Glands

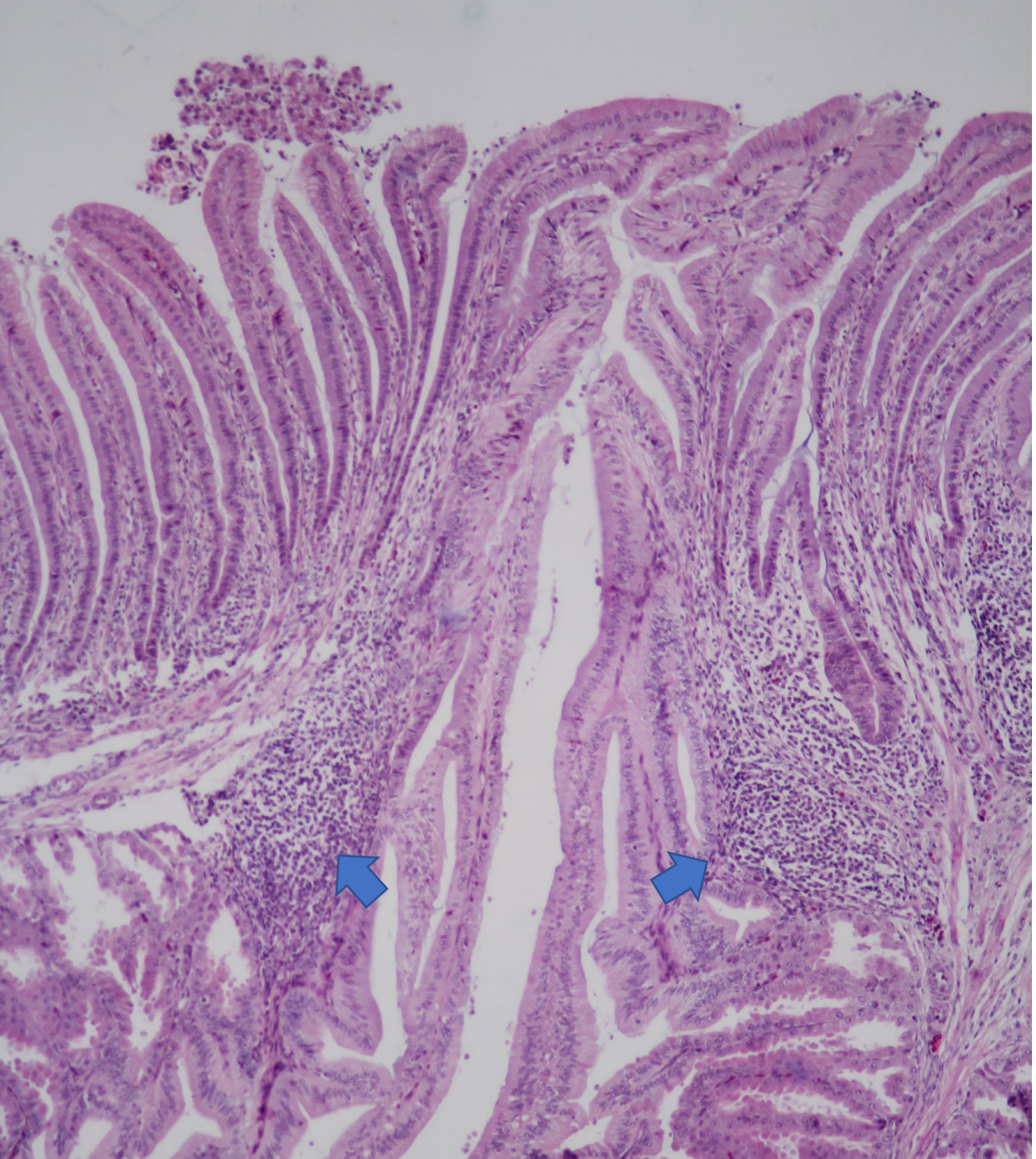




Salivary Glands- Periductal lymphoid tissue extends from tongue, down esophagus to include esophageal glands, to crop entrance

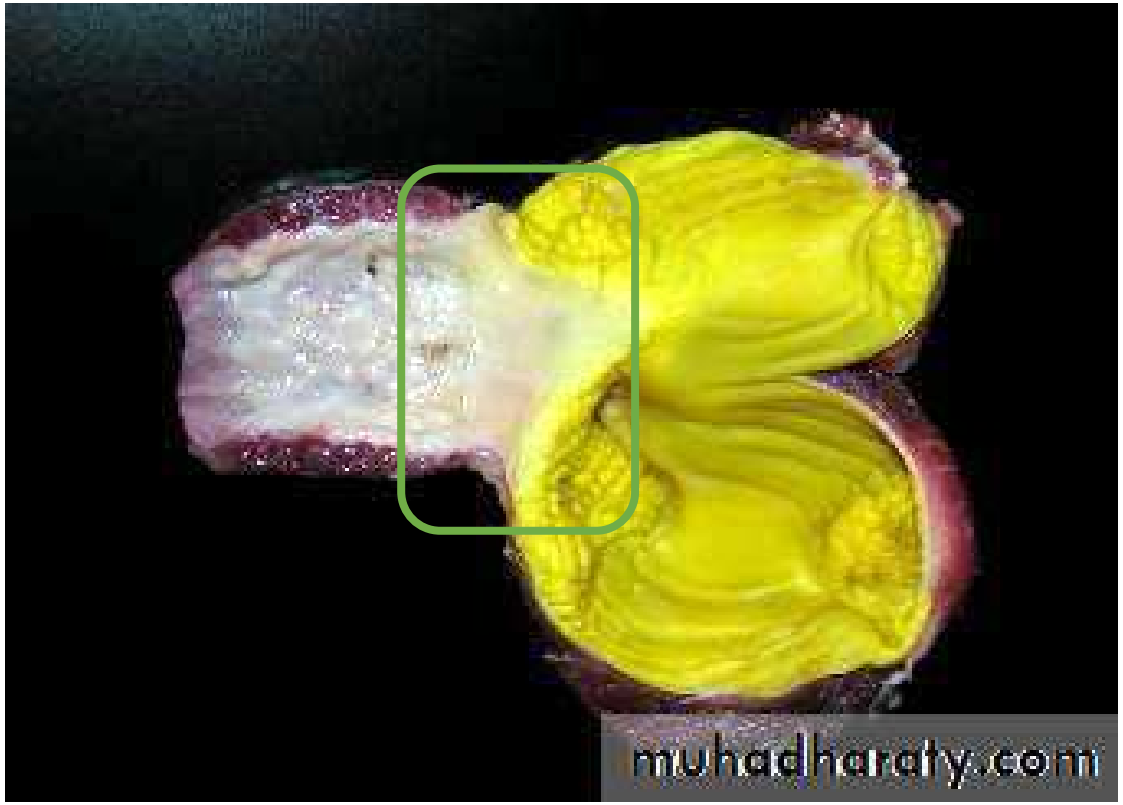
Tongue: Salivary Glands - Lymphoid Tissue, Plasma Cells, Russell Bodies

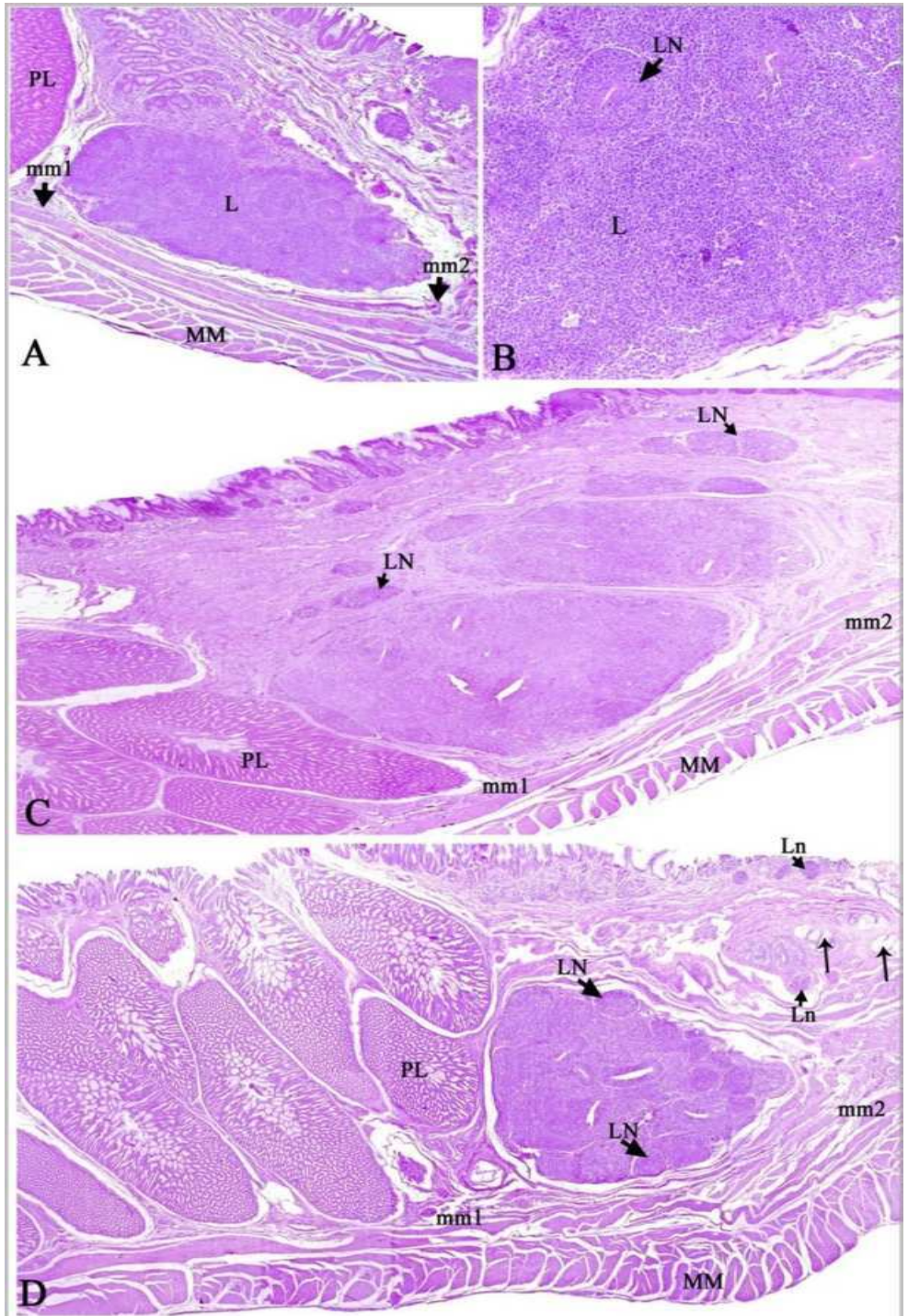


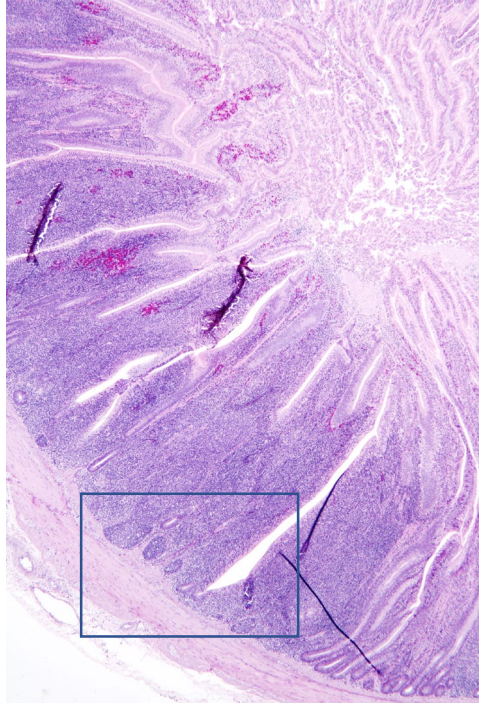


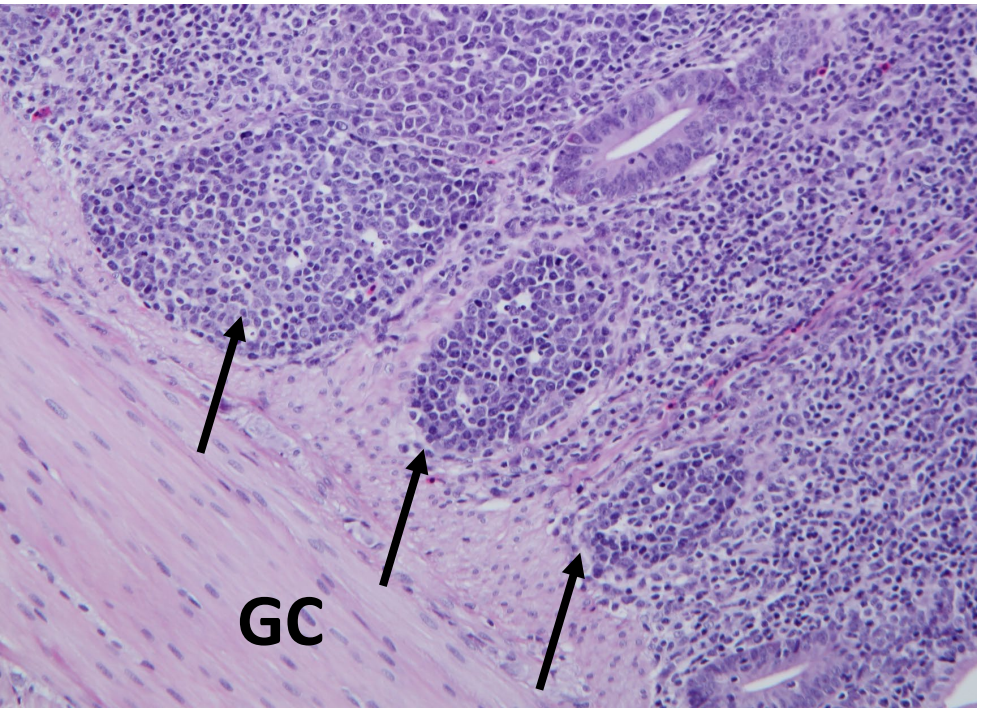
Proventriculus Papillae: Normal Lymphoid Tissue

Proventriculus-Gizzard Lymphoid Tissue (Tonsil-like)





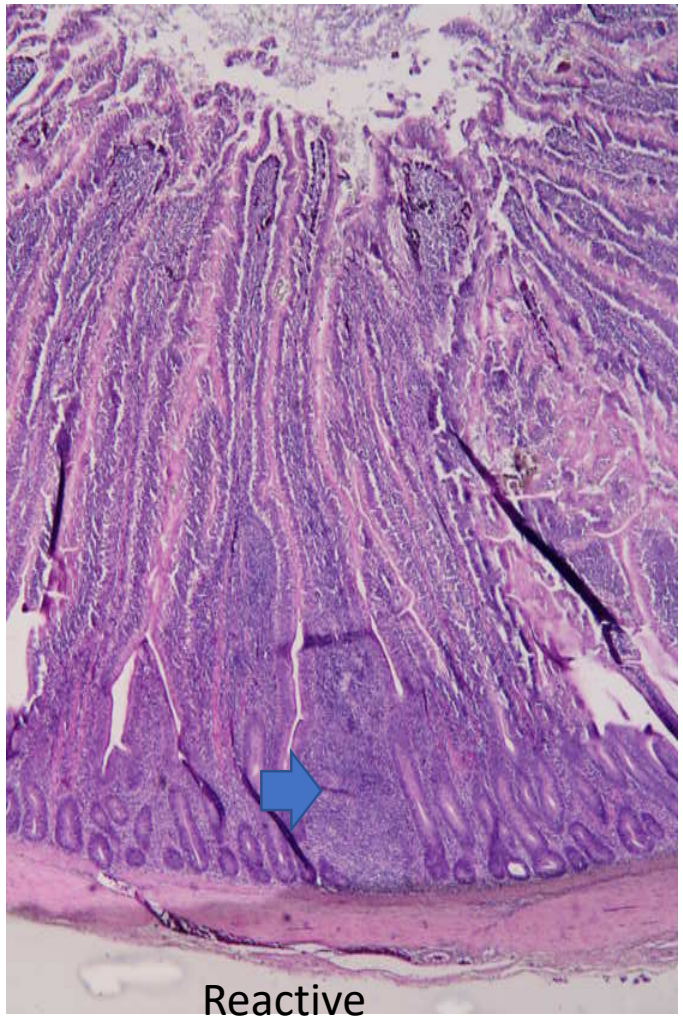




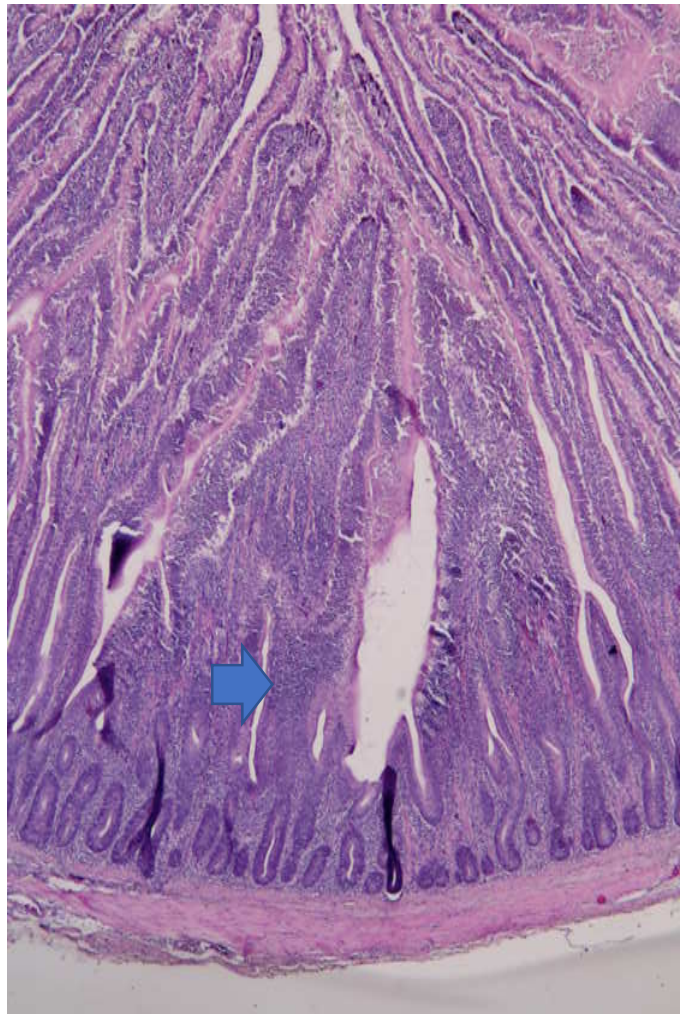
Peyer's Patch, Duodenum. Germinal centers (B cells) and diffuse lymphoid tissue (T-cell rich)

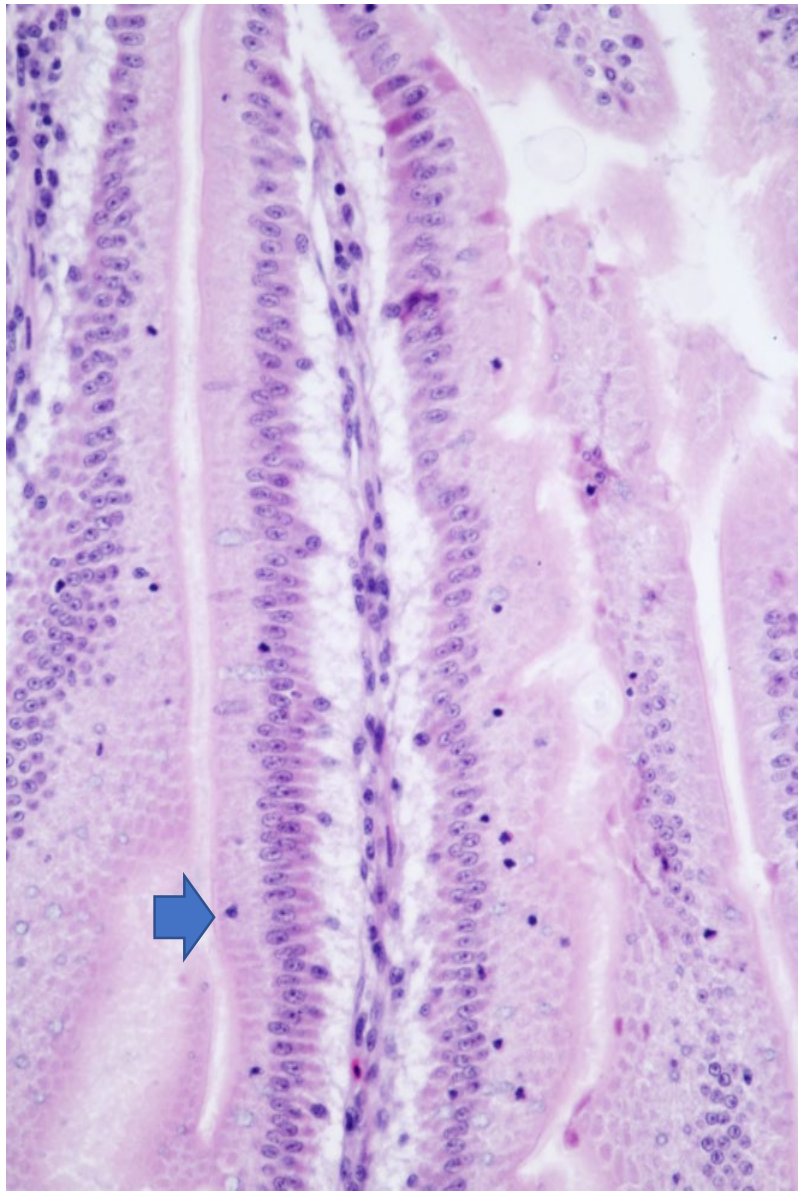
GALT: Gut Associated Lymphoid Tissue - Reactive

Nonreactive

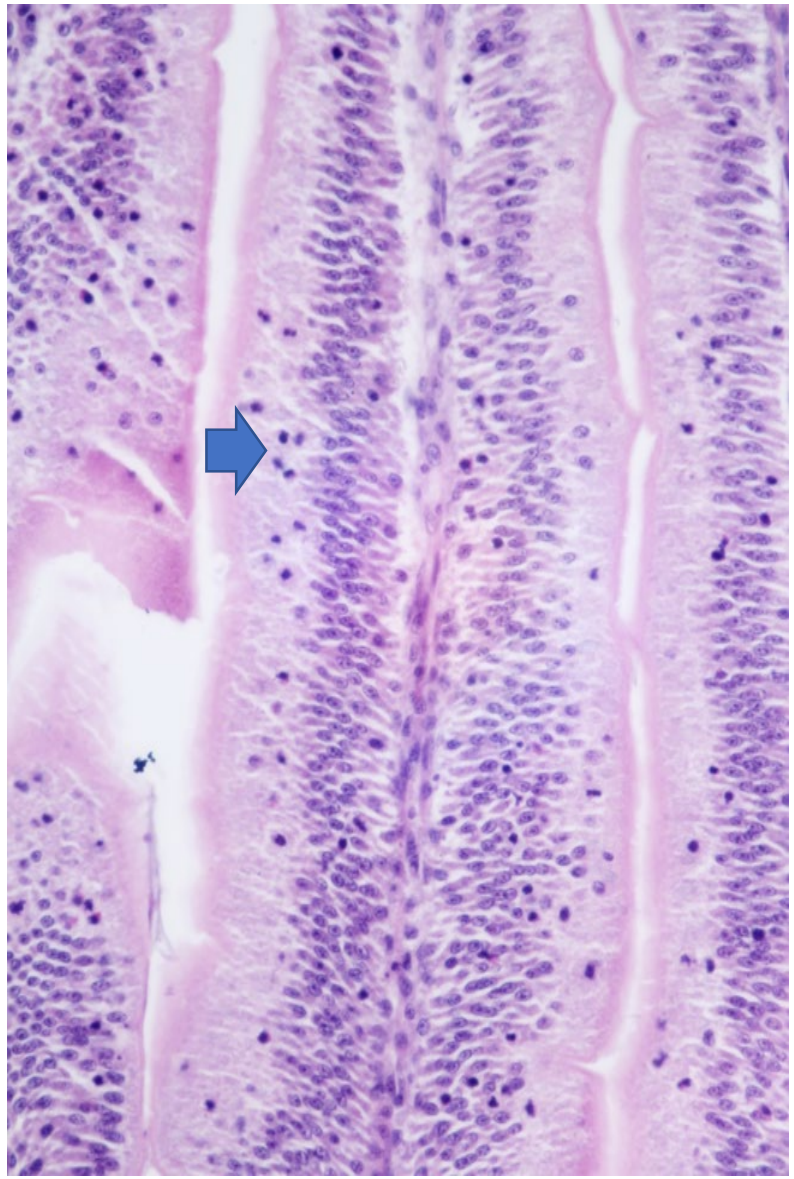


Reactive

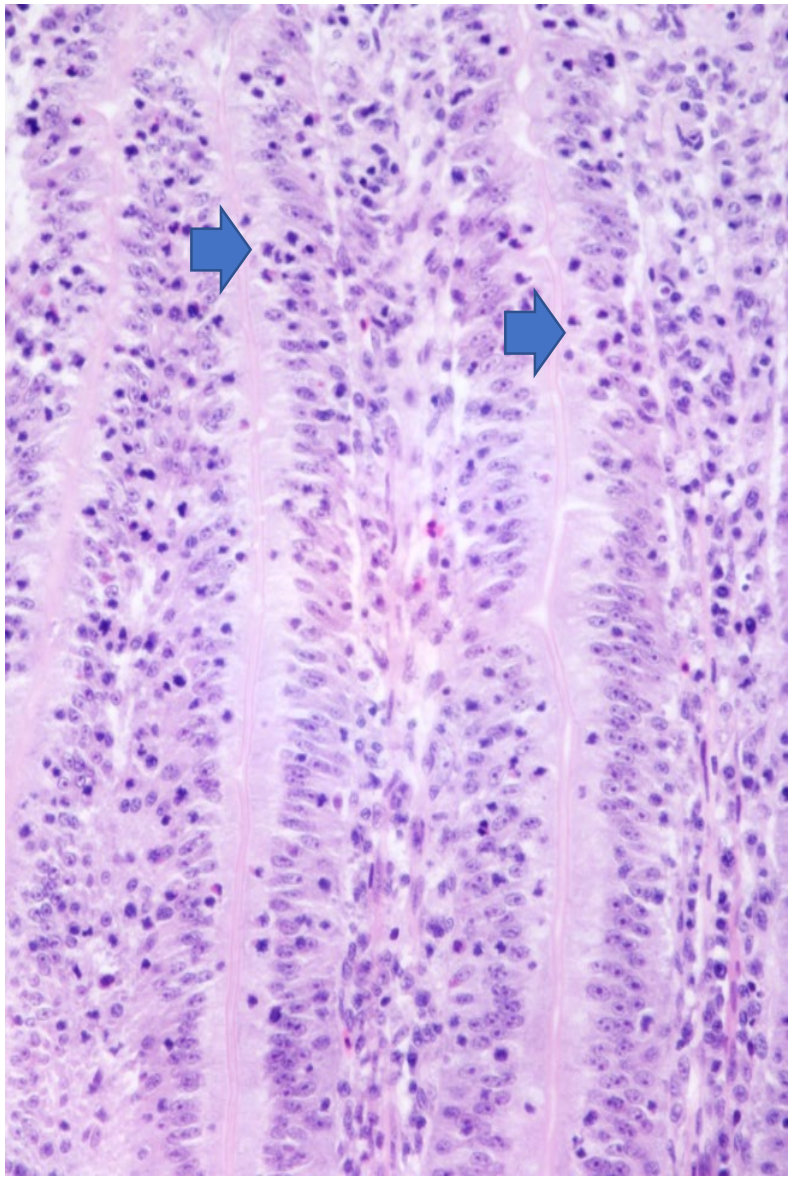




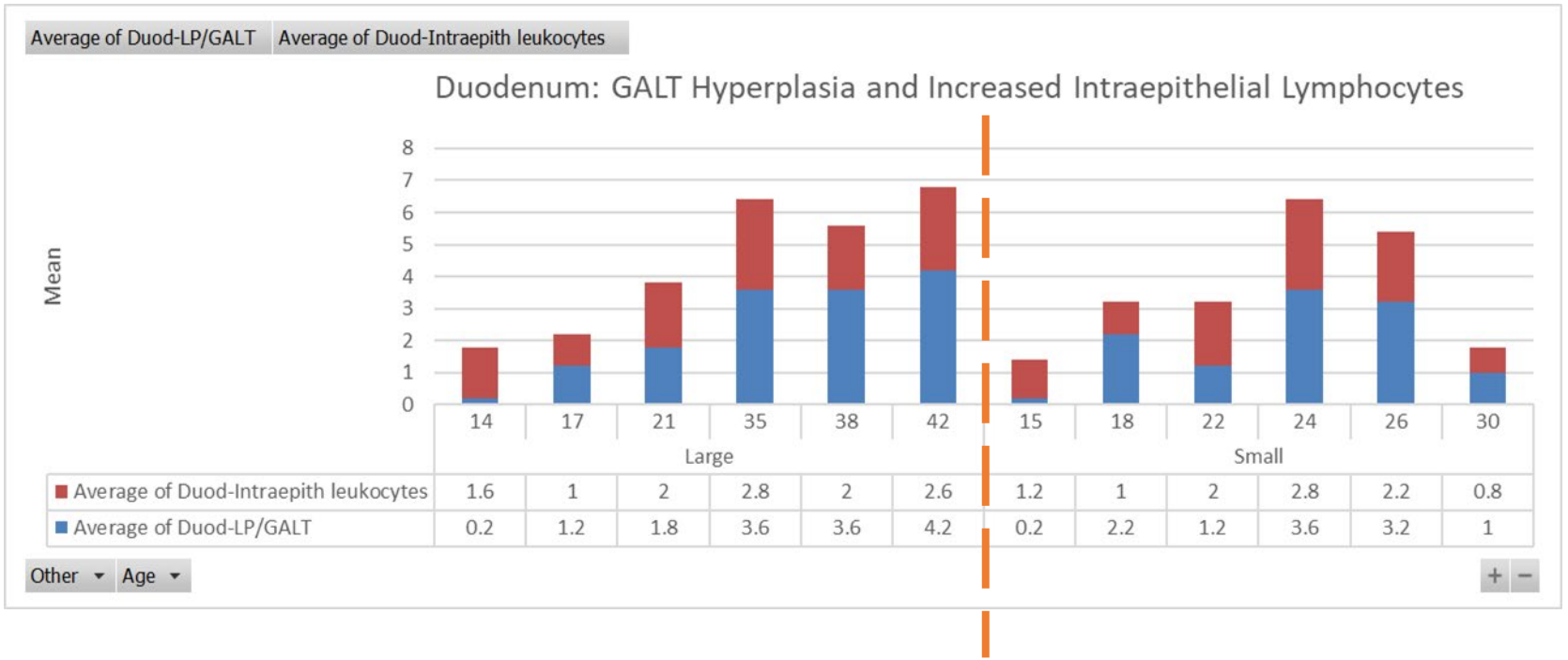
Duodenum. Intraepithelial lymphocytes, grade 0, within normal limits.



Duodenum. Intraepithelial lymphocytes, grade 1, minimal increase.

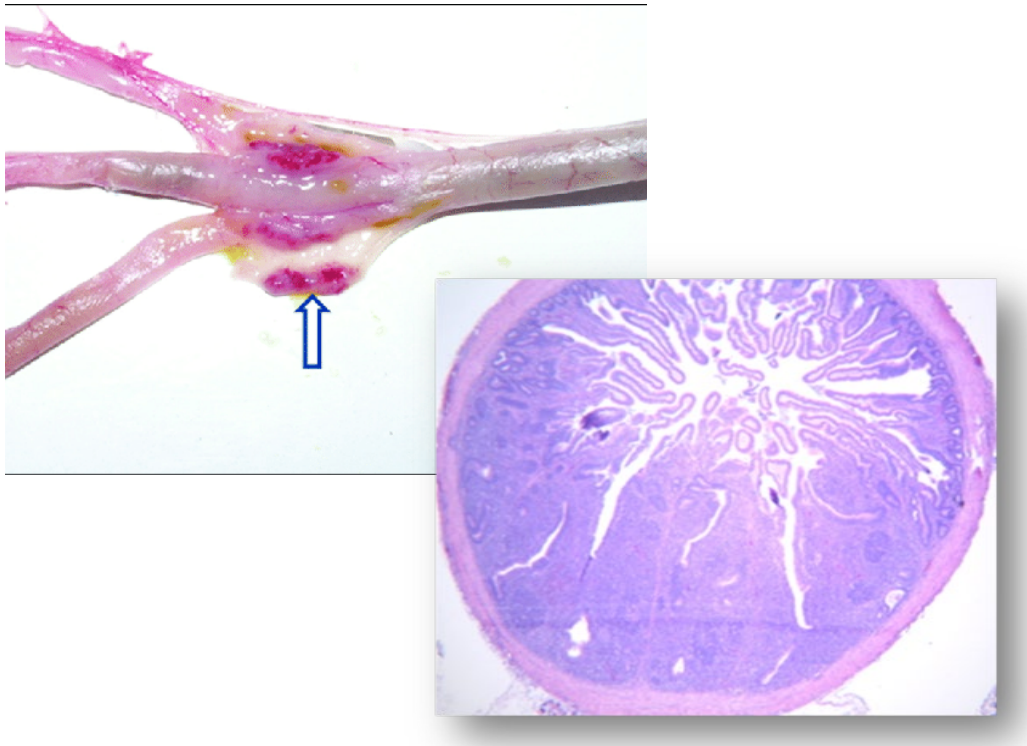


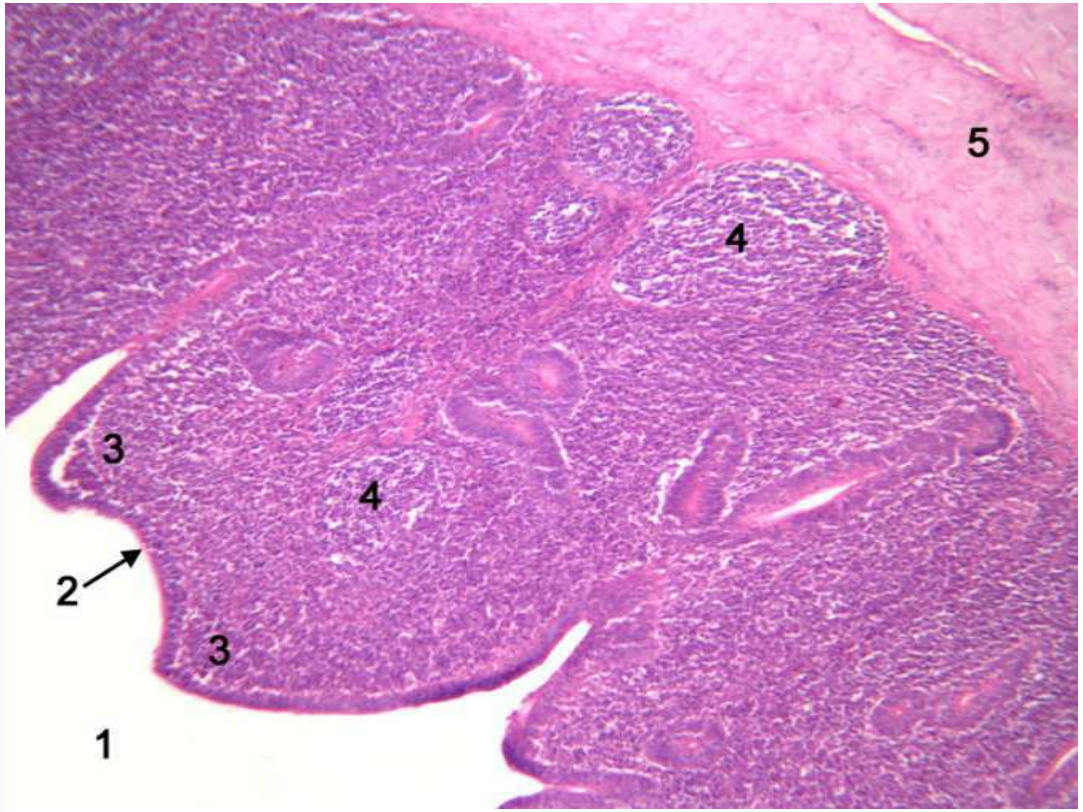
Duodenum. Intraepithelial lymphocytes, grade 2, mild increase



GALT Hyperplasia and intraepithelial lymphocytes generally increase with age in young chicken, and reflect antigenic stimulation in the digestive tract.

Cecal tonsil

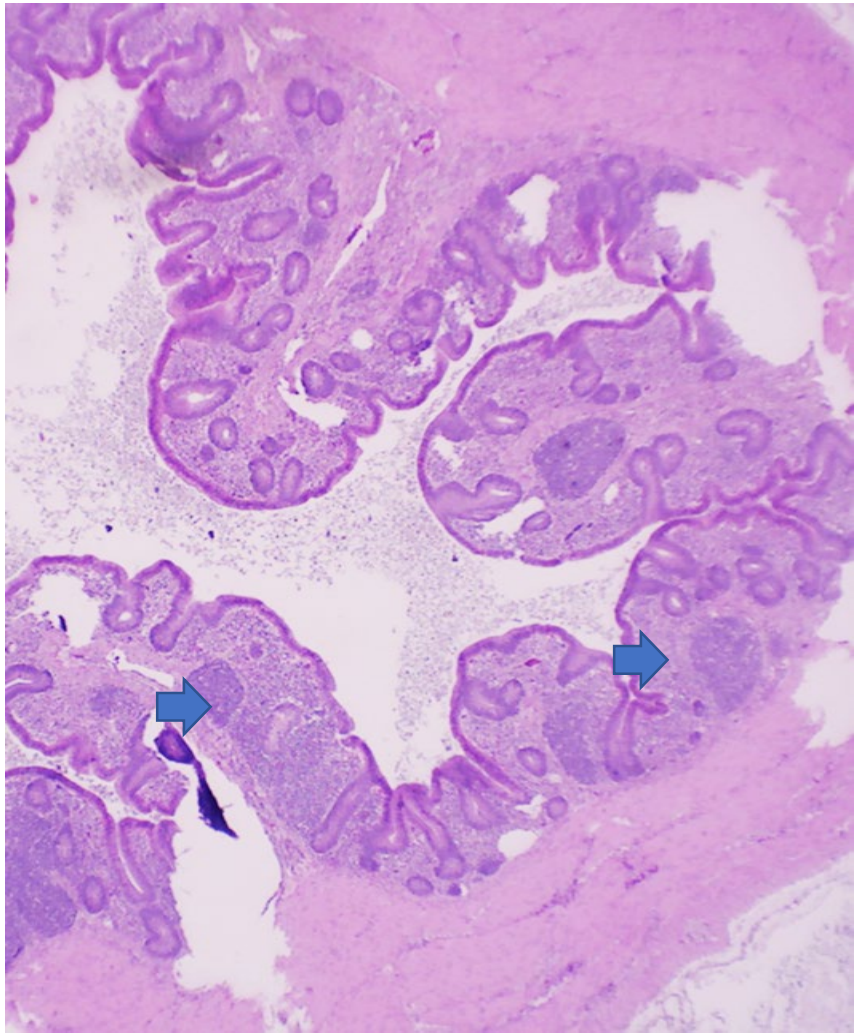


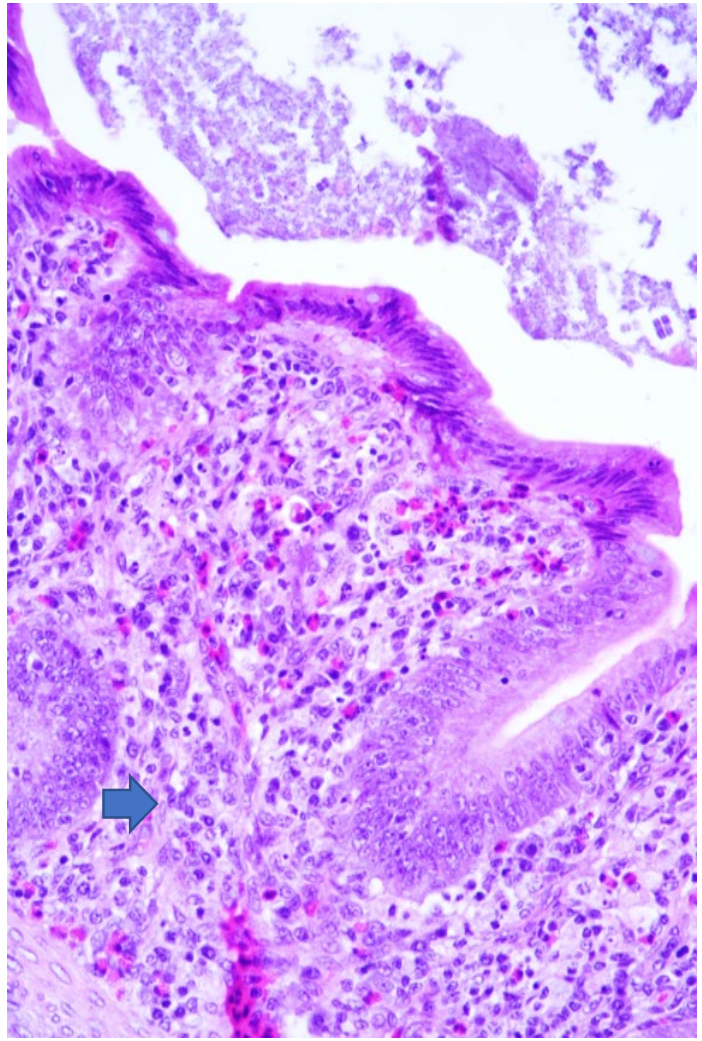


Diffuse lymphoid tissue and germinal centers.

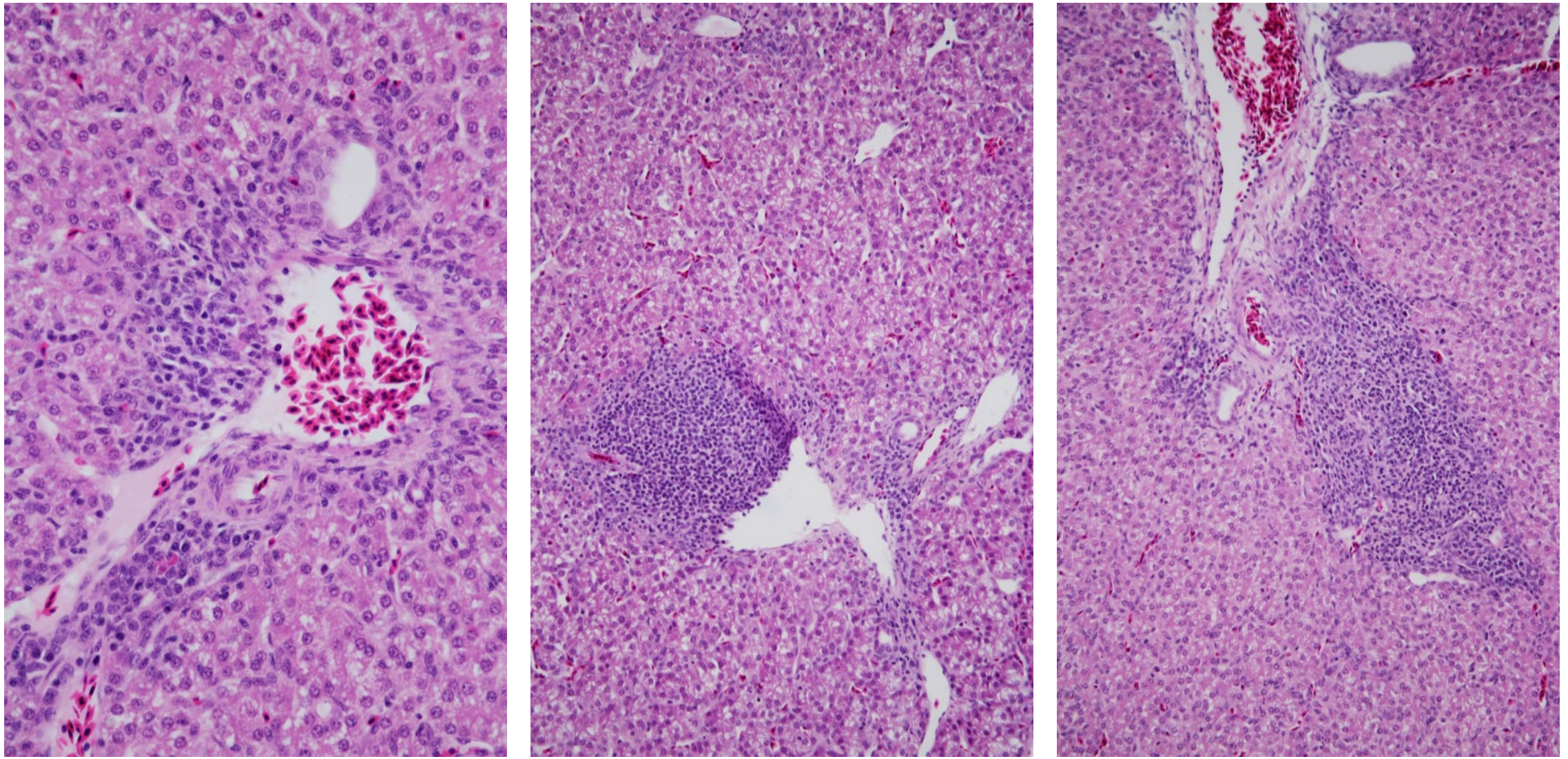
https://www.researchgate.net/figure/Figure-3-Hemorrhages-in-cecal-tonsils-arrow-with-up-head_fig4_315363722

Cecum: Reactive GALT Hyperplasia and Inflammation

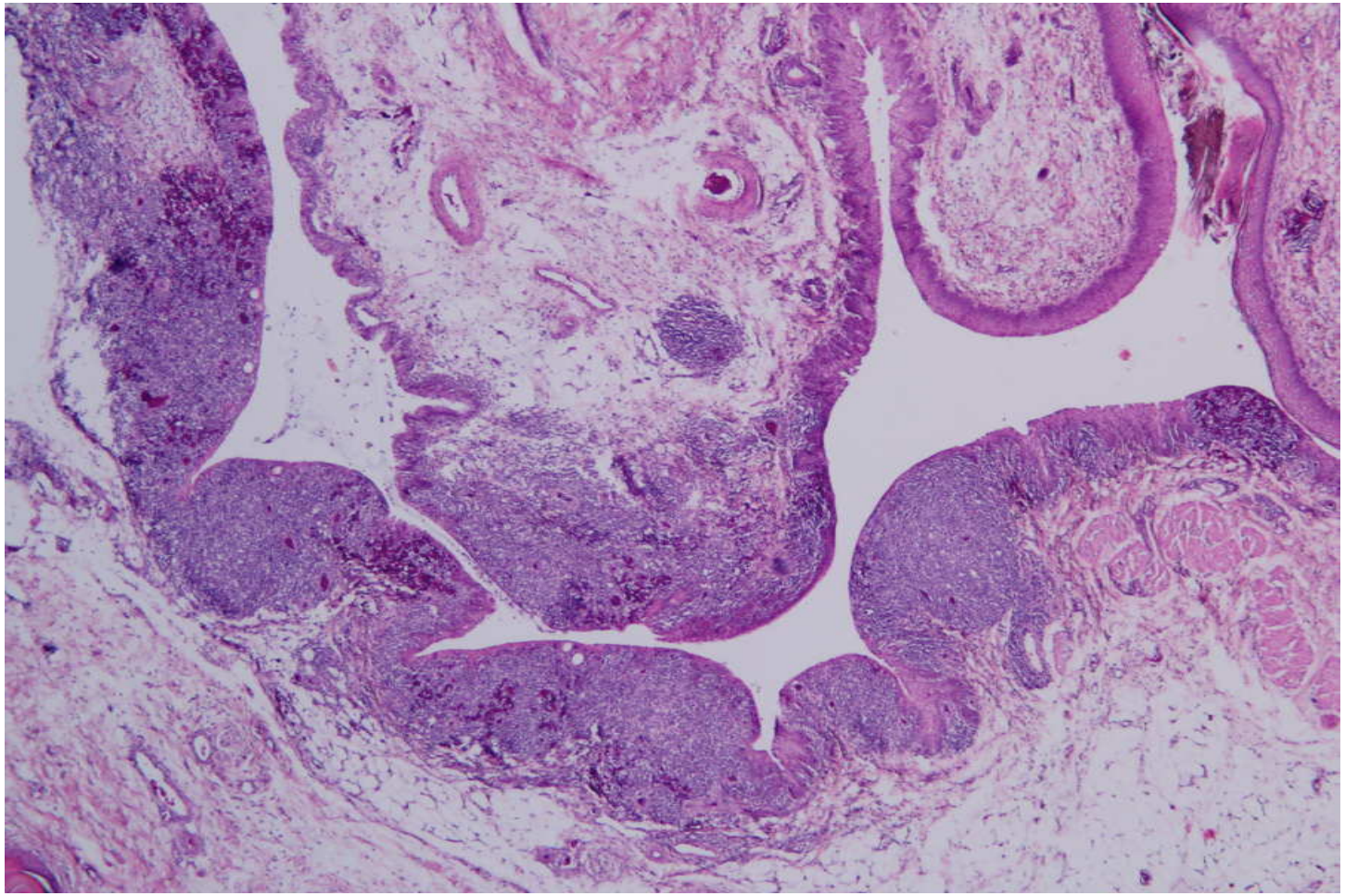




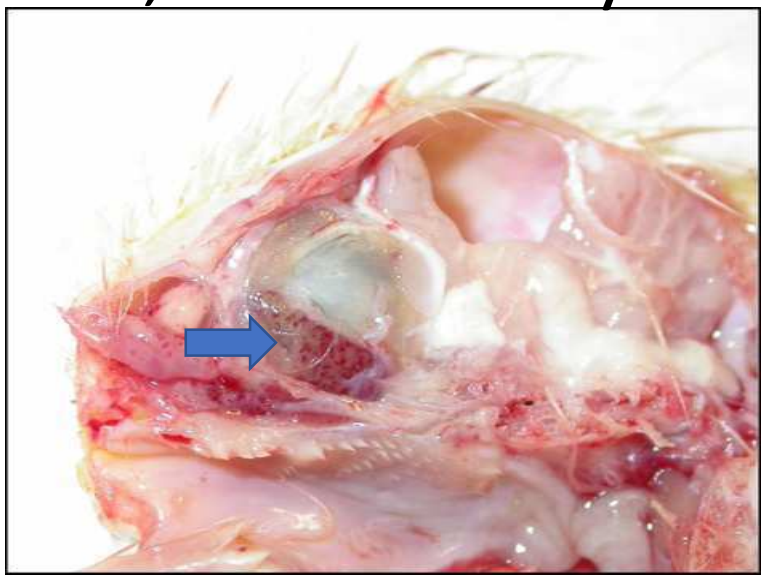
Liver, periportal diffuse lymphoid tissue

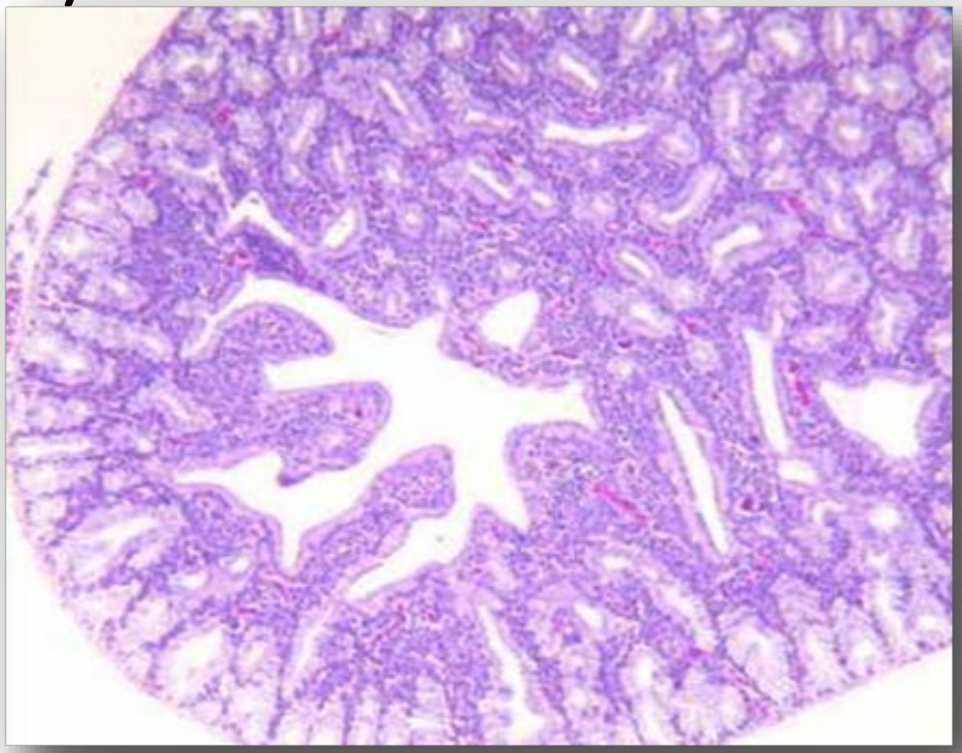


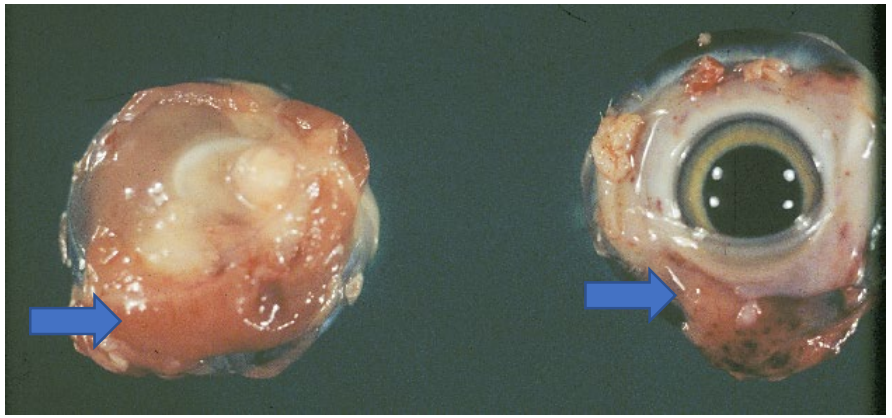
Conjunctiva associated lymphoid tissue with hyperplasia



Harderian Gland: lymphocytes and plasma cells; secretory antibody



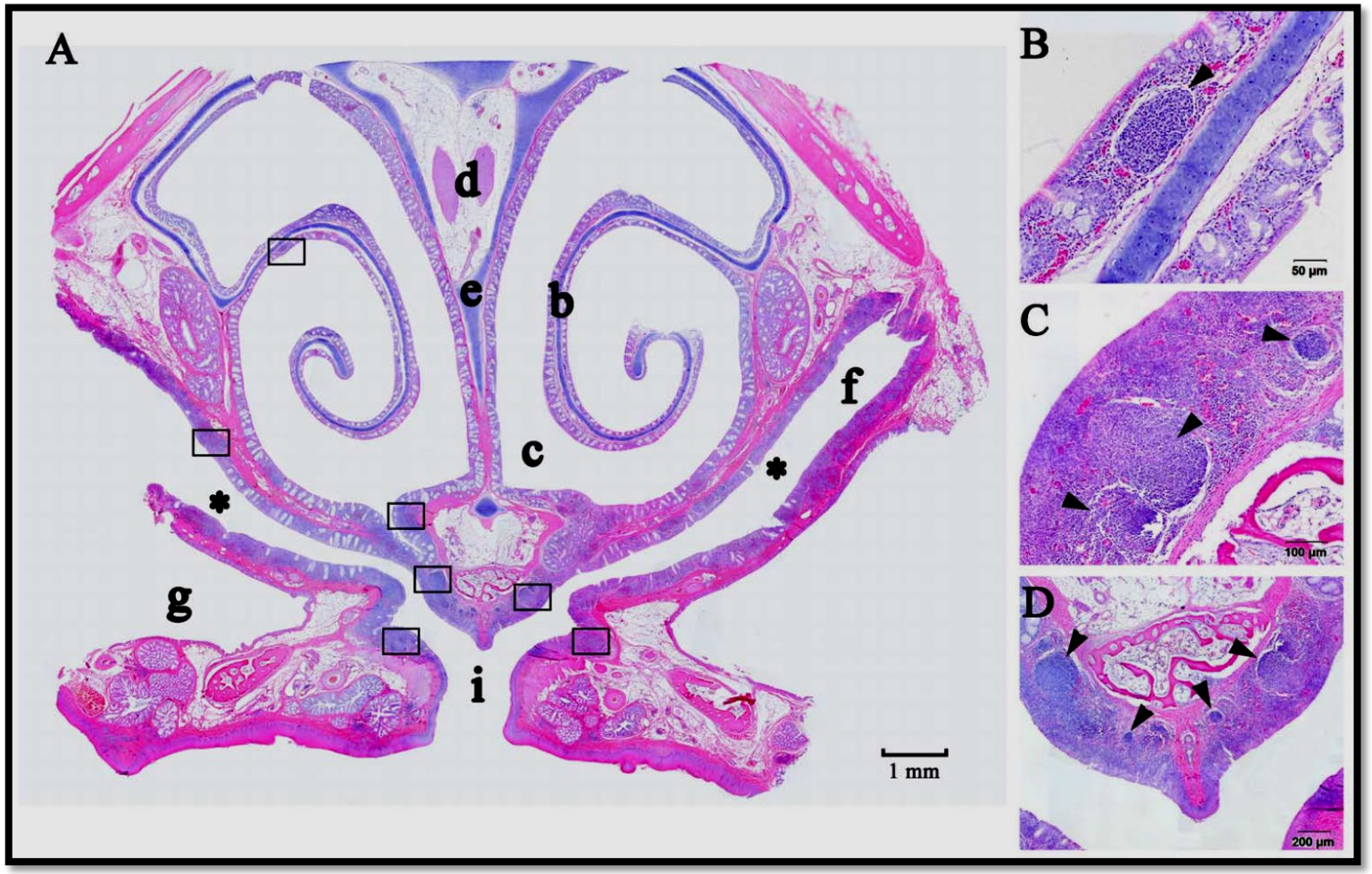




Haroldo Toro

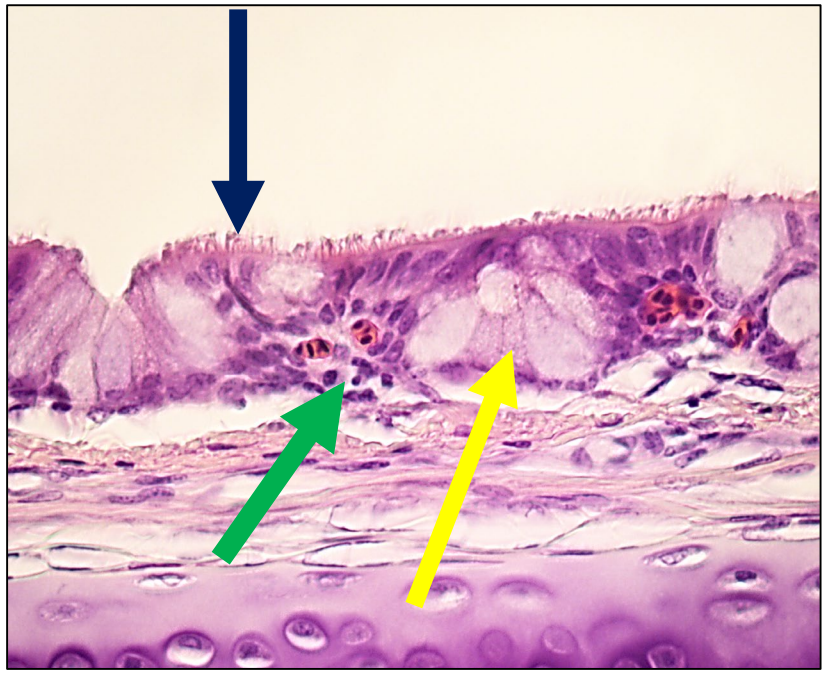
Upper respiratory lymphoid tissue

- With the Harderian gland, these tissues are essential for the function of aerosol vaccination.
- Portions lined with lymphoid tissue absorb antigen (inactivated influenza virus), more than non-lymphoid regions.

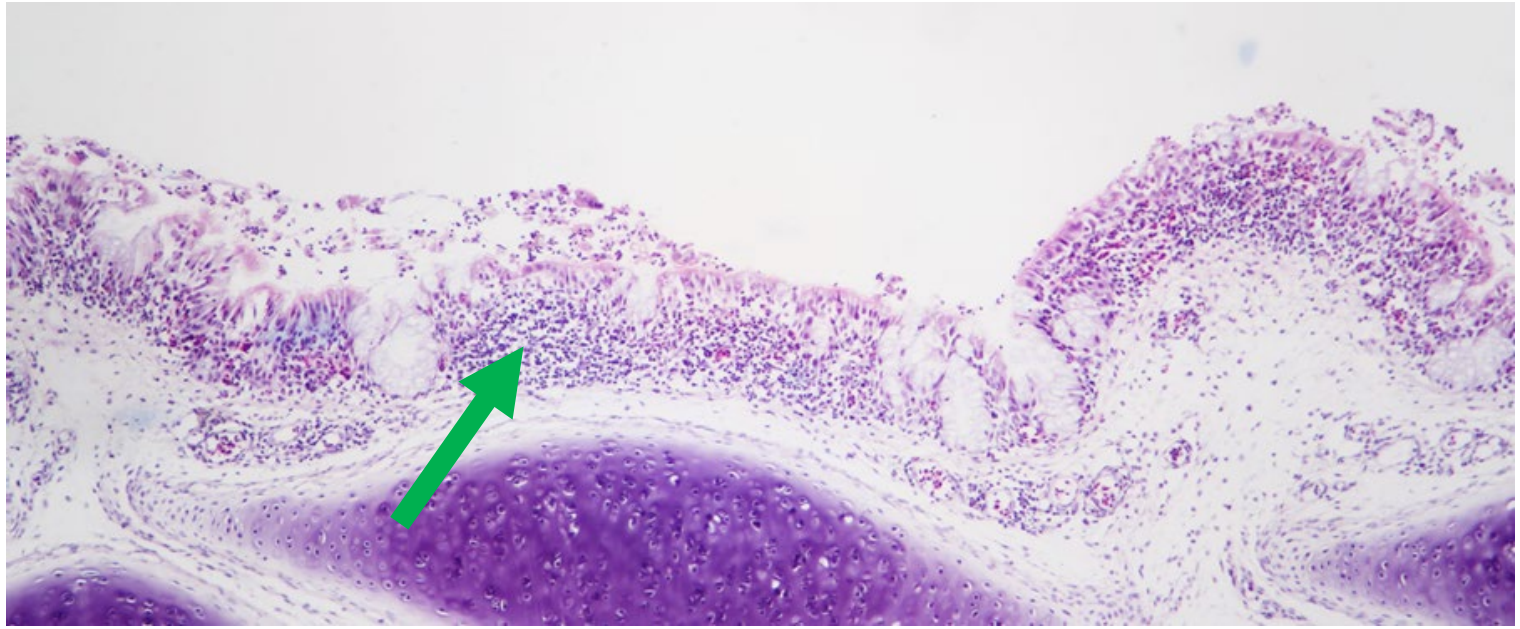


Citation: Kang H, Yan M, Yu Q, Yang Q (2013) Characteristics of Nasal-Associated Lymphoid Tissue (NALT) and Nasal Absorption Capacity in Chicken. PLoS
ONE 8(12): e84097. doi:10.1371/journal.pone.0084097

Trachea lymphoid tissue



Normal Trachea. Mucosal surface almost completely covered with cilia (black arrow), intact trachea gland (yellow arrow) & very mild lymphocytic infiltration of lamina propria (green arrow) (photo, Floyd Wilson).

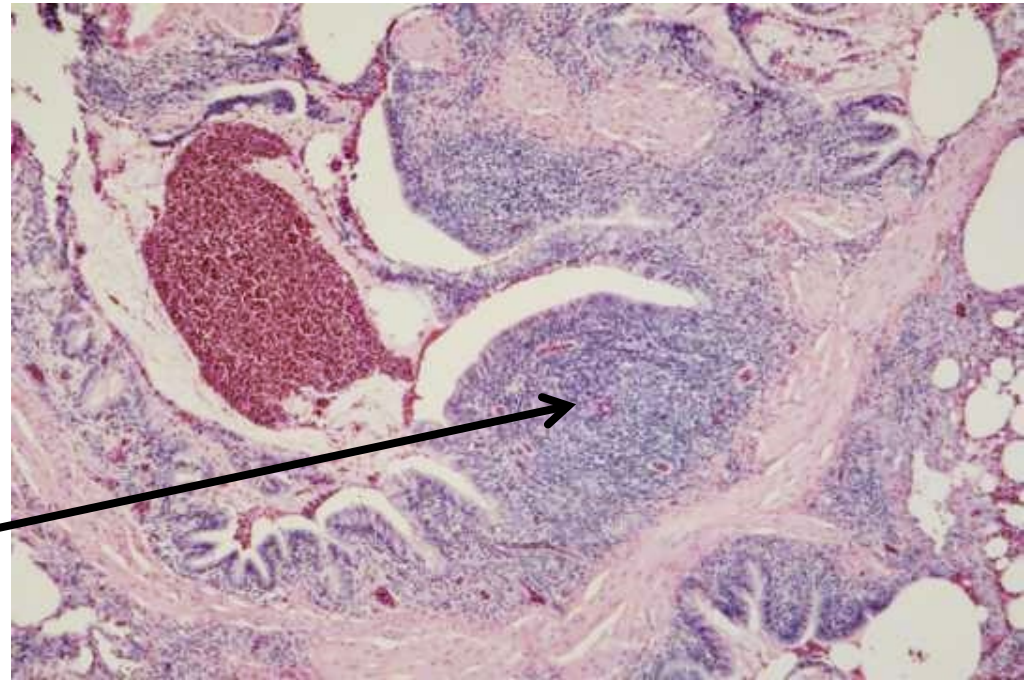


23-day trachea, lymphocytic infiltration of mucosa, consistent with reaction to attenuated live virus vaccine, within normal limits for production environment..

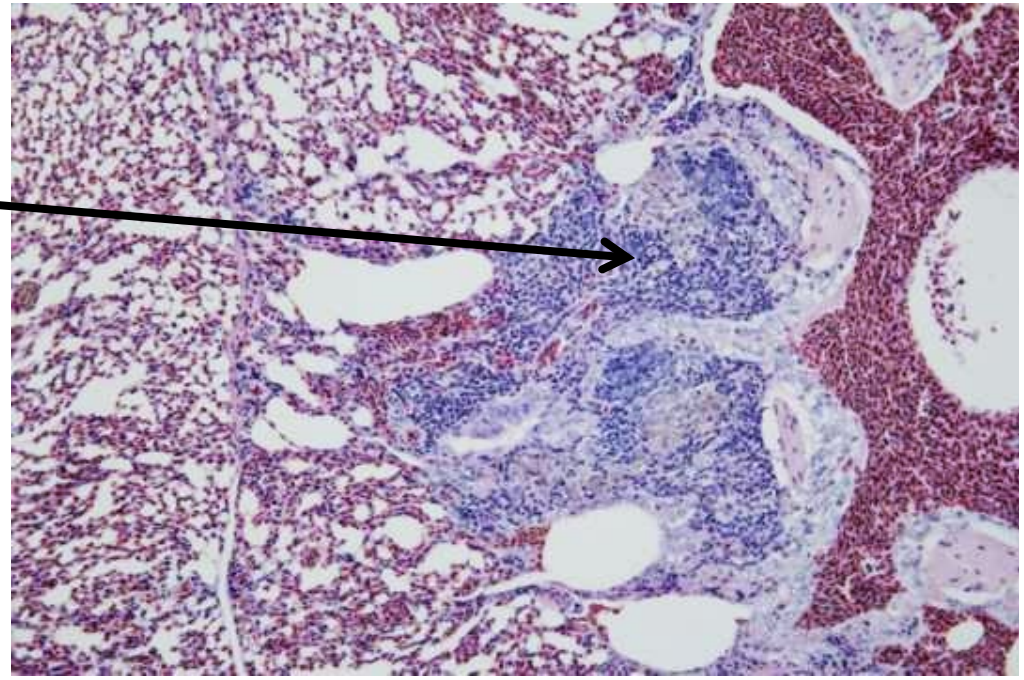
Lung – BALT

Bronchial associated
lymphoid hyperplasia (BALT)

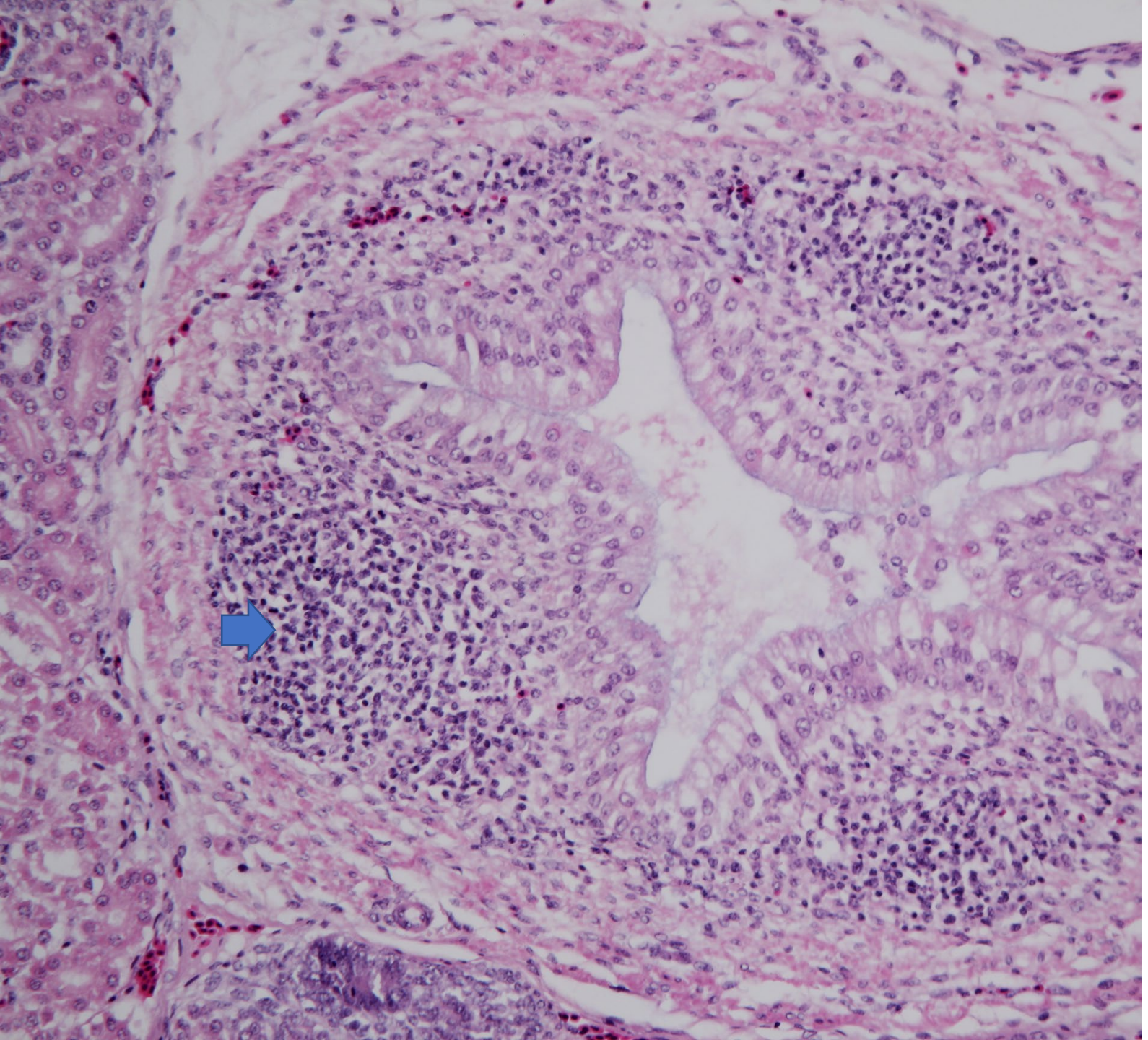
Secondary bronchus



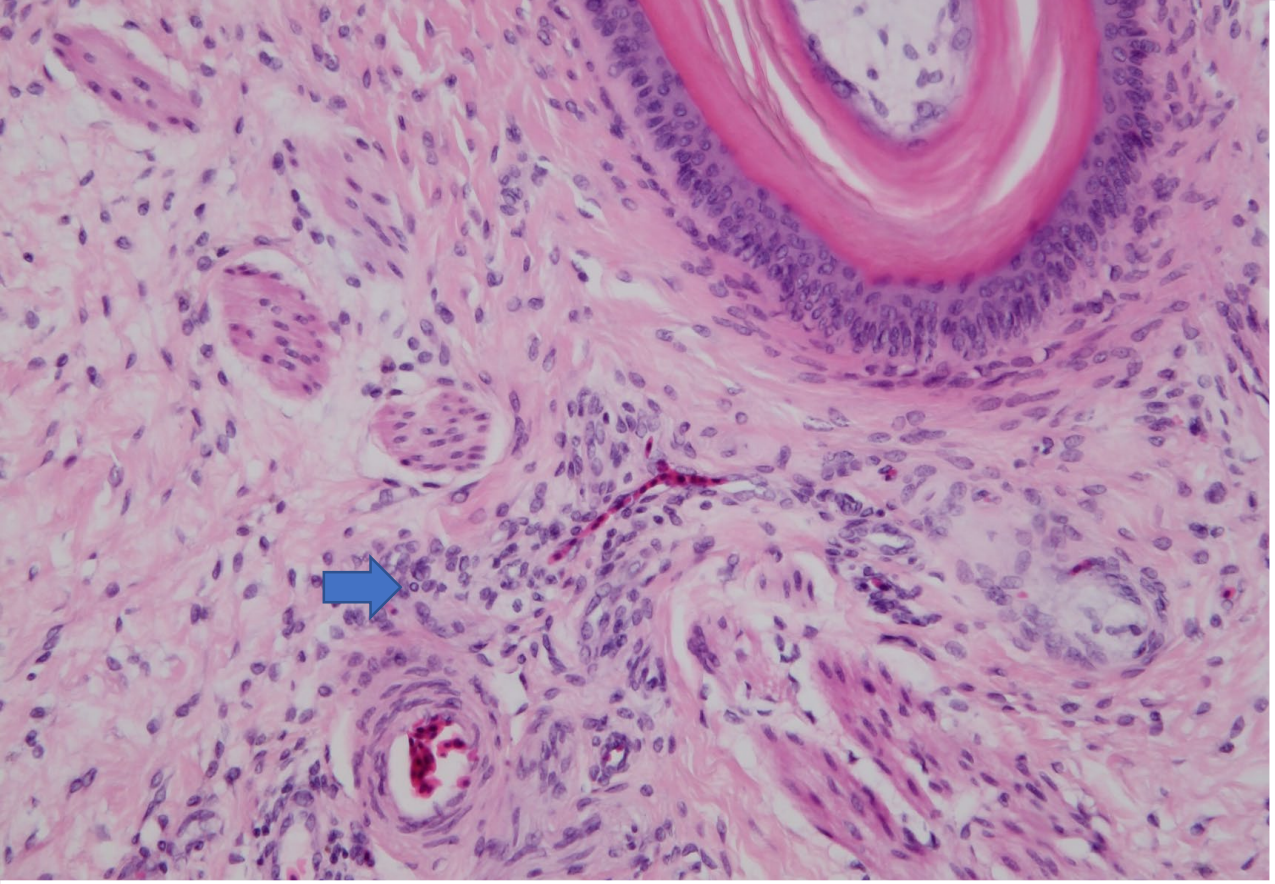
Tertiary bronchus,
atrium

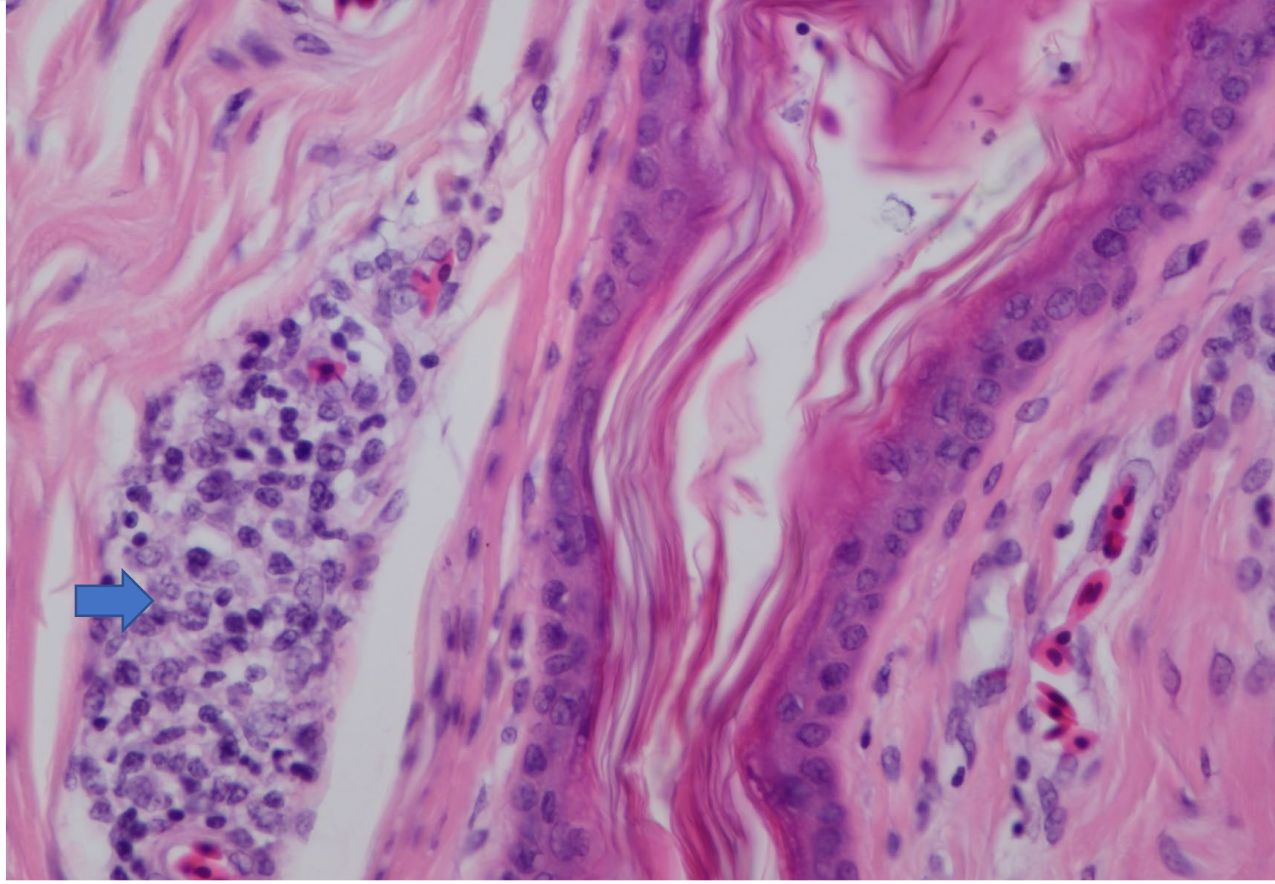


F. J. Hoerr 2011



Urinary Lymphoid Tissue: Lower Urinary Tract - Ureter

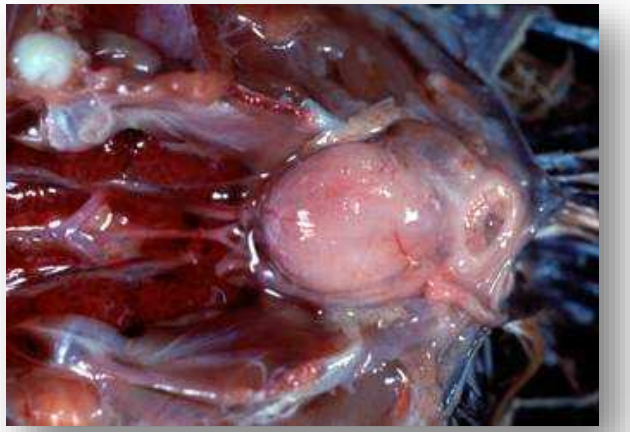


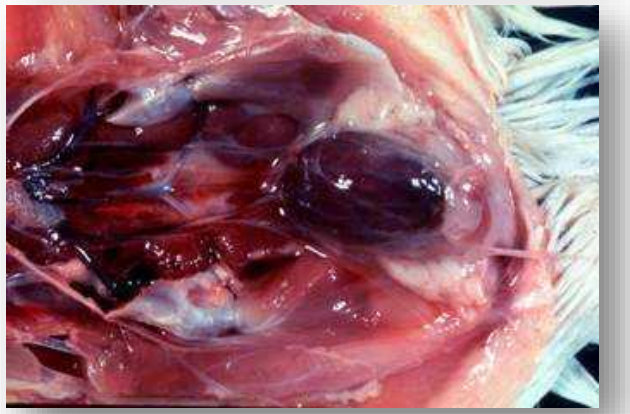


Skin – perifollicular and perivscular

Infectious Bursal Disease – Classical and Variant

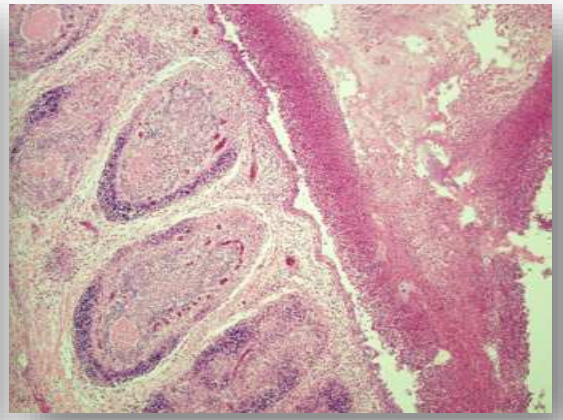
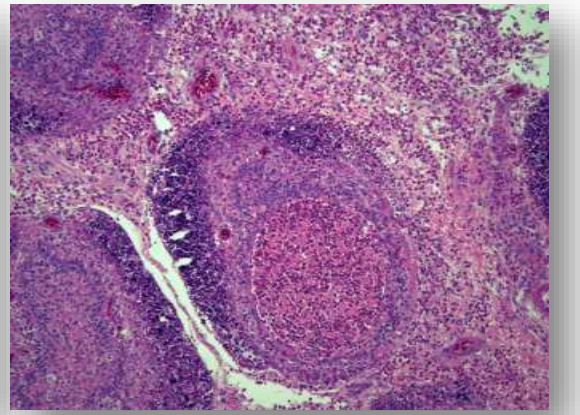
R. W. Winterfield

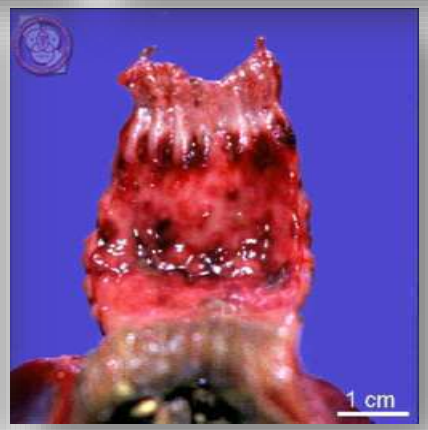


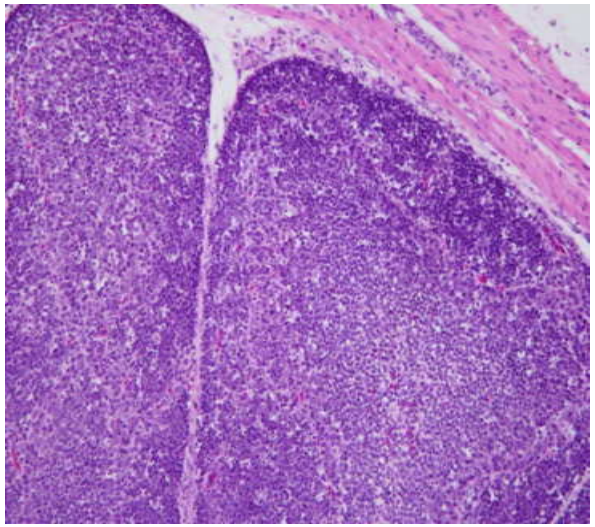


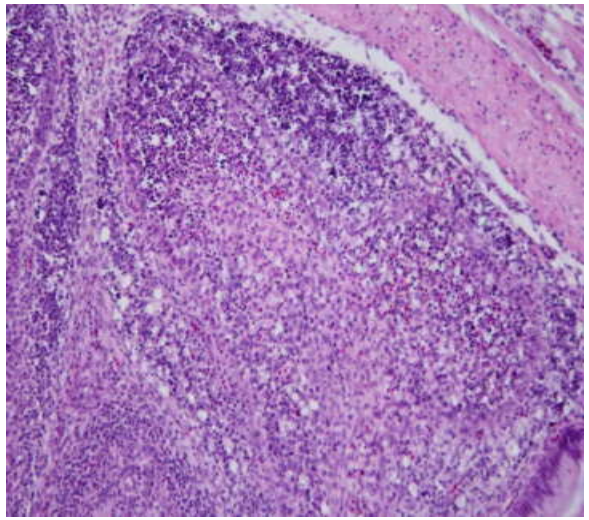
Delaware Variant

Very Virulent Bursal Disease (vvIBD)







Stages of Bursal Disease: Histopathology

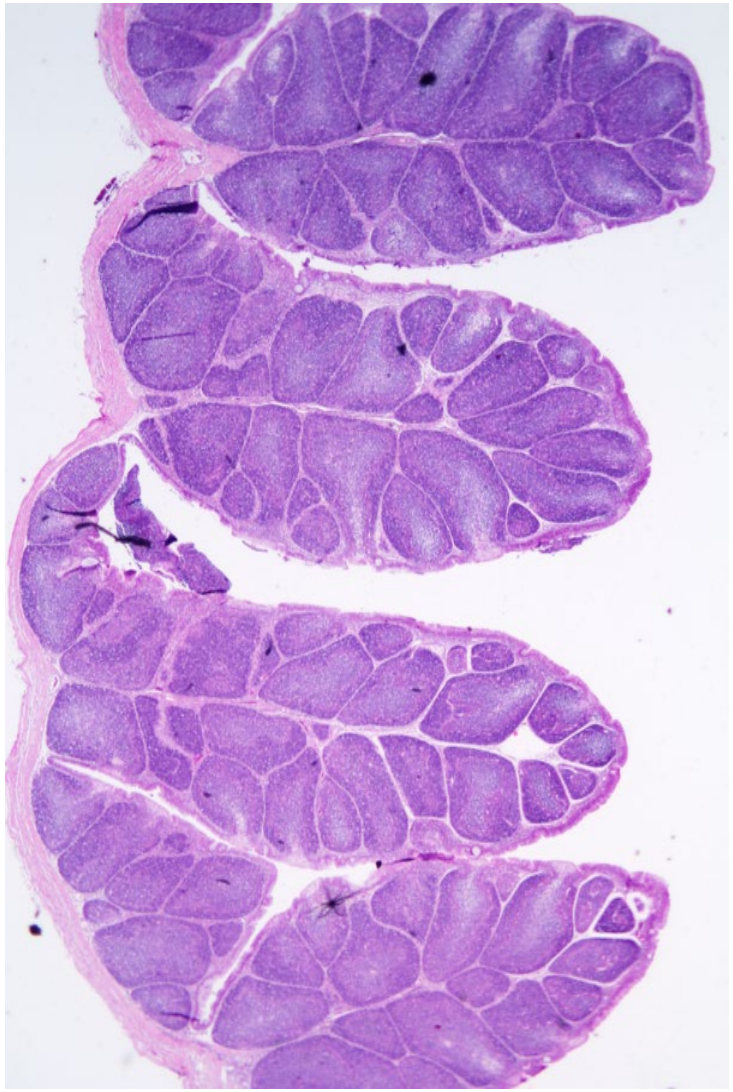
- **Normal Bursa**
- **Virus infection, acute necrotizing phase**
  - Lymphocyte necrosis/apoptosis –rapid progression
  - Strain variable
    - Apoptosis and lymphoid depletion, absent or minimal inflammation
    - Hemorrhage, edema, granulocytic and histiocytic inflammation
  - Cytotoxic T cells rapidly enter and associated with decline of viral load
  - End point: the lymphocyte population is destroyed
- **Follicular restitution phase**
  - Absent to substantially complete
  - Small undifferentiated follicles, lower immunocompetence
  - Large differentiated follicles, higher immunocompetence

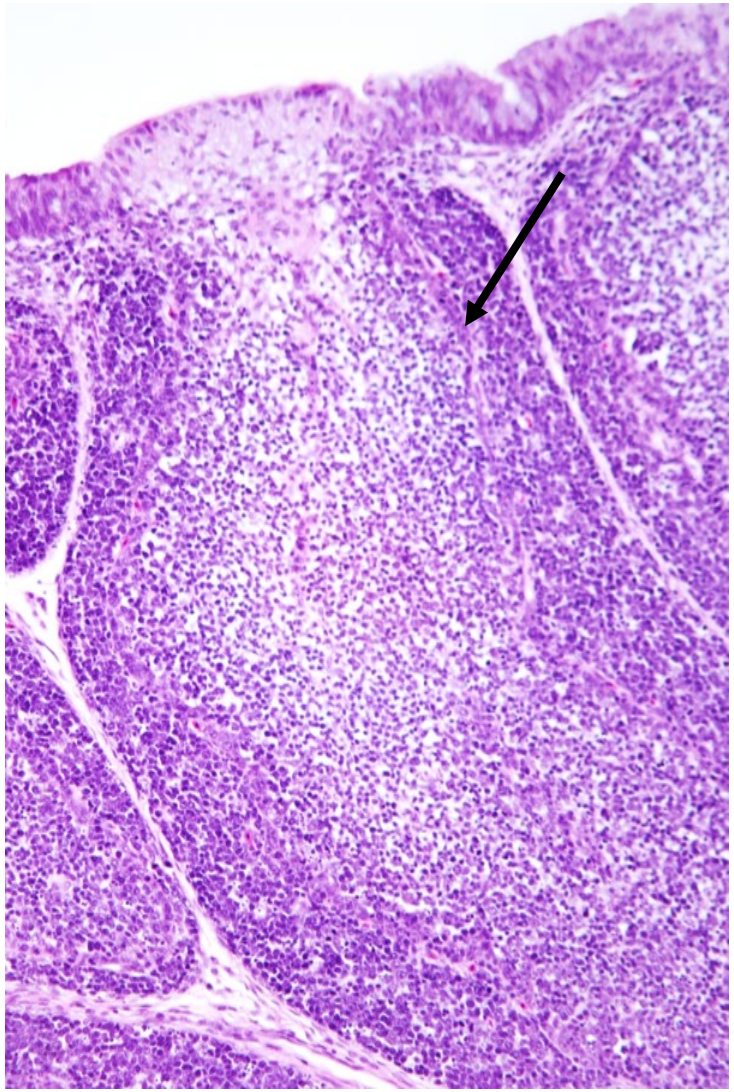
NORMAL

ACUTE PHASE – Necrosis/Apoptosis

RECOVERY PHASE – Follicular Restitution

0 1 2 3 4 5 5 4 3 2 1 0



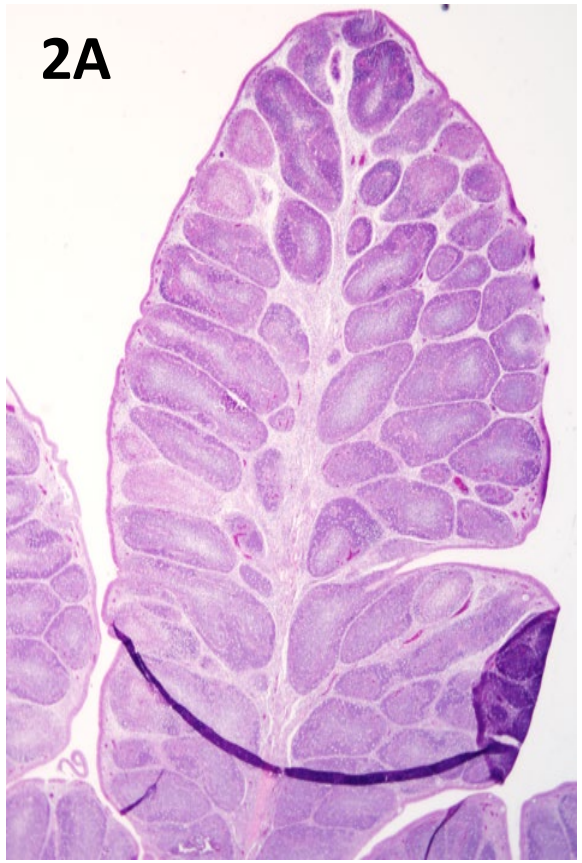


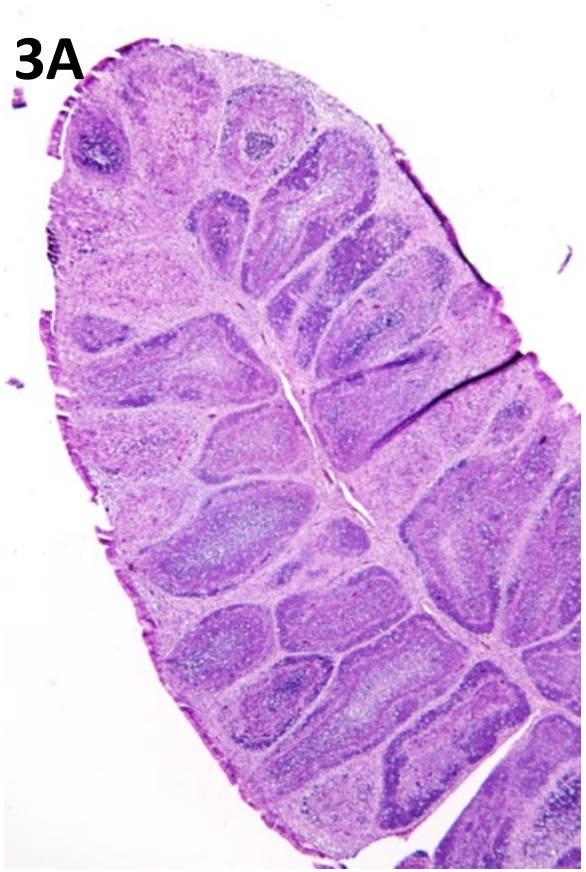
NORMAL

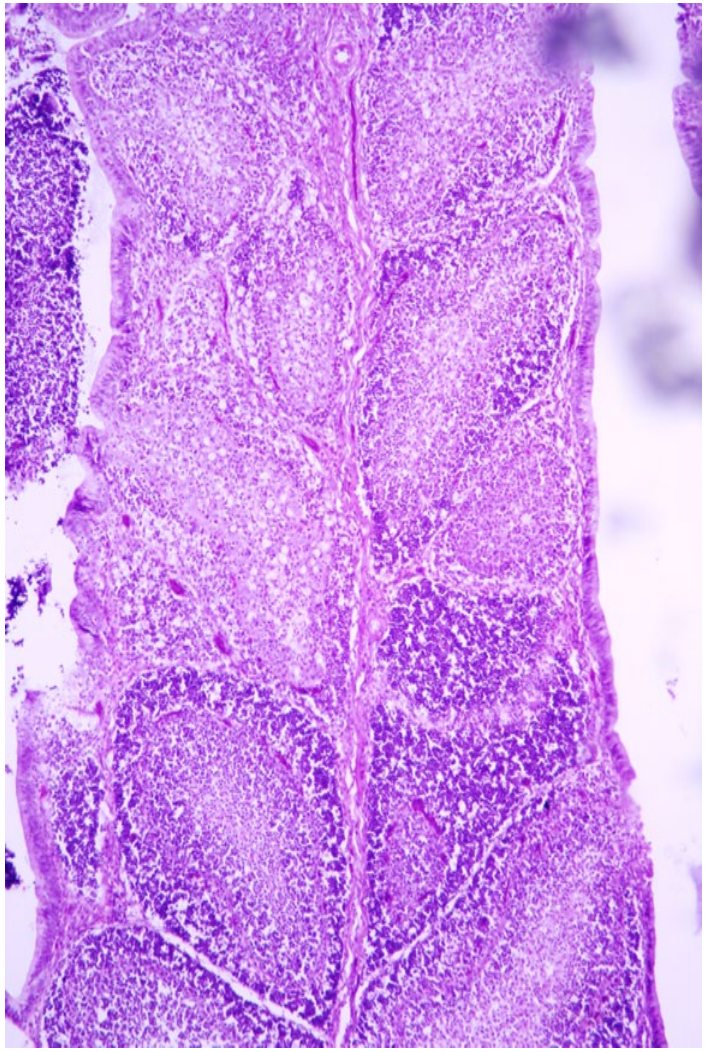
ACUTE PHASE – Necrosis/Apoptosis

RECOVERY PHASE – Follicular Restitution

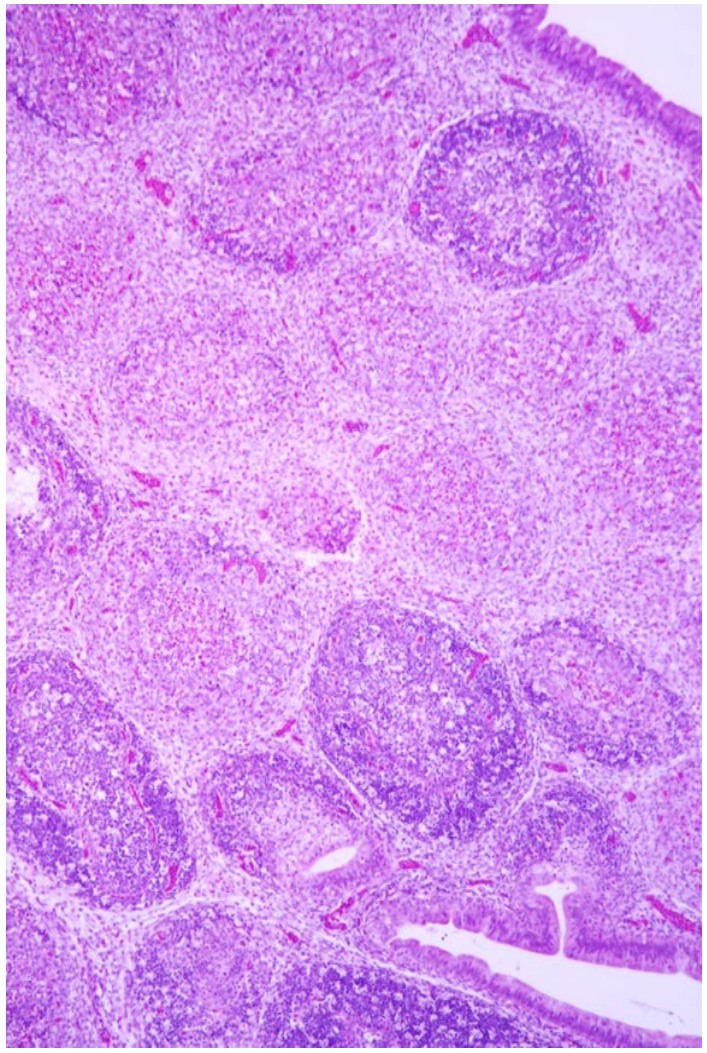
0 1 2 3 4 5 5 4 3 2 1 0



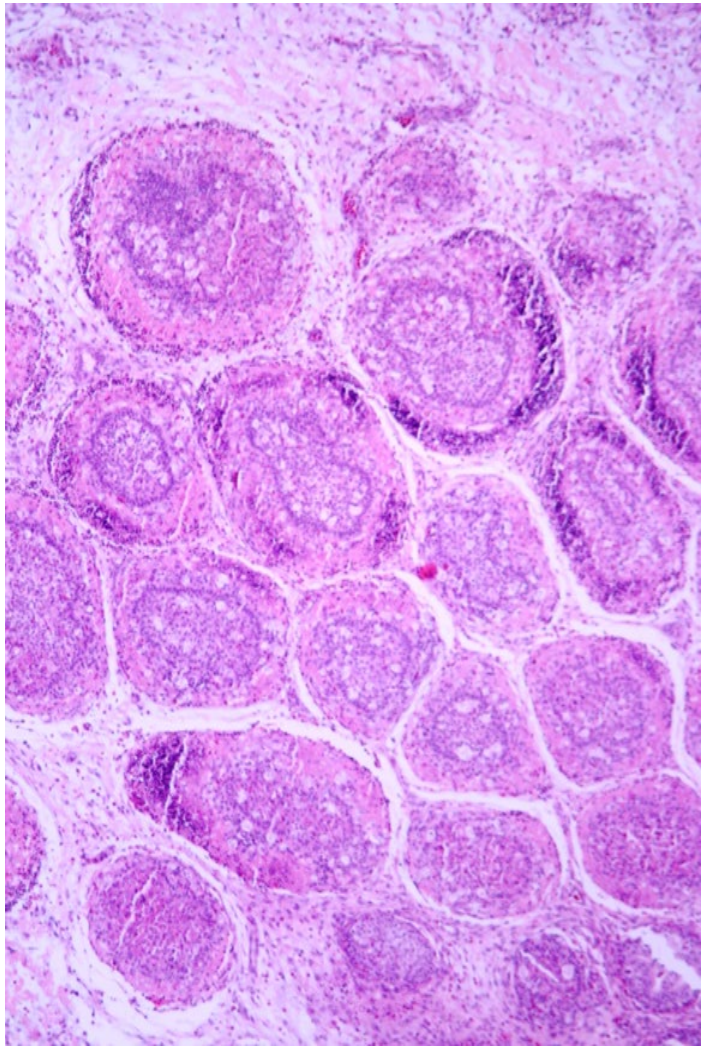




Delaware-type; minimal inflammation.



Classical Type, edema and heterophils

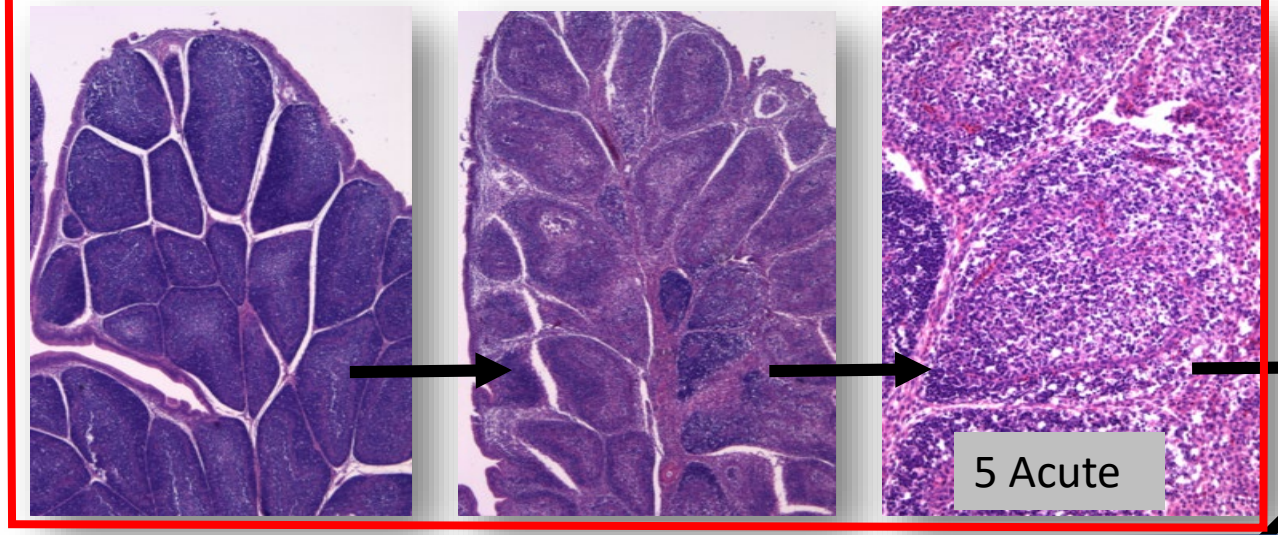


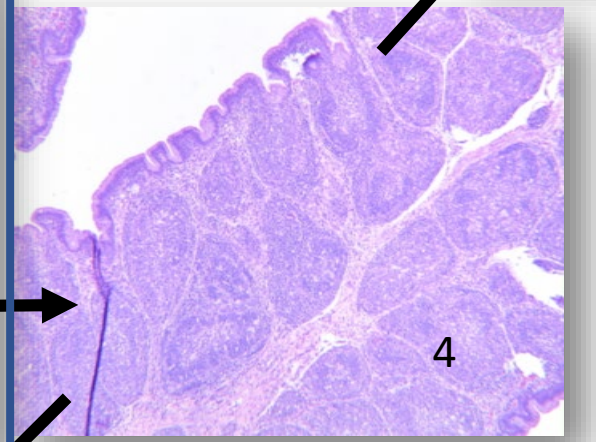
Very Virulent Suspect, liquefactive necrosis, edema

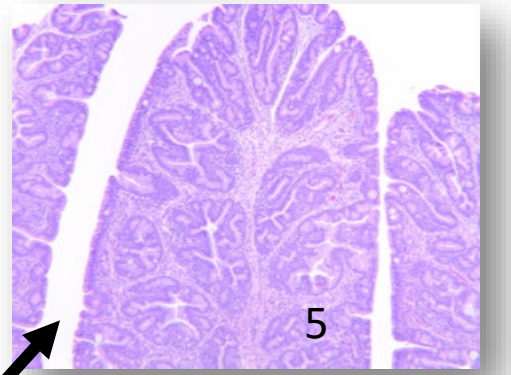
Stages of Infectious Bursal Disease

Acute Phase

Recovery Phase





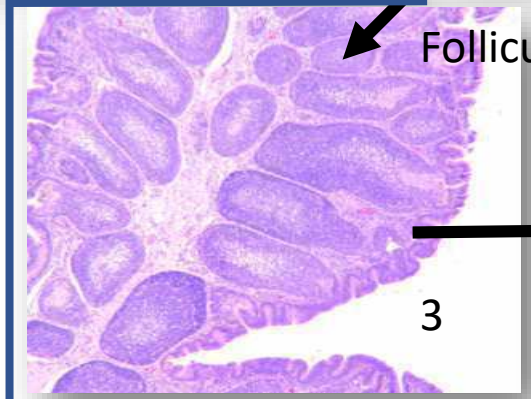


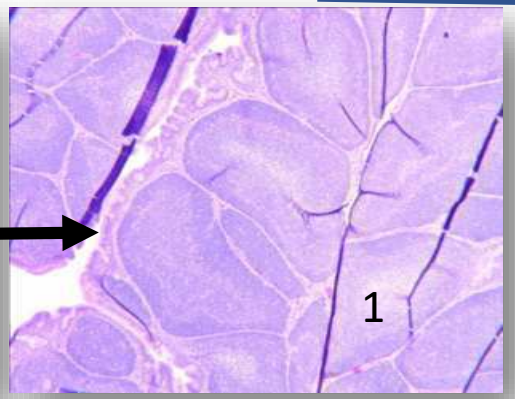
Normal

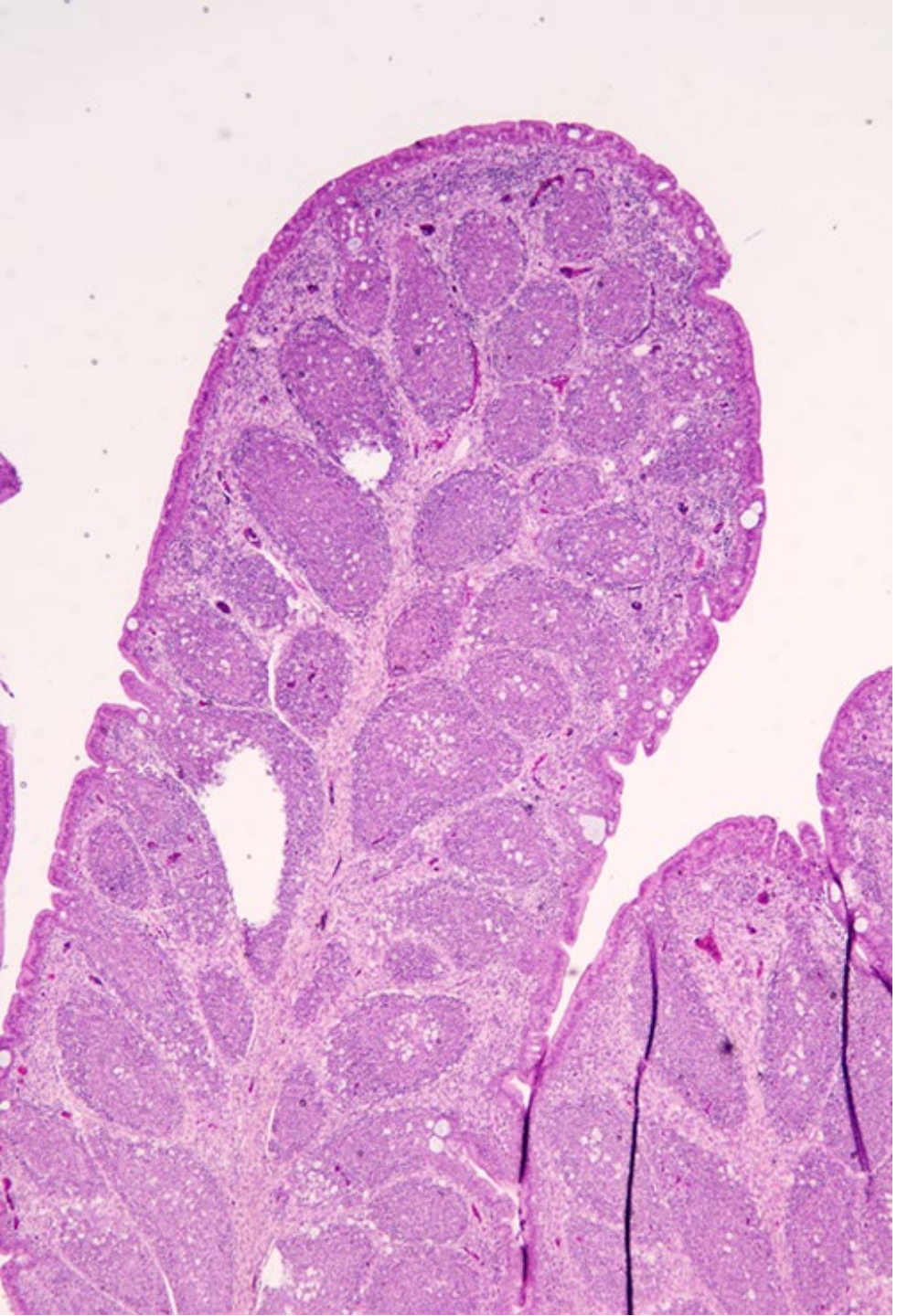
Score 0

Diffuse necrosis
(Score 5, acute)

Follicular Restitution







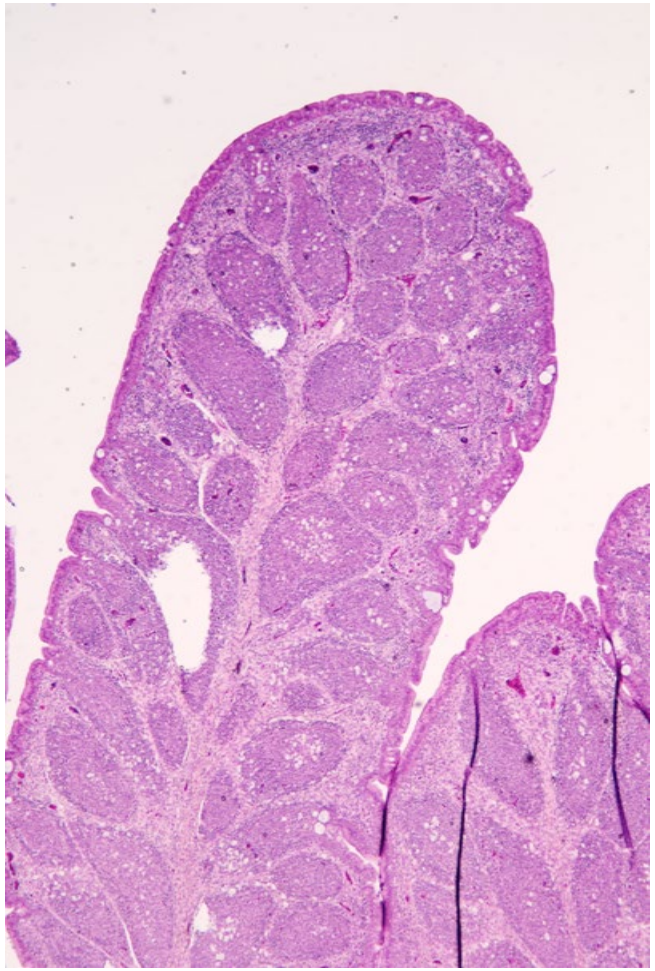
Transition: acute to recovery phase

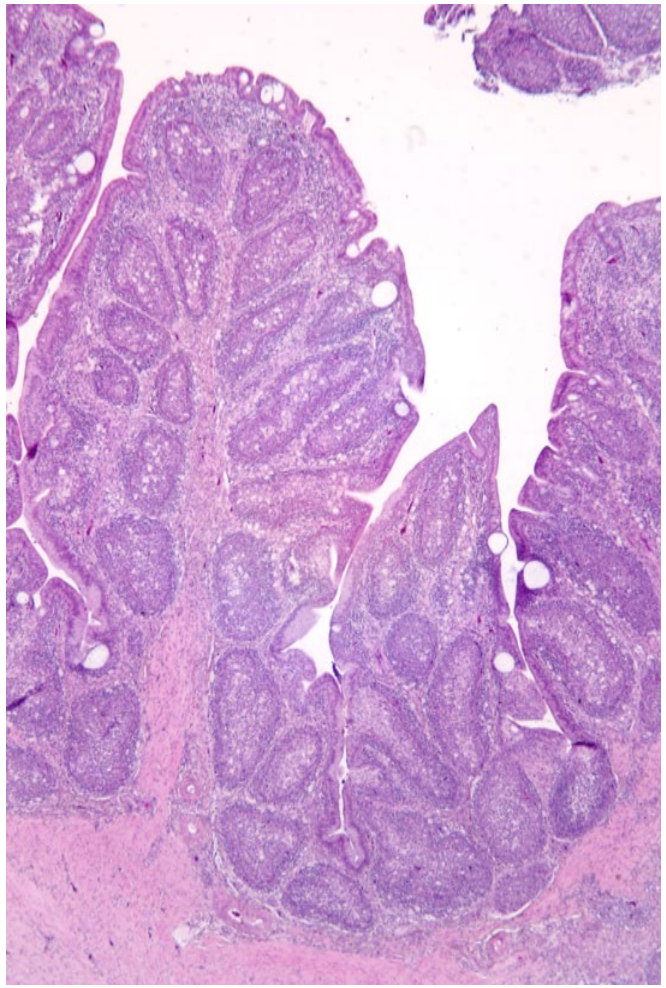
- Collapsed, lymphocyte depleted follicles
- Reactive macrophages & cytotoxic T cells
- Resolving inflammation

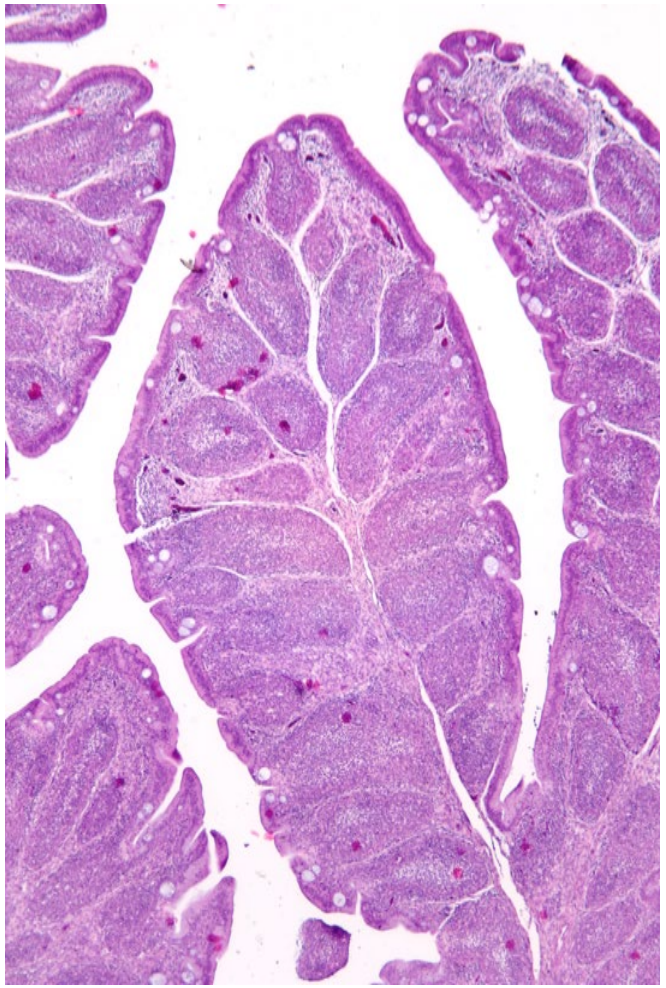
Recovery Score 4

NORMAL ACUTE PHASE – Necrosis/Apoptosis RECOVERY PHASE – Follicular Restitution

0 1 2 3 4 5 5 4 3 2 1 0







NORMAL ACUTE PHASE – Necrosis/Apoptosis RECOVERY PHASE – Follicular Restitution

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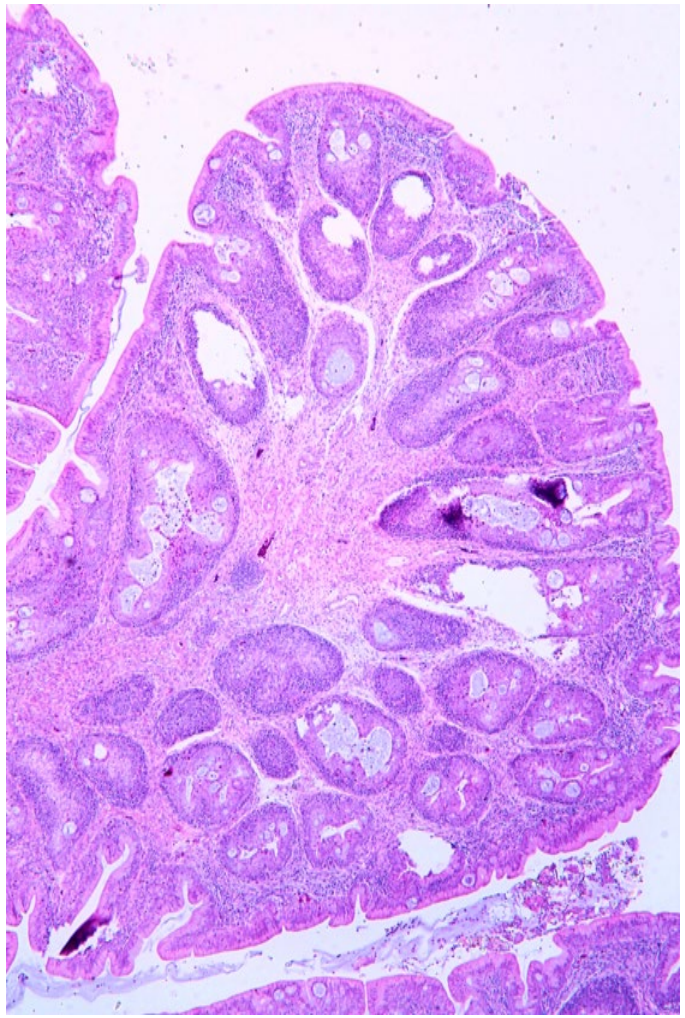

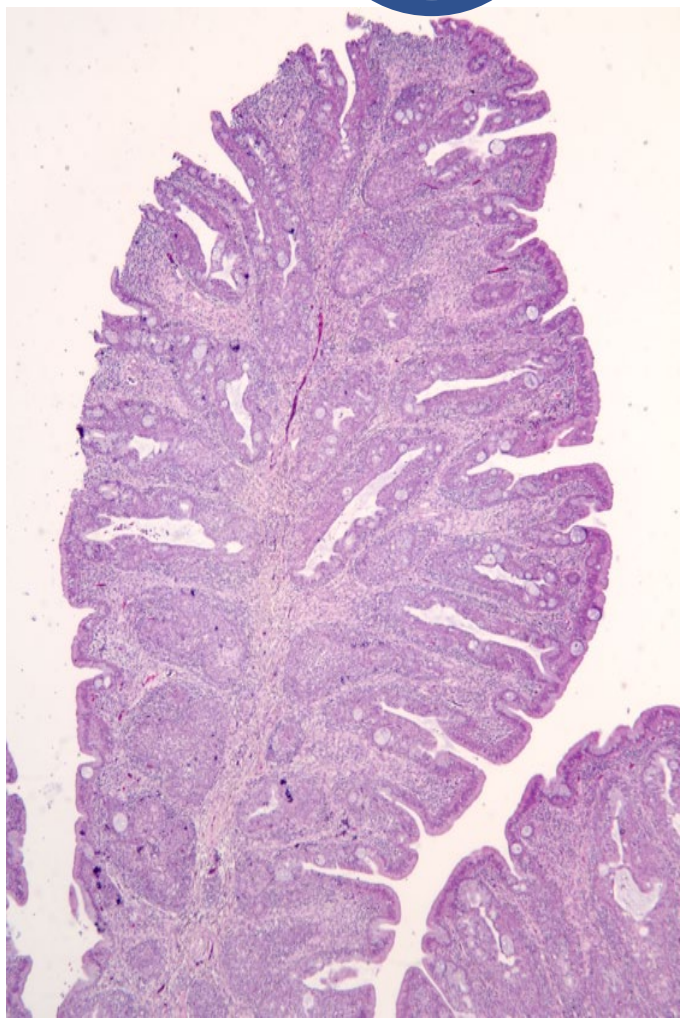

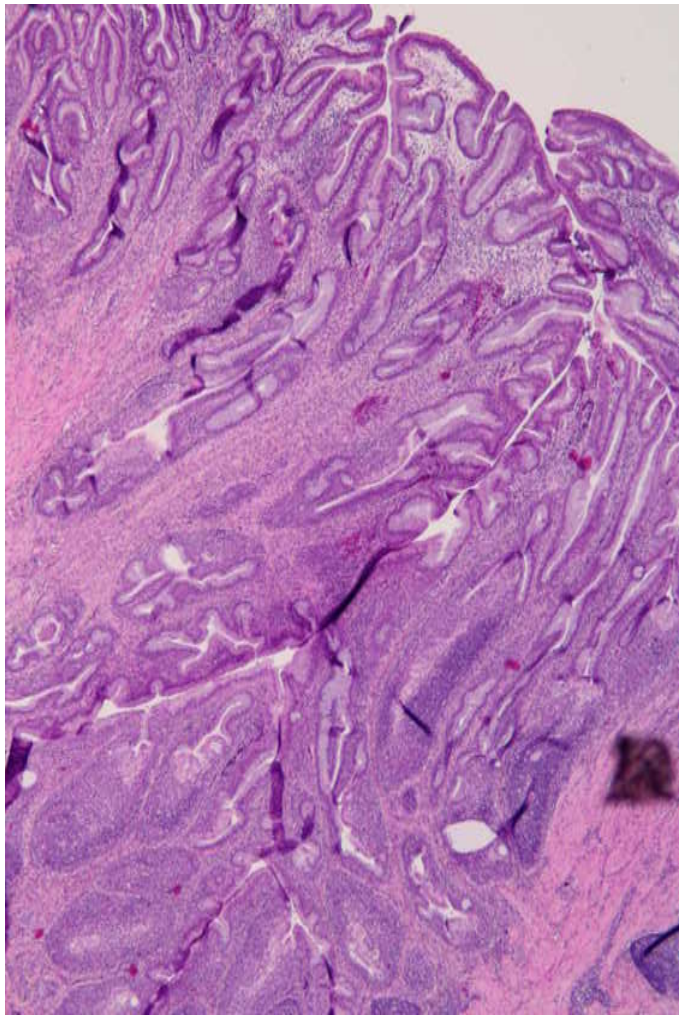

NORMAL ACUTE PHASE – Necrosis/Apoptosis RECOVERY PHASE – Follicular Restitution

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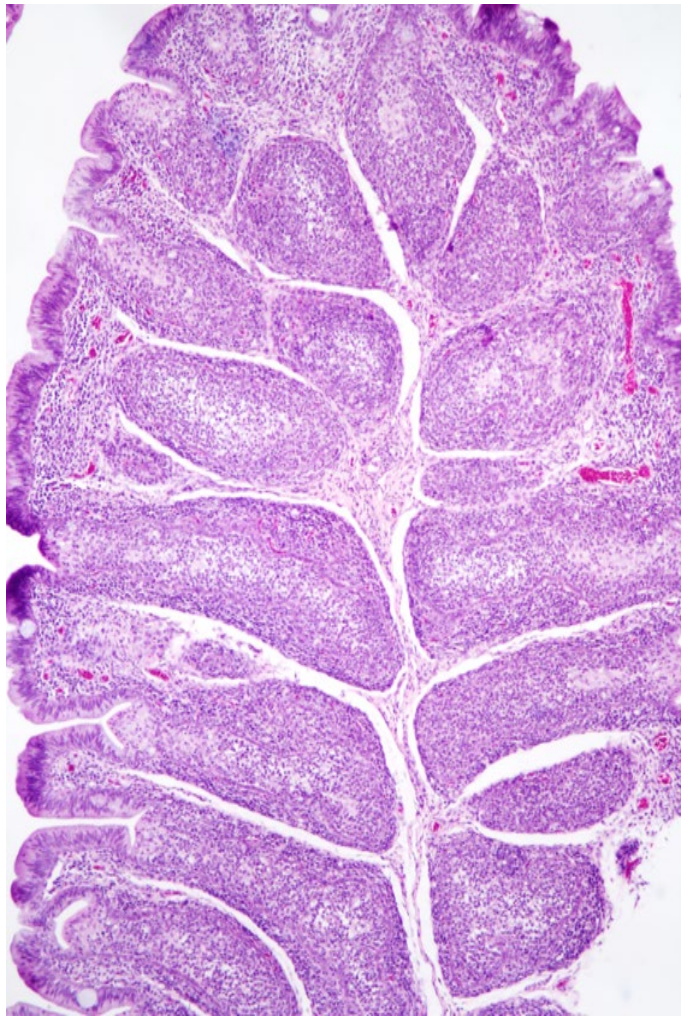

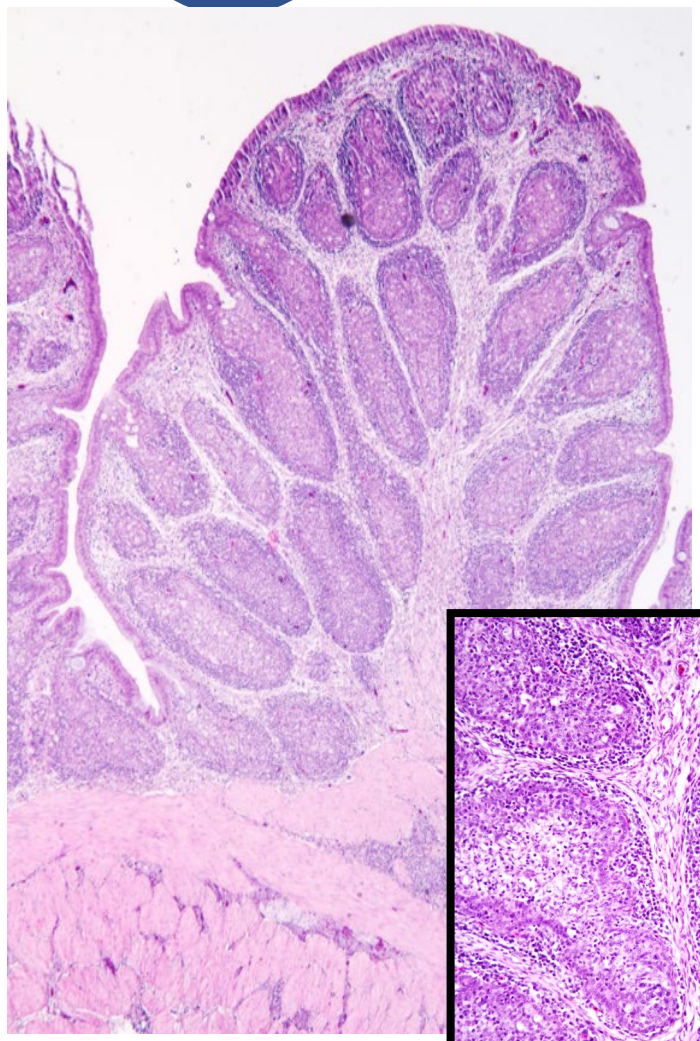

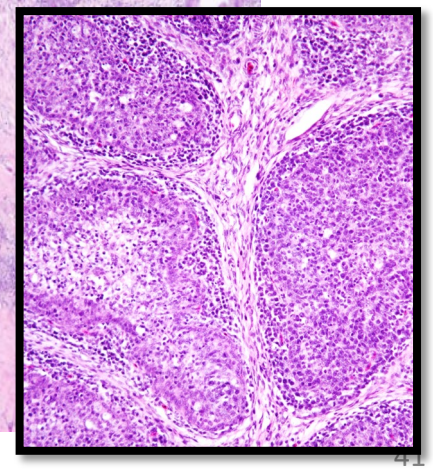

NORMAL ACUTE PHASE – Necrosis/Apoptosis RECOVERY PHASE – Follicular Restitution

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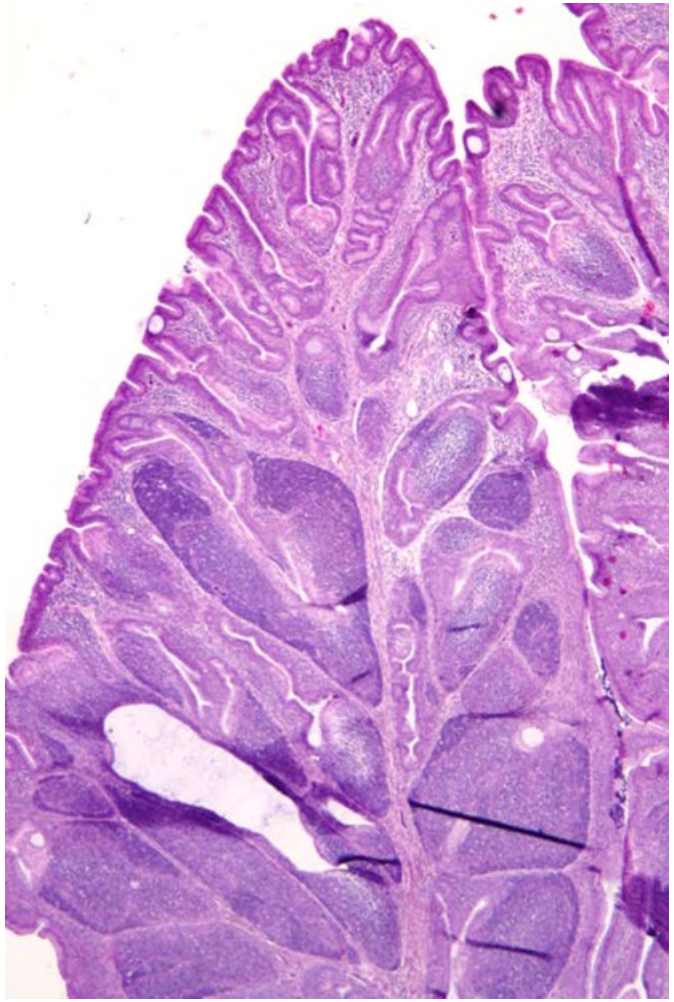

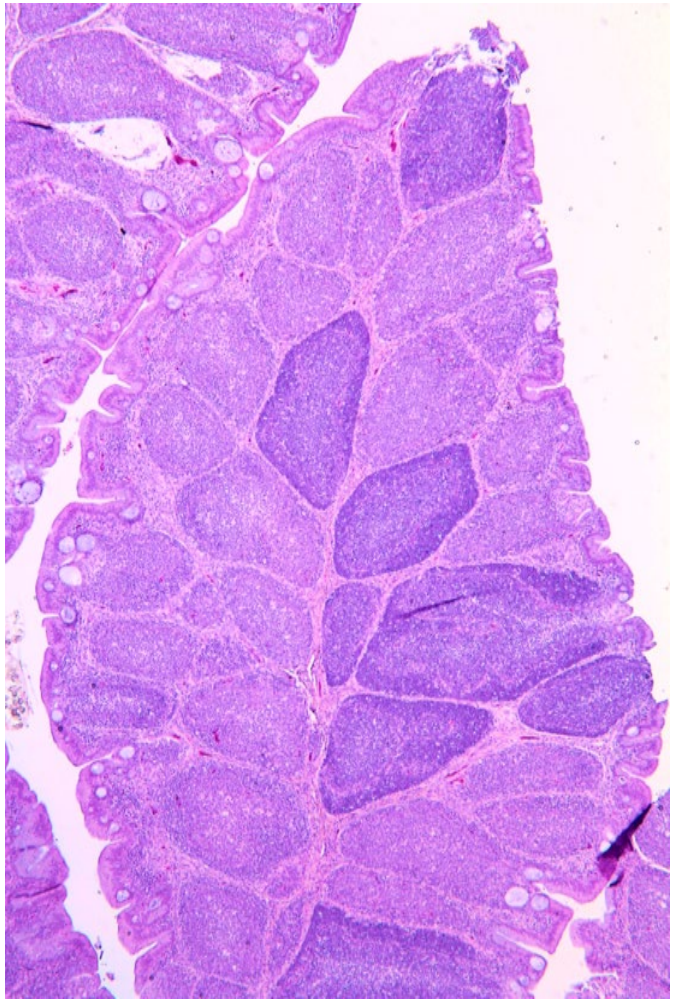

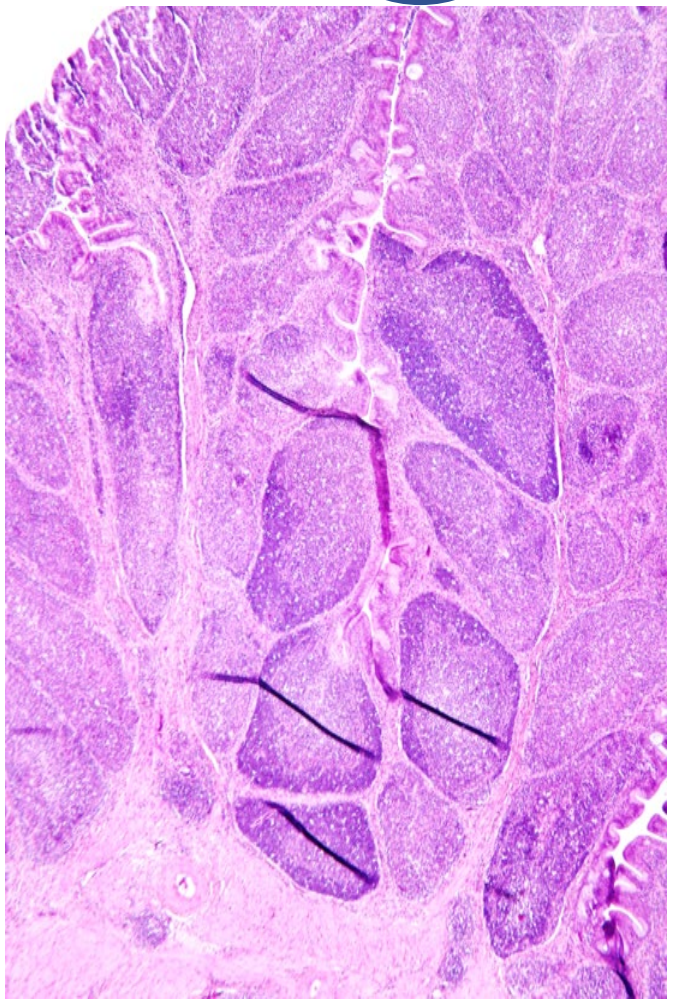

NORMAL ACUTE PHASE – Necrosis/Apoptosis RECOVERY PHASE – Follicular Restitution

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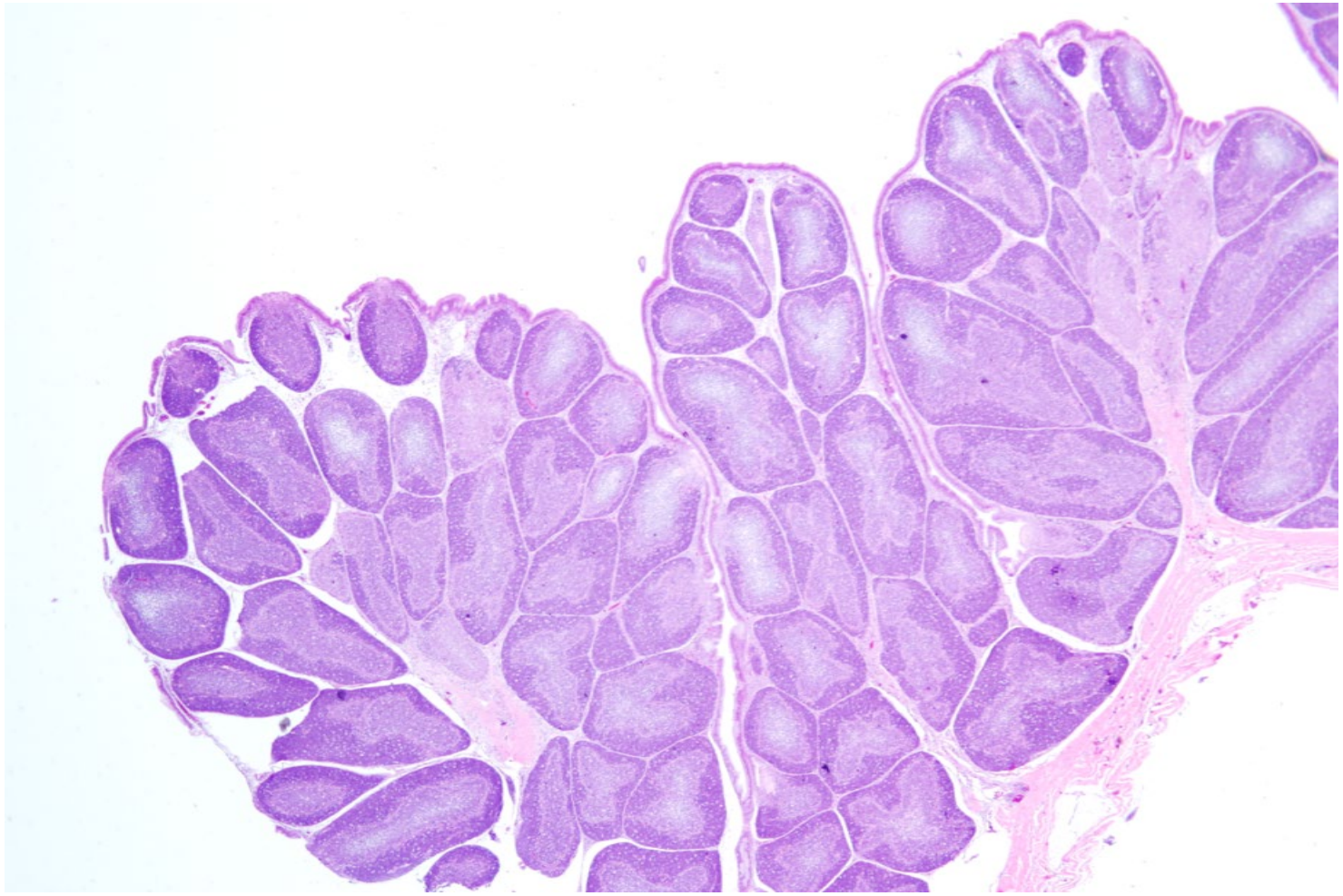
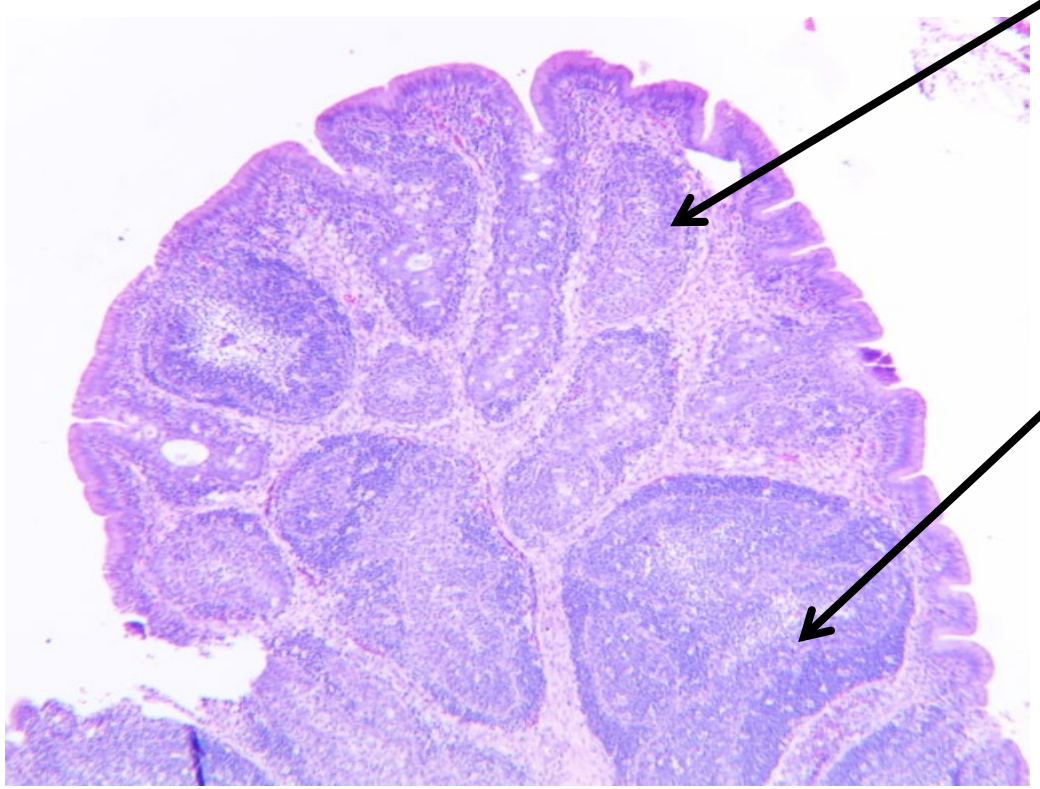


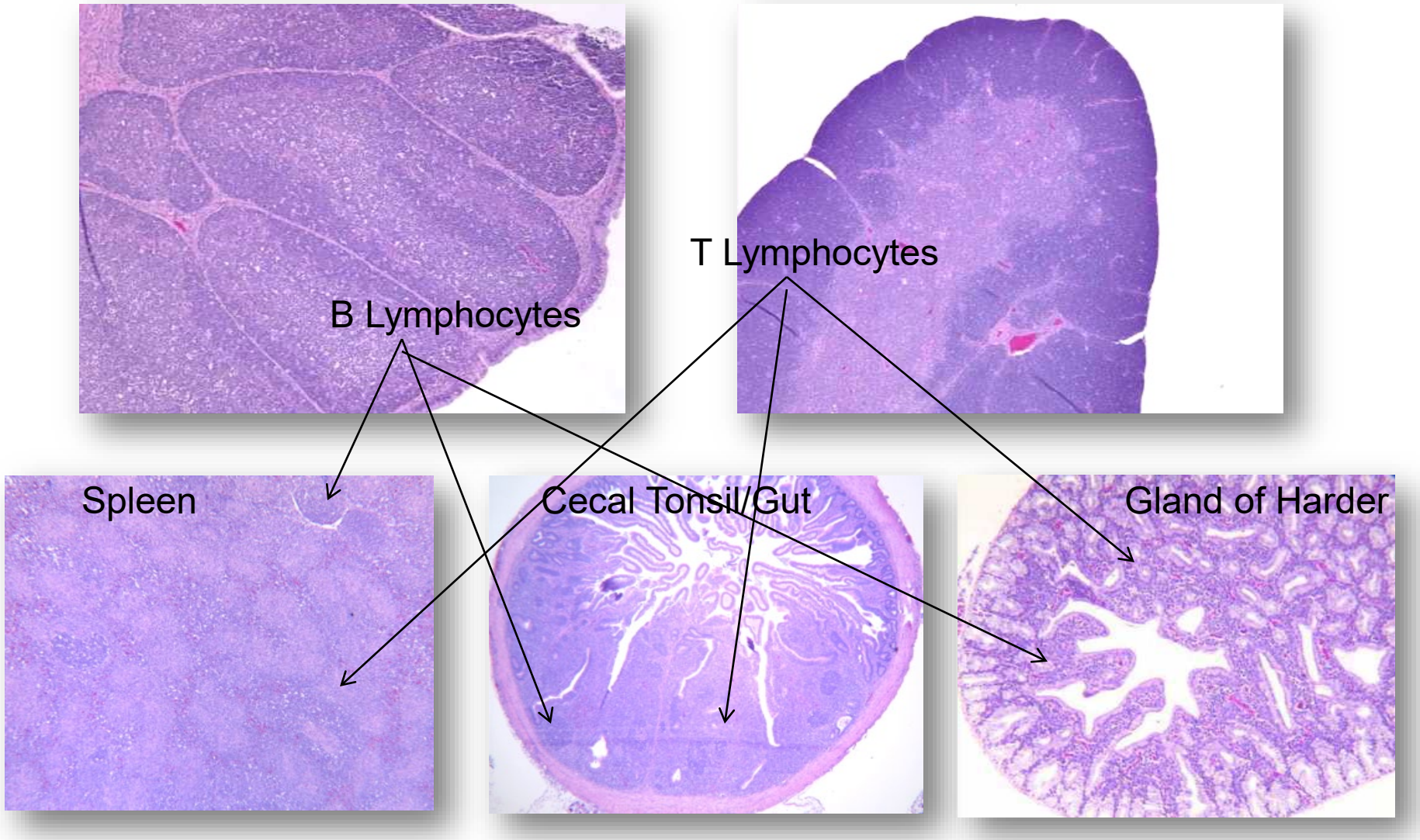
Post Bursal Disease Follicular Restoration Small vs. Large Follicles



- **Small undifferentiated follicles**
  - Lymphocyte restoration from medullary stem cells
  - Minimal or no immune response capability
- **Large follicles**
  - Rapidly proliferating B cells
  - Correlated with partial recovery of antibody response
    - *Withers et al. Immunology 117, 558-565, 2006*
  - The antibody response likely does not occur in the bursa
    - *M. E. Ifrah et al. Poultry Science 96, 2017*

Bursa and Thymus: First 14 Days

Primary Lymphoid Organs Seed Secondary Organs



2016 Bursa Survey: 6 flocks, 14 – 41 days

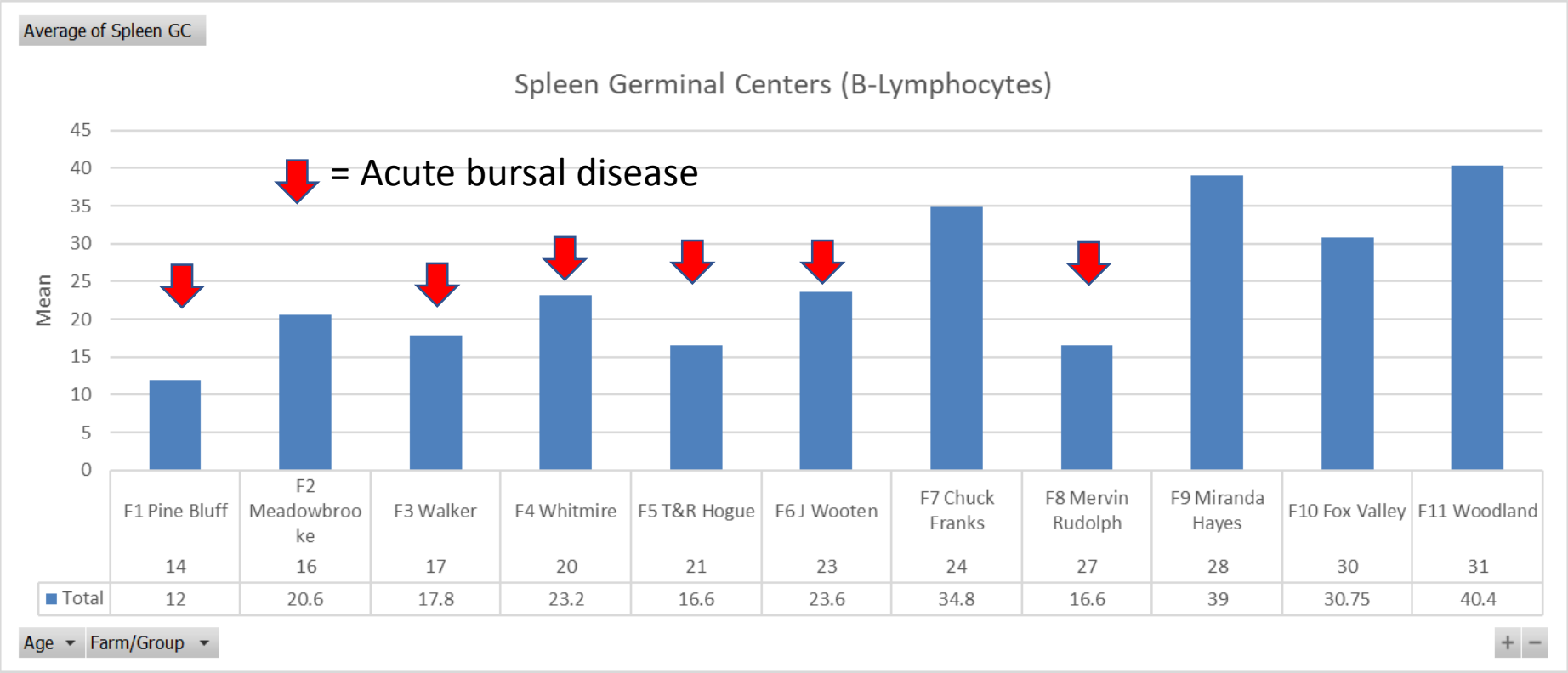
Age	Units	B1	B2	B3	B4	B5	B6	Mean	Virus Detect	#Nec	Comment
14	days	1	1	1	1	1	1	1		0	Within normal limits
21	days										Tissue not available
29	days	3.5	3.5	3.5	3.5	2	2	3	2	0	Recovery IBD
34	days	3	3	3	3.5	3	1	2.75	2	0	Recovery IBD
41	days	3	3	3	3	3	1	2.6667	2	0	Recovery IBD
14	days	1	1	1	1	1		1			Within normal limits
21	days	1	1	1	1	1		1			Within normal limits
28	days	4	4	1	4	4		3.4	1	4	Acute bursitis/IBD
33	days	3.5	3.5	3.5	3	3.5		3.4	2	0	Recovery IBD
41	days	3	3	3	3	3		3	2	0	Recovery IBD
41	days	3	3	3	3	3		3	2	0	Recovery IBD
14	days	1	1	1	1	1		1			Within normal limits
21	days	4	4	3.5	1	1		2.7	1	3	Acute bursitis/IBD
28	days	4	3.5	4	3	3		3.5	2	0	Recovery IBD
33	days	3	3	3	3	3		3	2	0	Recovery IBD
41	days	2.5	3	3	3.5	3		3	2	0	Recovery IBD
14	days	1	1	1	1	1		1			Within normal limits
22	days	1	1	1	1	1		1			Within normal limits
29	days	3	3	3.5	3.5	3.5		3.3	2	0	Recovery IBD
34	days	3	3	3.5	3	3		3.1	2	0	Recovery IBD
41	days	3	3	3	3	2.5		2.9	2	0	Recovery IBD
41	days	3	2.5	3	3	3		2.9	2	0	Recovery IBD
14	days	4	1	1	1	1		1.6	1	1	Acute bursitis/IBD
21	days	3	3.5	3.5	3.5	3.5		3.4	2	0	Recovery IBD
28	days	3.5	3.5	3.5	3.5	3.5		3.5	2	0	Recovery IBD
32	days	2.5	2.5	2.5	2.5	3		2.6	2	0	Recovery IBD
41	days	2.5	2.5	2	2	2.5		2.3	2	0	Recovery IBD
41	days	1	2.5	2.5	2.5	2.5		2.2	2	0	Recovery IBD
14	days	1	1	2	1	1		1.2	1	1	Acute bursitis/IBD
21	days	4	4	4	4	3.5		3.9	1	4	Acute bursitis/IBD
28	days	3	3	3	3	3		3	2	0	Recovery IBD
33	days	2.5	4	2.5	3	3		3	2	0	Recovery IBD
41	days	2.5	2.5	1	2.5	2.5		2.2	2	0	Recovery IBD
41	days	2.5	1	2.5	2.5	2.5		2.2	2	0	Recovery IBD

Bursa Survey: 6 flocks – Spleen Germinal Centers (B-cell centers)

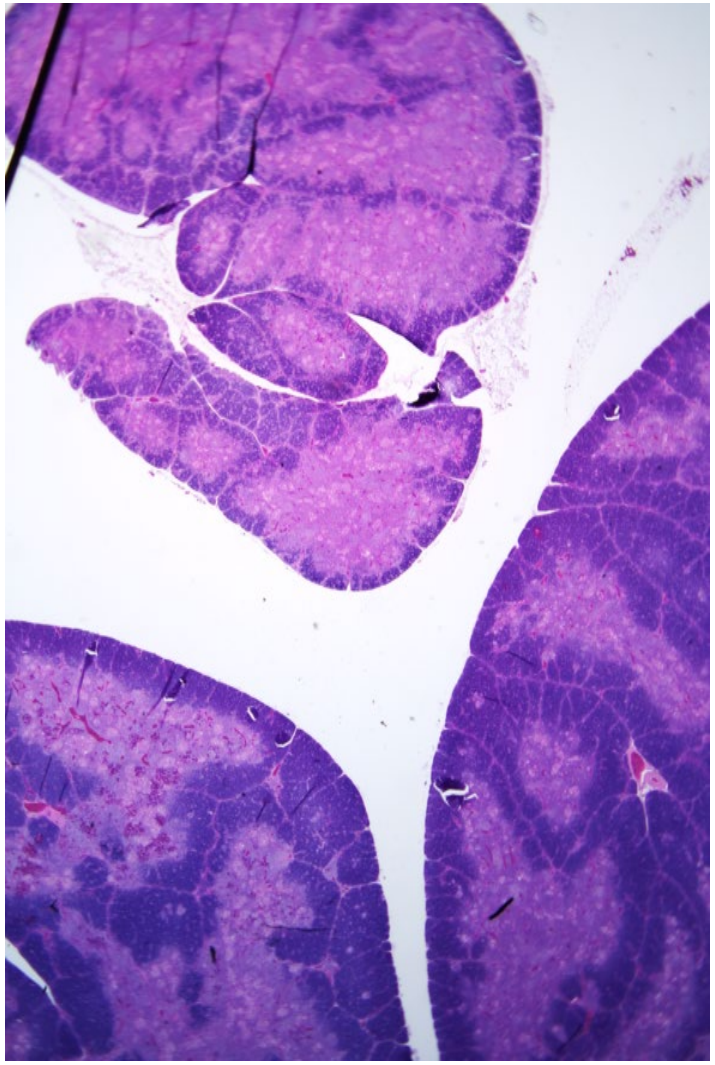
Age	Units	B1	B2	B3	B4	B5	B6	Mean	Virus Detect	#Nec	Comment	
14	days	1	1	1	1	1	1	1		0	Within normal limits	
21	days										Tissue not available	
29	days	3.5	3.5	3.5	3.5	2	2	3	2	0	Recovery IBD	
34	days	3	3	3	3.5	3	1	2.75	2	0	Recovery IBD	
41	days	3	3	3	3	3	1	2.6667	2	0	Recovery IBD	**25.8**
14	days	1	1	1	1	1		1			Within normal limits	
21	days	1	1	1	1	1		1			Within normal limits	
28	days	4	4	1	4	4		3.4	1	4	Acute bursitis/IBD	
33	days	3.5	3.5	3.5	3	3.5		3.4	2	0	Recovery IBD	
41	days	3	3	3	3	3		3	2	0	Recovery IBD	
41	days	3	3	3	3	3		3	2	0	Recovery IBD	**16.6**
14	days	1	1	1	1	1		1			Within normal limits	
21	days	4	4	3.5	1	1		2.7	1	3	Acute bursitis/IBD	
28	days	4	3.5	4	3	3		3.5	2	0	Recovery IBD	
33	days	3	3	3	3	3		3	2	0	Recovery IBD	
41	days	2.5	3	3	3.5	3		3	2	0	Recovery IBD	**18.8**

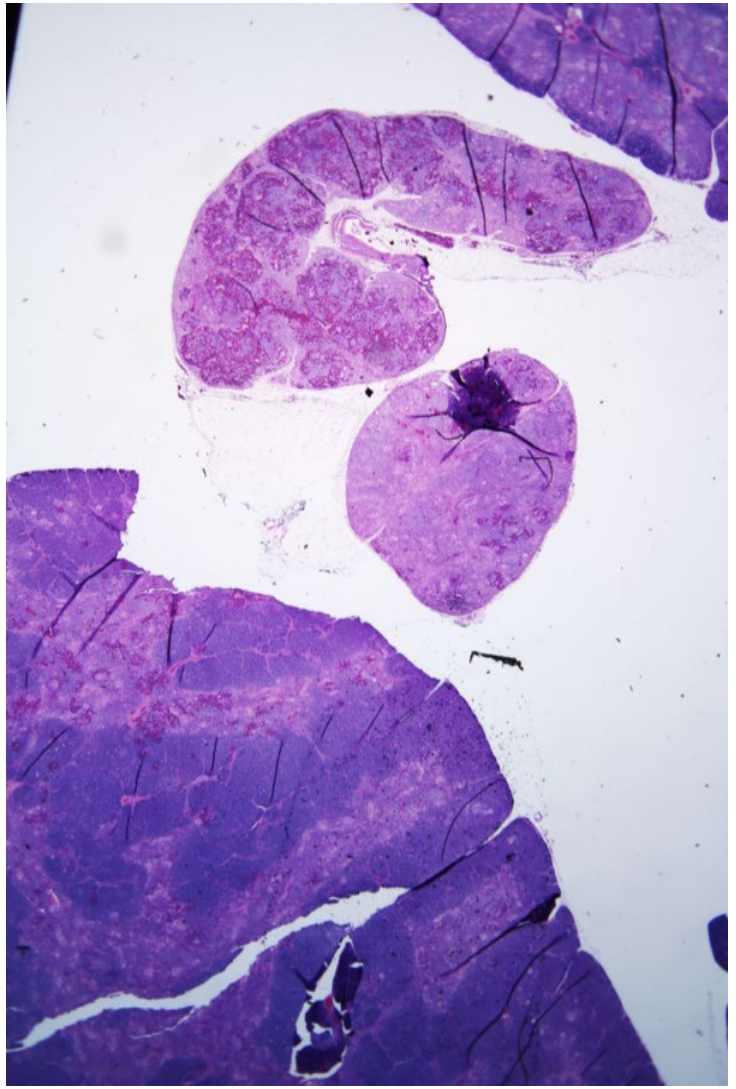
14	days	1	1	1	1	1		1			Within normal limits	
22	days	1	1	1	1	1		1			Within normal limits	
29	days	3	3	3.5	3.5	3.5		3.3	2	0	Recovery IBD	
34	days	3	3	3.5	3	3		3.1	2	0	Recovery IBD	
41	days	3	3	3	3	2.5		2.9	2	0	Recovery IBD	
41	days	3	2.5	3	3	3		2.9	2	0	Recovery IBD	**11.6**
14	days	4	1	1	1	1		1.6	1	1	Acute bursitis/IBD	
21	days	3	3.5	3.5	3.5	3.5		3.4	2	0	Recovery IBD	
28	days	3.5	3.5	3.5	3.5	3.5		3.5	2	0	Recovery IBD	
32	days	2.5	2.5	2.5	2.5	3		2.6	2	0	Recovery IBD	
41	days	2.5	2.5	2	2	2.5		2.3	2	0	Recovery IBD	
41	days	1	2.5	2.5	2.5	2.5		2.2	2	0	Recovery IBD	**4.2**
14	days	1	1	2	1	1		1.2	1	1	Acute bursitis/IBD	
21	days	4	4	4	4	3.5		3.9	1	4	Acute bursitis/IBD	
28	days	3	3	3	3	3		3	2	0	Recovery IBD	
33	days	2.5	4	2.5	3	3		3	2	0	Recovery IBD	
41	days	2.5	2.5	1	2.5	2.5		2.2	2	0	Recovery IBD	
41	days	2.5	1	2.5	2.5	2.5		2.2	2	0	Recovery IBD	**7.2**

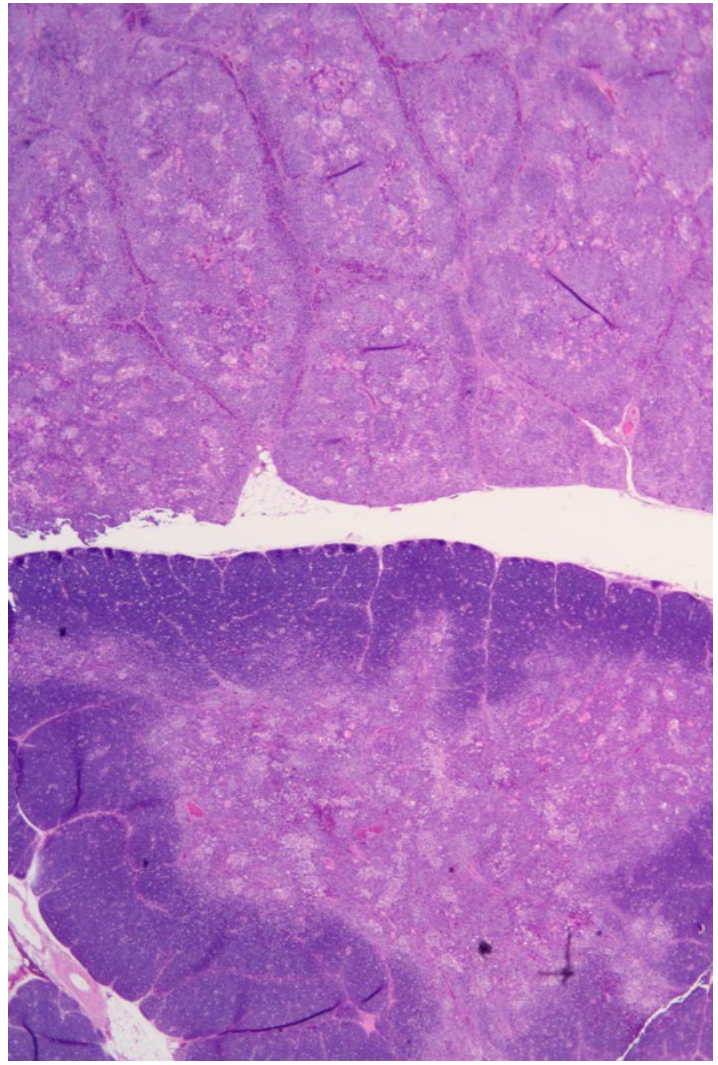
Following age-appropriate increases in germinal centers, acute bursal disease can transiently depress germinal center numbers in the spleen.



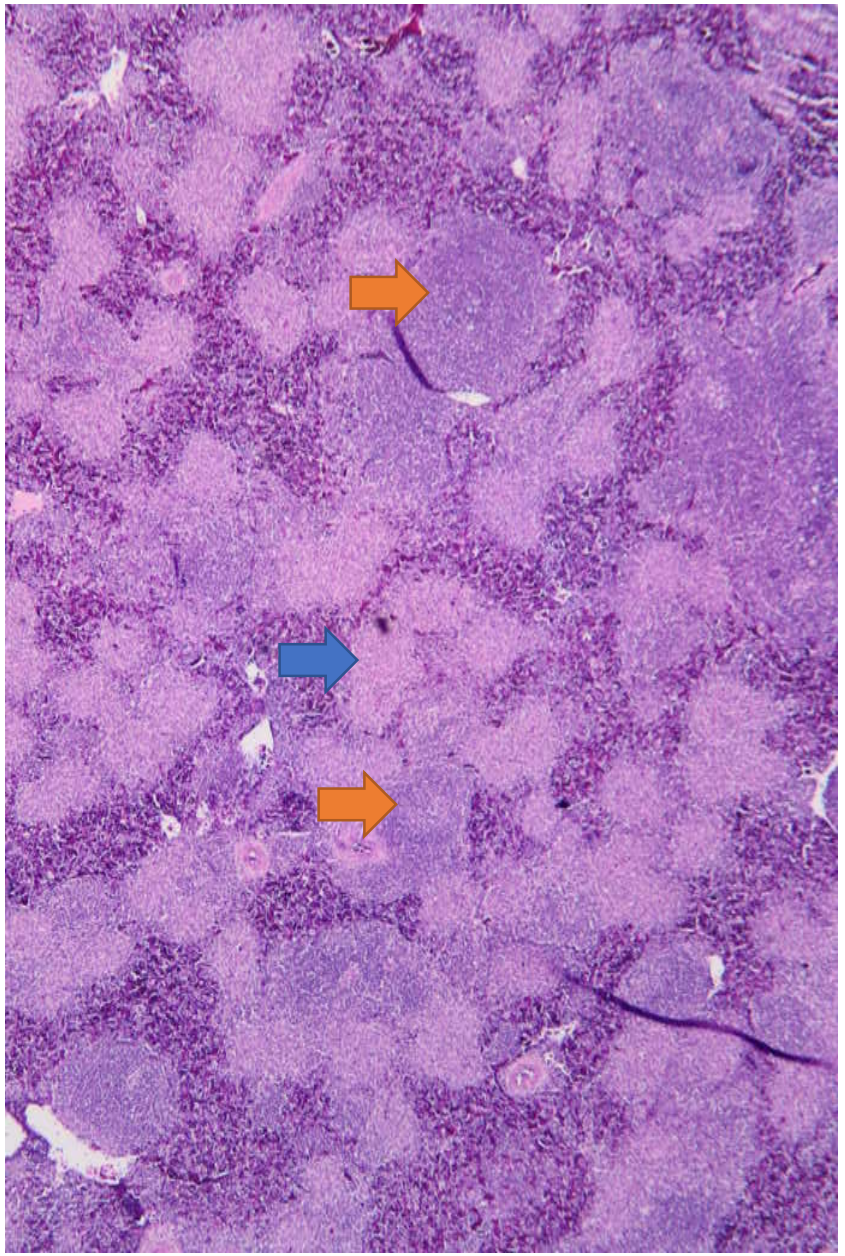
Thymus Pathology: Lymphocyte Depletion

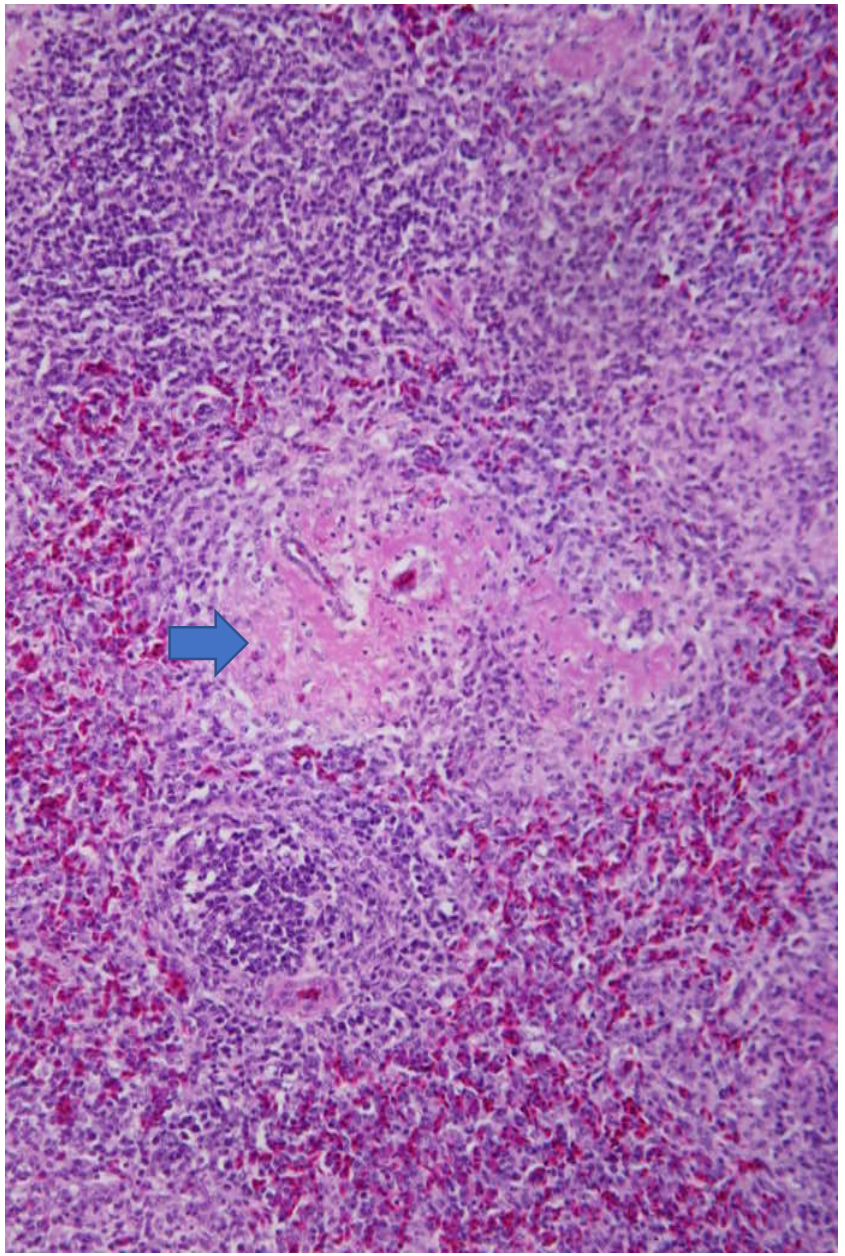




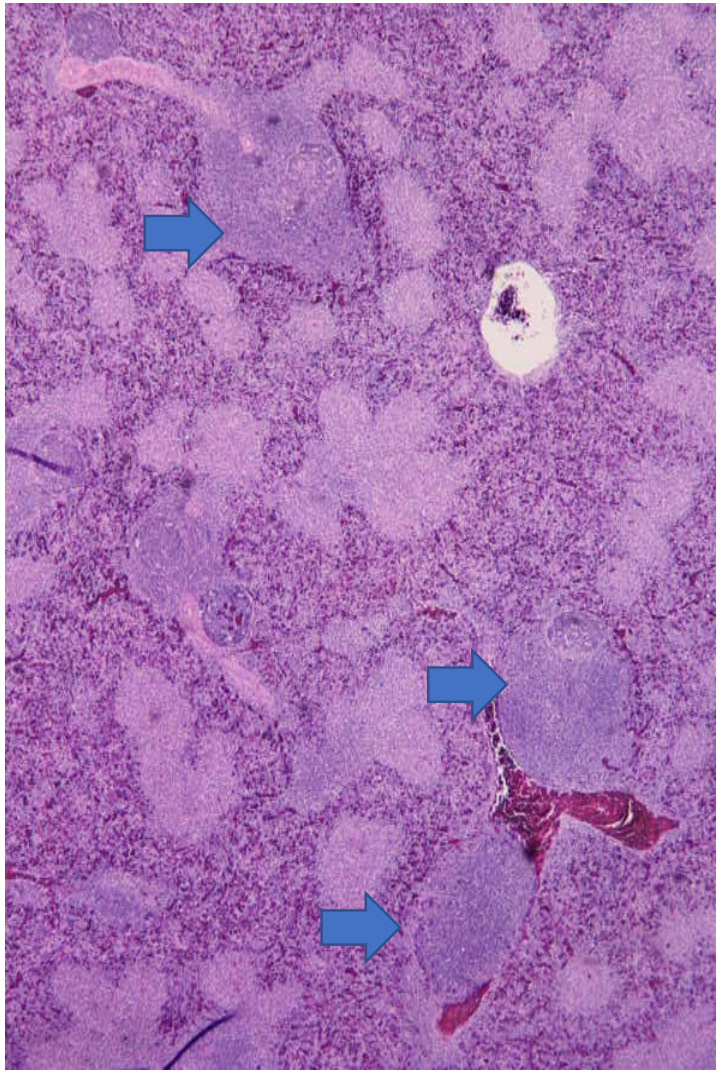


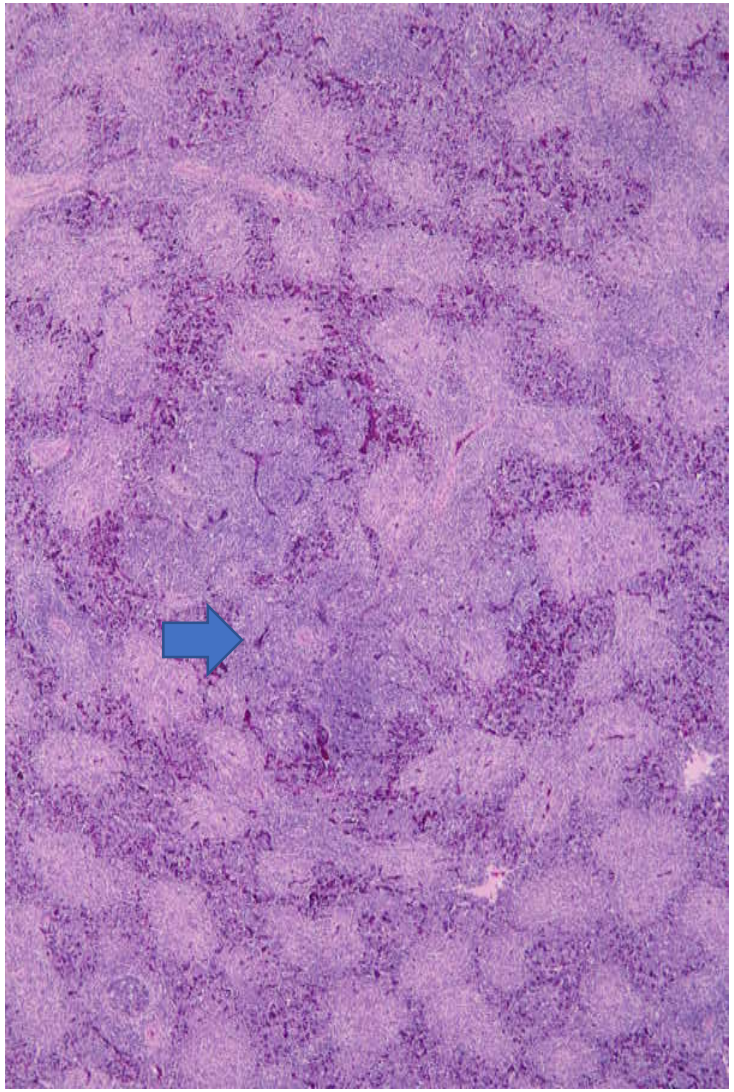
28 days, spleen. The spleen has fibrinoid necrosis of the ellipsoids (blue arrow) accompanied by moderate hyperplasia of perivascular lymphoid tissue (orange arrows). This lesion reflects the systemic effects of a systemic bacterial or viral infection, or toxemia. The spleen is central to pathogen processing and to development of the immune response to pathogens.



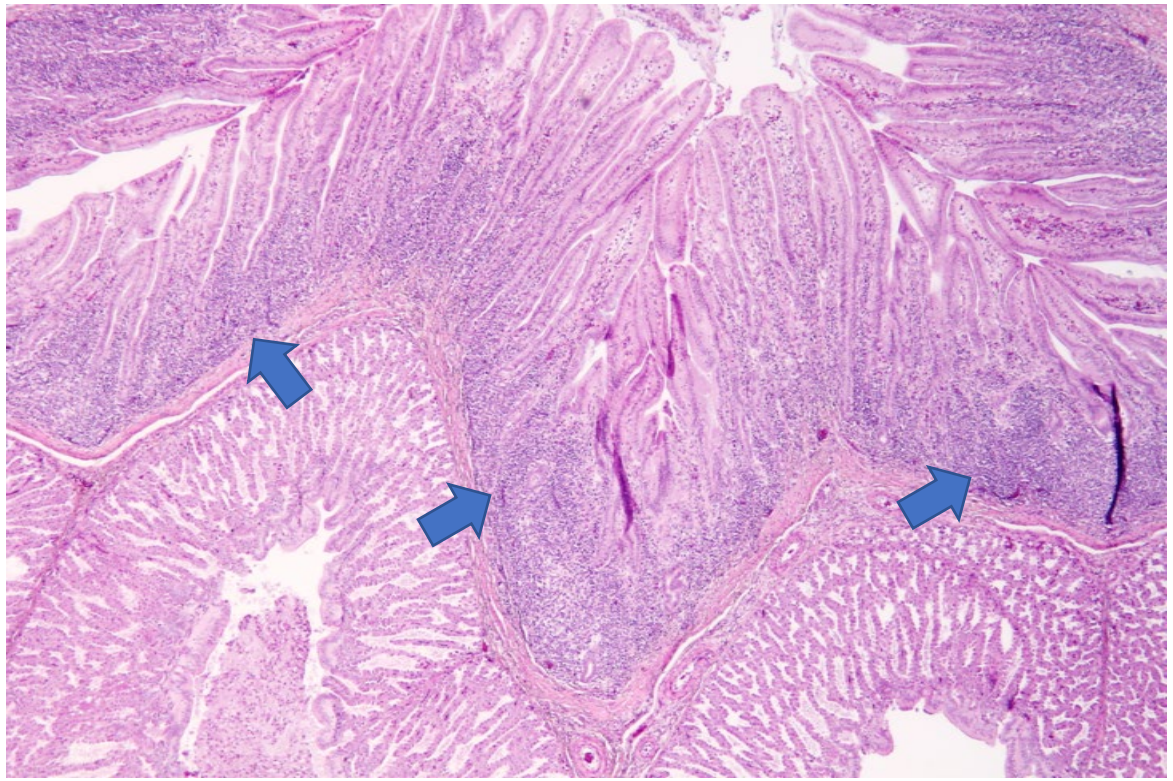


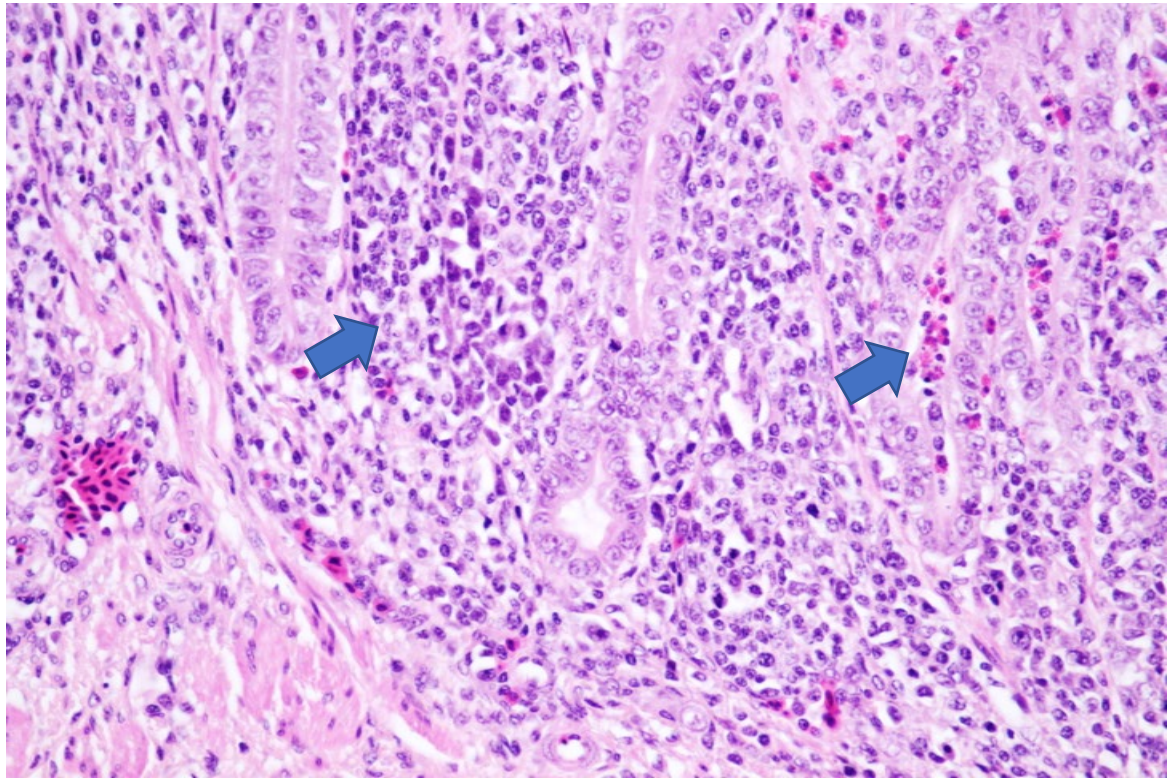
Spleen: lymphoid hyperplasia

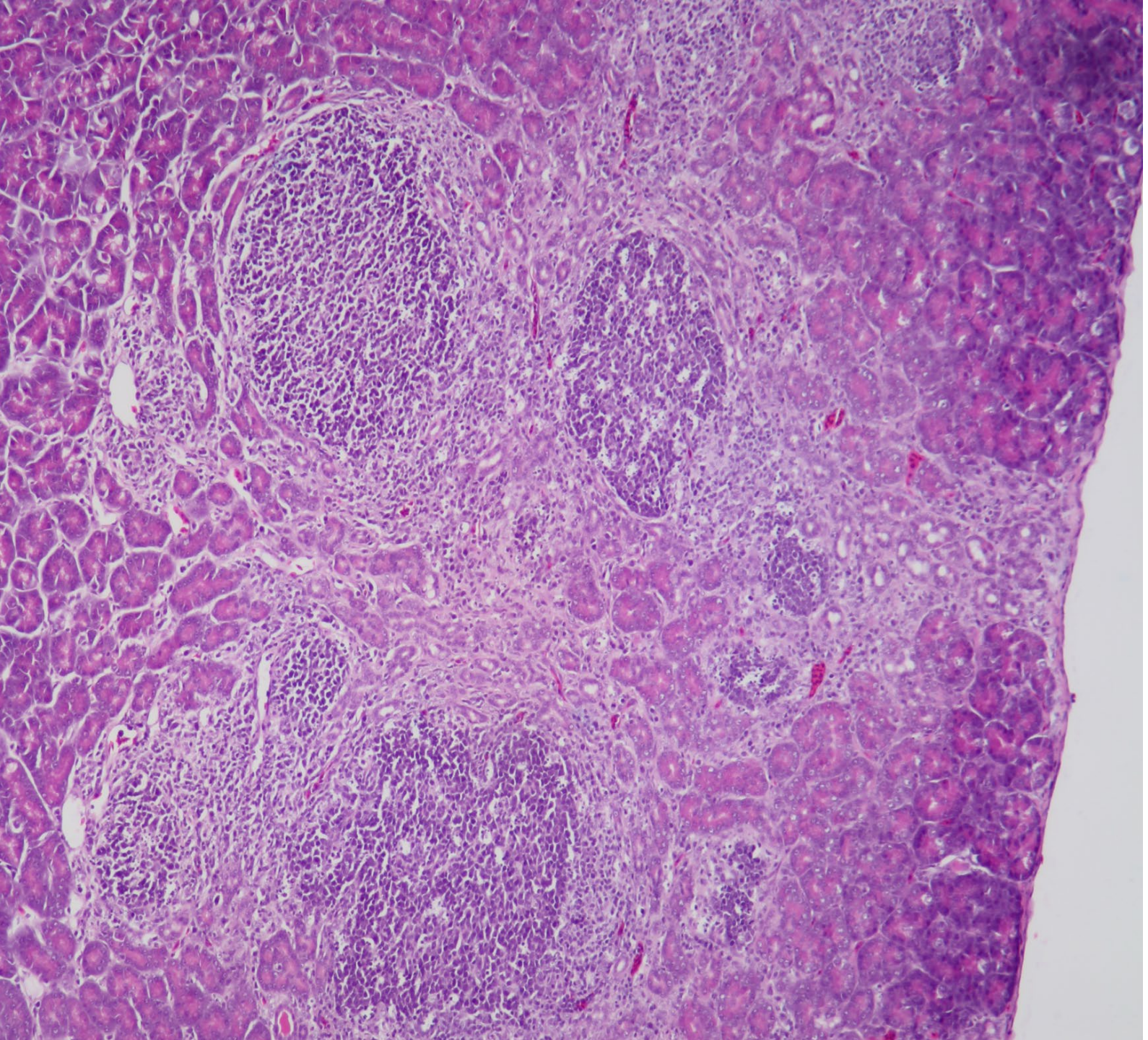




Proventriculus: diffuse mucosal proventriculitis, lymphocytic and heterophilic

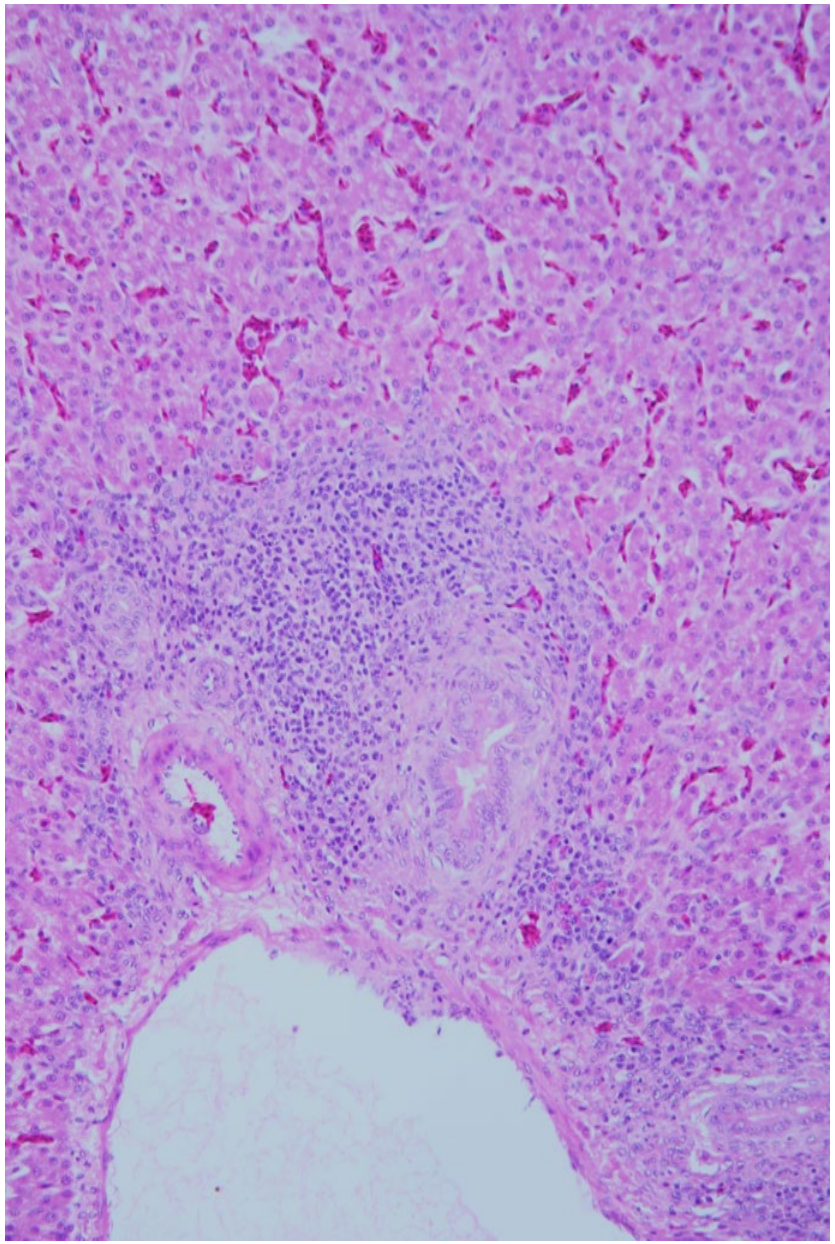


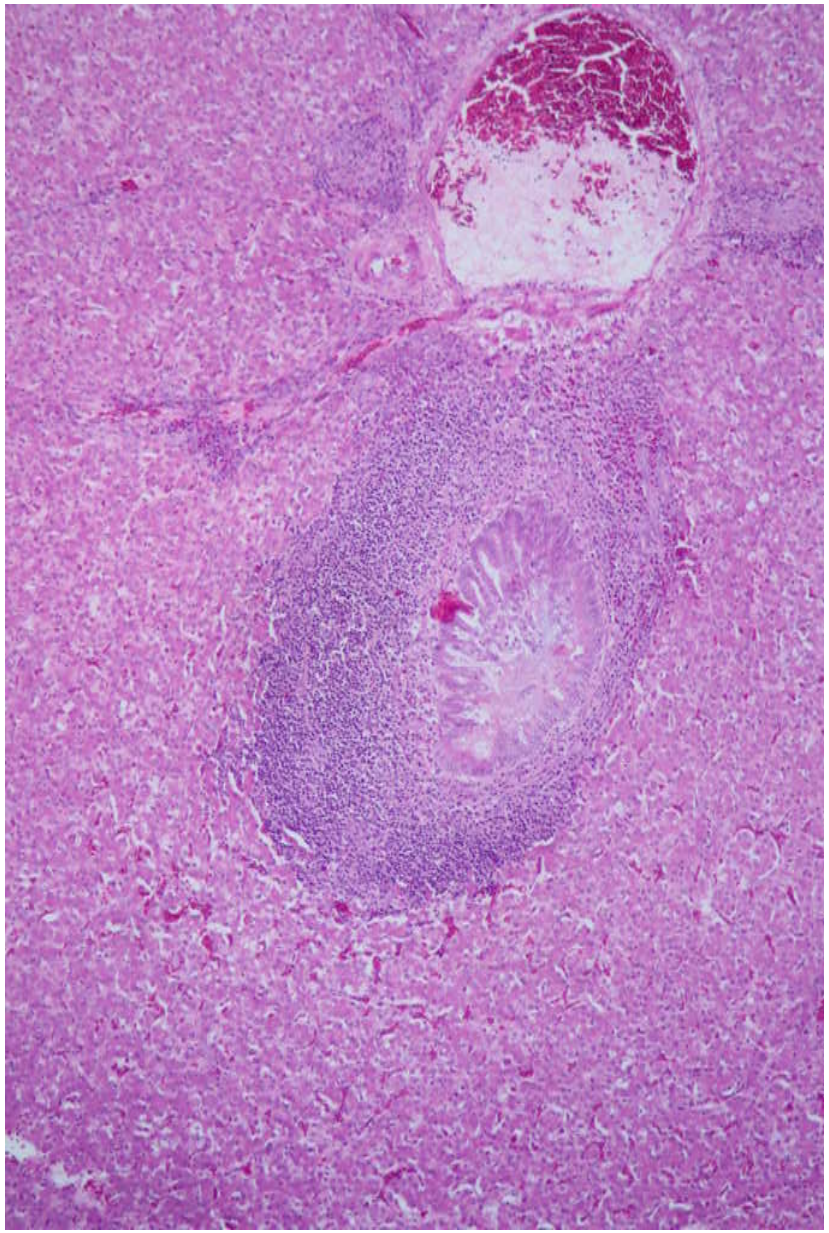


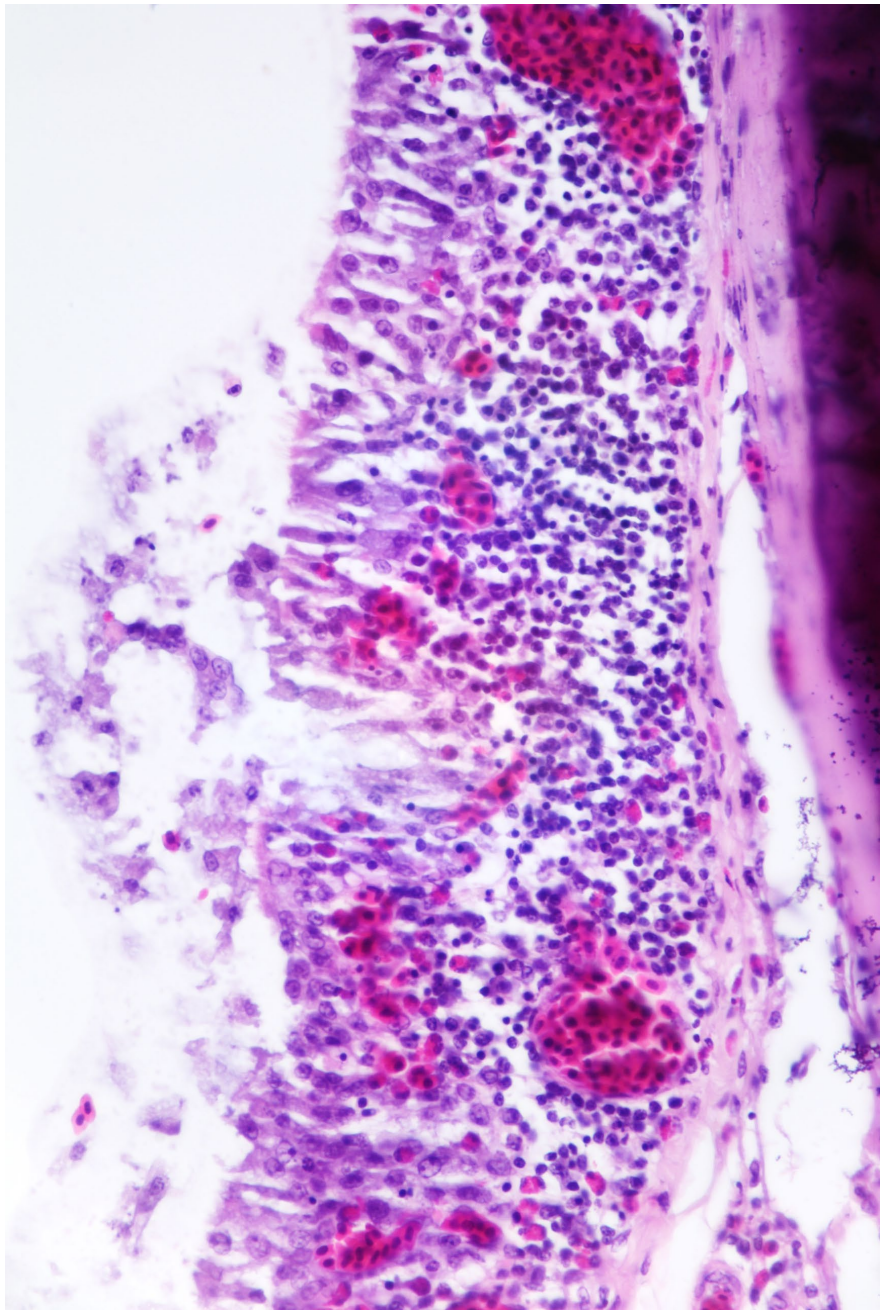


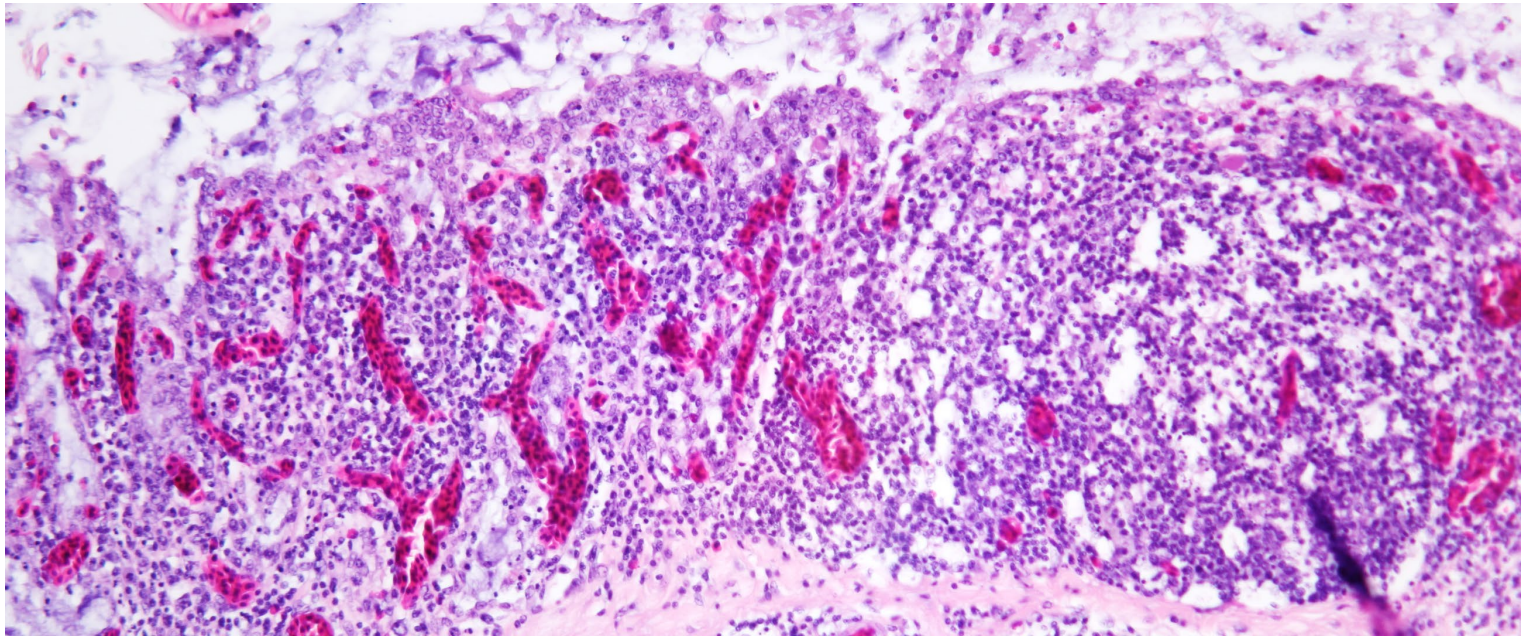
Pancreas: Lymphoid hyperplasia, sequela to viral infection

Liver, periductular lymphocytes and heterophils; cholangiohepatitis

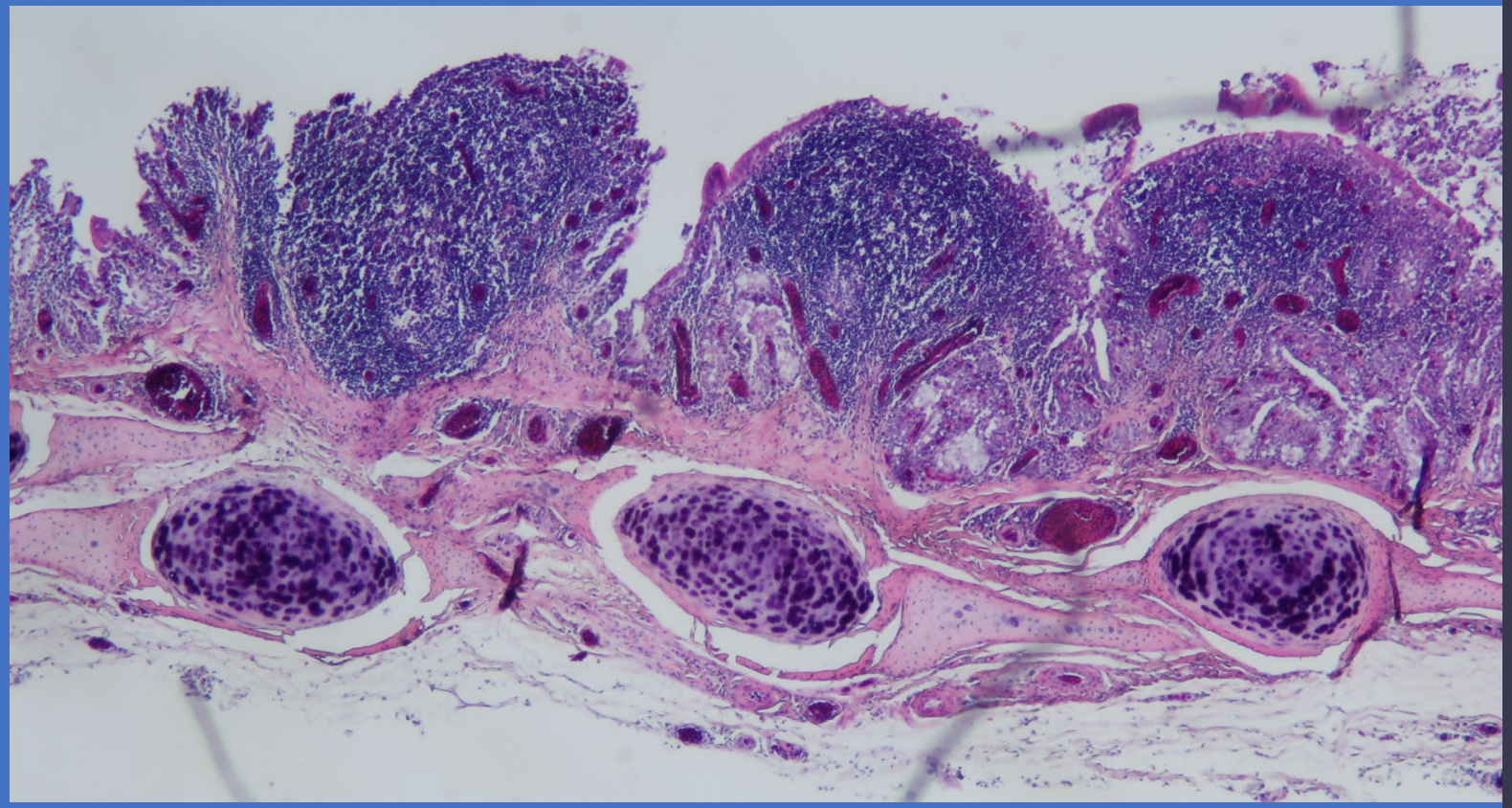






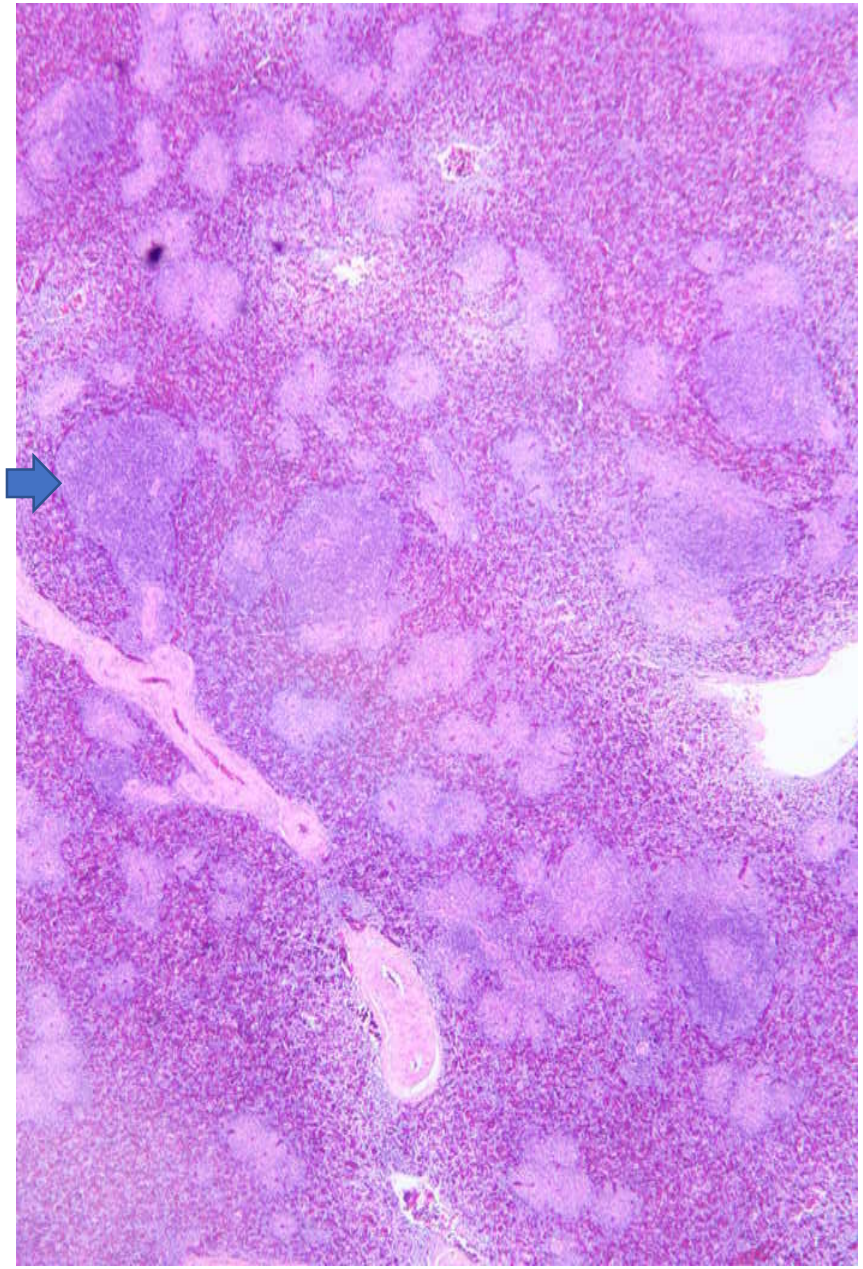


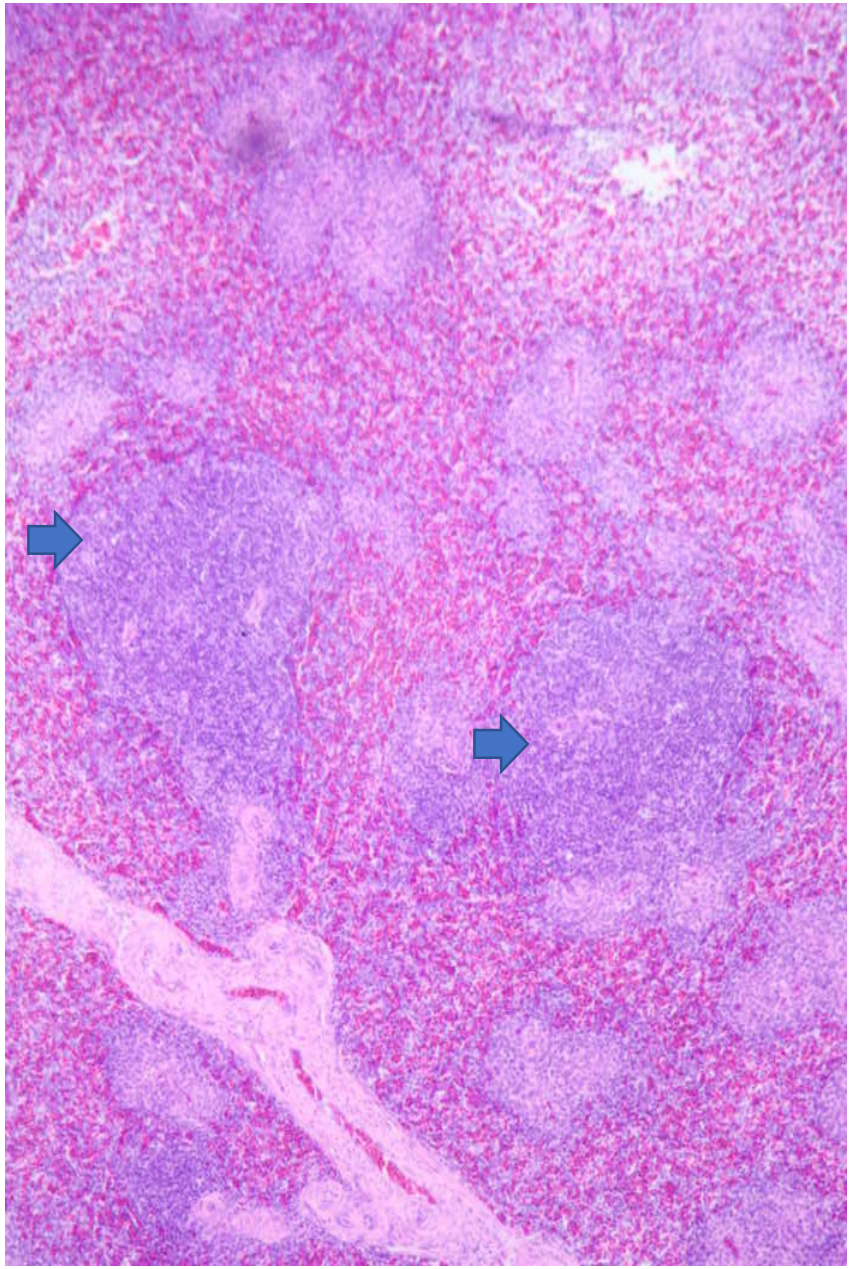
Lymphocytic tracheitis, with lymphoid hyperplasia: MG



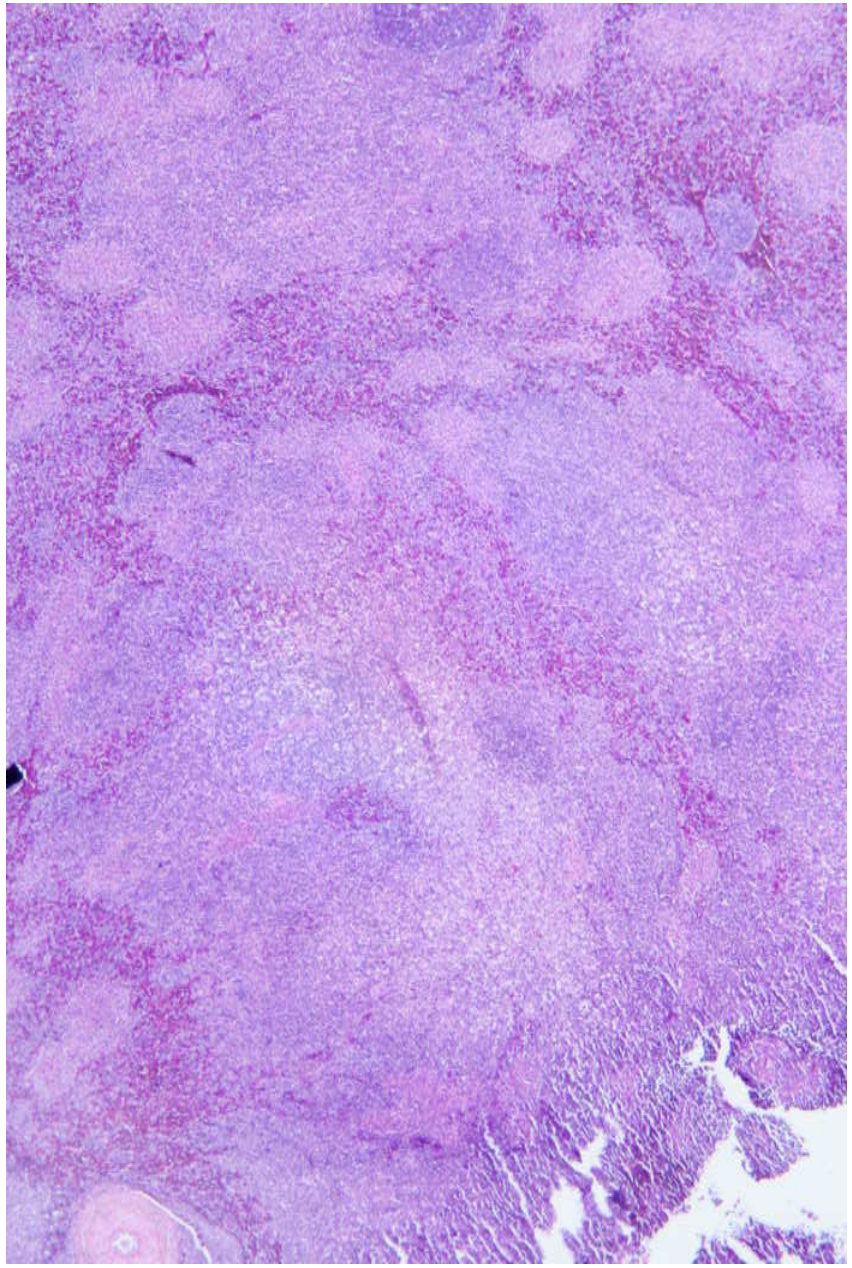
Trachea: Lymphocytic tracheitis and lymphoid hyperplasia; *Mycoplasma gallisepticum*

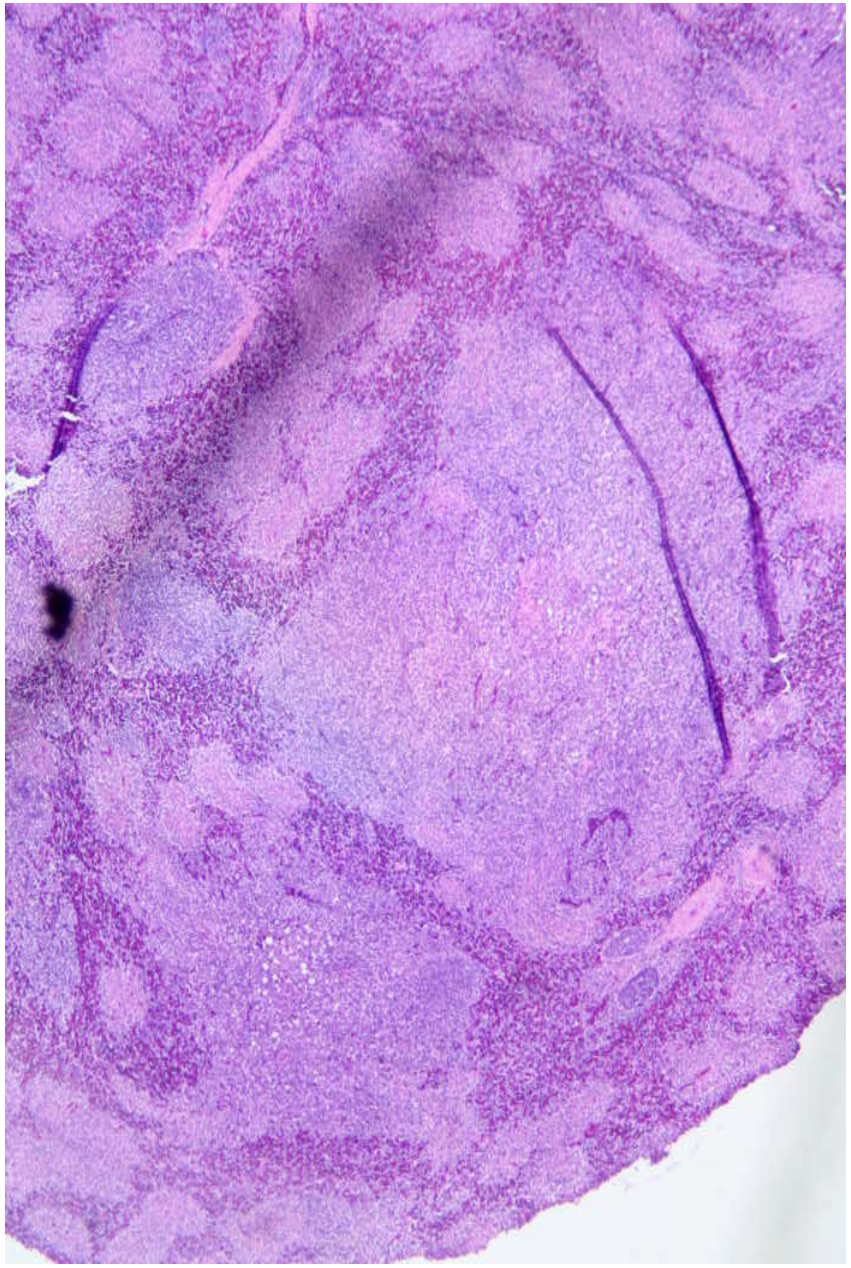
Broiler spleens: white foci at processing. Hyperplasia or initial neoplasia?



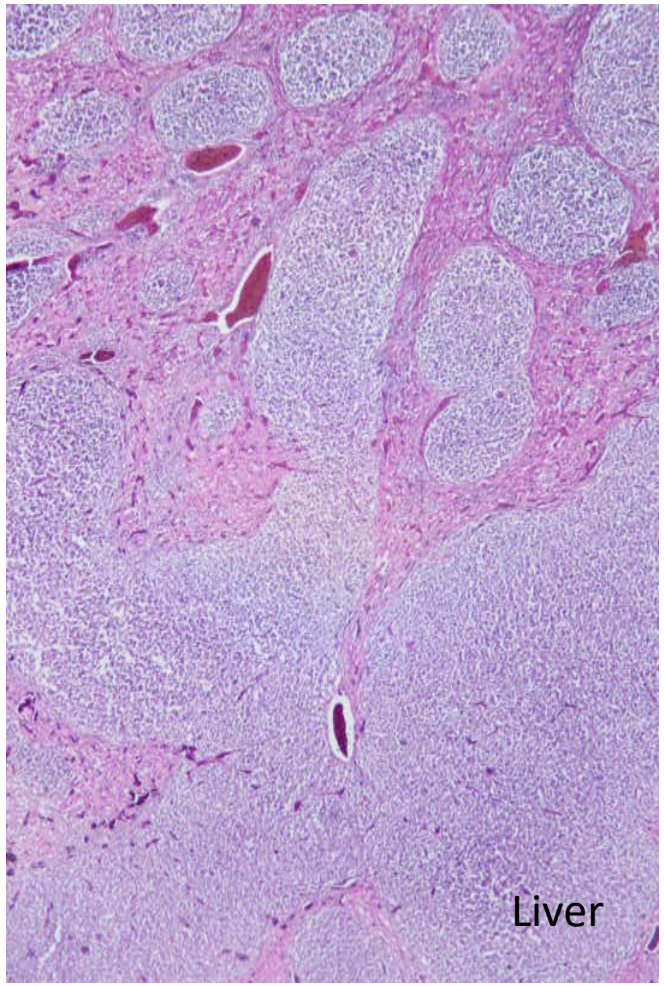


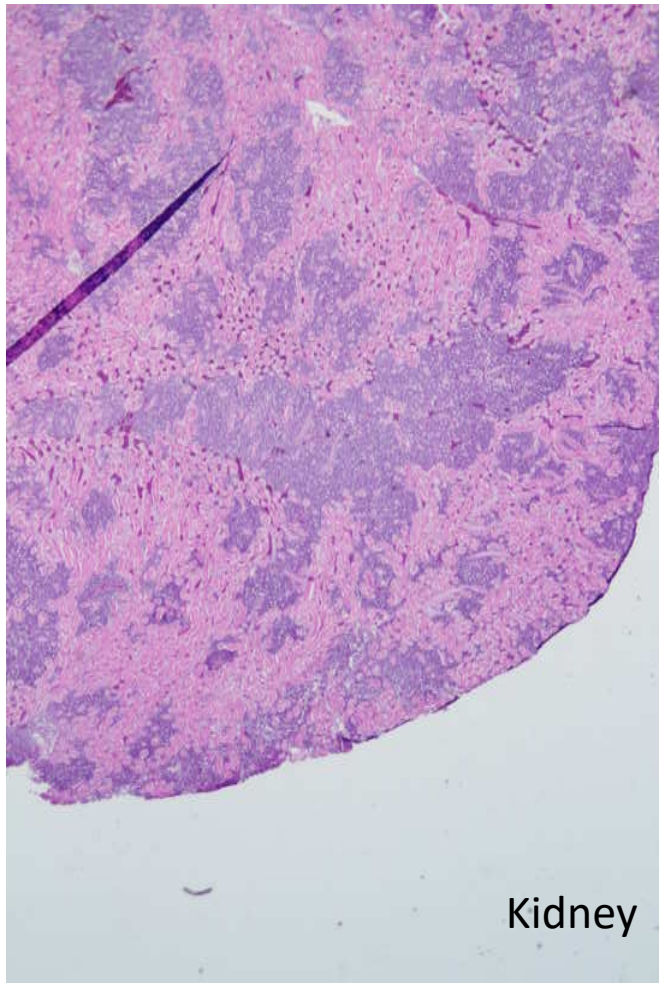
Broiler spleens: white foci at processing. Hyperplasia or Neoplasia?

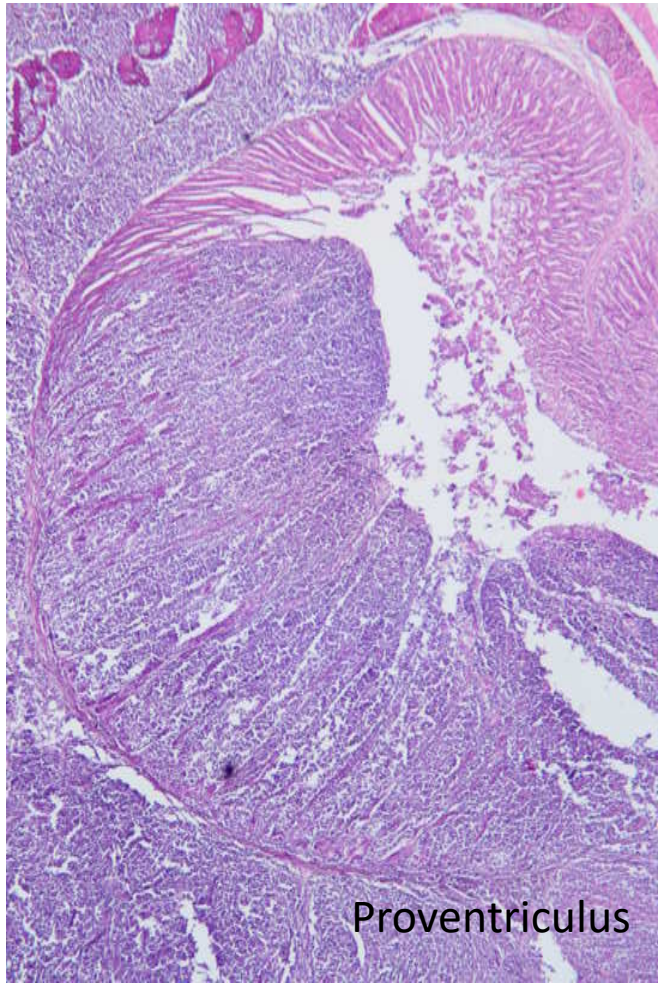




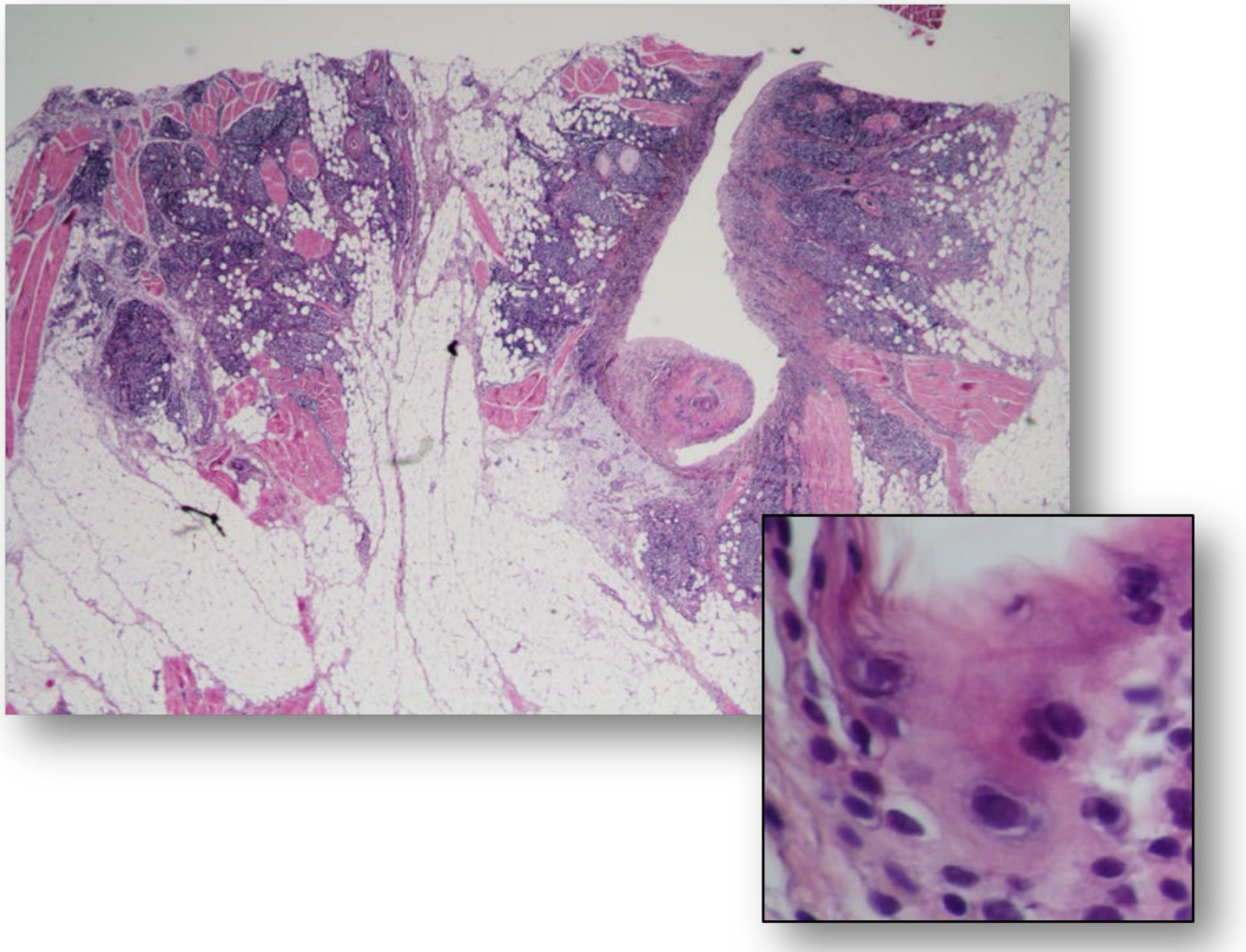
Lymphoid Neoplasia







Marek’s Disease, skin



END

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