European Society for Veterinary Clinical Pathology
MYSTERY SLIDE SESSION 2020
CASE 4

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SPECIMEN: Abdominal fluid smears, photomicrographs, data

SIGNALMENT: An 8-year-old, male border terrier.

HISTORY AND CLINICAL FINDINGS:
Nils had an acute onset of vomiting yellow green, somewhat slimy vomit (Day 1 = Dec 19 2018). He was tense and painful in the abdomen and appeared worried.

LABORATORY DATA:

Clinical Chemistry results Day 1

Test	Patient		Reference values
C-reactive protein	90	mg/L	< 7
S-Protein	47	g/L	56 -75
S-Albumin	18	g/L	27- 37
S-Glucose	5,8	mmol/L	3,5 -5,8
S-Fructosamine	208	umol/L	250 -400
S-Creatinine	83	umol/L	46 -115
S-Urea	10,2	mmol/L	2,5- 8,8
S-ALAT	3,0	ukat/L	0 -1,3
S-ALP	80,4	ukat/L	0 -2,2
S-GT	2,9	umol/L	0 -0,15
S-Bile acids fasted	552	umol/L	0 -12
S-Bilirubin, total	75,2	umol/L	0-3,2
S-Calcium total	2,3	mmol/L	2,3-2,8
S-Phosphate	2,9	mmol/L	0,8 -1,9

HEMATOLOGY DATA Advia 2120 Automated and Manual Results Day 1:

Test	Patient	Reference Values
Hematocrit	59 %	38-57
WBC	19.1 x 10^9 /L	5.8-16
Neutrophils seg	16.4 x 10^9 /L	3-11.5
Neutrophils band	0.2 x 10^9 /L	0-0.3
Lymphocytes	0.4 x 10^9 /L	1.4-4.8
Monocytes	2.1 x 10^9 /L	0.2-1.4
Platelets	540 x 10^9 /L	170-490

Urinalysis

Physical exam: 1.038, brown-green color
Chemical exam: 3+ protein, 2+ blood, 1+ glucose. ketones negative
Microscopic exam: 10 granular casts/lpf, 3 WBC/hpf, 2 RBC/hpf, moderate number of bilirubin crystals

Abdominal Fluid Analysis

Day 1 Bloody fluid with gross clumps in it so no WBC count performed. WBC differential count was 91 % neutrophils, 9 % macrophages and occasional mesothelial cells. No bacteria were seen. See figures 1-4.

Day 14 (January 2, 2019) WBC count Advia Baso: 10.5 x 10^9 /L; Advia Perox 11.9 x 10^9 /L. See figure 5 and slide in the slide set.

Questions

1 What is/are the material(s) found in the background of the abdominal fluid cytological smears taken 2 weeks apart (Figures 1-5)? Explain the difference morphologically, chemically and pathophysiologically.

a. Fibrin clots
b. Cryoglobulin
c. Cryofibrinogen
d. bile
c. amyloid

2. Why was the albumin low? Serum albumin was on day 1: 18 g/L, day 3: 13 g/L day 4: 11 g/L, day 5 19 g/L (RI 27- 37). The veterinarians in charge of the case were very concerned and asked us to analyze it often.

a. Protein loss in the urine.
b. Blood loss
c. Hepatic failure
d. Abdominal fluid loss via drainage tube

Figure 1
Gross photo of 2 May Grunwald Giemsa stained, direct smears of abdominal fluid day 1

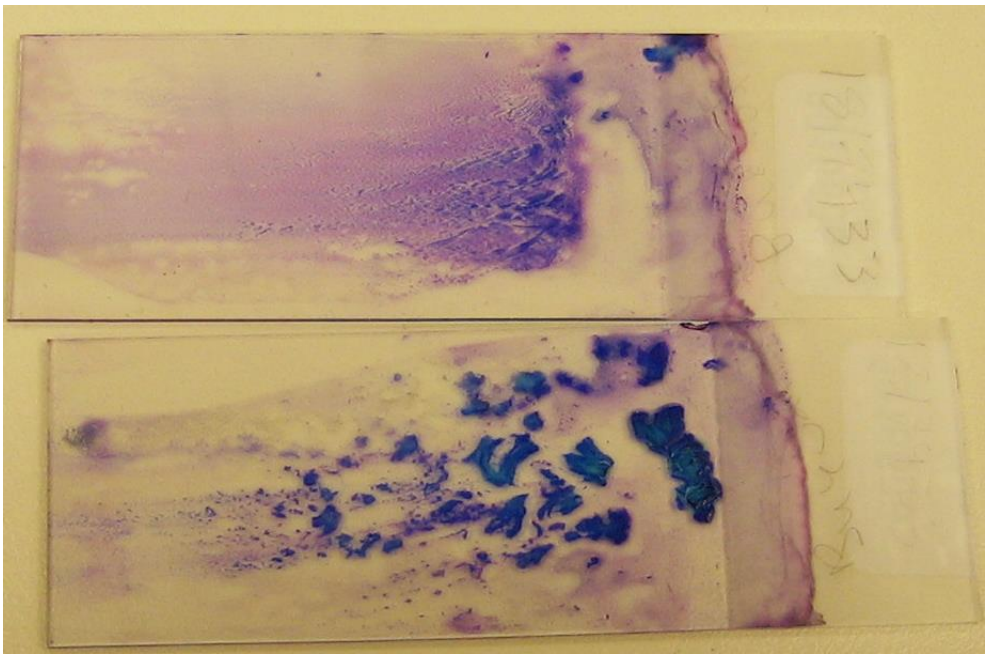


Figure 2 Abdominal fluid smear day 1, May Grunwald Giemsa original magnification 20X. Use erythrocytes (7 um) for size comparison.

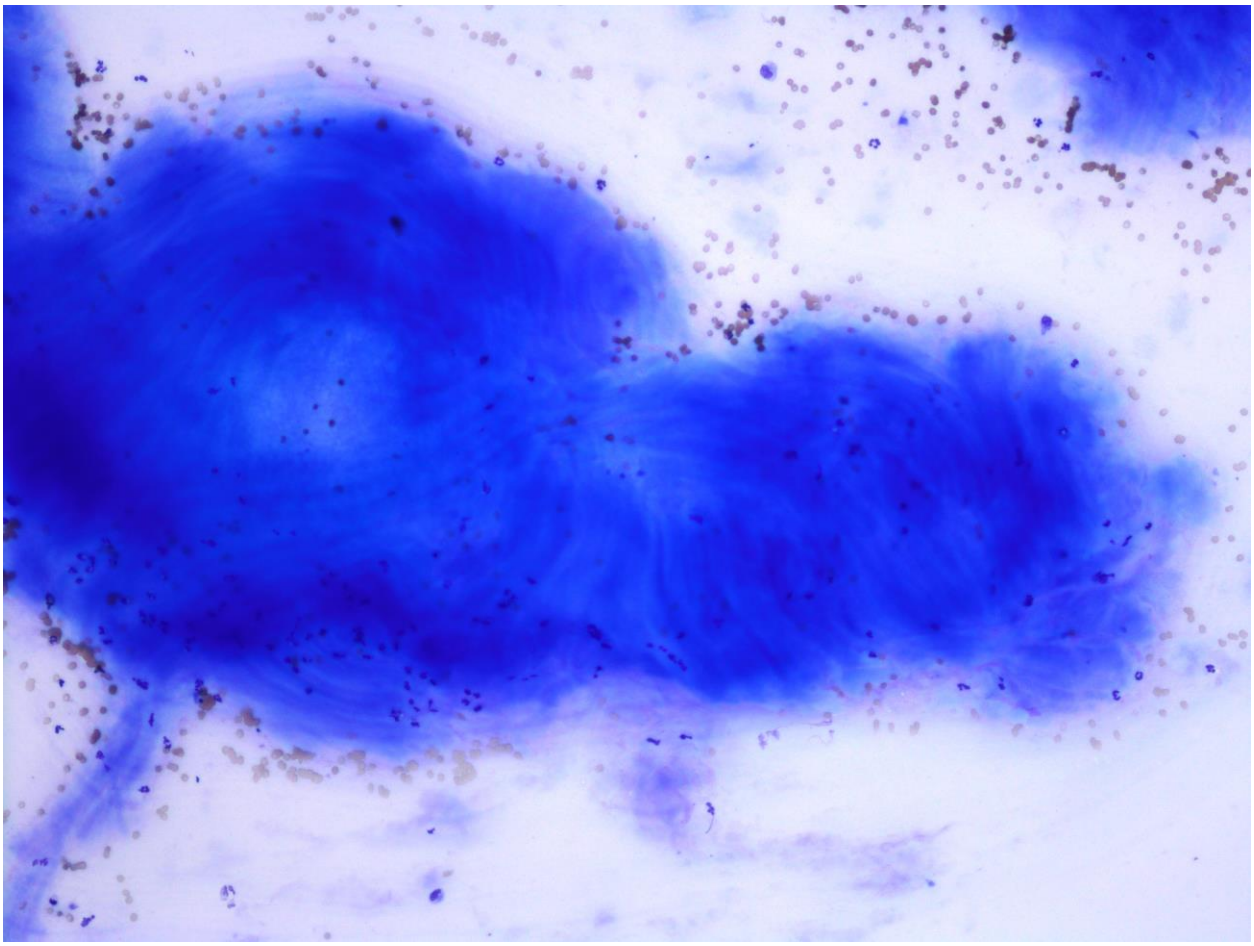


Figure 3 Abdominal fluid smear day 1, May Grunwald Giemsa original magnification 100X. Use erythrocytes (7 um) for size comparison.

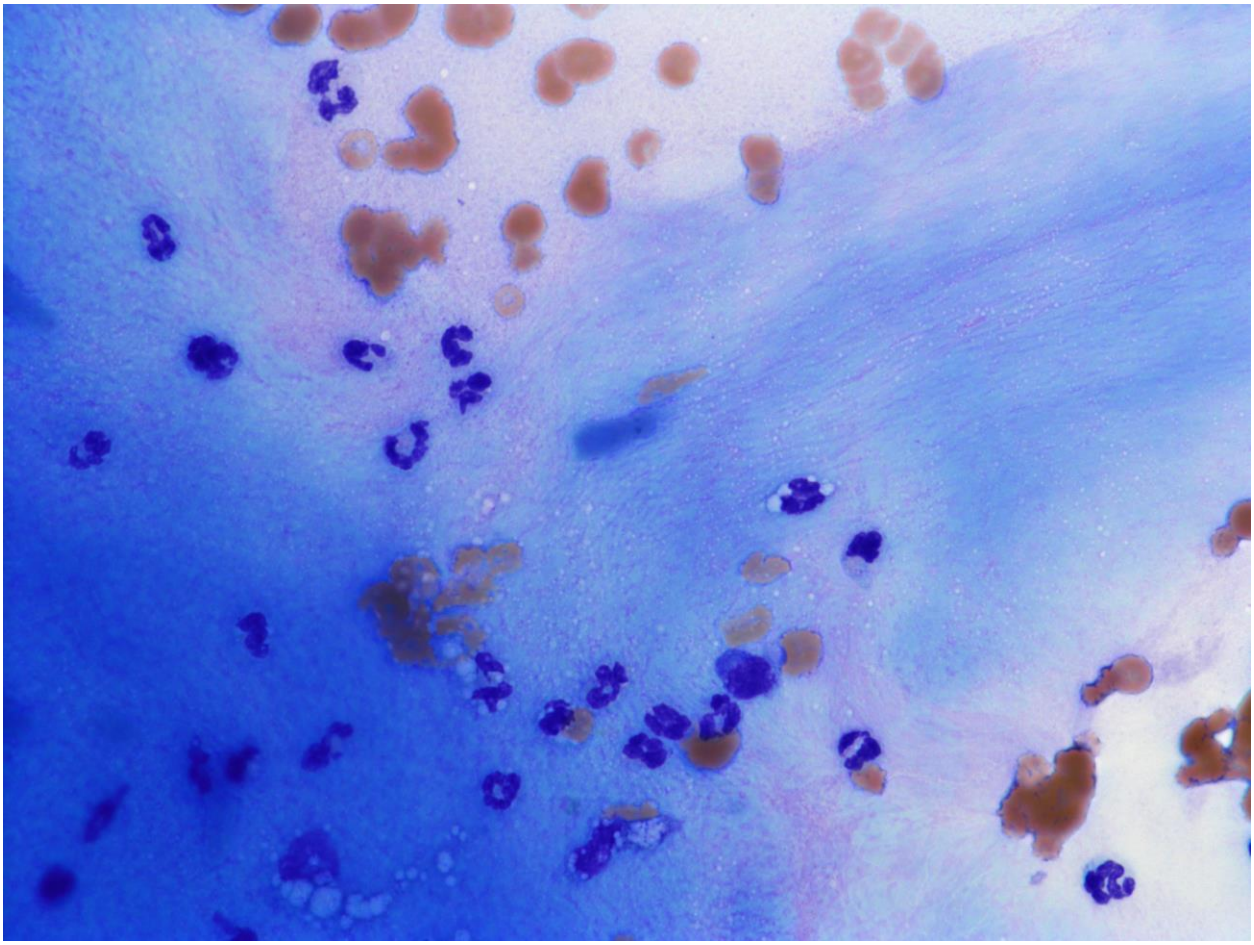


Figure 4 Abdominal fluid smear day 1, May Grunwald Giemsa original magnification 100X. Use erythrocytes (7 um) for size comparison.

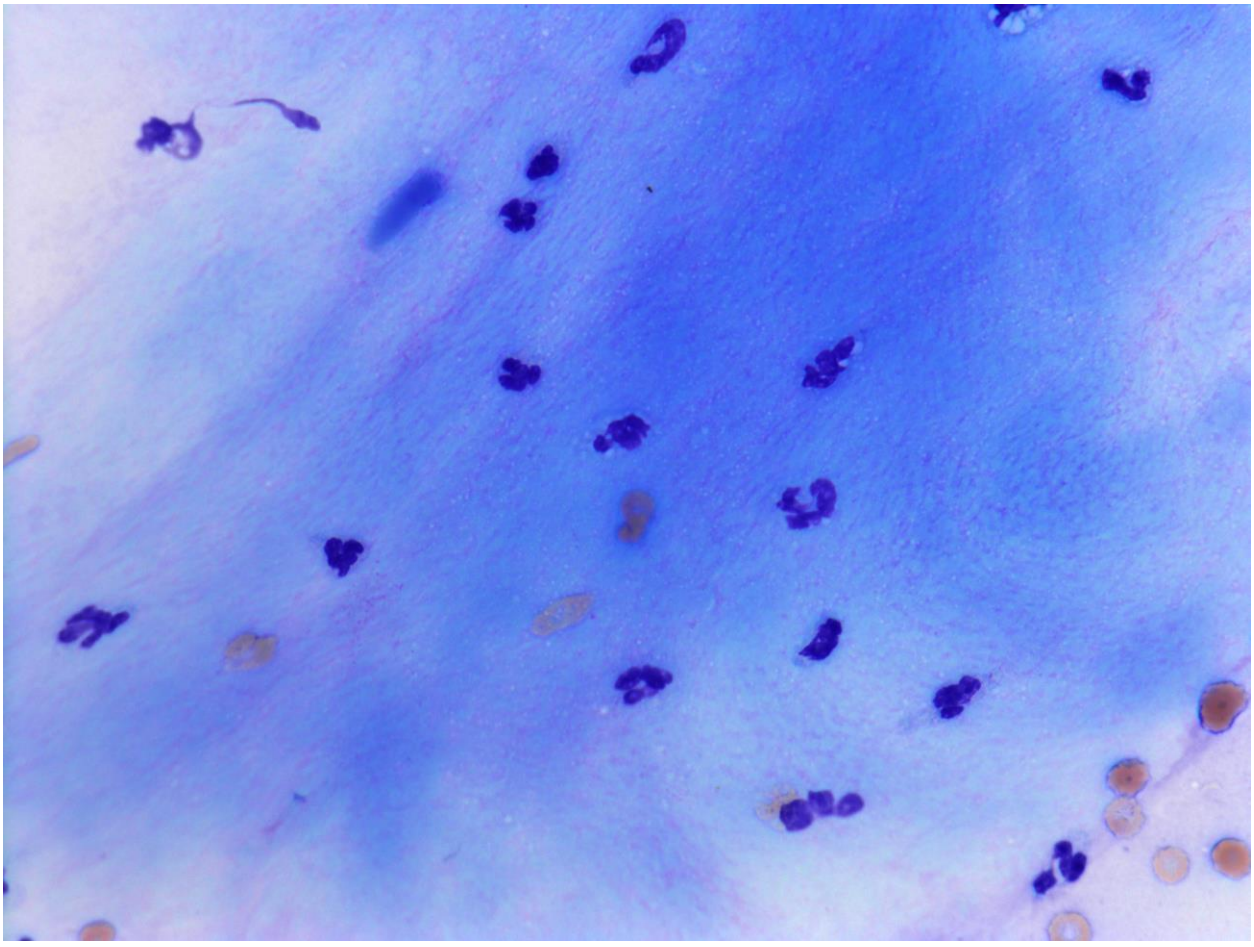
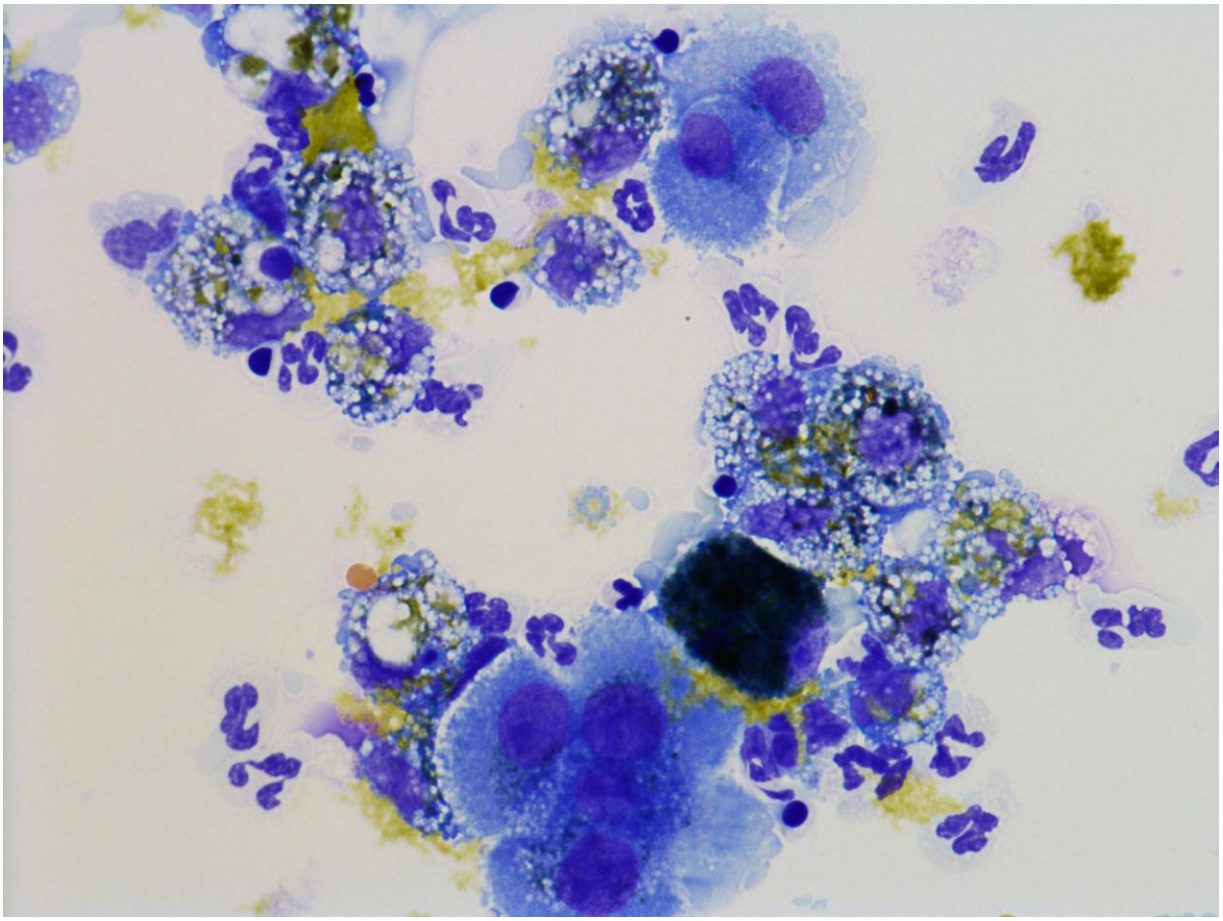


Figure 5 Abdominal fluid smear day 14, May Grunwald Giemsa original magnification 600X. Use erythrocytes (7 um) for size comparison.



DIAGNOSIS: Ruptured gallbladder mucocele with massive amounts of white bile in the abdominal cavity causing peritonitis acutely on day 1. Peritonitis persisted with the presence of more classical green and yellow bile in the abdominal fluid on day 14.

CLINICAL OUTCOME/FOLLOW-UP: The dog presented with acute bile peritonitis from a ruptured mucocele. The gall bladder was surgically removed and the abdominal cavity was drained and flushed for about a week in the hospital. The dog was sent home for about 1 week but returned with persistent bile peritonitis from continued leakage of bile. The dog was then euthanized.

ANSWERS TO QUESTIONS:

The material on day 1 was white bile. After 2 weeks it became more classical green and yellow bile (see discussion).

Hypoalbuminemia was likely due to multiple causes including all listed in the question. Life is not a question with only 1 correct answer permitted!

DISCUSSION:

The original abdominal fluid sample had unusually huge amounts of “white bile” and exceptionally good morphology showing the fibrillary, light blue color resembling mucus. The term white bile is incorrect because this form of bile is colorless. A more descriptive name would be low bilirubin bile. The theory for its formation is that pressure in the somewhat obstructed gall bladder prevents normal hepatocyte secretion of bilirubin and bile acids into the bile ducts.[1] Bile duct epithelial cells produce mucus which becomes the main component of “low bilirubin bile”. This stains positive for mucin with Alcian blue-PAD and Mayar’s mucicarmine stains.[1]

After 2 weeks of leakage of bile into the abdominal cavity and a high bilirubin concentration in the abdominal fluid the second cytological sample showed the more classical yellow, green and dark pigment. This gave the fluid a green color grossly which has been called black bile or yellow bile. Bilirubin on day 2 in the abdominal fluid was 120.6 umol/L. Bilirubin in serum was 28.0 umol/L. This was more than a four-fold increase in abdominal fluid. Greater than a doubling supports a diagnosis of bile peritonitis.[2] Gallbladder mucocele is becoming more commonly diagnosed in dogs.

Bile is mainly water and about 1-5 % bile salts and bilirubin, plus other substances such as fats and salts. Bile salts are organic solutes which act somewhat as surfactants which emulsify lipids and aids in fat absorption. Bile secretion is the main way to excrete bilirubin and bile salts that are too large for the kidney to excrete.. Lipophilic toxins are excreted via bile and divalent heavy metal cations like mercury, lead, and cadmium.[4] The 2 primary bile salts are cholic acid, and chenodeoxycholic acid. But they are metabolized by gut bacteria and return to the liver by the enteroheptic circulation resulting in about 12 major conjugated bile salts in blood.[4]

Bilirubin is yellow which causes the yellow color of icterus. Biliverdin is green. Biliverdin is formed by catabolism of heme. Biliverdin is converted to bilirubin but when bilirubin is oxidized it reverts to biliverdin. Urobilinogen is colorless. Urobilinogen is oxidized to form urobilin and stercobilin. Urobilin give urine its yellow color and stercobilin give feces its brown color.

The bile peritonitis created a large volume of abdominal fluid which was drained and flushed out ot the abdominal cavity. One of many comments in the record was “Drained 240 ml of bloody abdominal fluid with clots.” Removal of bloody exudate from the abdominal cavity is a major source of protein loss replaced by protein poor fluids like lactated Ringers or drinking water that would dilute remaining albumin in blood. Renal status was not well worked up in Nils. There was 3+ protein in the first and only urinalysis performed. Ten casts/lpf indicates renal damage perhaps due to hypovolemia and shock. Creatinine and urine specific gravity (1.038) do not indicate renal failure on day 1. Day 1 had total protein of 47 g/L, albumin of 18 and thus globulin of 29 g/L and A/G of 0.6. This can support more loss of albumin than globulins. Decreased hepatic synthesis of protein was likely a minor factor.

REFERENCES:

1. Owens SD, Gossett R, McElhaney MR, Christopher MM, Shelly SM. Three cases of canine bile peritonitis with mucinous material in abdominal fluid as the prominent cytologic finding. Vet Clin Pathol. 2003;32:114-20.

2. Ludwig LL, McLoughlin MA, Graves TK, Crisp MS. Surgical treatment of bile peritonitis in 24 dogs and 2 cats: a retrospective study (1987-1994). Vet Surg. 1997;26:90-8.

3. Hyo-Min K, Hye-Jin J, Ji-Houn K, et al. Atypical Bile Peritonitis Associated with White Bile in a Dog with Ruptured Gallbladder Journal of Veterinary Clinics 2018;35:17-18.

4. Boyer JL. Bile Formation and Secretion. Comp Physiol. 2013; 3:1035–1078.