Analysis of fluids

- What parts are useful for the referring veterinarian? What parts have the potential to mislead?
- What do I find are the difficult or controversial parts?
- Fluid analysis combines the numbers with the images, so I need to ensure my reasoning includes both!

Animal Health Australia

Australian Animal Pathology Standards Program (AAPSP) 2013 Roadshow

THE UNIVERSITY OF SYDNEY

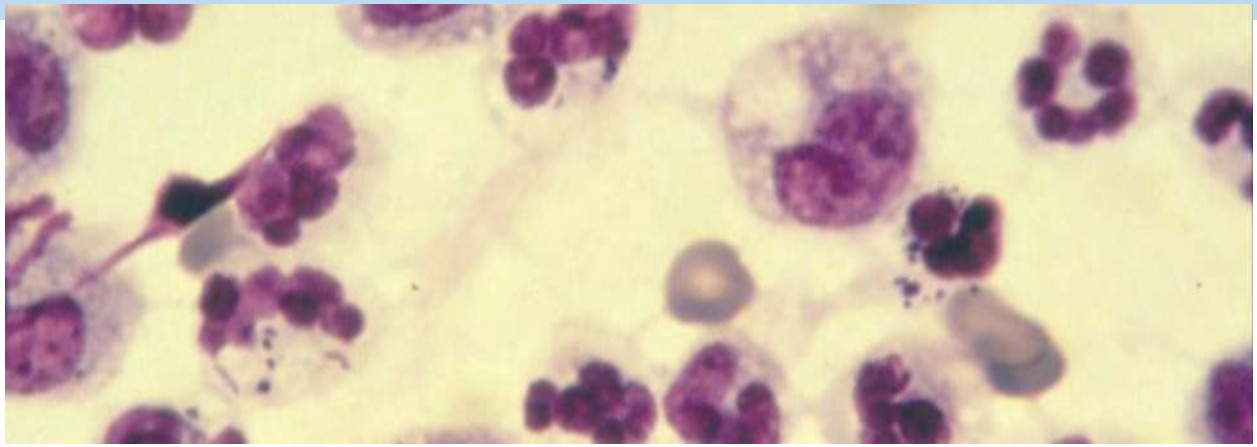
Professor Emeritus Paul Canfield, Faculty of Veterinary Science, University of Sydney

Usefulness of Body Fluid Analysis for the referring veterinarian?

- It helps in identifying the pathological process
  - Non-inflammatory (degenerative, haemorrhagic etc)
  - Inflammatory
  - Neoplastic

THE BIG TWO!

- The pathological process will have implications for **pathophysiology/pathogenesis** (ie fluid and disease development), including **aetiology** (cause)



One of the few parts of clinical pathology that my pathological processes approach is accepted! But do I like the simple classification for body cavity effusions based on pathological processes?

Large Body Cavity Effusions:

1. Non-inflammatory in origin

- Pure transudate
- Modified transudate
- Chylous/ (pseudochylous)
- Haemorrhagic

2. Inflammatory

- Non-septic exudate
- Septic exudate

3. Neoplastic

The numbers: protein, total cells, nucleated cell differential
The images: gross characteristics and the smear

Trends in values for diagnosis (mainly peritoneal, but has been applied to thoracic and pericardial)

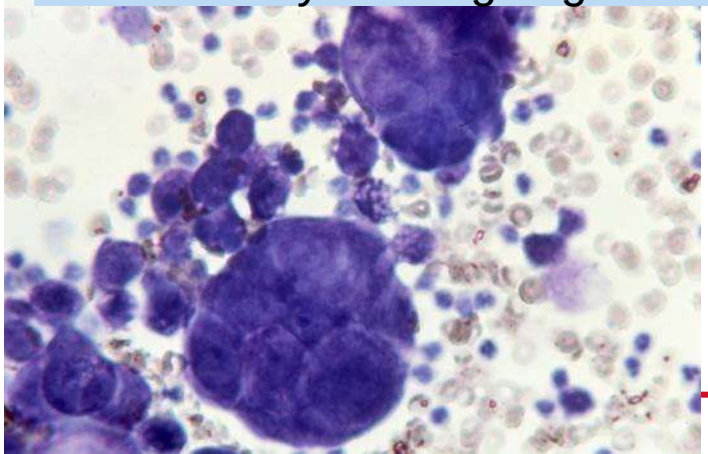
	Pure Transudate	***Modifed Transudate***	***Chylous***	***Haemorrhagic***	***Non-septic exudate***	***Septic exudate***
Gross characteristics	Clear, colourless (SA) or pale yellow (H,B)	Variable cloudiness, sero-sanguineous	Pink/white opaque	Red/brown opaque	Sero-sang, cloudy	Sero-sang or purulent, cloudy/flocculent
Protein (refract) g/l	<25 (SA, H); <30 (B)	25-50 (SA); 25-30 (H)	>50 (inaccurate)	Usually >40	>30-70 (SA & H); 20-60 (B)	>30-70 (SA & H); 20-60 (B)
NCell count x10$^{6/L}$; RBC?	500-1500 (SA); 1500-10,000 (H, B)	500-5000 & a few RBCs (SA); up to 12,000 (H)	500-5000 & variable RBCs (SA)	Variable NCC; numerous RBCs (PCV 5% or greater)	>5000 & variable RBCs (SA); >12,000 (H); 1-200,000 (B)	>5000 & variable RBCs (SA); >12,000 (H) ; 1-200,000 (B)
Smear	**Mo/Mac, L , N (nl) (SA, H); E (B - up to 30%)**	**Mes, Mac, L, N(nl) (usually increased), E (B)**	**L**, variable others (SA, H, B)	**As for blood** + Mes, Mac (red cell digestion)	**N(nl)**, Mes, Mac; E% drop in bovine	**N (l),** Mac, rare Mes, **bacteria;** E% drop in bovine

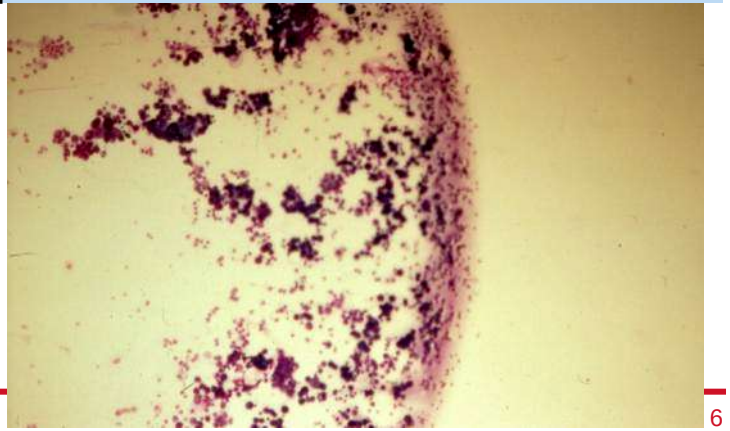
Some notes on effusions

- **Protein levels** are not only dependent on pathological process but also blood levels – don't get caught like I do! The refractometer may give false high protein readings for birds, but what about mammals? Who uses the biuret method for fluids?
- **Modified transudates** may progress to **Non-septic exudates** (non-microbial irritants or non-bacterial infectious agents may cause directly) – *on the numbers, how do you truly distinguish between the two? Does it really matter (eg FIP effusion has been variably placed because of the low nucleated cell counts?)*
- Cattle abdominocentesis: vast variance reported in nucleated cell counts (5 or 10,000 is normal?) and protein values, but general trends apply; eosinophils prominent in transudate smears (not in thoracic fluid), but reduce in exudates. Fluid from healthy cattle may clot apparently (not related to fibrinogen content?)
- Categories hard to apply to coelomic cavity fluid analysis in birds and reptiles. Yolk 'peritonitis' may have low cells! Exfoliative neoplasia, haemorrhage and exudates (non-septic and septic – most have high heterophils) all occur.

My approach to making smears from body cavity fluid?

- Always good to make a **direct smear** of the fluid at the time of collection
  - In case of delays in processing leading to cellular degeneration or microbial contamination
  - Make as for a peripheral blood film, but slower!
  - Always look towards the feathered edge at the end of the smear for larger cells
- Should you concentrate before smearing?
  - If cellularity low and you are particularly looking for neoplastic cells
  - Other times of low cellularity (eg transudates), the smear may not be that useful
  - Useful if you are going to do ICC on a smear or pellet





What are the major issues for me for large body cavity fluid analysis?

1. Is the classification system stifling or misleading to the referring veterinarian?
2. Identifying those cells – particularly reactive versus neoplastic mesothelial!
3. Reminding myself that not all tumours exfoliate
4. Reminding myself to always consider culture if inflammatory (irrespective of cell types and appearance)

A case: a two year old Siamese cat with fever and abdominal distention

Pattern recognition? But what evidence do you need to back it up and what else is on your list of DD's?

Male Siamese from a breeding colony. It had a 4 w history of URT disease and 2 w history of lethargy, inappetance, weight loss and abdominal distension

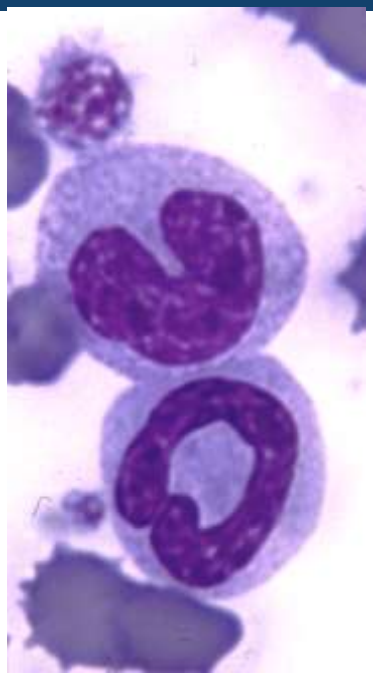
On presentation, it had naso-ocular discharge, abdominal fluid distension and a temp of 39.7 degrees C

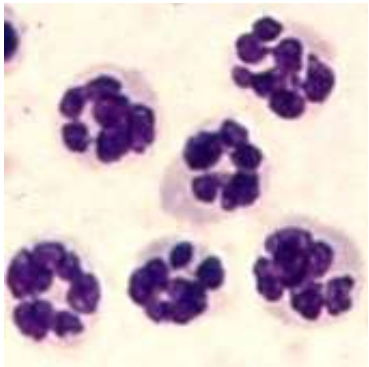
"One should always look for a possible alternative, and provide against it. It is the first rule of …investigation…You should never lose sight of the alternatives." *Sherlock Holmes in The Adventures of Black Peter*

"No, no: I never guess. It is a shocking habit,--destructive to the logical faculty." Sherlock Holmes in The Sign of The Four

A two year old Siamese cat with fever and abdominal distension - Haematology

PCV L/L	**0.22**	0.30-0.45	Nseg x 10^9/L	**11.2**	4-10
TPP g/L	**95**	59-78	Nband x 10^9/L	**1.0**	0-0.2
Retics %	0.4	0-1	Lymph x 10^9/L	**1.2**	1.6-7.0
			Mono x 10^9/L	**0**	0.1-0.7
WBC x 10^9/L	13.6	8-14	Eos x 10^9/L	0.2	0.2-1.4
Smear: Some hypersegmented neutrophils present					



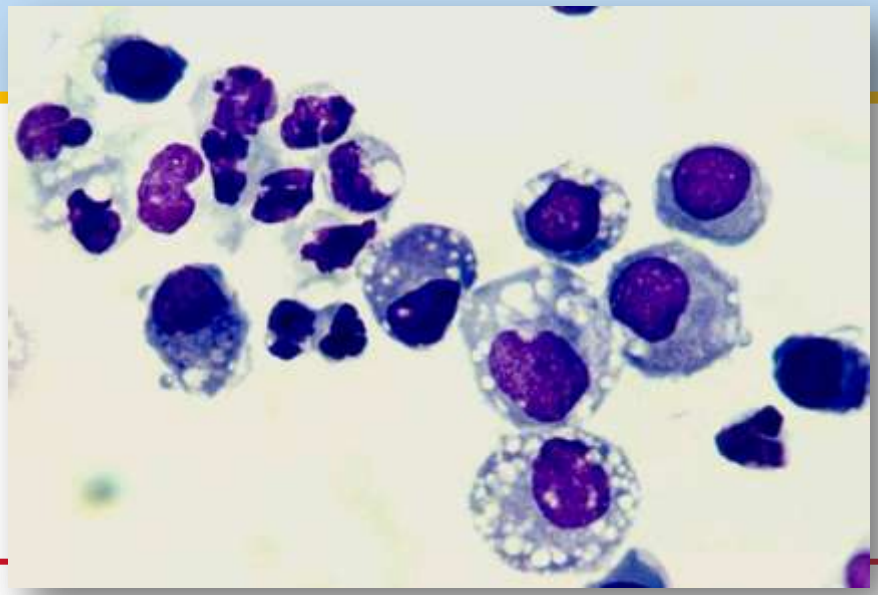


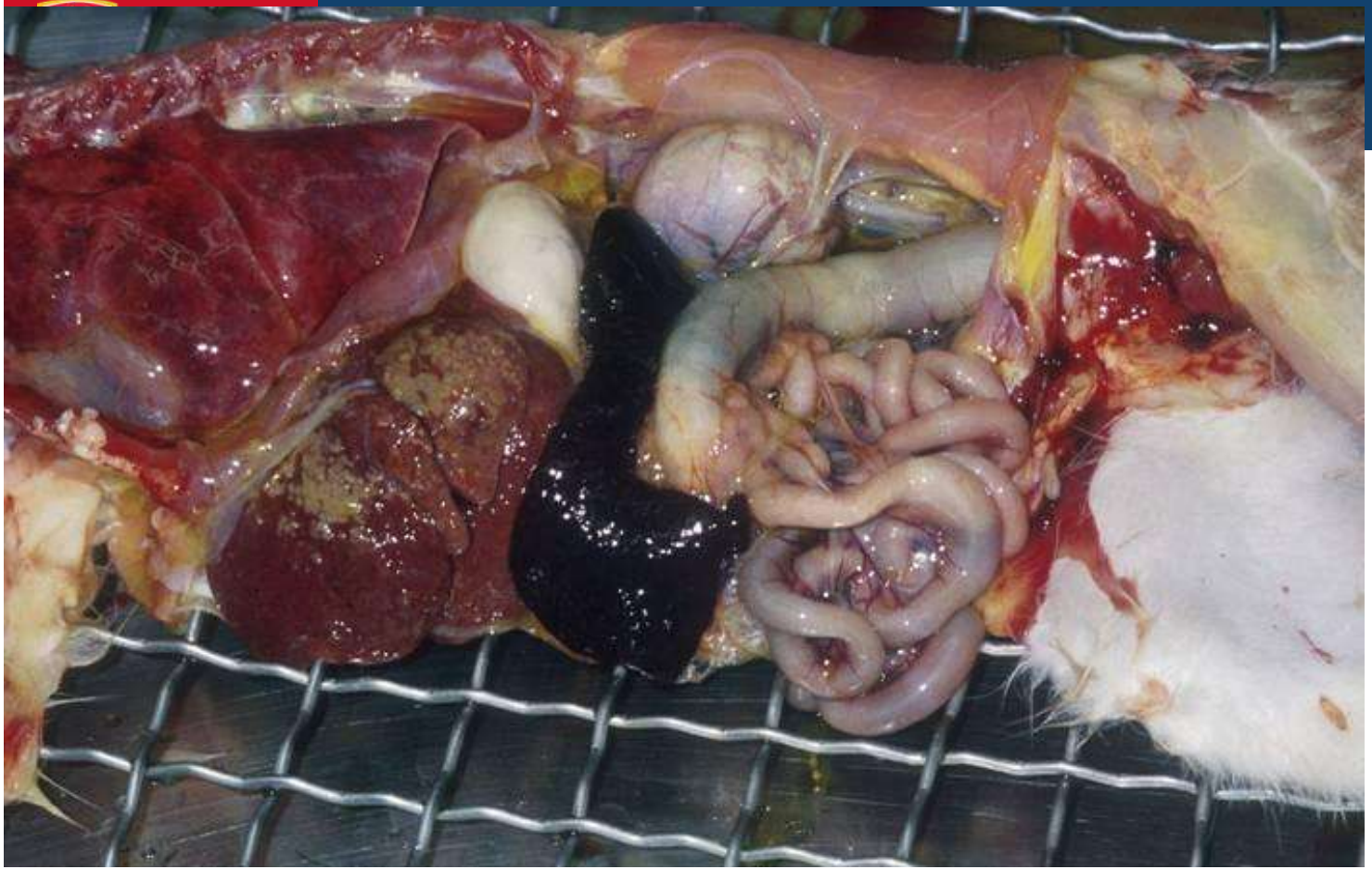
A two year old Siamese cat with fever and abdominal distention

- **Abdominal fluid analysis**:
  - pale yellow and cloudy;
  - protein was 80 g/l (0-25);
  - RBCs 100 (0);
  - TNC 4,900 (<1500).

How would you classify this? Modified transudate or non-septic exudate? Depends if you use cut off points for the categories!

- **Differential: 65% non-lytic neutrophils, 10% lymphocytes, 25% macrophages (some could have been degenerate mesothelial cells – another issue for me!)**





Necropsy findings – how do you diagnose FIP ante-mortem?

FIP viral antigen detection on cytological preparations or biopsy (ICC, IHC)

An old cat with respiratory distress

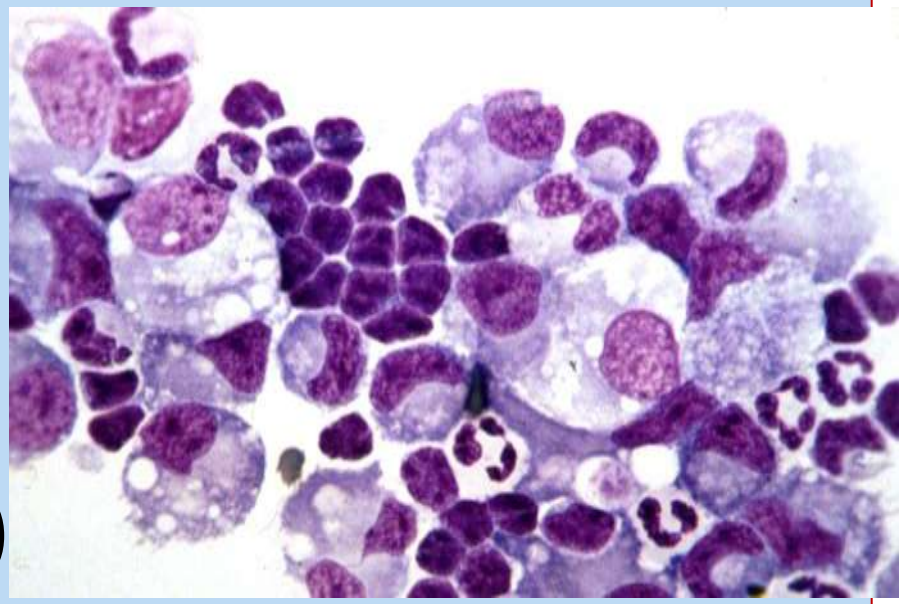
Pleural fluid analysis:

Gross: turbid, white

Protein: 36 (0-25)

RBCs: 7600 (0)

TNC: 6140 (<1500)



- Pleural fluid smear revealed: 58% L, 29% non-lytic N and 13% Macrophages and Mesothelial cells

Chylous effusion

The pleural fluid

Who does trigs and cholesterol analysis in suspected chylous effusions??

- Pleural fluid triglycerides: 3.2 mmol/l (plasma level: 0.9)
- Pleural fluid cholesterol: 1.3 mmol/l (plasma level:2.6)
- **So what, how does knowing the fluid is chylous help the referring veterinarian in understanding pathogenesis and cause? What is the most common reason for pleural chylous effusion in the cat (common things occur commonly – a useful heuristic, and don't forget Father Ockham)?**

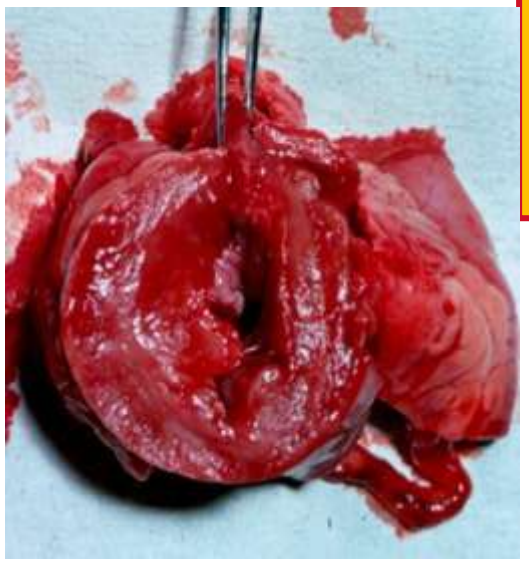
Or is it idiopathic?

Cardiac disease?

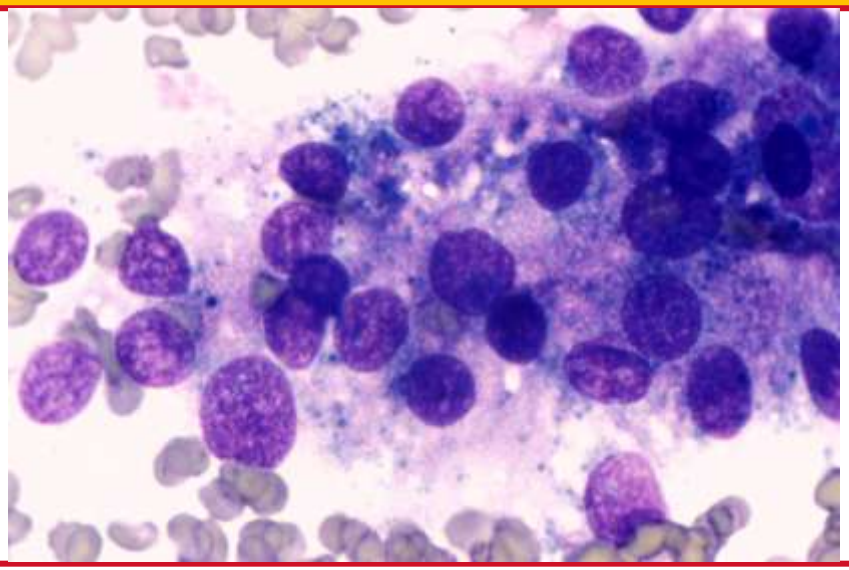
Plasma: pleural trigs and cholesterol are reverse for pseudochylous effusions (ie higher cholesterol and lower trigs in fluid compared to plasma levels). But who has seen any?

By the way, the old cat with respiratory distress had –

- T_4: 102 nmol/l (20-40) predisposing to cardiomegaly/cardiomyopathy



Hyperthyroidism due to functional adenoma



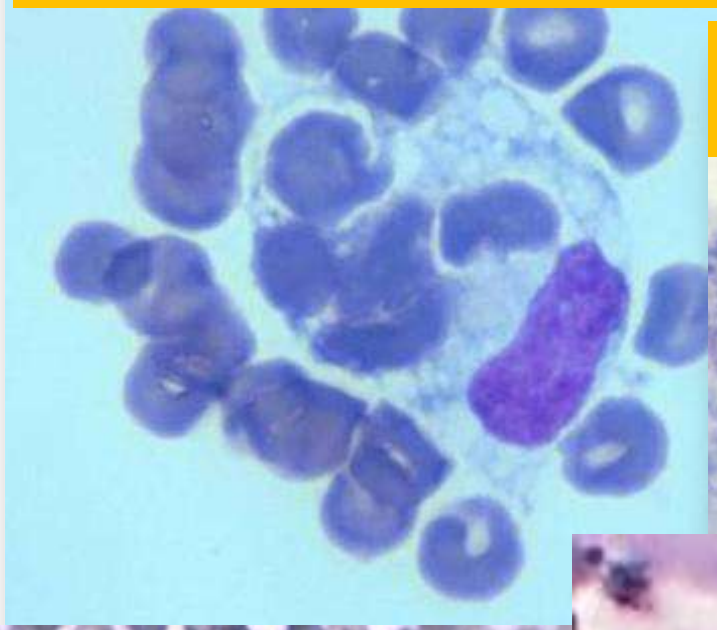
10 YO Labrador with weight loss and coughing

- **Pericardial fluid analysis**:
  - red and cloudy;
  - protein: 62 (0-25);
  - RBCs: 1,660,200 (0); PCV: 0.06 (0);
  - TNC: 8,175 (<1500);
  - Smear: 22% non-lytic N, 26% macrophages and mesothelial cells and 52% neoplastic epithelial cells.
- **A (haemorrhagic) neoplastic effusion?**

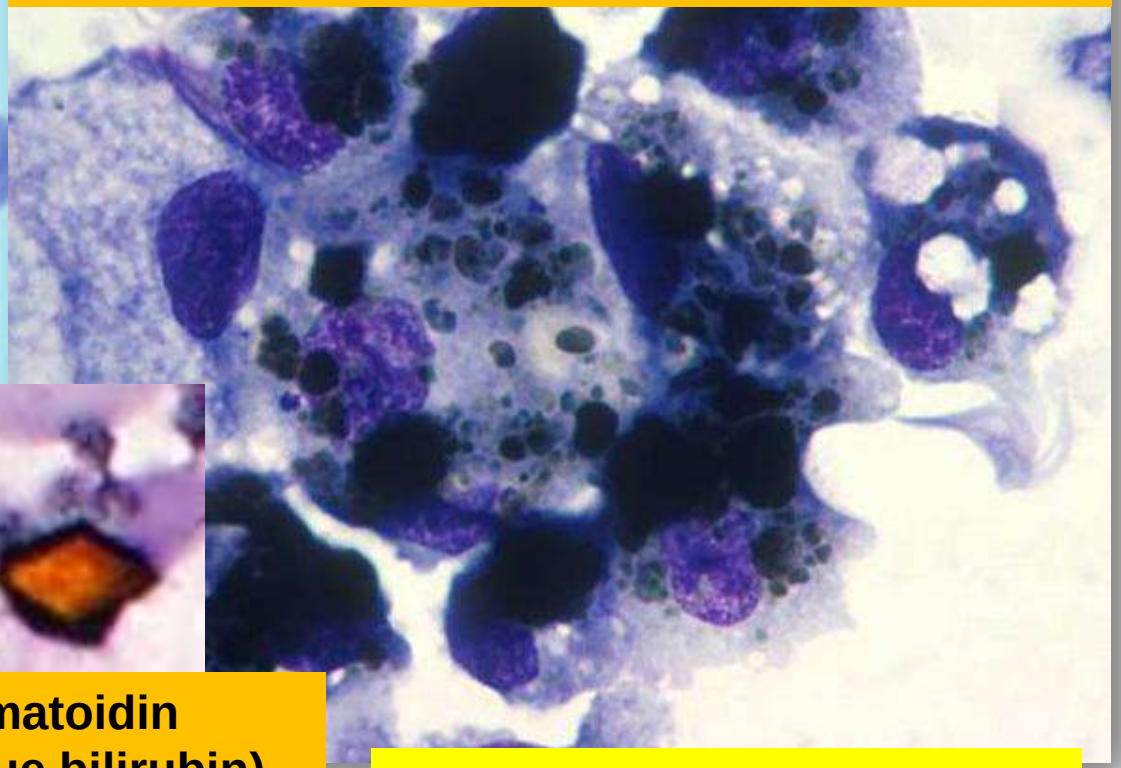
An opportunity to talk about haemorrhage and neoplasia

How long does it take for erythrocytes to be reduced to haemosiderin and haematoidin? It depends on the tissue!

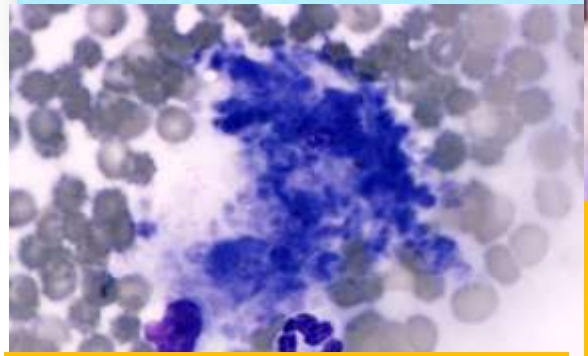
Recent erythophagocytosis (0-24 hrs)



Past erythrophagocytosis (perhaps 4 days to 1 week plus? – 1 day plus in lung?)



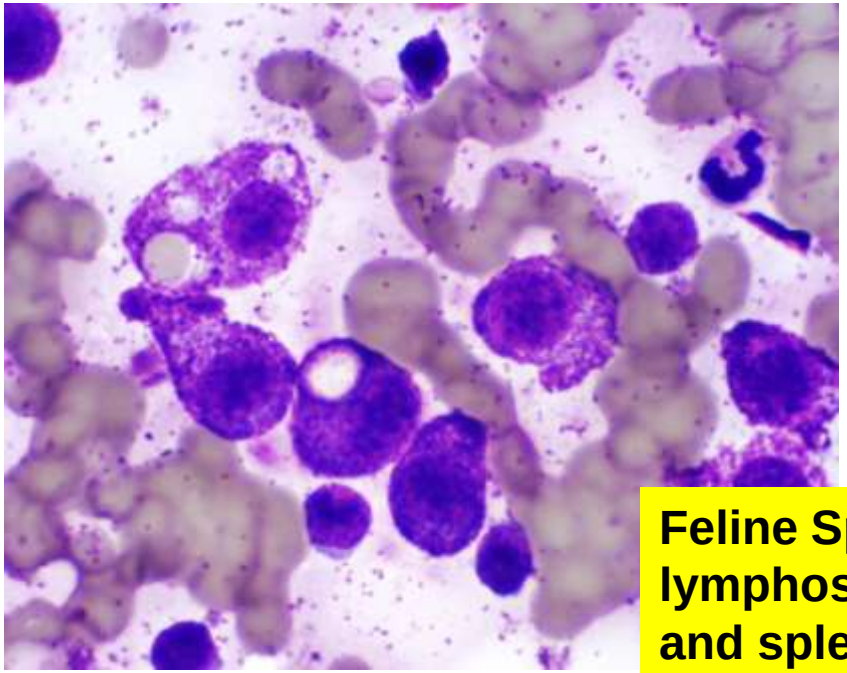
Haematoidin (tissue bilirubin) crystal – 9-10 days plus?

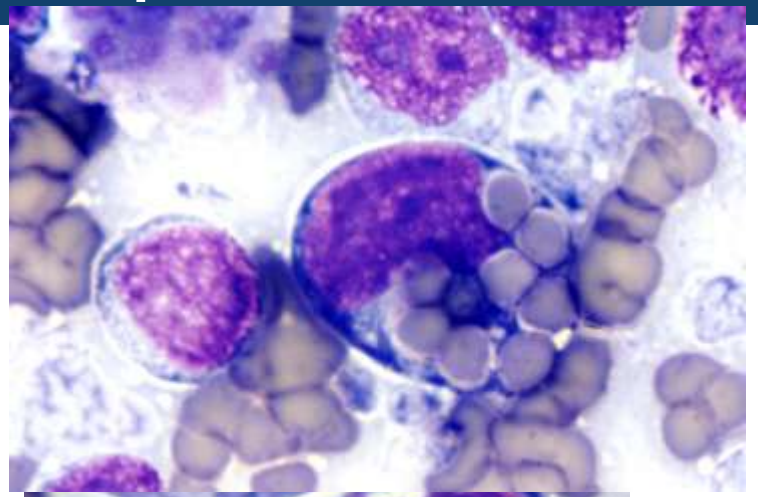


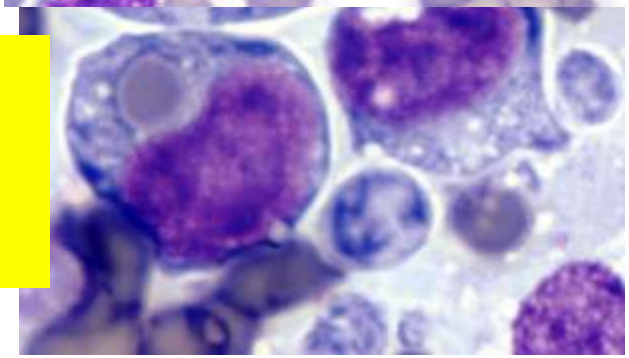
Clot – iatrogenic or recent bleeding?

Interpreting bruises at necropsy. JClinPathol 2001;54:348–355. P Vanezis.

Erythrophagia (or is it a variant form of emperipolesis?) by neoplastic cells



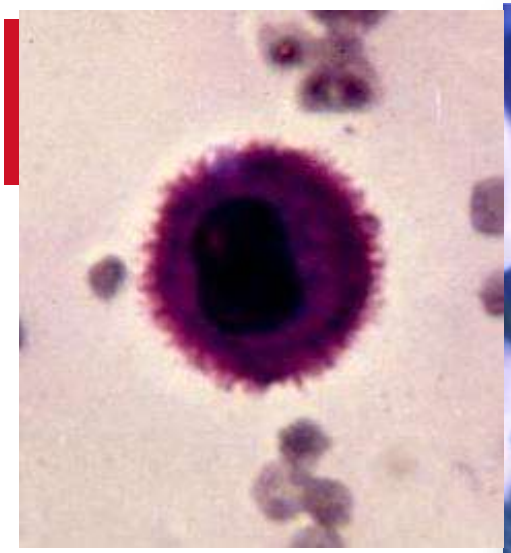


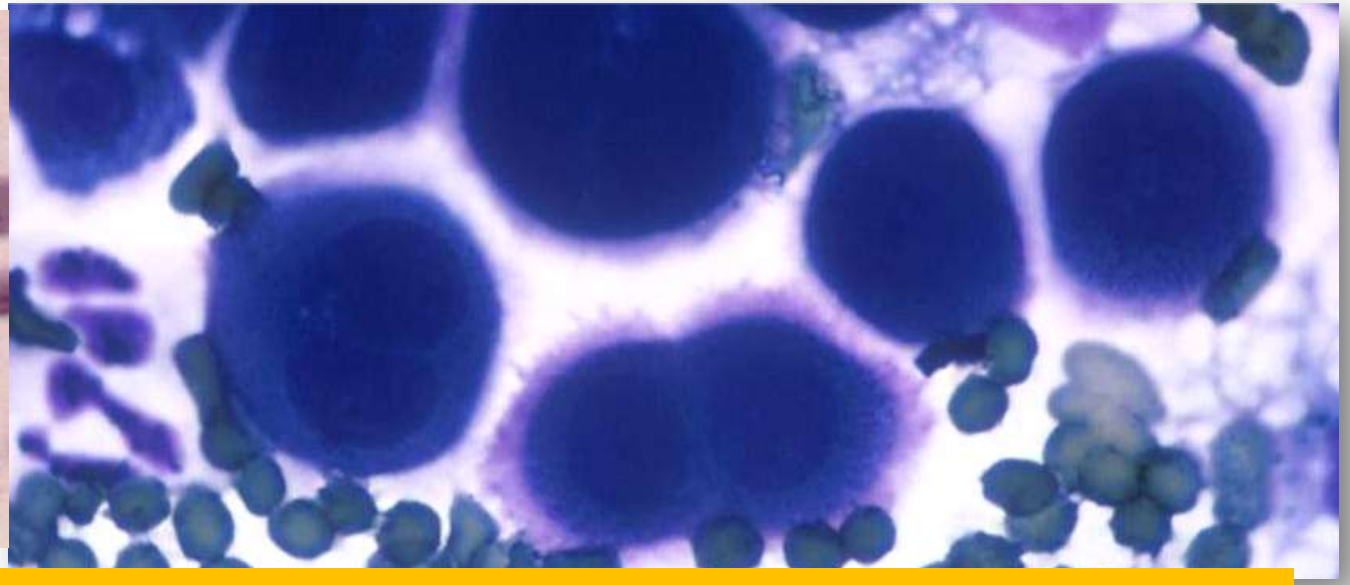


Feline Splenic lymphosarcoma and splenic mast cell neoplasia

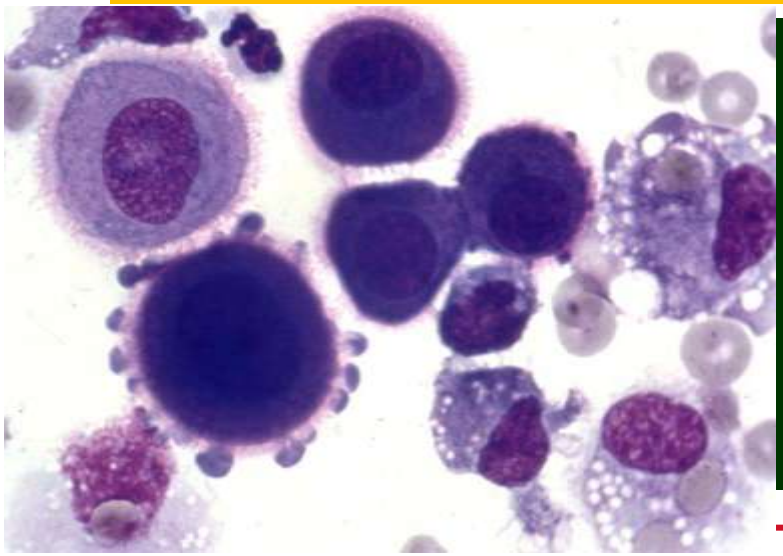
Apart from histiocytic malignancies, engulfed erythrocytes can occur in a wide variety of neoplasms, including haemangiosarcoma, osteosarcoma, lymphosarcoma and mastocytoma

Hemophagocytic syndrome, which involves cytokine mediated activation of macrophages, can also occur in malignancies – all blood cells can be involved

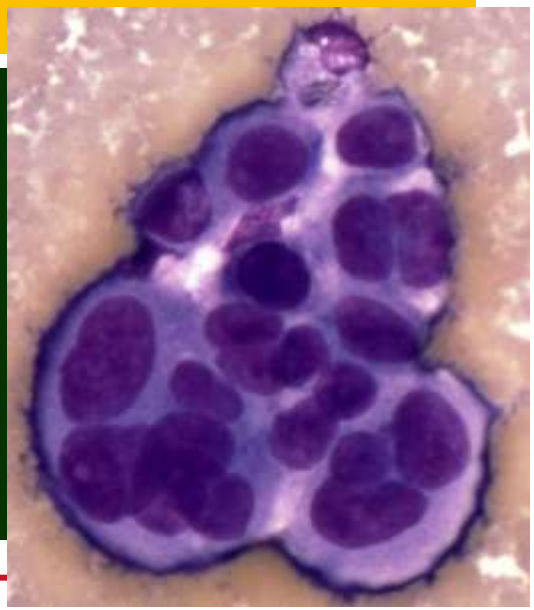




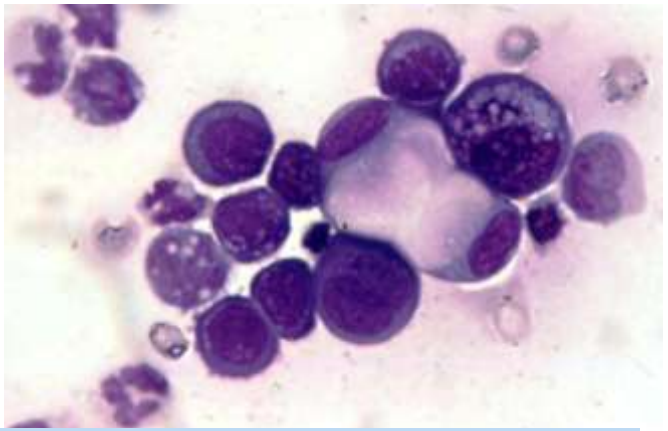
Reactive mesothelial cells with ‘corona’. But if they don’t have the corona how difficult are they to distinguish from neoplastic mesothelial cells?



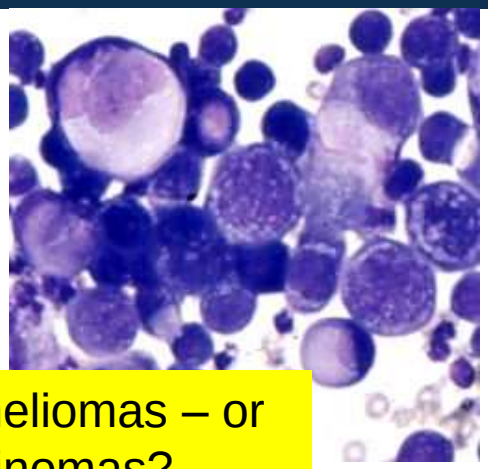
Urban myth or true for body cavity neoplasia: if mesothelial cells are seen with a corona then it is unlikely to be mesothelioma?



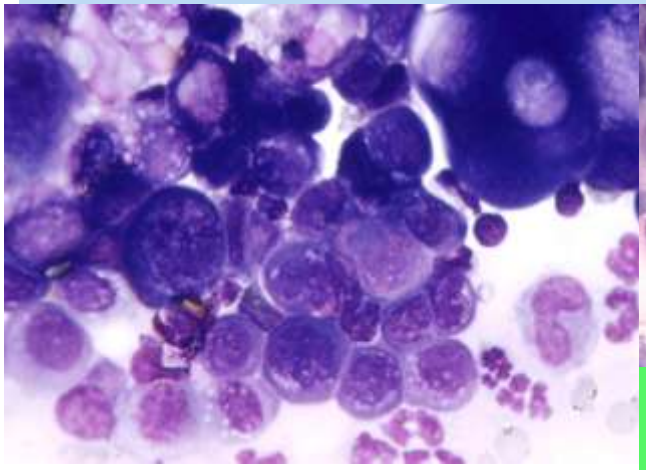
Those abnormal cells – adenocarcinoma or mesothelioma? Does ICC help (cytokeratins, vimentin, desmin)? Do you use any to positively identify mesothelial cells? Big problem in all species!

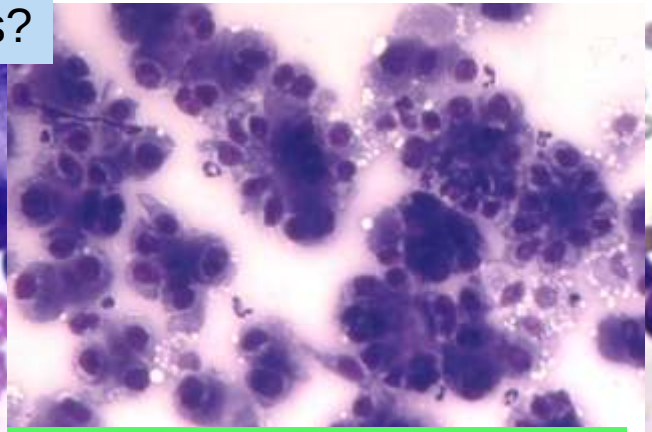


Canine pleural carcinomas – or were they mesotheliomas?

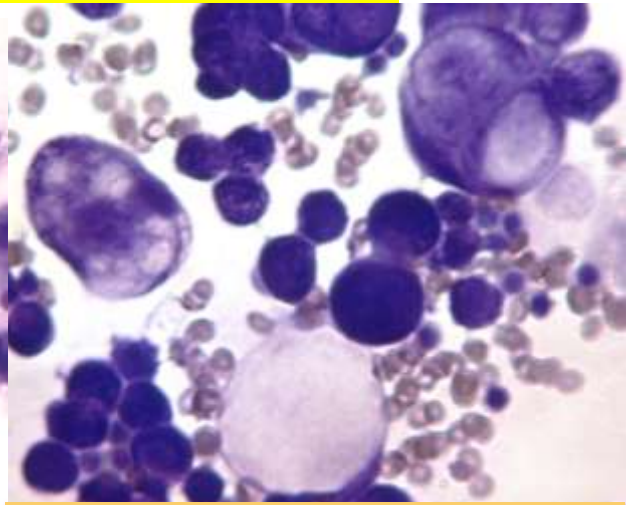


Canine mesotheliomas – or were they carcinomas?

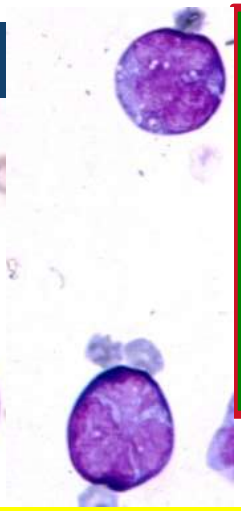




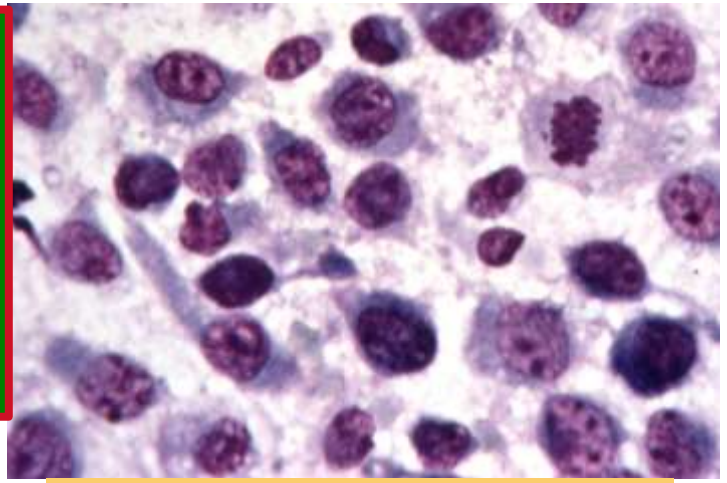
Canine pulmonary carcinoma – definite!



Canine bile duct carcinoma – definite!

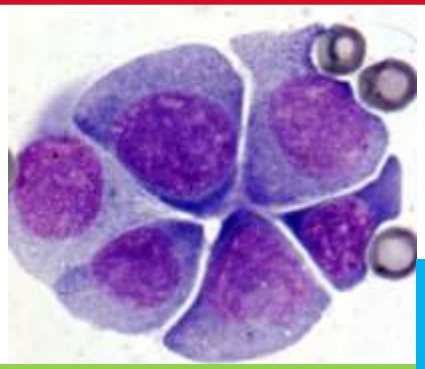


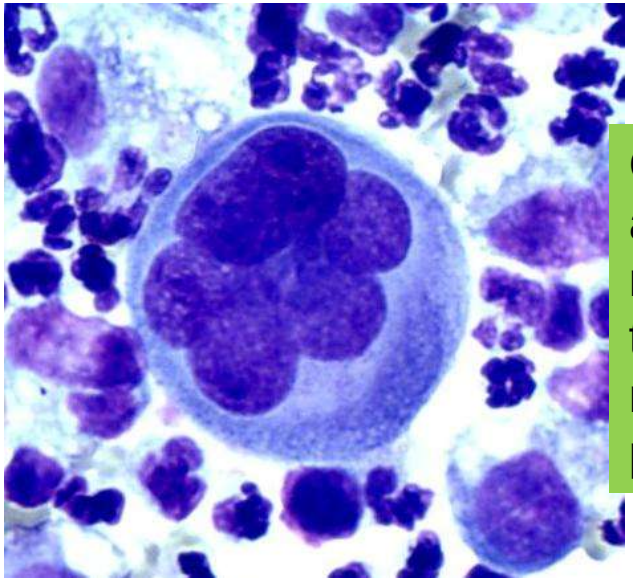
Most exfoliative neoplastic cells round up in the fluid and either remain discrete or clump



Canine pleural sarcoma – spindle!

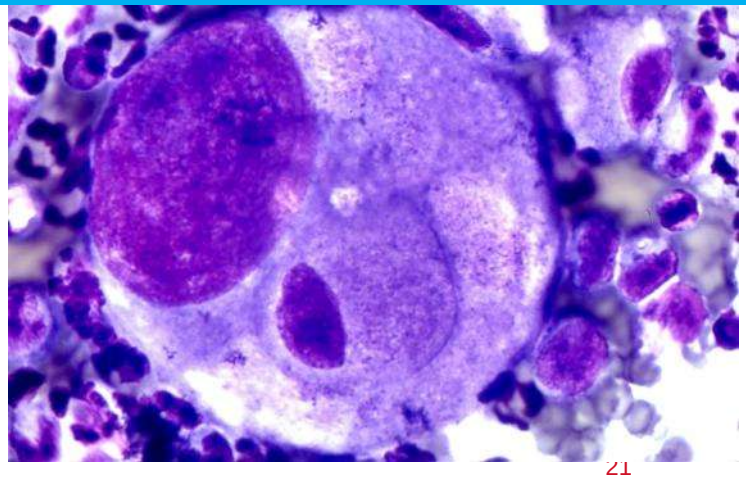
Canine pleural lymphosarcoma – those nuclei!





Canine pleural amelanotic melanosarcoma – tough unless melanophages present:

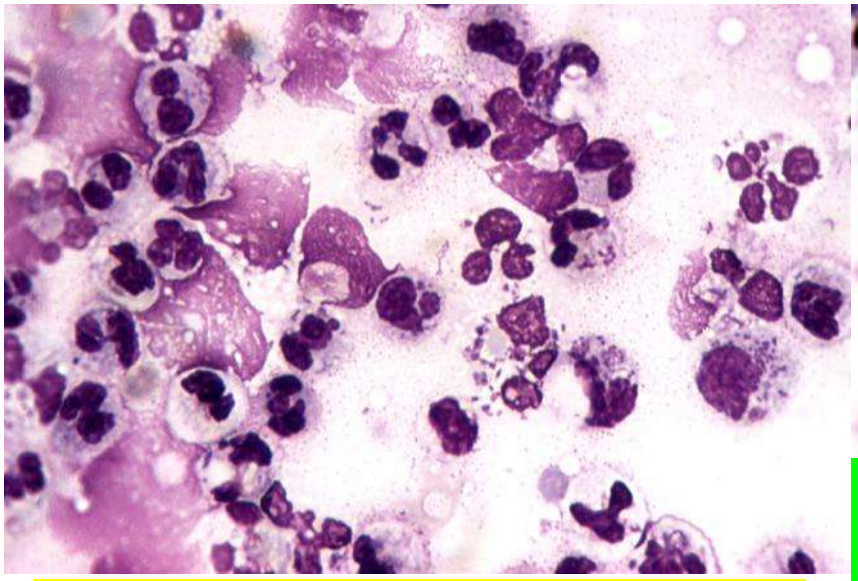
Feline abdominal adenocarcinoma – emperipolesis or phagocytosis?



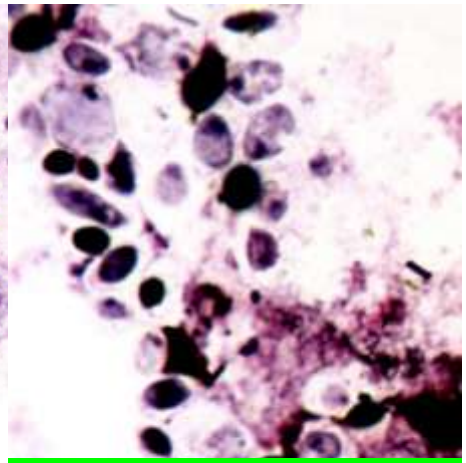
Feline pleural adenocarcinoma – inflammation!

Some reminders on septic exudates that help me

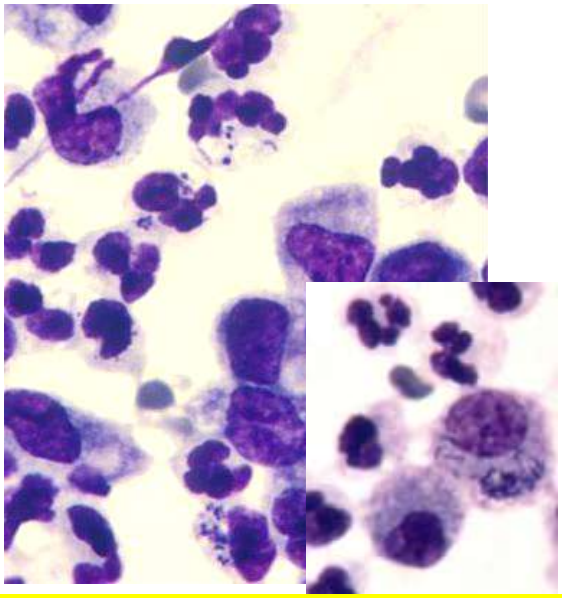
•Cell counts in septic exudates can vary markedly because of lysis of neutrophils.
•Bacteria have variable capacity to cause lysis of neutrophils (lytic/degenerate – ***what do you call them***?)
•The hallmark of a septic exudate is the presence of bacteria (beware contaminants!)
•Exudates are worthwhile culturing (at least do a Gram), even if bacteria are not obvious, unless cumulative data suggest a non-bacterial aetiology (the advantage of that direct smear!)



Feline Pyothorax – high toxicity



A reminder about gut taps!



Canine Pyothorax – low toxicity

Synovial fluid analysis

What is useful to the referring veterinarian? Is it a local problem (and what type and cause) or a systemic manifestation?

Issues:

- Great variability in reference intervals amongst joints
- Apart from sepsis rarely gives an exact diagnosis - need to combine with other tests
- Categories are still basic pathological processes: acute and chronic degeneration (**non-inflammatory** – includes joint haemorrhage), **non-septic and septic inflammation**, **neoplastic**
- Neutrophils in septic inflammation may be variably lytic depending on the toxicity of the microbe and protective functions of synovial fluid
- In SA often only enough fluid to make a smear

Common synovial fluid changes in disease

	Acute degenerative/ traumatic conditions	Low grade/Long term degenerative conditions	Primary inflammatory (non-septic)	Primary inflammatory (septic)
Volume (dependent on joint and species)	Increased	normal to increased	increased	increased
Colour (colourless or light yellow in horse)	normal to discoloured (red)	normal to discoloured (red-brown)	discoloured	discoloured
Turbidity (clear)	slight to marked	clear to slight	slight to marked	marked
Protein (<25 g/L D,C; <15 - H, <30 -B) Fibrin clot (-ve)	normal to increased -ve to +ve	normal to sl increase -ve	increased +ve	increased +ve
Viscosity (viscid)	Variable	normal to sl decrease	decreased	decreased
Mucin clot test (normal is good)	fair to poor	good to fair	fair to very poor	poor to very poor
Total nucleated cells (variable: <3000 x 10^6/L - D; <1000 - C; <500 H,B)	slight to moderate	normal to slight increase eg up to 10,000 for D, up to 5000 in H	moderate to marked increase (eg 10,000-100,000 for D; >5000 for H)	
Differential cell count (mainly mononuclear – less than 10% neutrophils)	Mainly mononuclear, erythrocytes (neutrophils increase because of bleeding)	Mainly mononuclear, macrophages (cartilage fragments may be seen), slight increase in neutrophils (usually up to 20% of total)	mainly neutrophils (30-80%); non-lytic (non-degenerate) for non-septic, usually lytic (degenerate) for septic (some protection from lysis); plus erythrocytes (via diapedesis), macrophages and lymphocytes (chronicity); (**microbes for septic – may not always be easy to see – CULTURE!**)	

A 2 year old greyhound is presented with pyrexia and swollen peripheral joints of 2 weeks duration. Joint fluid was taken from the right hock. Synovial fluid analysis:

TEST	SAMPLE	REFERENCE INTERVALS
Appearance	**markedly turbid and amber**	clear
Protein (refract.)	**42 g/l**	<25 g/l
Total nucleated cells	**87,666 x 10^6/l**	<2,900 x 10^6/l
Erythrocytes	**24,311 x 10^6/l**	none
Smear	**96% non-lytic neutrophils, 2% lymphocytes, 2% monocytes and synovial cells**	>90% lymphocytes and monocytes

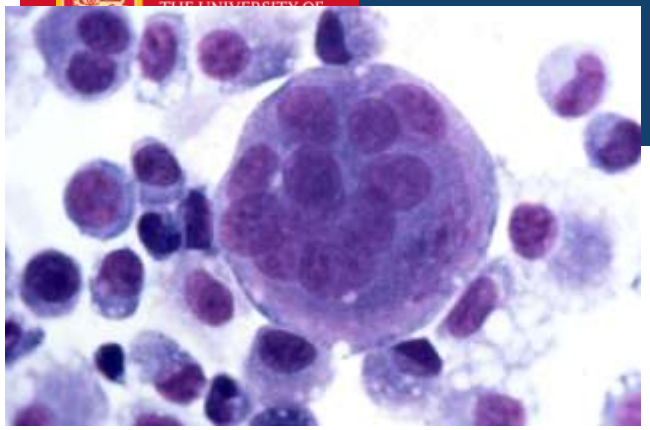
No bacteria detected on culture – what do you think about aetiology for this apparent non-septic exudate?

Four months female Thoroughbred foal with depression, swollen joints, pyrexia for over one week – septic exudate with systemic inflammatory response (left shift and toxic changes)

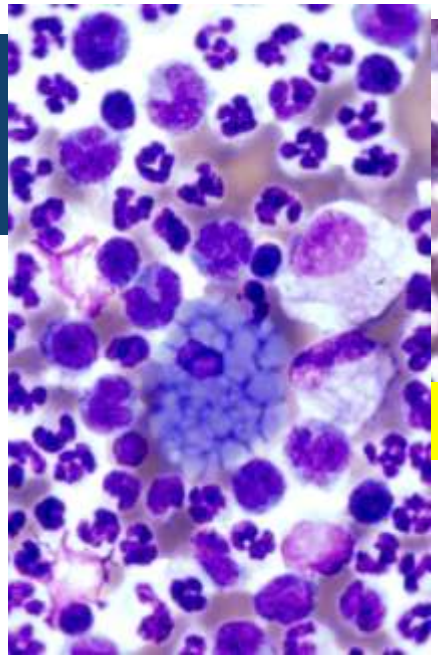
HAEMATOLOGY	SAMPLE	REFERENCE INTERVAL
Plasma appearance	Clear	Clear
PCV L/L	**0.23**	0.32-0.52
Plasma protein g/L	64	58-84
Haemoglobin g/L	**78**	110-190
Erythrocytes $x10^{12}$/L	**6.7**	8-12.5
MCV fl	**34?**	41-49
MCHC g/L	339	300-360
Leukocytes $x10^9$/L	**39**	6.0-13
Neutrophils (seg.) $x10^9$/L	**31**	2.5-7
Neutrophils (band) $x10^9$/L	**2**	0-0.2
Lymphocytes $x10^9$/L	4.5	1.6-5.4
Monocytes $x10^9$/L	**1.3**	0-0.7
Eosinophils $x10^9$/L	0.2	0.2-1
Basophils $x10^9$/L	0	0-0.4
Blood film: **vacuolation of neutrophils**, some Howell-Jolly bodies present		
Fibrinogen g/L	**10**	1-4

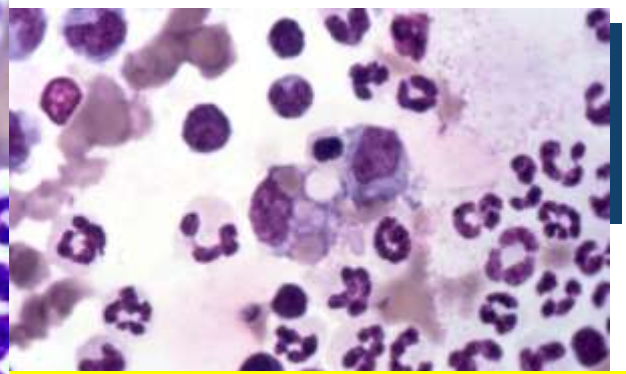
Appearance of **Joint fluid**:	slightly cloudy, yellow green (usually clear and light yellow)
Protein (g/L):	**45**(usually less than 15)
Total nucleated cells ($x10^6$/L):	**19,100 (**usually less than 500)
Erythrocytes ($x10^6$/L):	**32,000**(usually none)
Differential:	**97% (some lytic but many non-lytic) neutrophils with intracellular bacteria and 3% macrophages** (usually most cells are lymphocytes or monocytes with neutrophils being less than 10%)

***E. coli* recovered from joint fluid**

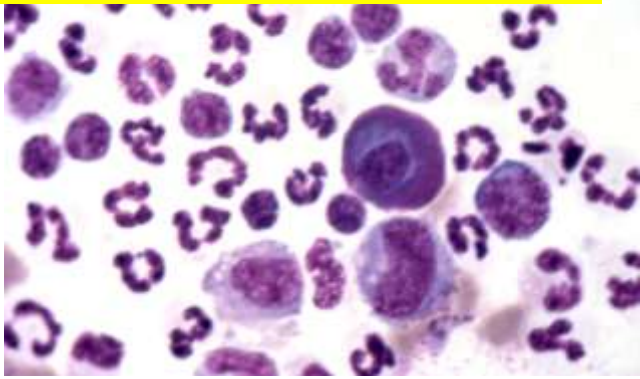


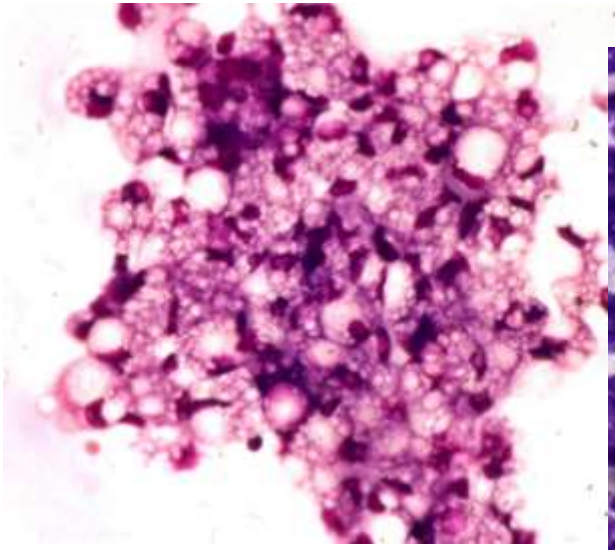
Canine chronic osteoarthropathy



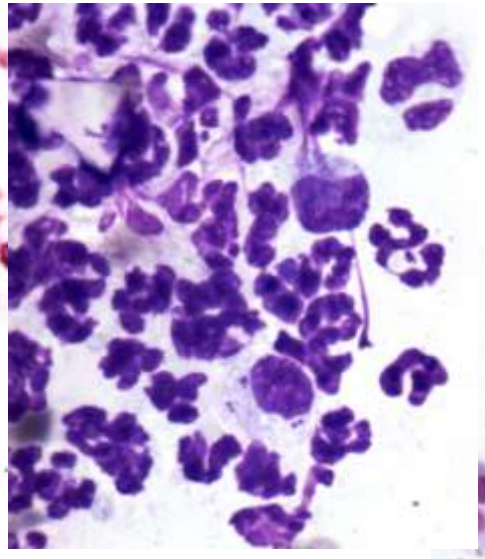


Canine non-septic arthritis





Equine non-septic athritis due to silicon injection



Canine *Salmonella meunchen* arthritis

Canine synoviosarcoma

Cerebrospinal fluid analysis

What is useful to the referring veterinarian? what type and cause of lesion? If no change do they keep looking (way forward)?

- **things I need to keep reminding myself :**
- Mainly of use in dog and cat, but reference values have been reported in horses and ruminants
- Subtle differences in reference values between CSF taken from cisterna magna (atlanto-occipital joint) and lumbo-sacral region (most ruminant samples are taken from the latter site)
- Subtle changes in total nucleated cell numbers and protein levels may have meaning (eg > 5 cells and > 0.4 g/L protein)! Analysis needs to be done quickly or else cells deteriorate and lyse (low protein contributes)
- Best changes occur for meningeal disease whilst few changes may be seen for deeper parenchymal disease
- Still looking for pathological processes (degenerative (non-inflammatory), inflammatory, neoplastic) as well as cause

CSF: colourless and clear for D, C, H, and ruminants.

Total NCC is < 5 for D, C, H (6-8 grey zone) and <6 for ruminants (7-12 grey zone)

Differentials generally 60-70% L, 20-30% large mononuclears and rare neutrophils.

Protein is less than 0.3 g/L for dog, 0.2 g/L for cat and 0.6-0.7 g/L for horse and ruminants. Most of the protein is albumin.

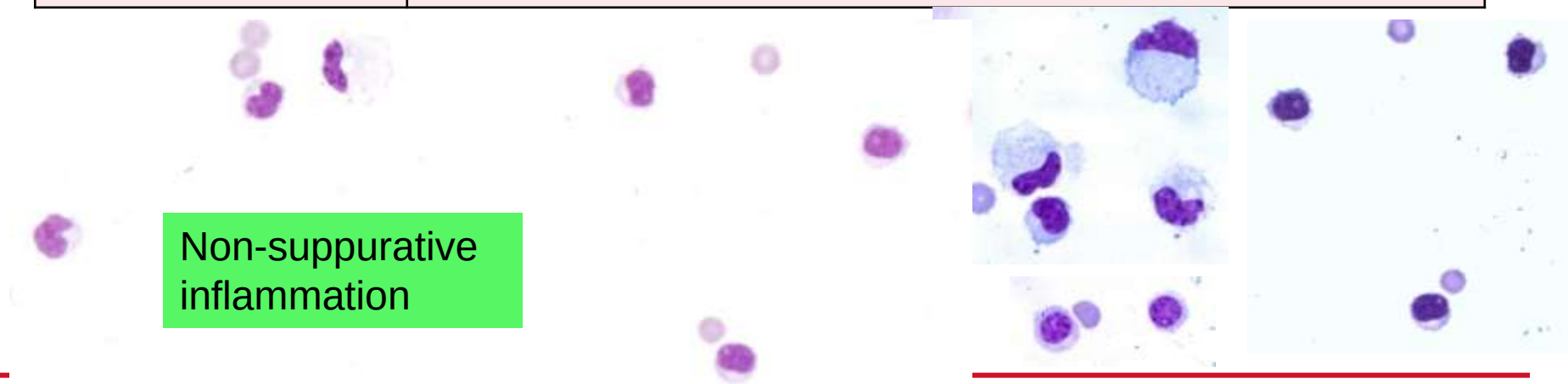
Birds and reptiles rarely have CSF taken for analysis

Values reported for ruminants are extremely variable

Six years old male cross Labrador with depression, fitting, ataxia

CEREBROSPINAL FLUID ANALYSIS:

Gross characteristics	clear and colourless (normal)
Protein	**0.54 g/L** (reference interval < 0.3 g/L)
Cells	erythrocytes **9 x 10^6/L**; nucleated cells **47 x 10^6/L** (reference interval for nucleates < 5 x 10^6/L, no erythrocytes normally seen)
Differential	65% lymphocytes, 35% monocytes and macrophages (normal - usually a mixture of mononuclear cell types)



Non-suppurative inflammation

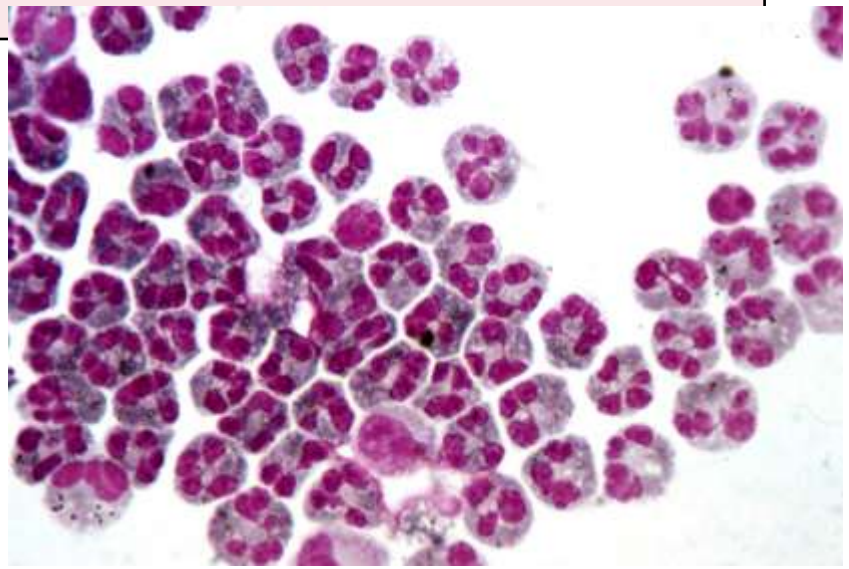
Ten month old Great Dane with a 2 day history of hindlimb weakness and ataxia

CEREBROSPINAL FLUID ANALYSIS:

Gross characteristics	**Cloudy** (abnormal) and colourless (normal)
Protein	**0.46 g/L** (reference interval < 0.3 g/L)
Cells	erythrocytes **120 x 10^6/L**; nucleated cells **800 x 10^6/L** (reference interval for nucleates < 5 x 10^6/L, no erythrocytes normally seen)
Differential	**80% eosinophils, 8% neutrophils**, 7% lymphocytes, 5% monocytes and macrophages (normal - usually a mixture of mononuclear cell types)

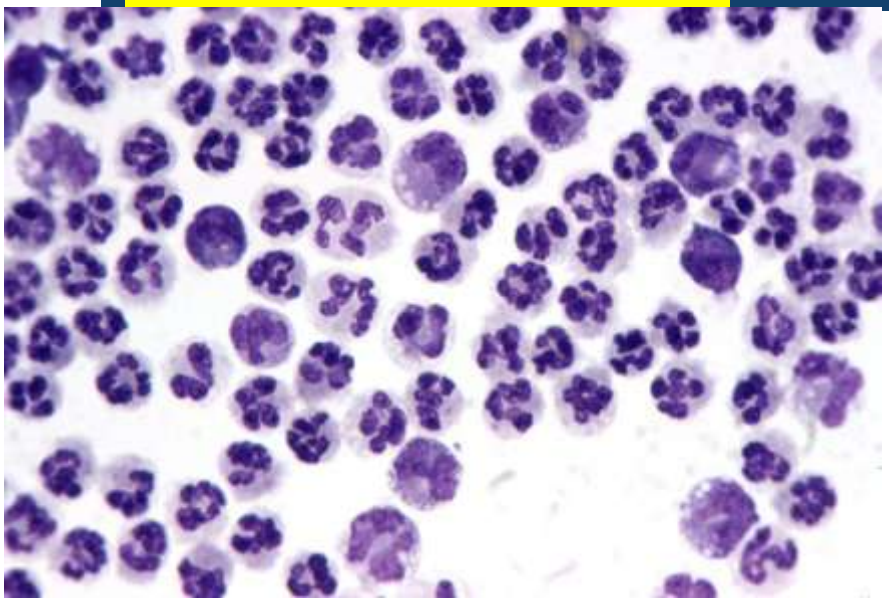
Eosinophilic meningoencephalomyelitis

- Idiopathic – immune-mediated?
- Fungal?
- Protozoal (apicomplexans)?
- Multicellular parasite?
  - *Dirofilaria immitis?*
  - *Toxocara canis?*
  - *Angiostrongylus cantonensis?*

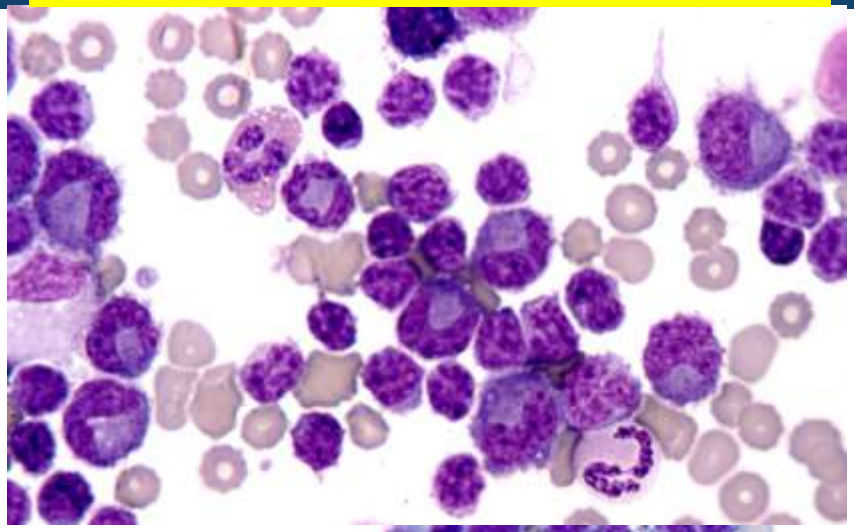


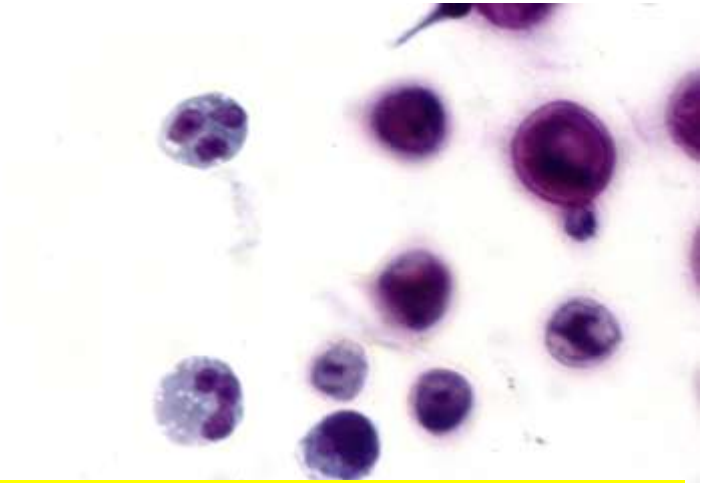
CSF in dogs

Canine suppurative (neutrophilic) meningitis

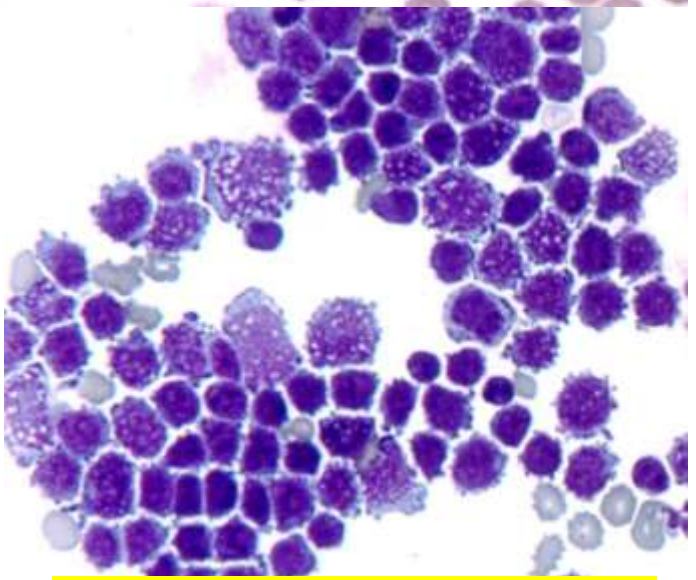


Canine granulomatous meningitis





Canine CNS cryptococcosis



Canine lymphosarcoma

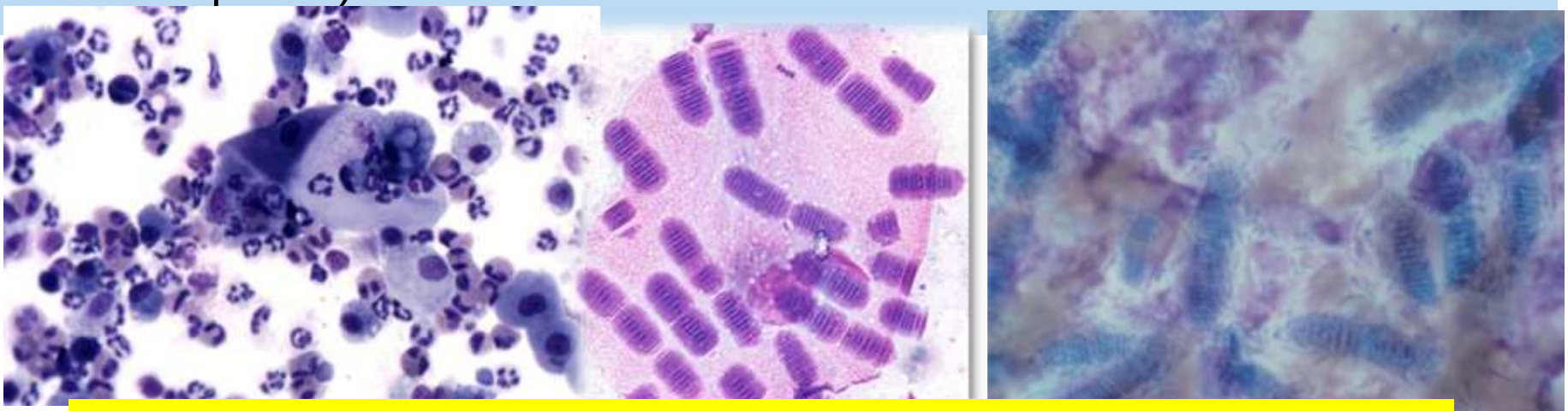
Respiratory Washes (transtracheal aspirates and bronchoalveolar lavages)

What is useful to the referring veterinarian? Is it a local problem (and what type and cause) or a systemic manifestation? How can they move forward?

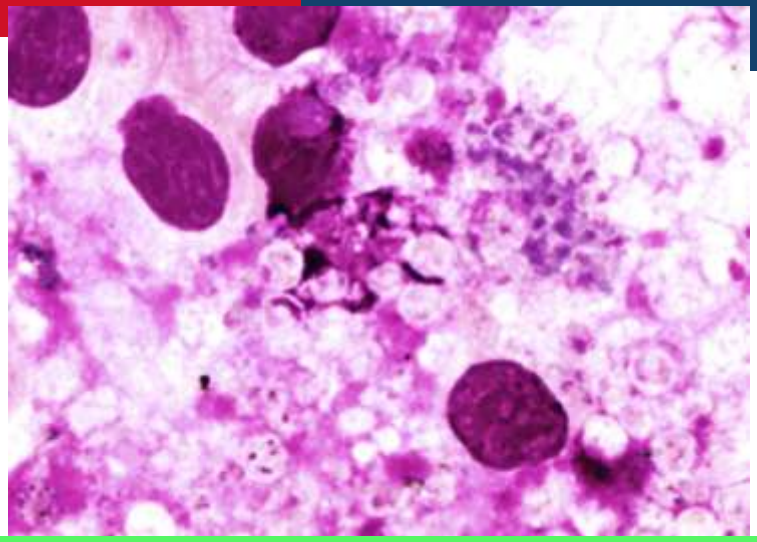
- Dog, cat, horse and ruminants (the last, mainly group problems): BAL most commonly done as it obtains a sample from deep in the pulmonary parenchyma, but sometimes not sterile because of oropharyngeal contamination. TTA ideal for culture
- Often separated into:
  1. **Chronic, non-specific**
  2. **Inflammatory (septic and non-septic; mycotic; parasitic; allergic)**
  3. **Haemorrhagic (particularly for the horse)**
  4. **Neoplastic**

Things I need to keep reminding myself:

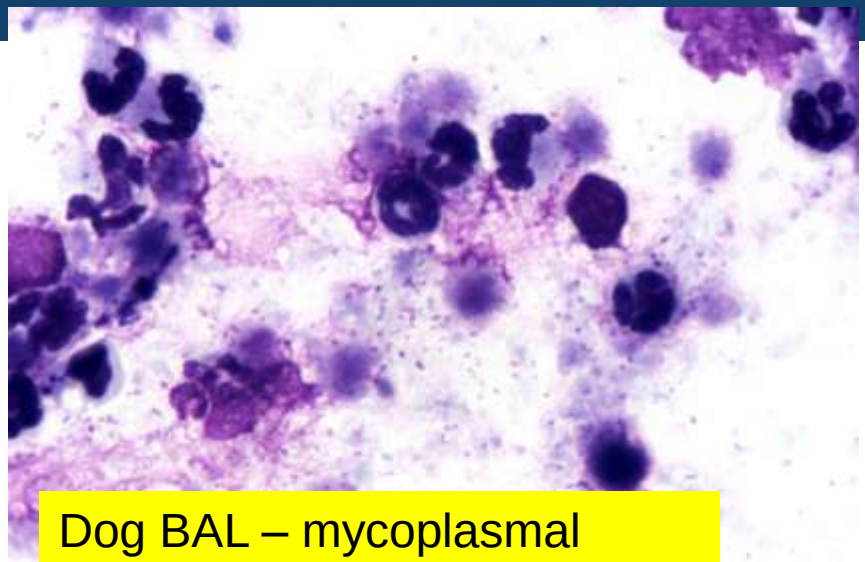
- Has the vet got a sample from deep in the lung (ie are alveolar macrophages present)?
- Has the vet given me the best stuff from the sample (if smears submitted)?
- Is the sample contaminated from upper oro-pharynx (ie are squamous epithelium and microbes present)?
- Not all pulmonary tumours exfoliate
- Eosinophils are common in ‘healthy’ cat BALs and TTAs (up to 15% - but now disputed!)



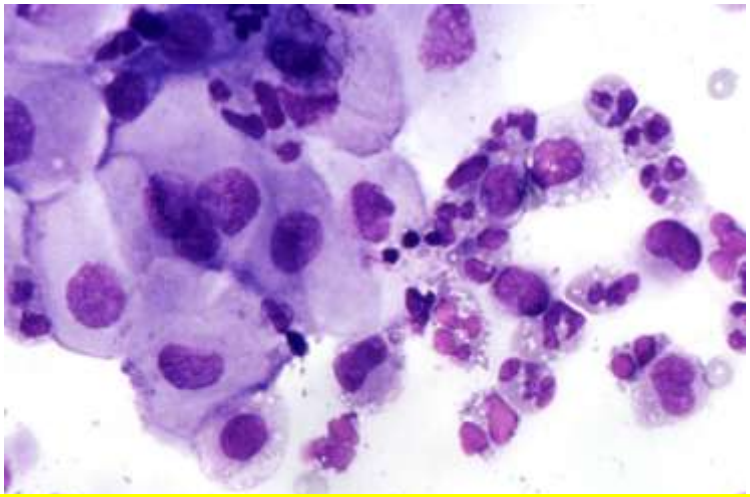
Cat BAL – oropharyngeal contamination –epithelium and *Simonsiella* sp



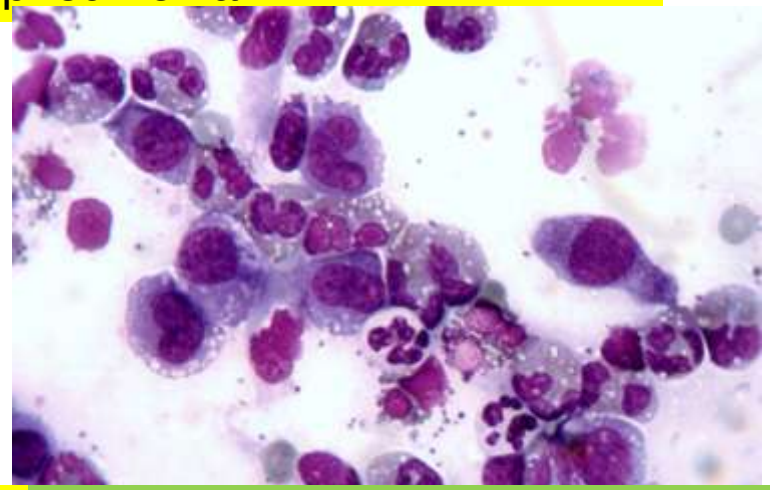
Dog BAL – *Pneumocystis carinni*



Dog BAL – mycoplasmal pneumonia



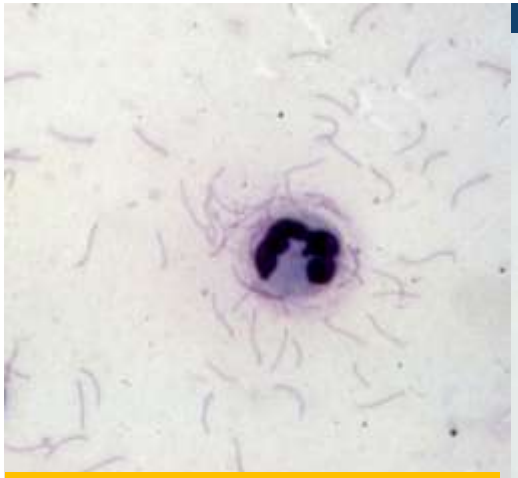
Canine TTA – eosinophilic inflammation and squamous metaplasia



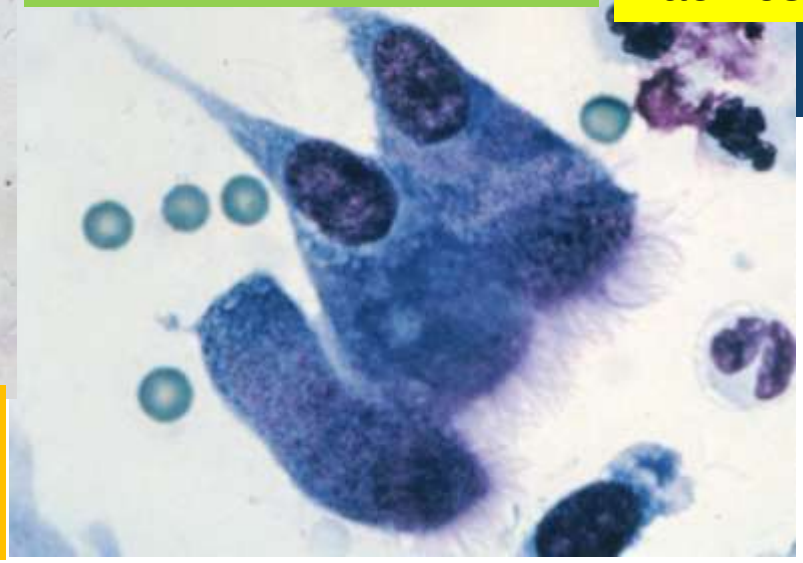
Dog TTA eosinophilic inflammation

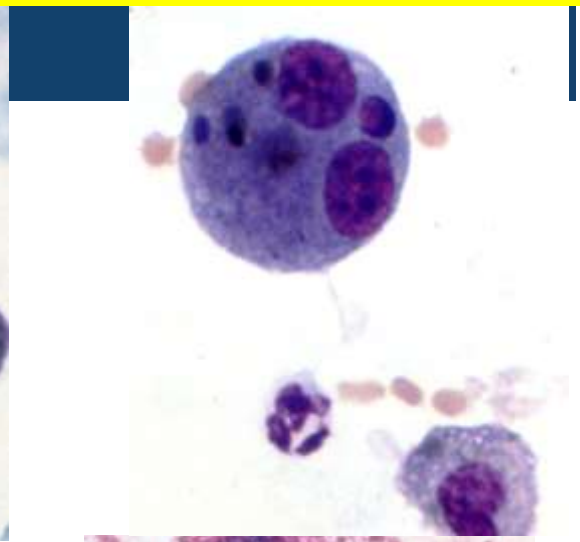
Equine ciliated respiratory epithelium

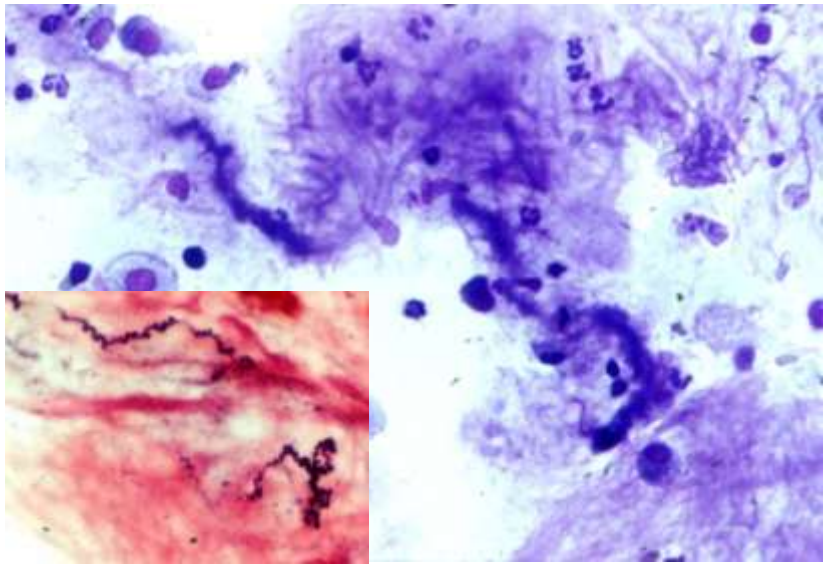
Equine alveolar macrophage with haemosiderin

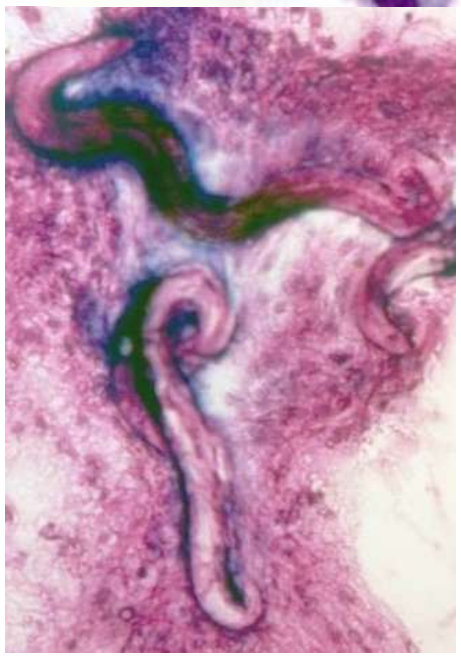


Free respiratory cell cilia









Curschmann's spiral in canine chronic bronchitis

Capillaria and lungworm larvae in a cat

Winter at the University of Sydney?

Cases for discussion

- Can work through the cases on your own, in couples or more
- Use your own style, whether it be pattern recognition and working back or sequential, problem-oriented working forward
- Discussion will be along the lines:
    1. Can a diagnosis be offered and if so what are the key pieces of supporting information?
    2. What results can't be explained by the diagnosis?
    3. If a diagnosis can't be gleaned can you think of a way forward for the referring veterinarian to get a diagnosis (optional)?

A 10 years old thoroughbred pregnant (near term) mare was presented for depression, lethargy and apparent pyrexia over the past 48 hrs. She was out in a field with other mares, which were all healthy. She had had a foal the year previously without complications. On examination, the mare was depressed and had injected mucous membranes, a capillary refill time of 4 secs (RI 1-2) a heart rate of 80 b/m (RI 25-45), a respiratory rate of 24 b/m (RI 8-16), a temperature of 37.5 degrees Centigrade (RI 36.5-38.5) and cold limbs. There was a strong suspicion of increased abdominal fluid.

TEST	SAMPLE	REFERENCE VALUES
GGT IU/L	17	<36
AST IU/L	92	<400
CK IU/L	54	<400
Lactate mmol/L	**3.3**	<2.0
Serum protein (biuret) g/L	69	60-76
Albumin (BCG) g/L	**20**	29-38
Globulins g/L	**49**	26-40
A:G ratio	**0.41**	0.62-1.46
Bilirubin total µmol/L	**142**	<50
Glucose mmol/L	6.1	4.5-6.3
Urea mmol/L	**14**	3.7-8.2
Creatinine µmol/L	**243**	87-149
Calcium mmol/L (uncorrected)	**2.53**	2.8-3.3
Inorganic phosphate mmol/L	1.72	0.80-1.77
Sodium mmol/L	133	132-150
Potassium mmol/L	3.8	2.8-5.0
Chloride mmol/L	**94**	99-110
Bicarbonate mmol/L	25	24-32
Anion gap	17	12-20

TEST	SAMPLE	REFERENCE VALUES
Plasma appearance	Clear	Clear
PCV L/L	**0.57**	0.32-0.52
Plasma protein g/L (refractometer)	70	55-75
Hb g/L	**202**	110-190
Erythrocytes $x10^{12}$/L	**13.2**	6.5-12.5
MCV fL	43	34-58
MCHC g/L	354	300-390
MCH g/L	15	12-18
Leukocytes $x10^9$/L	**4.7**	6.0-13.0
Neutrophils (seg.) $x10^9$/L	**2.1**	2.5-6.9
Neutrophils (band) $x10^9$/L	**1.1**	0-0.24
Lymphocytes $x10^9$/L	**1.3**	1.6-3.4
Monocytes $x10^9$/L	0.19	0-0.72
Eosinophils $x10^9$/L	**0.05**	0.2-0.96
Basophils $x10^9$/L	0.00	0-0.36
Platelets $x10^9$/L	229	80-300
Fibrinogen g/L	**9**	2.0-4.0
Blood film: **neutrophils appear to have moderate cytoplasmic granulation.** Everything else appears normal		

ABDOMINAL FLUID TEST	SAMPLE	REFERENCE VALUES
Appearance	Yellow and markedly turbid	Clear and light yellow
Lactate mmol/L	**10.5**	<2.0
Total protein g/L	**56**	<25
Erythrocytes x 10^6/L	None	None
Nucleated cells x 10^6/L	**107,000**	<10,000
Smear	**96% lytic (degenerate) neutrophils, 0% small lymphocytes, 4% monocytes/macrophages. Possible bacterial rods and cocci within some neutrophils**	Scattered mix of mononuclear cells and non-lytic (non-degenerate) neutrophils

Likely conclusions: the mare has a septic peritonitis which could be due to either a abdominal viscus leakage/rupture or a penetration wound (uterus or gut most likely?). The prognosis for the animal is guarded, especially in light of the blood/peritoneal lactate ratio, the overwhelming inflammatory demand and possible endotoxaemia, and the electrolyte and protein changes.

Postscript: the animal deteriorated rapidly and was destroyed at the owner's request. At **necropsy** the mare had severe peritonitis and a large cystic mass in lumen of left ventral colon coming from wall and covered in mucosa (no histopathology done on the mass). There was an area of necrosis in the pelvic flexure of the colon, which may have been due to pressure from the growing mass.

In horses, lactate levels in peritoneal fluid may be analysed in conjunction with plasma levels to assess abdominal problems. In healthy horses lactate levels in peritoneal fluid are usually lower than in plasma (most is mammalian L(+) isomer of lactate, which is the usual form measured (indicates anaerobic glycolysis); D (-) isomer lactate is produced solely by bacteria as is DL lactate, *but some gut bacteria can also produce L-Lactate* . With abdominal crises L(+) lactate levels in peritoneal fluid exceed those in plasma (especially when there is gut hypoperfusion [eg strangulation] or sepsis), and the wider the gap, the poorer the prognosis . D (-) and DL Lactate can be measured and may prove better indicators for prognosis.

Reference: Lactate Production and Measurement in Critically Ill Horses. BS Tennent-Brown. Vetlearn.com, December 2011, Compendium: Continuing Education for Veterinarians E1-E7

Possible reasons for abnormalities: the mild hyperlactataemia (probably only mammalian L lactate measured – 2-5 mmol/L is regarded as mild) suggests mild hypoxaemia and/or reduced tissue perfusion (ie increased anaerobic glycolysis). The hypoalbuminaemia in acute disease could be partly related to the fact that it is a negative acute phase protein, but is most likely due to redistribution in the abdominal fluid (it is an exudate). Perhaps the horse has been sicker than what was reported and the hypoalbuminaemia could be related to chronic illness?. The hyperglobulinaemia could be due to acute phase protein increases, but we cannot exclude gamma globulin increase with longer occurring inflammatory disease. The A:G ratio just reflects the low albumin and high globulin. The borderline hypocalcaemia (corrected value is 2.78) may be due sweating, gut malabsorption or due to a diet low in calcium. The mild azotaemia (both urea and creatinine) could be prerenal due to increased urea catabolism and decreased renal blood flow due to dehydration (maybe suggested by the extended capillary refill time; also the horse does have haemocentration). The hypochloridaemia may be partly related to redistribution due to the abdominal effusion, and since corrected chloride (Corrected Cl = (normal Na/measured Na) x measured Cl) is normal suggests that it follows the borderline low Na.

. The neutropenia with left shift might suggest overwhelming inflammatory demand. This is likely related to the septic exudates (endotoxaemia may be a part of that). The neutrophilic toxic changes fit in well with the sepsis and hyperlactataemia. The lymphocytopenia and eosinopenia are probably mainly corticosteroid induced. The hyperfibrinogenaemia is related to inflammation and degeneration. There is a septic exudate and peritoneal lactate compared to blood lactate is 3x (wider the gap, the poorer the prognosis – this is probably only L lactate measured and not D or DL isomers that are only produced by certain gut bacteria – it is likely elevated because of decreased viability of gut [ie decreased Oxygen delivery or utilisation]).

Likely conclusions: the mare has a septic peritonitis which could be due to either a abdominal viscus leakage/rupture or a penetration wound. Since the latter was not detected then the former is most likely. The viscus involved is not obvious at the moment, but both the uterus and gut should be strongly considered, as they would allow microbes to enter the abdominal cavity (the uterus is commonly sterile, but at term the cervix becomes open and may allow microbes to enter). The prognosis for the animal is guarded, especially in light of the blood/peritoneal lactate ratio, the overwhelming inflammatory demand and possible endotoxaemia, and the electrolyte and protein changes.

Plan for further investigation: indication of bacterial production of D and DL lactate in the abdominal fluid and perhaps leakage into plasma might have been more useful to determine sepsis and prognosis? It certainly would be worthwhile culturing the abdominal fluid and determining antibiotic susceptibility. You will probably also wish to do serial lactates (for prognosis) and perhaps repeat the salient haematological and biochemical tests.

(**Postscript**: the animal deteriorated rapidly and was destroyed at the owner's request. At **necropsy** the mare had severe peritonitis and a large cystic mass in lumen of left ventral colon coming from wall and covered in mucosa (no histopathology done on the mass). There was an area of necrosis in the pelvic flexure of the colon, which may have been due to pressure from the growing mass.)

A 2-years-old desexed male domestic short hair cat was presented with a suspected abdominal effusion of unknown time period. The cat was lethargic, depressed, anorexic and in poor body condition. Temperature was normal.

TEST	SAMPLE	REFERENCE VALUES
CK IU/L	180	<200
ALT IU/L	**156**	<60
ALP IU/L	**165**	<50
Total bilirubin µmol/L	**32.5**	2.5-3.5
Cholesterol mmol/L	3.7	1.9-3.9
Glucose mmol/L	**10.4**	3.6-6.6
Urea mmol/L	**4.4**	7.2-10.7
Creatinine µmol/L	**49**	90-180
Calcium mmol/L	1.9	1.7-2.6
Inorg phosphate mmol/L	1.5	1.3-2.3
Sodium mmol/L	**144**	147-156
Potassium mmol/L	4.6	4.0-4.6
Chloride mmol/L	115	115-130
Total bile acids µmol/L	**200**	<50

TEST	SAMPLE	REF VALUES
Plasma appearance	yellow	Clear
PCV L/L	0.35	.30-.45
Plasma protein g/L	59	59-78
Hb g/L	132	80-140
Erythrocytes x1012/L	8.00	6-10
MCV fl	44	40-45
MCHC g/L	377	310-350
Leukocytes x109/L	**21.3**	8-14
Neutrophils (seg.) x109/L	**19.00**	3.8-10.1
Neutrophils (bands.) x109/L	0	0-0.42
Lymphocytes x109/L	**1.1**	1.6-7.0
Monocytes x109/L	0.6	0.1-.6
Eosinophils x109/L	0.6	0.1-1.4
Basophils x109/L	0	0-.14
Platelets x109/L	Clumped	300-700
Reticulocyte % (uncorrected)	0	0-1.0
Blood film: **2-3+ poikilocytosis of erythrocytes**		

ABDOMINAL FLUID TEST	**RESULT**	**REFERENCE VALUES**
Appearance	**Yellow**	clear and colourless
Total protein g/L	22	<25
Erythrocytes x 10^6/L	None	none
Nucleated cells x 10^6/L	**1500**	<1000
Smear	**51% non-lytic (non-degenerate) neutrophils, 9% small lymphocytes, 20% eosinophils, 20% monocytes, macrophages mesothelial cells**	Scattered mix of mononuclear cells and non-lytic (non-degenerate) neutrophils

Likely conclusion: ongoing chronic liver disease giving rise to hypoproteinaemia and abdominal effusion. Possibility of corticosteroid influences. (**Postscript:** the animal was treated with fluids but failed to respond. A subsequent albumin revealed 15 g/L [low] and a poor prognosis was given. Consequently, the owner elected for euthanasia. The necropsy revealed end stage liver disease with significant fibrosis and biliary hyperplasia. The cause was not determined but could have been due to chronic toxicity. There was also extensive pancreatic fibrosis).

Possible reasons for changes: the mildly elevated ALT suggests some limited hepatocellular damage while the moderately elevated ALP suggests cholestasis (difficult to equate rise with the degree of cholestasis in the cat). An increased bilirubin (in the absence of anaemia) could either suggest hepatic or post-hepatic disease. The high bile acids suggest either hepatic disease or problems with blood or bile flow. The cause of the hyperglycaemia is unclear, but could be partly due to stress (there is neutrophilia and lymphocytopaenia)? Hyperglycaemia can occasionally occur in chronic liver disease, but often after eating. There could also be the possibility of early diabetes, but the glucose value has not yet reached renal threshold levels. The low urea could be related to the low protein or decreased liver conversion of ammonia, whilst the low creatinine could be due to poor muscle mass, decreased intake or high bilirubin influencing the assay method. The hyponatraemia (and borderline low chloride) is also difficult to explain, but could be due to dispersion in the abdominal fluid? The borderline hypoproteinaemia could be a reflection of prolonged anorexia, but could be due to decreased production (liver disease) or increased loss in gut or kidneys. The elevated MCHC could be laboratory error or due to interference from the elevated bilirubin. The neutrophilia could be due to true demand or due to stress. Poikilocytosis could be non-specific and related to chronic disease (liver disease is a common reason for significant poikilocytosis in the cat). The abdominal effusion can be classified as a modified transudate. This could be related to liver disease. The hypoproteinaemia is not severe enough to cause oedema (which usually produces a pure transudate).

Likely conclusion: indications are that there is a possibility of ongoing chronic liver disease giving rise to hypoproteinaemia and abdominal effusion.

(**Postscript:** the animal was treated with fluids but failed to respond. A subsequent albumin revealed 15 g/L [low] and a poor prognosis was given. Consequently, the owner elected for euthanasia. The necropsy revealed end stage liver disease with significant fibrosis and biliary hyperplasia. The cause was not determined but could have been due to chronic toxicity. There was also extensive pancreatic fibrosis [a contributor to the hyperglycaemia?]).

Jeremy Allen DAFWA: 30 out of 1000, 6 to 9 month old Merino wethers have died and another 20 looking weak and wobbly or recumbent. The mob has been grazing wheat stubble for the last six weeks. One jaundiced wether was submitted for PM examination. Liver was swollen and mottled

TEST	SAMPLE	REFERENCE VALUES
CK IU/L	283	<500
ALT IU/L	**37**	<30
GGT IU/L	**133**	<67
GD (GLDH) IU/L	**43**	<20
Total serum protein (biuret) g/L	**75.9**	60-75
Albumin g/L	32.0	28-34
Globulins g/L	**43.9**	30-42
A:G ratio	0.70	0.6-1.1
Haptoglobin mg/mL	**3.68**	<0.6
Total bilirubin µmol/L	**307**	<15.0
Total conjugated bilirubin µmol/L	**139**	<5.0
Cholesterol mmol/L	1.92	1.2-2.6
Beta hydroxybutyrate (BHB)	0.61	<0.7
Urea mmol/L	**47.0**	3.3-8.0
Creatinine µmol/L	**289**	50-150
Calcium mmol/L	2.43	2.2-3.0
Inorganic phosphate mmol/L	**4.11**	0.9 – 2.5
Magnesium mmol/L	1.15	0.8 - 1.44
Iron µmol/L	28.1	33-36

Possible reasons for biochemistry result changes (my words): hepatic cellular damage (GLDH and ALT elevations; elevated total bilirubin) cholestasis (GGT and marked increase in conjugated bilirubin), renal dysfunction (elevated urea and creatinine [some could be due to pre-renal factors], hyperphosphataemia). The increased total protein and globulins could be partly due to dehydration and partly due to ongoing hepatopathy (antigenic stimulation and increase in APP). The elevated haptoglobin (acute phase reactant) is most likely related to both the hepatic and renal disease.

Necropsy results (DAFWA):

Morphological Diagnosis: moderate, multifocal, subacute, cholangio-hepatopathy with acicular clefts (steroidal saponins); tubular nephrosis associated with acicular clefts

Aetiological Diagnosis: saponin-associated hepatopathy and nephropathy

Comments: the lesions in the liver and kidney are typical of Caltrop (*Tribulus terrestris*) toxicity.

Aslani, M.R., Movassaghi, A.R., Mohri, M., Pedram, M. and Abavisani, A., 2003. Experimental *Tribulus terrestris* poisoning in sheep: clinical, laboratory and pathological findings. *Veterinary Research Communications*, **27(1)**, 53–62

BIOCHEMISTRY	SAMPLE	REFERENCE INTERVAL
Urea mmol/L	**31**	3-10
Glucose mmol/L	**3.1**	3.3-6.4
Sodium mmol/L	**122**	137-150
Potassium mmol/L	**7.1**	3.3-4.8
Chloride mmol/L	**91**	105-120

HEMATOLOGY	SAMPLE	REFERENCE INTERVAL
Plasma appearance	Clear	Clear
PCV L/L	**0.35**	0.37-0.50
Plasma protein g/L	68	55-75
Hemoglobin g/L	119	100-150
Erythrocytes $x10^{12}$/L	5.9	5-7
MCV fL	**59**	60-75
MCHC g/L	340	300-360
Leukocytes $x10^{9}$/L	12.6	7-12
Neutrophils (seg.) $x10^{9}$/L	6.3	4.1-9.4
Neutrophils (band) $x10^{9}$/L	0	0-0.24
Lymphocytes $x10^{9}$/L	**4.9**	0.9-3.6
Monocytes $x10^{9}$/L	0.6	0.2-1.0
Eosinophils $x10^{9}$/L	0.8	0.14-1.2
Basophils $x10^{9}$/L	0	0-0.4
Blood film: normal		

A 7 years old female neutered corgi with anorexia and weight loss, occasional vomiting, and clinically dehydrated.

Many of the results could be consistent with hypoadrenocorticism. Vague clinical signs often accompany hypoadrenocorticism. The changes are related to variable decreases in aldosterone and/or cortisol. Consequently, the laboratory findings are variable. In this case the hyponatraemia and hyperkalaemia are highly suggestive of a lack of aldosterone. The ratio of approximately 17 to 1 is well below the 23 to 1 that some workers accept as being unequivocal evidence for hypoaldosteronism (although some think 23-18 to 1 is a grey zone). The changes are due to urinary loss of sodium (the chloride loss follows this) and urinary retention of potassium

Diagnosis and postscript: Hypoadrenocorticism (adrenal insufficiency) was confirmed by measuring baseline cortisol levels (low).

Possible general interpretation, conclusions, further investigation and implications for management: many of the results could be consistent with hypoadrenocorticism (**conclusion and diagnosis)**. Vague clinical signs often accompany hypoadrenocorticism. The changes are related to variable decreases in aldosterone and/or cortisol. Consequently, the laboratory findings are variable. In this case the hyponatraemia and hyperkalaemia are highly suggestive of a lack of aldosterone (**conclusion)**. The ratio of approximately 17 to 1 is well below the 23 to 1 that some workers accept as being unequivocal evidence for hypoaldosteronism (although some think 23-18 to 1 is a grey zone). The changes are due to urinary loss of sodium (the chloride loss follows this) and urinary retention of potassium. Some gastrointestinal disturbances may affect the Na:K ratio in a similar manner which may create difficulty in diagnosis as some hypoadrenocortical dogs may present with diarrhoea.

The azotaemia (moderately elevated urea) is consistent with hypoaldosteronism (80% of cases) and is due to pre-renal factors such as protein catabolism, hypovolaemia mainly due to electrolyte disturbances and subsequent reduced renal perfusion. Some animals may have impaired tubular concentrating ability with a specific gravity less than 1.025. This often means that primary renal failure is one of the diagnoses under consideration. We are unable to comment on this case and **further investigation** for renal disease may be warranted. The mild hypoglycaemia is common in cases of hypoadrenocorticism and is due to the lack of cortisol affecting gluconeogenesis and possibly through enhanced insulin-mediated uptake by muscle cells.

The mild anaemia (only on PCV) has not been categorised but is borderline microcytic, normochromic and possibly worse than it seems if the animal is haemoconcentrated. In hypoadrenocorticism, a mild non-regenerative anaemia can occur due to cortisol lack (**conclusion**), which is an essential hormone for erythropoiesis. The hyponatraemia may cause microcytosis, which may be the reason why only the PCV is within the anaemia range? The lymphocytosis in a sick animal, which possibly should be stressed, is unusual and a further indication that there may be a lack of cortisol release. In some cases of adrenal insufficiency eosinophilia may also occur.

All these laboratory changes are inconsistent in adrenal insufficiency (eg up to 26% of dogs with confirmed adrenal insufficiency can have normal electrolyte levels). Therefore, it is often necessary to measure baseline cortisol levels and levels after exogenous ACTH administration to confirm the diagnosis of adrenal insufficiency (**further investigation)**. Of course, if it is a primary lack of aldosterone then the values may be normal but most cases of primary adrenal insufficiency do involve abnormalities of both cortisol and aldosterone.

Diagnosis and postscript: Hypoadrenocorticism (adrenal insufficiency) was confirmed by measuring baseline cortisol levels (low). The animal was treated with corticoid replacers.

A 14 years old thoroughbred gelding was presented for lethargy, nasal discharge and intermittent anorexia over the past ten weeks. On examination, the mare appeared dehydrated, had a poor coat and significant muscle wasting. Vital signs (heart rate, respiratory rate and temperature) were within reference intervals.

TEST	SAMPLE	REFERENCE VALUES
GGT IU/L	15	<36
AST IU/L	282	<400
CK IU/L	357	<400
GD (GLDH) IU/L	3.1	0.9-4.7
Serum protein (biuret) g/L	**93**	60-76
Albumin (BCG) g/L	**28**	29-38
Globulins g/L	**65**	26-40
A:G ratio	**0.43**	0.62-1.46
Bilirubin total µmol/L	**51.8**	<50
Glucose mmol/L	**6.4**	4.5-6.3
Urea mmol/L	**12.2**	3.7-8.2
Creatinine µmol/L	**181**	87-149
Calcium mmol/L (uncorrected)	**3.39**	2.8-3.3
Inorganic phosphate mmol/L	0.8	0.80-1.77
Sodium mmol/L	**151**	132-150
Potassium mmol/L	3.6	2.8-5.0
Chloride mmol/L	**111**	99-110

TEST	SAMPLE	REFERENCE VALUES
Plasma appearance	Clear	Clear
PCV L/L	0.52	0.32-0.52
Plasma protein g/L (refractometer)	**96**	55-75
Leukocytes $x10^9$/L	**31.5**	6.0-13.0
Neutrophils (seg.) $x10^9$/L	**0.9**	2.5-6.9
Neutrophils (band) $x10^9$/L	**3.5**	0-0.24
Lymphocytes $x10^9$/L	**19.2**	1.6-3.4
Monocytes $x10^9$/L	**7.9**	0-0.72
Eosinophils $x10^9$/L	**0.00**	0.2-0.96
Basophils $x10^9$/L	0.00	0-0.36
Platelets $x10^9$/L	223	80-300
Fibrinogen g/L	**6.9**	2-4

Blood film: **Some lymphocytes appear large with prominent nucleoli and ample basophilic cytoplasm. Some large lymphocytes are difficult to distinguish from monocytes. Neutrophils have prominent cytoplasmic granulation.** Everything else appears normal

Likely conclusions: there is evidence for hypertonic dehydration (with low albumin!), inflammation and lymphoid leukaemia. Considering the commonness of lymphosarcoma in horses, this may be a case of ‘stage 5’ (leukaemic manifestation). If so then the hypercalcaemia may be a paraneoplastic phenomenon. The high globulin may well be due to production of a whole or partial gamma globulin.

Postscript: Multicentric lymphosarcoma (on necropsy).

Possible reasons for changes: dehydration and wasting might partly explain the protein changes (hyperproteinaemia due to hyperglobulinaemia [nb albumin low], hence a reduced A:G)but there could be a true increase in globulins, the hyperbilirubinaemia is probably related to anorexia, the mild hyperglycaemia may be due to corticosteroid release, the azotaemia could be due to dehydration (only mild) and increased protein catabolism (only urea), the borderline hypernatraemia and hyperchloridaemia are probably related to the dehydration. The hypercalcaemia may be related to the possible lymphoid leukaemia. The neutropenia with left shift and hyperfibrinogenaemia could be related to inflammatory demand and bone marrow disease, the toxic granulation of neutrophils may accompany the tissue damage and inflammation (metabolic reason). The large nucleolated lymphoid cells are abnormal and indicate leukaemia (the increased monocytes may be false). Absolute eosinopenia could be due to corticosteroid release.

Likely conclusions: there is evidence for hypertonic dehydration, inflammation and leukaemia. Considering the commonness of lymphosarcoma in horses, this may be a case of 'stage 5' (leukaemic manifestation). If so then the hypercalcaemia may be a paraneoplastic phenomenon (tumour production of parathormone-like protein, prostaglandins, Vitamin D-like steroid and osteoclast activating factor - hence the name *pseudohyperparathyroidism.* Stimulation of release of parathyroid hormone because of paraproteins binding to ionised calcium may contribute to the hypercalcaemia. The high globulin may well be due to production of a whole or partial gamma globulin.

Further testing: check for lymphoid masses in thorax, abdomen and enlargement of peripheral lymph nodes. Check for secondary infections in case the inflammatory changes are not just due to tumour/tissue necrosis. Further calcium monitoring (ionised calcium?) and SPE to look at reason for elevated globulins (monoclonal or biclonal gammopathy?). Monitor all blood values and perhaps take bone marrow sample to assess extent of myelophthisis?

Postscript: Multicentric lymphosarcoma (on necropsy).

In horses, lymphosarcoma has variable cell morphology and the multicentric form is commonly B cell in origin (although they may be associated with many mature T lymphocytes). Subcutaneous and alimentary forms are also usually composed of B lymphocytes, whilst the mediastinal form is composed of neoplastic T lymphocytes. Lymphocytosis and leukemia are uncommon, but a monoclonal gammopathy has been recorded in the multicentric form. Anaemia (due to either immune-mediated destruction or chronic disease) is often the most common laboratory finding and may be the presenting complaint.