The Final Session

- Urinalysis : 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?
- Do I have time for anything else?

Australian Animal Pathology Standards Program (AAPSP) 2013 Roadshow

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

Urinalysis

- The vet realises that this can detect **systemic**, **renal** and **lower urogenital disease;** and often includes it in a general workup of a case or an indication of response to therapy
  - **BUT** they often forget that the method of collection can affect results (gross, biochemical and microscopic) and sometimes don't tell you!
  - The veterinary practitioner sometimes struggles with sediment identification or doesn't bother doing it. An opening for the clinical pathologist!

What can we provide?

Reminding the vet how the components of urinalysis (namely, gross characteristics, solute concentration, chemical analysis and sediment examination) are inter-dependent (eg cloudiness and urinary sediment; Sp Gr and proteinuria, RBC,WBC; red urine, positive blood strip and haematuria)

- Identifying important structures down the microscope (ie distinguishing between and determining significance of, elements of sediment)
- A way forward (ie further investigation after finding abnormality)

What do I need to keep reminding myself?

- Normal gross characteristics do not exclude abnormal urinalysis findings! AND
  - **Colour can vary markedly (**normally colourless to yellow to light amber depending on concentration). That horse's urine turns brown on standing!
  - **Turbidity can be due to anything (** may be due to crystals, mucin, cells. May develop with bacterial growth)
  - **Odour may mislead me** as volatile fatty acids are stronger in the male and in concentrated urines and need to be distinguished from ketone bodies and ammonia

Things I need to remember about the use of the refractometer for USG

- USG is inversely proportional to volume except in cases of renal tubular function disturbance and advanced diabetes mellitus
- USG is enhanced by protein and glucose (commonly 1-4 units)
- Adequate concentrating ability is >1.030-35 (dog), >1.035-40 (cat), and >1.025 (horse and cow). Is the difference between dog and cat partly because some veterinary refractometers have different scales for USG for dog and cat? **Your thoughts? What is it based on?**

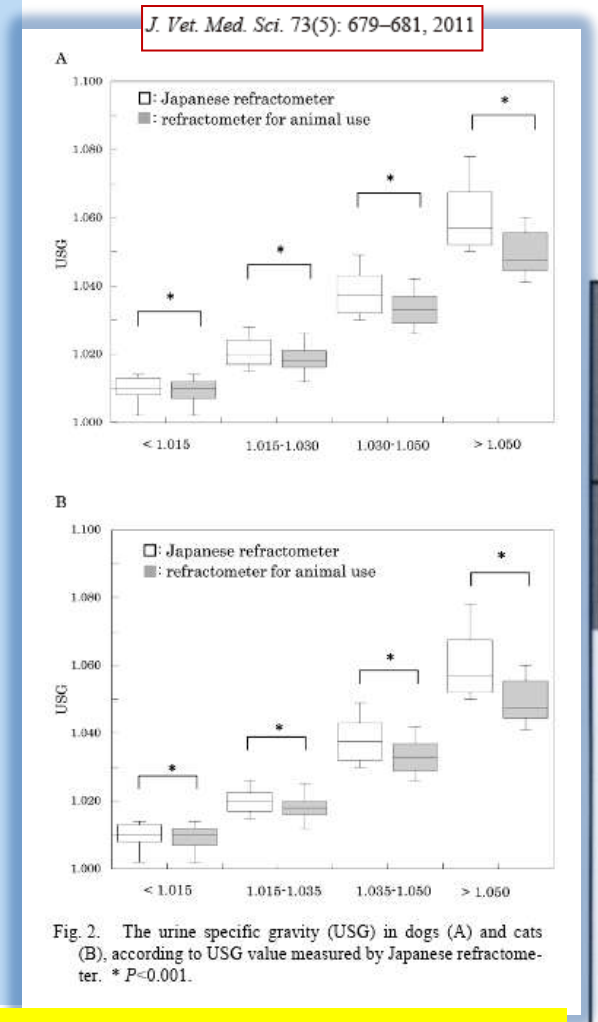
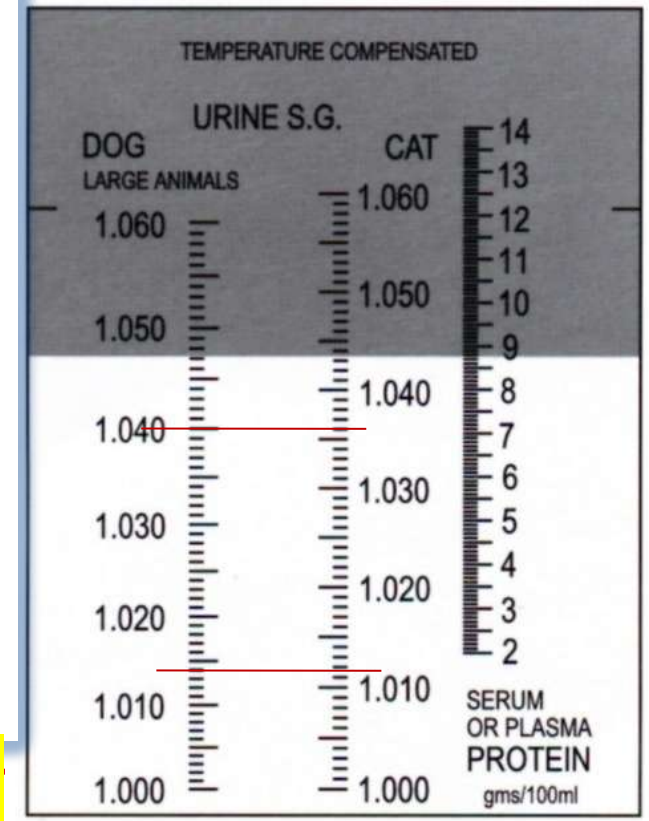


Fig. 2. The urine specific gravity (USG) in dogs (A) and cats (B), according to USG value measured by Japanese refractometer. * $P<0.001$.



Rubini ME, **Wolf AV**. **Refractometric determination** of **total solids** and **water** of **serum** and **urine**. **J Biol Chem 1957**;**225**:**869-876**

About the dipstix for urinalysis?

- Remembering that the dipstix is only useful for protein, glucose, ketones, bilirubin, pH and blood for companion animals.
- Trying to remember all the reasons for false positives and false negatives eg protein strip can be affected by pH (especially alkaline)!
- Easy to remember that the bilirubin strip is hopeless if the urine is pigmented!
- Reminding myself that alterations may be **transitory (**eg proteinuria due to fever or exercise) but I am more concerned about the **persistent alterations** (always pathological – eg glomerular/tubular disease causing proteinuria)

Anything else ?

What do I need to remind myself about urinary sediment?

- Semi-quantitative or direct counting of cells can be done, but for them to be useful for diagnosis, they have to be related to USG or if the urine has been concentrated
- I wont be able to recognise everything in a wet prep - and if I have a problem, then I need to move to dry preparations (stain with rapid Romanowsky type stain and examine with oil objective)
- I need to recognise all the elements of urinary sediment and relate them to potential disease issues
  - elements, apart from the artefacts and contaminants, reflect either **normality** or a **pathological process** affecting the body or urogenital tract.
  - In interpretation think pathological process then cause

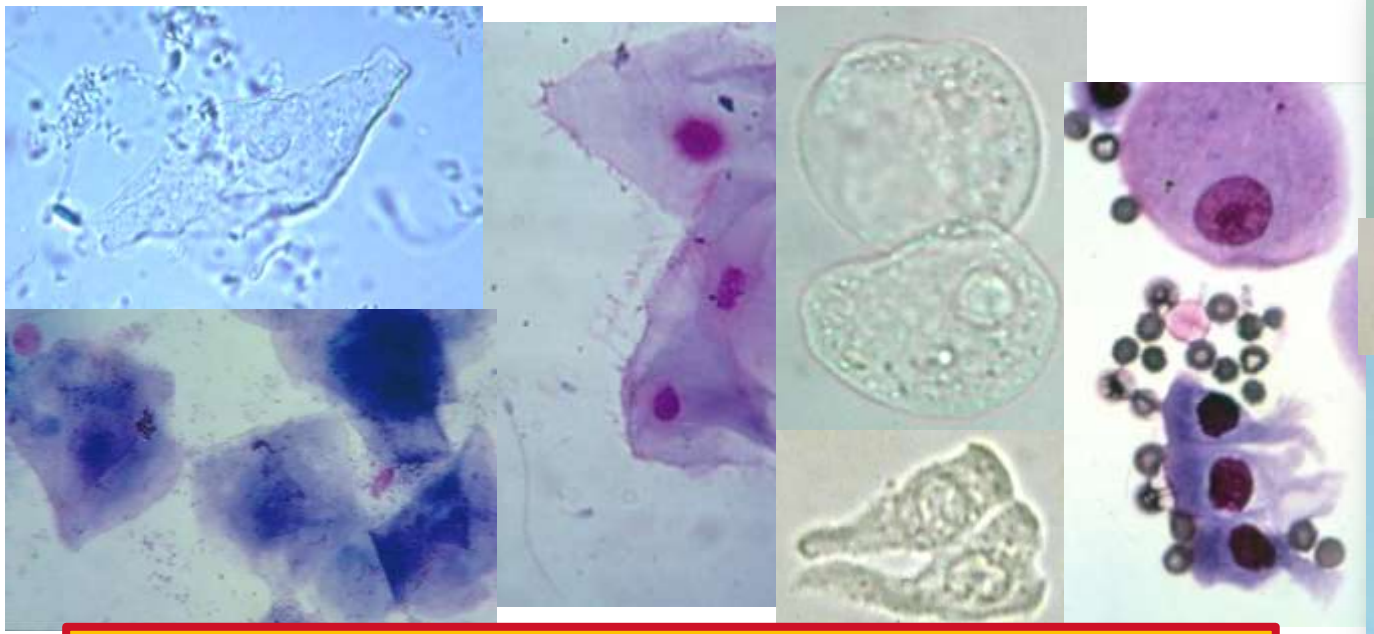
How do I approach urinary sediment?

- **I simplistically divide elements into 3 groups:**
  - Group I includes those items that are normally present in urine and which increased levels usually have no significance *unless* indicated by other information
  - Group II includes those elements that are commonly present in urine in low numbers, and which increased levels often indicate disease.
  - Group III includes those items not normally found in urine and whose presence usually indicates disease.

Groups 1 and II structures are influenced by mode of collection!

Group I includes those items that are normally present in urine and which increased levels usually have no significance *unless* indicated by other information

- **Epithelial cells** – mainly squamous (usually contaminants), transitional and caudate
- **Crystals** - Struvite, Ca oxalate (dihydrate), urates, silicates, carbonates (*increased numbers may or may not be associated with urolithiasis*). Amorphous phosphates rarely of importance
- **Miscellaneous -** Fat, mucin, sperm and contaminants

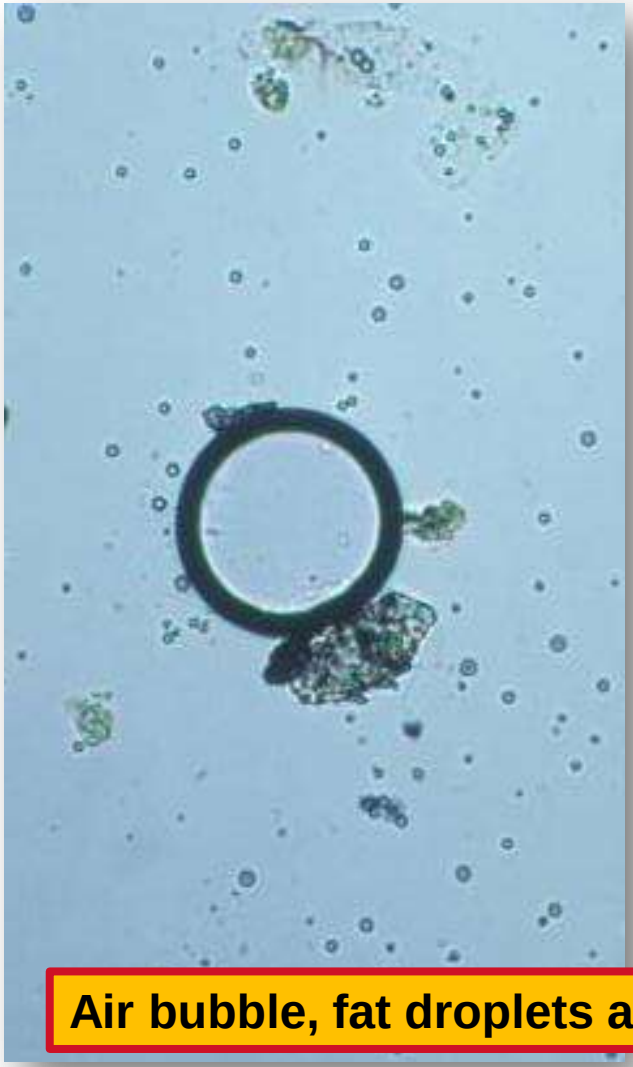


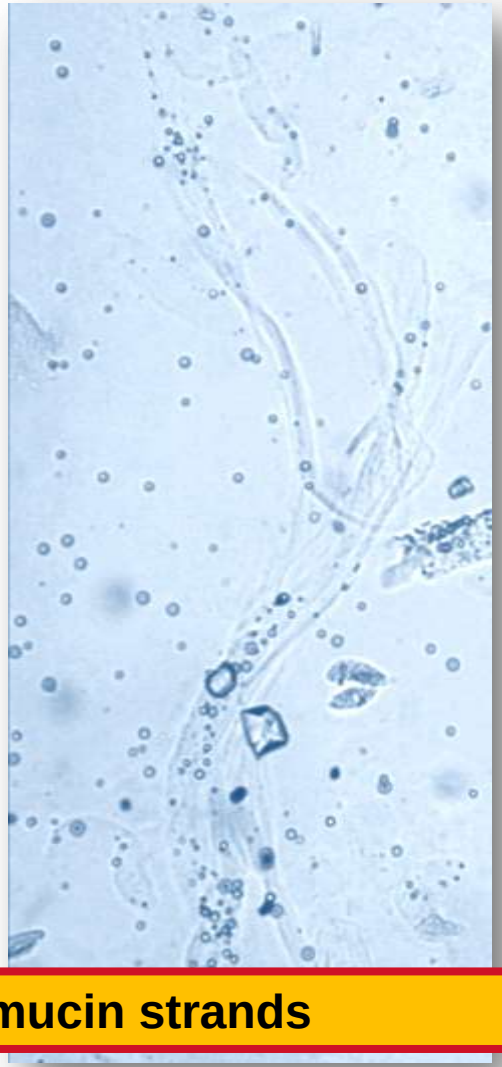
Squames, transitional and caudate epithelium

Crystals

Artefacts, contaminants and others

Alternaria mould spore contaminant

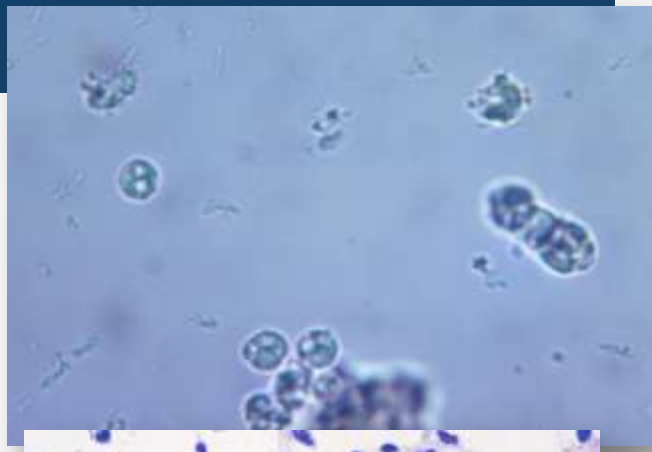


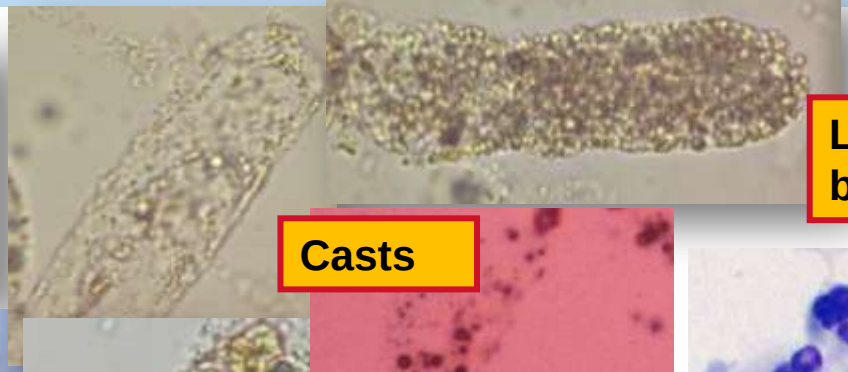


Air bubble, fat droplets and mucin strands

Group II includes those elements that are commonly present in urine in low numbers, and which increased levels often indicate disease.

- **Cells –** erythrocytes and leukocytes
- **Bacteria**
- **Casts –** hyaline, granular, fatty
- **Crystals** - bilirubin

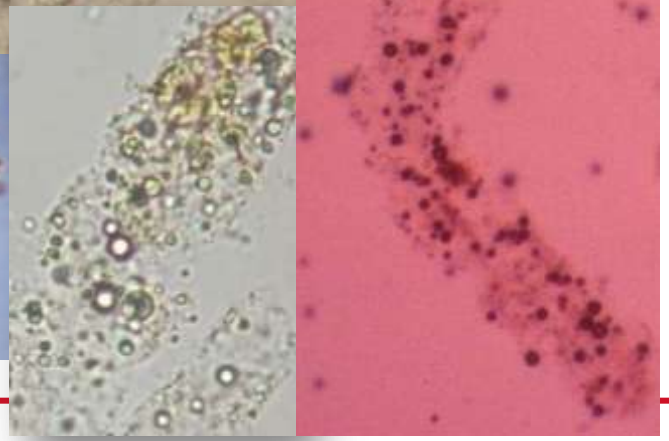


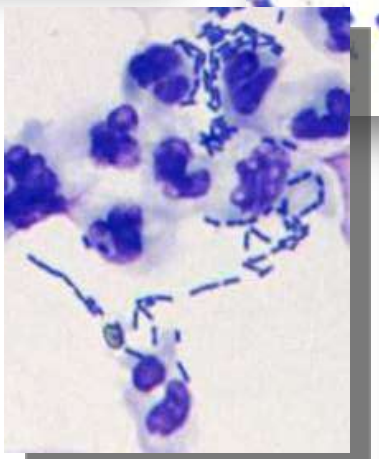


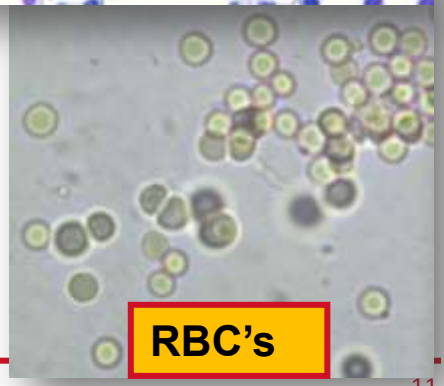
Leukocytes and bacteria

Bilirubin

Casts







RBC's

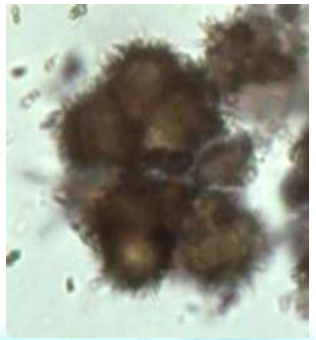
Group III includes those items not normally found in urine and whose presence usually indicates disease.

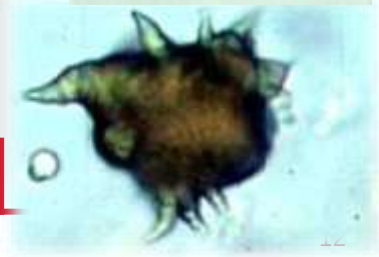
- **Cells –** renal epithelium in clusters, neoplastic cells
  - *Bacteria and fungi* on a cystocentesis sample
- **Casts** – waxy casts; casts with embedded cells
- **Crystals** – ammonium biurate, tyrosine, cystine, sulphonamide crystals

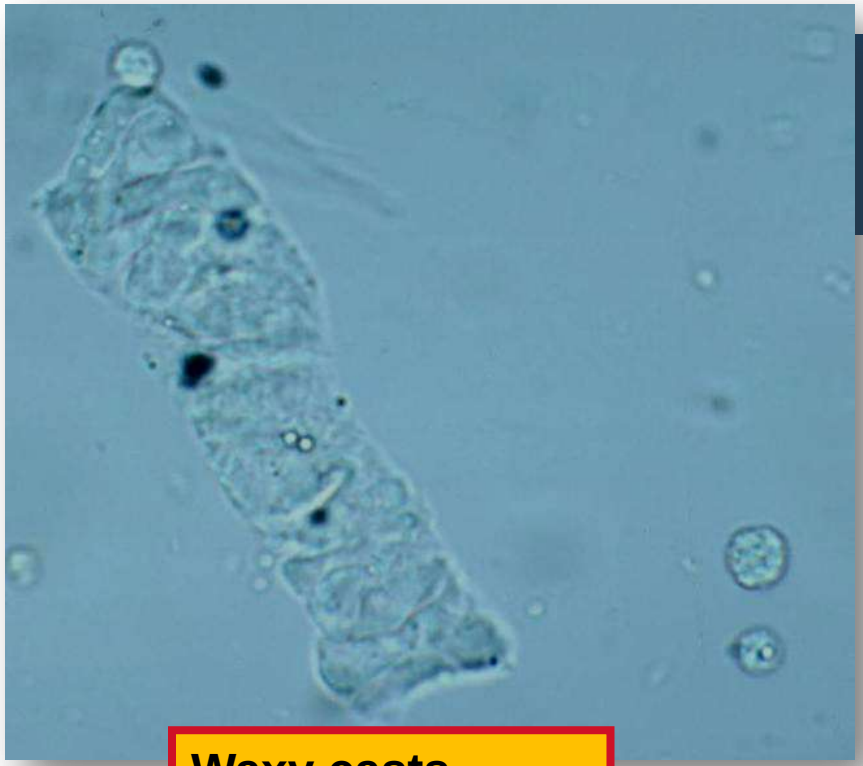
Calcium oxalate (monohydrate) crystals

Cystine crystals – canine urine

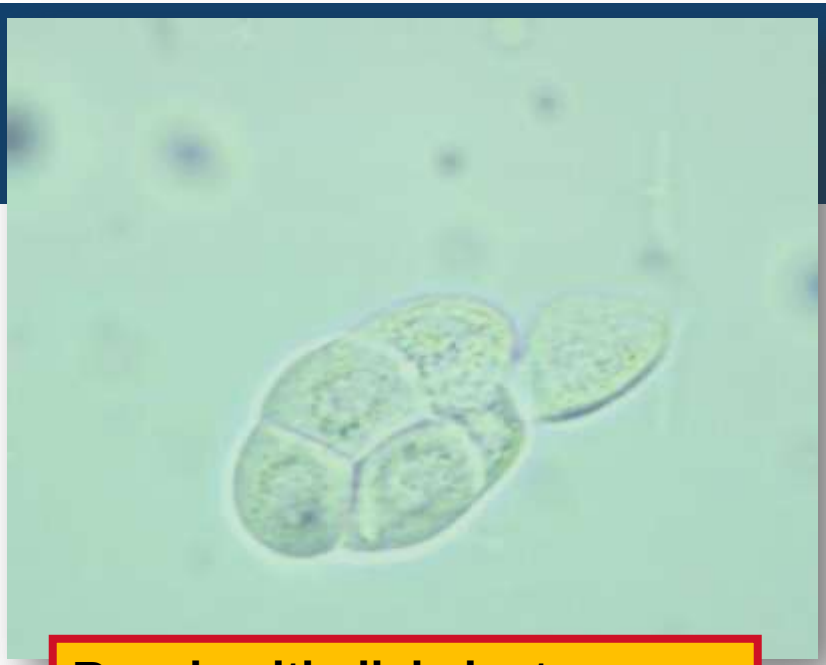
Ammonium biurate crystals



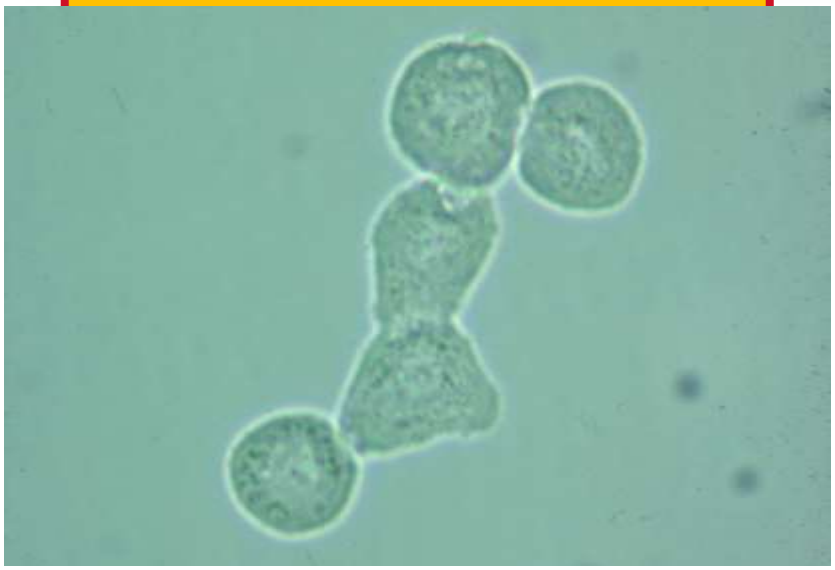


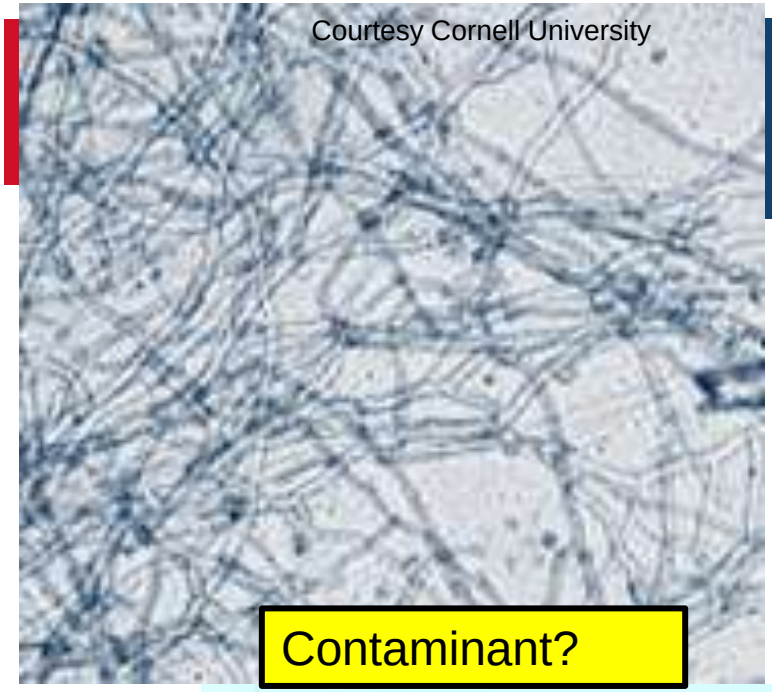


Waxy casts



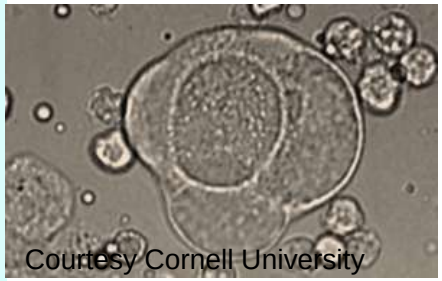
Renal epithelial cluster





Contaminant?

Fungal hyphae and eosinophils – host response evident!



Transitional cell carcinoma – not so easy to detect on wet prep!

What do I find are the major issues confronting me over interpretation?

- Neoplastic cells not so easy to detect on wet preps
- How to interpret haematuria?
- How to assess the importance of the presence of living agents, particularly bacteria – leukocytes useful but not always seen!
- What agents of disease predispose to cystitis and bacterial infection (useful for the vet)?
- How well can we assess renal disease/dysfunction through urinalysis?

Why does haematuria pose diagnostic problems?

- Haematuria can only be detected by sediment examination due to blood strip limitation
- Erythrocytes may lyse in urine that experiences aging, hyposthenuria and/or extremes of pH
- Haematuria is a vascular disturbance and can be due to a primary haemostatic disorder, but may be related to trauma through neoplasia, calculi or even severe infection

Eight YO female German Shepherd with suspected haematuria

- (Voided) cloudy, dark amber
- Sp. Gr. 1.056
- Protein **2+**
- pH 5.5
- Glucose -ve
- Ketones -ve
- Blood **4+**
- Bilirubin -ve

- Sediment: Occasional squamous cell and bacteria, **100-150 RBCs and 7 leukocytes per HPF**

Bladder haematuria related to struvite calculi

pH and Crystal formation

Crystal	Acid urine (pH<7)	Neutral urine (pH7-7.5)	Alkaline urine (pH>7.5)
Magnesium ammonium phosphate (MAP; other names are struvite and 'triple phosphate')	No	Yes	Yes
Amorphous phosphates and calcium carbonate	No	No	Yes
Calcium oxalate monohydrate/dihydrate	Yes	Yes	Rarely
Cystine and ammonium urate (biurate)	Yes	Yes	No
Uric acid/amorphous urates, tyrosine/leucine and sulphonamides	Yes	No	No
Bilirubin (non pH dependent)	Yes	Yes	Yes

Seven YO female German Shepherd with suspected haematuria

- (catheterised) cloudy, dark amber
- Sp. Gr. 1.039
- Protein **2+**
- pH 8.0
- Glucose -ve
- Ketones -ve
- Blood **3+**
- Bilirubin trace

- Sediment: many caudate and transitional epithelial cells, **80-150 RBCs and 30-50 leukocytes per HPF**, 1granular cast per LPF, **abundant bacteria**, struvite crystals

Secondary cystitis and haematuria due to calculi

The interpretation of quantitative urine culture for the dog and cat - growth is only significant if you are happy with the quality of the sample that was sent! Leukocytes help, but not always present!

	Contaminant	Suspicious	Significant
1. Dog			
Voided urine	<10,000*	10,000-90,000	>100,000
Catheterisation	<1000	<1000-10,000	>10,000
Cystocentesis	<100	100-1000	>1000
2. Cat			
Voided urine	<1000	1000-10,000	>10,000
Catheterisation	<100	100-1000	>1000
Cystocentesis	<100	100-1000	>1000

**Colony forming units per millilitre (CFUs/ml)*

Adapted from Cowell RL, *Veterinary Clinical Pathology Secrets*, Elsevier Mosby St Louis, Missouri 2004. P 163

Nine YO female collie with PD/PU. Now collapsed and vomiting

- (Catheterised) cloudy, yellow
- Sp. Gr. 1.051
- Protein trace
- pH 8.0
- Glucose **2%**
- Ketones **2+**
- Blood -ve
- Bilirubin trace

- Sediment: abundant transitional epithelial cells, **6-7 RBCs per HPF, 10-15 hyaline and granular casts per LPF**

Diabetic ketoacidosis with secondary mild nephrosis

Suspected chronic renal failure in a 10 yo male cocker spaniel

- (Voided) clear, light yellow
- Sp. Gr. 1.013
- Protein **1+**
- pH 6.5
- Glucose -ve
- Ketones -ve
- Blood -ve
- Bilirubin **1+**

- Sediment: occasional transitional epithelial cell, 1-2 leukocytes per HPF, **1-2 waxy casts per LPF**

Chronic renal failure

11YO Hereford X cow with haematuria, anorexia and significant dehydration

- (voided) cloudy, red
- Sp. Gr. 1.017
- Protein **4+**
- pH 7.0
- Glucose **2+**
- Ketones **1+**
- Blood **4+**
- Bilirubin -ve

- Sediment: occasional squamous and transitional epithelial cells, **90-200 RBCs and 3-6 leukocytes per HPF, 1-2 granular cast per LPF**

- **Perirenal haemorrhage and nephrosis**

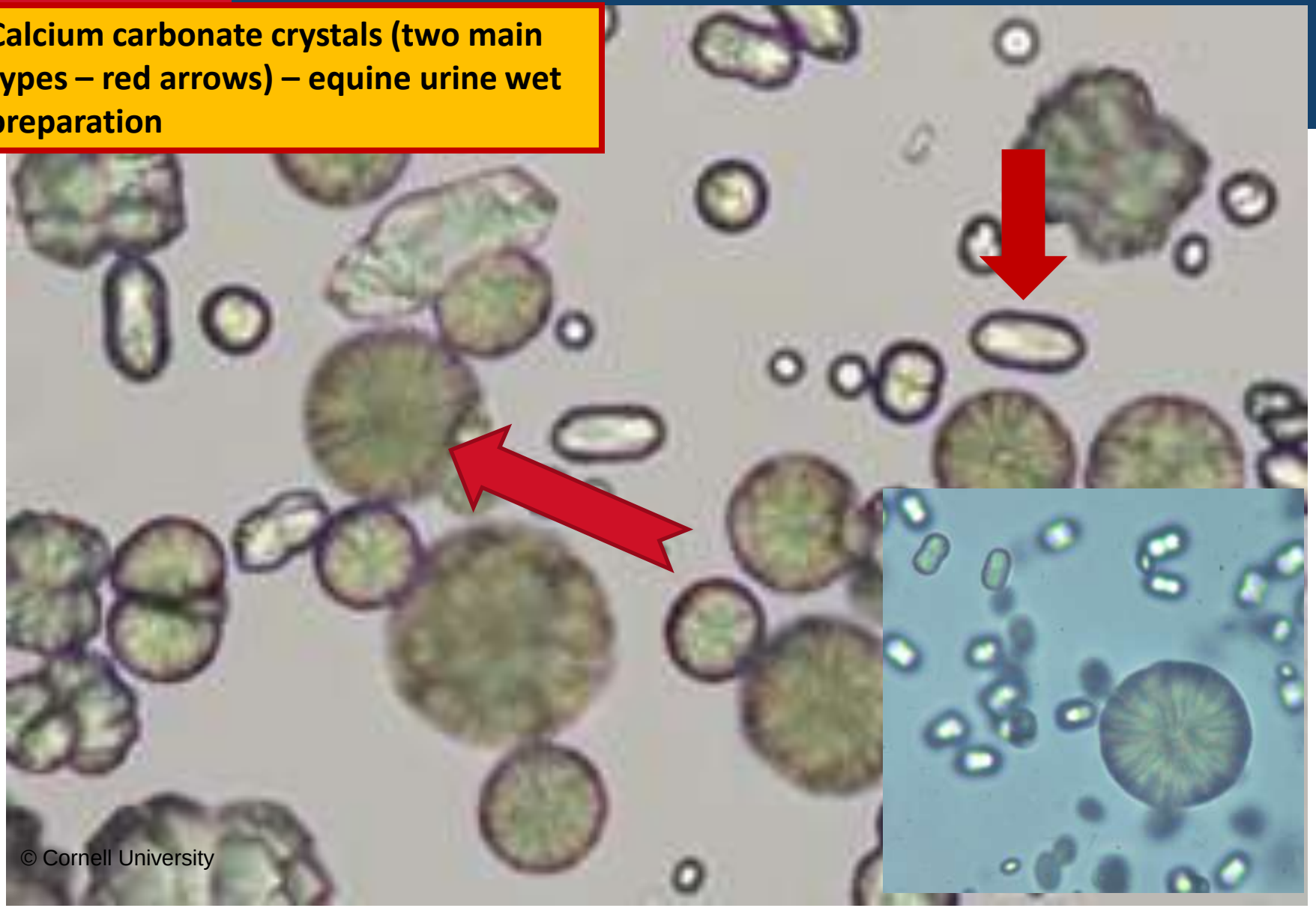
Six YO Mare with stiffness, muscular pain and oliguria

- (catheterised) cloudy, dark brown
- Sp. Gr. 1.019
- Protein **2+**
- pH 8.0
- Glucose -ve
- Ketones -ve
- Blood **4+**
- Bilirubin -ve

- Sediment: occasional caudate and transitional epithelial cells, 0-1 RBCs and 1-3 leukocytes per HPF, **2-3 granular cast per LPF**, numerous calcium carbonate crystals

Myonecrosis and nephrosis

Calcium carbonate crystals (two main types – red arrows) – equine urine wet preparation



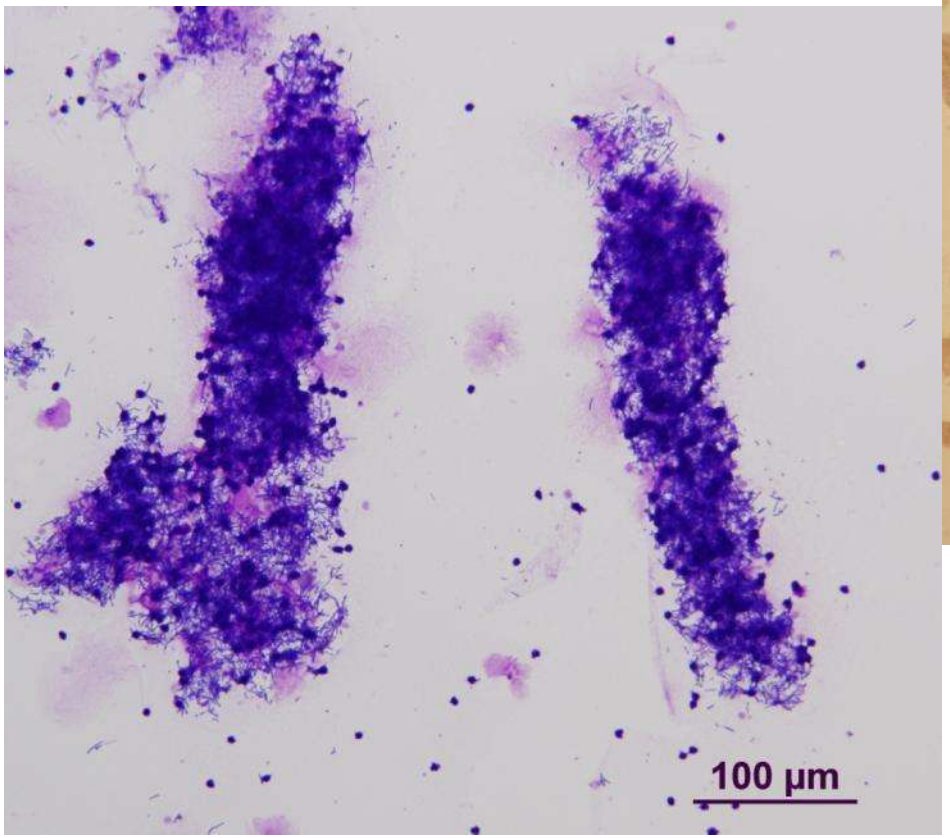
Weight loss and generalised oedema in 1 yo female DSH cat

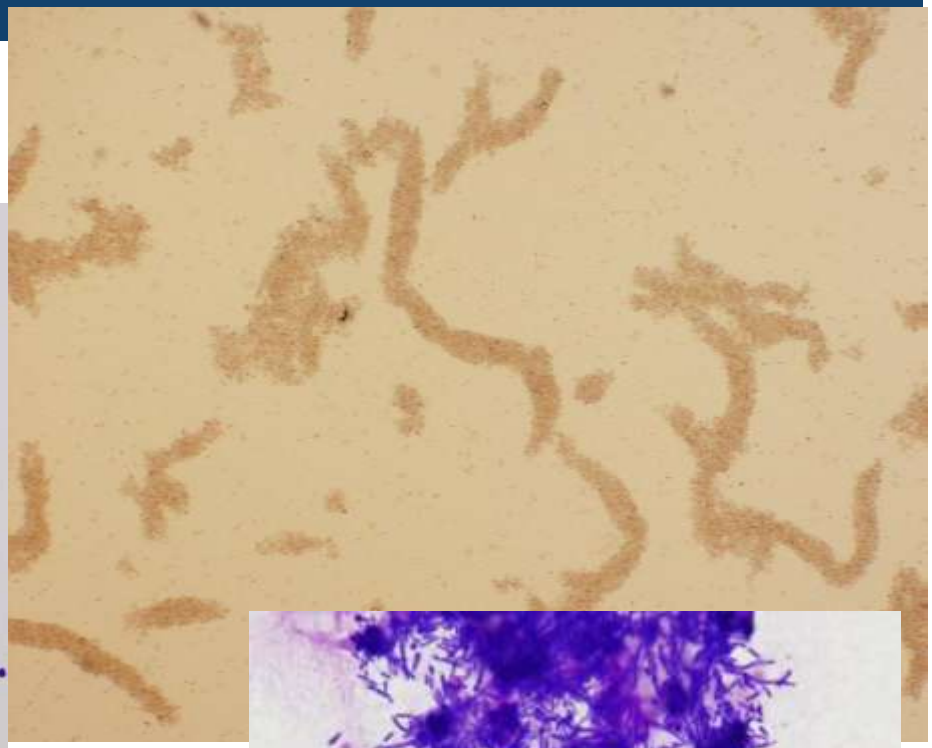
- (Voided) cloudy, light yellow
- Sp. Gr. 1.019
- Protein **4+**
- pH 7.0
- Glucose -ve
- Ketones -ve
- Blood -ve
- Bilirubin -ve

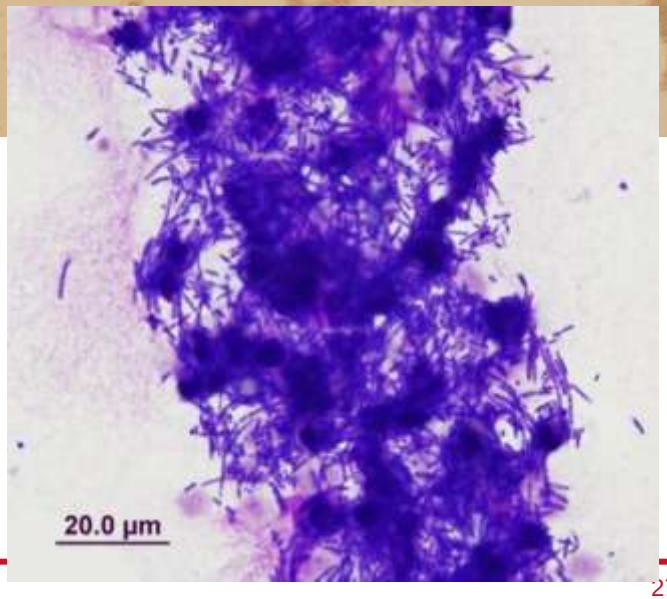
- Sediment: 1-2 leukocytes per HPF, much lipid, 1 fatty cast per LPF
- Urinary Protein to Creatinine ratio = **3.5**

Nephrotic syndrome

Pseudocasts







Urinalysis in birds and reptiles?

- Urinalysis is rarely done in birds and reptiles because of the problem of getting a clean sample (avoiding urates or faecal contamination).
- **Healthy birds:**
  - Uncontaminated urine samples usually have a specific gravity between 1.005-1020
  - The chemical dipstix strips are negative except for a trace protein (and a variable pH)
  - Sediment will likely contain spherical urate crystals, squames, few bacteria and usually less than 3 erythrocytes or leukocytes per HPF (40 x objective).

Final 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)?

Taronga Wildlife Hospital: Adult Male Bush Stone Curlew ('thicknee') presented in poor condition and with band constriction on right hindlimb. Deteriorated despite treatment

Haematology	Results (0 wk)	Result (6 wk)
RBC x 10^{12}/L (1.5-2.8)	1.53	**3.36**
Haemoglobin g/L (104-149)	ND	ND
PCV L/L (0.31-0.54)	0.34	**0.55**
MCV fL (130-200)	**222**	163
MCH pg (30-43)	ND	ND
MCHC g/L (260-310)	ND	ND
WBC x 10^{9}/L (2.5-23)	16.94	**76.1**
Band Heterophils (0)	0	0
Heterophils (0.7-12.4)	**14.9**	**60.1**
Lymphocytes (1.1-9.2)	**0.85**	6.1
Monocytes (0-07-1.2)	0.85	**3.8**
Eosinophils (0.2-1.3)	0.34	**6.1**
Basophils (0-06-1.4)	0	0
Plasma Protein (refract-26-53)	53	**74**
Thombocytes x 10^{9}/L (20-30)	**15.8**	**15.2**
Plasma colour: *clear* Smear: *+polychromasia (0 wk) +++polychromasia (6 wk). Toxic heterophils (0 and 6 wk)*		

Biochemistry (VetScan - Hep)	Results (0 wk)	Results (6 wk)
CK U/L (211-2400)	435	477
AST U/L (220-433)	162	152
Bile Acids µmol/L (<35)	<35	<35
Uric Acid mmol/L (0.327-0.809)	**0.069**	**1.173**
Glucose mmol/L (12.6-21.1)	**11.7**	13.3
Protein g/L (24-50)	**53**	**67**
Albumin g/L (9-20)	12	16
Globulin g/L (8-26)	**41**	**50**
Calcium mmol/L (2.3-3.0)	2.72	2.88
Phosphate mmol/L (0.3-2.3)	1.6	1.4
Sodium mmol/L (151-172)	**135**	**149**
Potassium mmol/L (0.8-3.2)	**4.4**	2.9

Reference intervals from ISIS (International Species Information System)

Likely conclusions: most haematological changes can be attributed to active chronic inflammation (bacterial sepsis?); some of the biochemical changes are likely due to tissue necrosis, inappetence and changes in hydration status

Destroyed due to chronic osteomyelitis (streptococcal organisms recovered)

Possible reasons for changes:

0wk- heterophilia with toxicity likely to be due to inflammatory demand associated with infection or toxicity. The lymphocytopenia could be due to stress, but there is nothing else to support this; so infection should be considered (partly due to stress but other mechanisms may be involved). The increased MCV could be laboratory error. The most common reason for thrombocytopenia in birds is sepsis (increased utilisation?). The +polychromasia could be normal (1-5% of RBCs are polychromatophilic in health [2-10% are reticulocytes] – the higher numbers cf to mammals are thought to be due to shorter life span [range is 28-45 days). The low uric acid may be related to inappetence, as can the low glucose. The increased globulins could be due to ongoing inflammation/infection. The low sodium is difficult to explain (possibly retention of water and dilution?; could the high protein be contributing?), but the hyperkalaemia may be related to tissue necrosis or perhaps metabolic acidosis (no test done to indicate).

6wk- the bird appears dehydrated (increased PCV, RBCs, TP and uric acid) and has significant increases in heterophils, monocytes and eosinophils (the latter two more likely related to chronic inflammation with antigenic stimulations [nb monocytosis can occur in acute infections]). The thrombocytopenia continues, as does the hyperglobulinaemia (although part could be due to dehydration). The animal still has hyponatraemia, but could this be due to wrong Ref values rather disease?

Likely conclusions: most haematological changes can be attributed to active chronic bacterial sepsis; some of the biochemical changes are likely due to inappetence and changes in hydration status

Destroyed due to chronic osteomyelitis (streptococcal organisms recovered)

VetScan VS2 for lower vertebrates biochemistry

Avian/Reptilian Profile Plus

This rotor provides the following 12 parameters - ALB, AST, BA, CA, CK, GLOB, GLU, K^+, NA^+, PHOS, TP, UA

Does haemolysis, lipaemia, or icterus affect the results? Yes!

The VetScan VS2 assesses and supposedly suppresses the impact of haemolysis, lipaemia, and icterus by determining assay-by-assay the level at which these substances could affect the reliability of the results.

Notes on blood biochemistry of birds and reptiles

Blood Biochemistry of Lower Vertebrates (ACVP Meeting13-Nov-2004). **TW Campbell**

- *Lymph fluid contamination* of blood in reptiles may drop protein & K (and haematol)
- Blood best collected in *lithium heparin* (for both haematol and biochemistry) because of the unpredictability of clotting (extrinsic and often prolonged)
- Plasma commonly *colourless*(exceptions occur mainly due to carotenoid pigments).
- **Uric acid** most useful in birds and reptiles for renal dysfunction, but is still inconsistent and can be increased for pre-renal reasons (eg dehydration, gout, necrosis). **Urea**, as well as uric acid, may be useful in some water turtles.
- **Na, K and Cl** level increases and decreases may occur for similar reasons to mammals. Disorders of the *salt gland*, in those birds and reptiles that possess it, may affect electrolyte balance. **Total calcium** can be 2-4x normal levels in female birds and reptiles producing eggs (increased protein bound fraction). Reasons for increases and decreases of Ca and IP are similar to those in mammals.
- **AST activity** suggests hepatic or muscle injury in birds or reptiles, **ALT** may be useful in carnivorous birds and reptiles. **CK** is for muscle damage. **Bile acids** useful in birds and perhaps in some reptiles
- **Proteins** are affected by similar mammalian mechanisms (nb oestrogen-induced globulin increases in yolk production)
- **Glucose** levels are extremely variable in health and are affected by similar mechanisms that occur in mammals (nb. *DM reported in birds*)

Taronga Wildlife Hospital: Juvenile Male Loggerhead Turtle (*Caretta caretta*) with suspected pneumonia

Haematology	Results (healthy)	Result (ill – 3 wk later)
RBC x 10^{12}/L (0.16-0.7)	0.39	0.70
Haemoglobin g/L (39-95)	ND	ND
PCV L/L (0.09-0.36)	0.21	0.23
MCV fL (257-1188)	538	328
MCH pg (158-216)	ND	ND
MCHC g/L (241-324)	ND	ND
WBC x 10^{9}/L (1.8-23.6)	6.6	**26.18**
Band Heterophils (0)	0	
Heterophils (0.04-18.4)	1.58	**19.64**
Lymphocytes (0.08-18.0)	4.88	1.05
Monocytes (0.06-0.69)	0.13	**5.5**
Eosinophils (0.09-1.4)	**0**	**0**
Basophils (0.06-0.92)	**0**	**0**
Plasma Protein (refract-?)	37	28
Thombocytes x 10^{9}/L (?)	23.17	17.82
Plasma colour: *clear* Smear: *++polychromasia (healthy and ill)* *+poikilocytosis (ill)*		

Biochemistry (VetScan Hep)	Results (healthy)	Results (ill – 3 wk)
CK U/L (660-4048)	**6004**	2385
AST U/L (74-1114)	347	318
Bile Acids µmol/L (?)	0	0
Uric Acid mmol/L (0.012-0.119)	0.034	0.036
Glucose mmol/L (5.8-9.3)	8.6	**15.0**
Protein g/L (12-55)	32	24
Albumin g/L (8-22)	12	**5**
Globulin g/L (2-33)	20	19
Calcium mmol/L (1.3-2.2)	1.79	**1.21**
Phosphate mmol/L (0.7-6.3)	2.73	1.75
Sodium mmol/L (147-159)	157	**123**
Potassium mmol/L (2.7-5.5)	**6.5**	**6.2**

Reference intervals from ISIS (International Species Information System)

Likely conclusions: most changes could be attributed to stress, inflammation and chronic disease

Died – necropsy revealed systemic mycobacteriosis, primarily affecting the lungs

Possible reasons for changes: compared to the healthy sample the sick result shows an increase in red cell mass, which may be related to haemoconcentration (but not supported by increased protein or uric acid increases). The heterophilia and monocytosis could go along with suspected pneumonia/infection (monocytes can increase in both acute and chronic infections). The lymphopenia (and therefore part of the heterophilia could go along with corticosteroid release (supported by the hyperglycaemia). The poikilocytosis of RBCS (probably referring to nuclear variation as well as cytoplasmic outlines) is non-specific and can occur in starvation, regeneration and inflammation – the latter is probably the cause. The high CK for the healthy may be related to more struggling on blood collection? The drop in protein cf to the healthy sample appears to be due to albumin decrease (negative acute phase protein? Inappetence?). The electrolyte changes are difficult to explain (fluid therapy?;K may be normal?; hyponatraemia in reptiles is most common caused by retention of water [salt gland issues?]; the hypocalcaemia [total measured] is likely due to the low albumin) but are probably of little significance.

Likely conclusions: most changes could be attributed to chronic disease, stress and inflammation

Died – necropsy revealed systemic mycobacteriosis, primarily affecting the lungs

Seven years old male Boxer presented for lethargy, lack of exercise tolerance and inappetence in excess of one month. On presentation, the dog had pale mucous membranes, possible splenomegaly and hepatomegaly.

HAEMATOLOGY	SAMPLE	R0EFERENCE INTERVAL
Plasma appearance	Clear	Clear
PCV L/L	**0.19**	0.37-0.50
Plasma protein g/L	**50**	55-75
Haemoglobin g/L	**72**	100-150
Erythrocytes $x10^{12}$/L	**2.9**	5-7
MCV fL	65	60-75
MCHC g/L	**379**	300-360
Leukocytes $x10^9$/L (corrected)	**58.5**	7-12
Neutrophils (seg.) $x10^9$/L	**43.9**	4.1-9.4
Neutrophils (band) $x10^9$/L	**5.4**	0-0.24
Lymphocytes $x10^9$/L	**4.1**	0.9-3.6
Monocytes $x10^9$/L	**4.8**	0.2-1.0
Eosinophils $x10^9$/L	0.3	0.14-1.2
Basophils $x10^9$/L	0	0-0.4
Blood film**: moderate polychromasia, moderate acanthocytes and schistocytes (schizocytes), 10 NRBC per 100 leukocytes**		
Platelets $x10^9$/L	**100**	200-900
Reticulocyte % (uncorrected)	**5**	0-1.5
Absolute Reticulocytes x 10^9/L	**145**	0-75

Likely conclusions: there is evidence that the regenerative anaemia is most likely related to a microangiopathic haemolytic process (although some internal bleeding could also be contributing). Considering also the clinical information (and the fact that this is an aging Boxer!), neoplasia is a strong possibility in either the spleen or liver (or both).

Diagnosis and postscript: the animal was investigated further by radiography and ultrasound. Multiple masses were detected in liver and spleen. A tentative diagnosis of neoplasia was given to the owners and the dog was euthanased. Necropsy and histopathology revealed **metastatic hemangiosarcoma**.

Possible reasons for changes: The regenerative anemia (corrected reticulocyte count for the level of anemia was 2.1%) was assumed to be due to blood destruction rather than blood loss due to the presence of the circulating nucleated erythroid cells and the strong neutrophilia with left shift. The lymphocytosis and monocytosis were considered the result of antigenic stimulation and erythrocyte destruction occurring over a period of time. The presence of moderate numbers of schistocytes usually indicates a microangiopathic hemolytic process (fibrin strands laid down in small vessels damage erythrocytes in transit). This can occur in vasculitis, disseminated intravascular coagulation (due to numerous microthrombi formation in small vessels), some splenic disorders and abnormal vascular proliferation (eg vascular tumours). Acanthocytes also occur in splenic and microangiopathic diseases. They are often common in hemangiosarcoma . The high MCHC were considered not to be due to laboratory error, but due to the presence of free hemoglobin (although this was not detected grossly in plasma) related to low grade hemolysis. The low plasma protein did not fit in with blood destruction (more with blood loss), but was considered to be due to chronic disease, inappetence and probable liver disease. The thrombocytopenia may have been partly due to increased consumption (although macroplatelets were not detected) or due to bone marrow suppression with neoplastic and chronic illness.
Likely conclusions: there is evidence that the regenerative anaemia is most likely related to a microangiopathic haemolytic process (although some internal bleeding could also be contributing). Considering the clinical information (and the fact that this is a Boxer!), neoplasia is a possibility in either the spleen or liver (or both).
Further investigation could involve diagnostic imaging of the spleen and liver. Fine needle cell aspiration or biopsy might be considered.

Diagnosis and postscript: the animal was investigated further by radiography and ultrasound. Multiple masses were detected in liver and spleen. A tentative diagnosis of neoplasia was given to the owners and the dog was euthanased. Necropsy and histopathology revealed **metastatic hemangiosarcoma**.
Hemangiosarcoma is one of the few diseases that commonly gives high levels of circulating nucleated erythroid cells. Other diseases include lead poisoning, erythroid neoplasia, other bone marrow disorders, occasional splenic disorders. In contrast to common causes of hemolytic anemia, where the levels of nucleated erythroid cells are always proportional to the levels of reticulocytes, these diseases may give disproportional levels. In hemangiosarcoma, for example, the anemia may be non-regenerative or regenerative, but circulating nucleated erythroid cells are common. This is probably due to the variable effects of this disease (it may cause some internal bleeding and/or it may cause bone marrow depression).

TEST	RESULT	REFERENCE INTERVAL
ALP u/L	96	<210
GGT u/L	18	5.6-22
Serum protein (refract.) g/L	63	53-76
Albumin (EPG) g/L	**27.1**	28-36
α globulins (EPG) g/L	11.3	8-13
β globulins (EPG) g/L	**15.1**	8-15
γ globulins (EPG) g/L	9.6	7-14
Glucose mmol/L	5.6	3.3-6.1
Urea mmol/L	**98**	3.6-6.5
Creatinine μmol/L	**1398**	110-170
Calcium mmol/L	**3.7**	2.8-3.4
Inorganic phosphate mmol/L	2.1	1-2.3
Sodium mmol/L	**118**	131-147
Potassium mmol/L	4.8	2.1-4.8
Chloride mmol/L	**86**	99-108

TEST	RESULT	REFERENCE INTERVAL
Plasma appearance	Clear	Variable
PCV L/L	0.38	0.32-0.53
Plasma protein g/L	66	55-78
Haemoglobin g/L	135	130-160
Erythrocytes $x10^{12}$/L	**6.4**	8-11
MCV fL	**59**	41-49
MCHC g/L	355	300-360
Leukocytes $x10^9$/L	11.9	6.5-12
Neutrophils (seg.) $x10^9$/L	**9.6**	2.5-7
Neutrophils (band) $x10^9$/L	0	0-0.2
Lymphocytes $x10^9$/L	2.1	1.6-5.4
Monocytes $x10^9$/L	0.2	0-0.7
Eosinophils $x10^9$/L	**0**	0.2-1
Basophils $x10^9$/L	0	0-0.4
Blood film: normal		

(voided urine sample)			
Appearance	Cloudy	pH	7.3
Colour	Brown	Glucose	-ve
Specific gravity	1.014	Ketones	-ve
Protein	**1+**	Blood	-ve
		Bilirubin	-ve
Microscopic findings: much mucin and calcium carbonate crystals			

A 13-15 years gelded male thoroughbred horse, was in poor condition (3 months duration) with a loss of muscle mass and a dry coat, and possibly polydipsic. The horse could have possibly been dehydrated with slightly sunken eyes. Vital signs (HR. Temperature, RR) were within reference intervals.

Likely conclusions: overall, the horse appears to have renal failure (the combination of dehydration, marked azotaemia and poorly concentrated urine suggests renal failure), which is likely to be chronic because of the history. Hypercalcaemia is not uncommon in CRF in the horse. There is little to indicate the pathological process affecting the kidneys.

This horse, at necropsy, had end stage renal disease due to the development of **polycystic kidneys**.

Possible reasons for changes: the marked azotaemia could be pre-renal and/or renal in origin. The poorly concentrated urine might be related to the polydipsia and, therefore renal disease.. The high MCV may be laboratory error in the measurement of PCV and erythrocytes. Anaemia is only indicated on a low erythrocyte count. The leukocyte changes are difficult to interpret and probably of little significance. The mild neutrophilia, low normal lymphocytes and absolute eosinopenia could be due to the release of corticosteroids but the changes are not convincing and do not fit in with chronic illness.
The mild hypoalbuminaemia could possibly be related to urine loss (1+ proteinuria at a sp. gr. of 1.014 is significant) and chronic disease cachexia. The SPE shows an increase in beta globulins, which could be due to increases in lipoproteins found in this band (beta lipoprotein can increase in renal failure in small animals). The hyponatraemia could be related to renal loss (polydipsia and presumably polyuria?). The low chloride is probably following the sodium, but could also be due to some movement into the stomach. The high calcium has been reported in horses with chronic renal failure. Apparently, the horse's kidney is important for calcium excretion and interference can lead to hypercalcaemia. Like most changes, this is not consistently present in renal failure in the horse (probably depends on the dietary intake of calcium). It is commonly accompanied by decreased inorganic phosphate (not the case here).
Apart from a sp. gr. close to isosthenuria and proteinuria, there is little else abnormal about the urine. Mucin and calcium carbonate crystals are normally found in horse's urine. The brown colour probably developed while the urine was waiting to be analysed (oxidation of compounds present).
Likely conclusions: overall, the horse appears to have renal failure (the combination of dehydration, marked azotaemia and poorly concentrated urine suggests renal failure), which is likely to be chronic because of the history. The animal does not show a prominent anaemia, which might be expected in chronic renal failure in most species. There is little to indicate the pathological process affecting the kidneys.
Possible further investigation: Image analysis of the kidneys could be undertake (especially ultrasound examination), but it is also important to assess the size of the kidneys through rectal palpation. A renal biopsy might be considered for prognostic purposes. Further laboratory testing for acid/base imbalances.

This horse, at necropsy, had end stage renal disease due to the development of **polycystic kidneys**.

A 14-years-old, 15.5 kg, female Border collie presented for inappetence, loss of weight, loose stools and difficulties sitting and climbing stairs over a prolonged period. She had a normal pulse, resp. rate 60/min and temp. 39.1 ºC, unilateral otitis externa and conjunctivitis. She had a distended bladder, wide-based stance, reduced patellar reflexes and reduced hind proprioception .

TEST	SAMPLE	REF VALUES
AMYLASE IU/L	**1704**	<1400
ALP IU/L	**3301**	<110
ALT IU/L	**539**	<60
CK IU/L	91	<200
Serum protein (biuret) g/L	70	50-70
Albumin (BCG) g/L	38.8	23-43
Globulins g/L	31.3	27-44
Total cholesterol mmol/L	5.24	1.4-7.5
Glucose mmol/L	5.85	3.3-6.4
Urea mmol/L	**21.55**	3.0-10
Creatinine µmol/L	103	40-120
Calcium mmol/L	2.71	2.1-2.9
Inorg. phosphate mmol/L	**1.63**	0.8-1.6
Sodium mmol/L	146.6	137-150
Potassium mmol/L	4.2	3.3-4.8
Chloride mmol/L	112.4	105-120
Bicarbonate (TCO2) mmol/L	**24.6**	18-24
Anion gap mmol/L	13.8	15-25

TEST	SAMPLE	REF VALUES
Plasma appearance	Clear	Clear
PCV L/L	**0.35**	0.37-.50
Plasma protein g/L (refract.)	**78**	55-75
Haemoglobin g/L	128	100-150
Erythrocytes x1012/L	5.3	5-7
MCV fL	66	60-75
MCHC g/L	**366**	300-350
MCH pg	24.2	20-25
Leukocytes x109/L	**12.2**	7-12
Neutrophils (seg.) x109/L	**9.88**	4.1-9.4
Neutrophils (band) x109/L	0.24	0-.24
Lymphocytes x109/L	0.98	.91-3.6
Monocytes x109/L	0.73	.2-.96
Eosinophils x109/L	0.37	.14-1.2
Basophils x109/L	0	0-.36
Platelets x109/L	**925**	200-600
Reticulocyte % (uncorrected)	0.2	0-1.5
Blood film**: Some neutrophils hypersegmented. Some toxic granulation of neutrophils. One+ macroplatelets**		

Urine (cystocent) appearance: slightly cloudy	PH: 6.0
Colour: pale yellow	Glucose: -ve
Specific gravity: 1.015	Ketones: -ve
Protein (SSA**): trace**	Blood: trace
Centrifuged: not done	Bilirubin**: +**
Microscopic findings: **moderate bacteria, 10-20 white blood cells per high power field, 10 Erythrocytes per HPF.**	

Likely conclusions: the dog certainly has strong indications of multisystemic disease. Many could be due to old age (eg degenerative joint/spinal disease). The likely conclusions from the clinical pathology results are *hepatic disease, renal disease and cystitis.* Pancreatic/gut disease has not been assessed effectively. The possibility of respiratory disease has not been assessed.

(**Postscript:** Ultrasound of the abdomen revealed an irregularly-shaped liver with variable echogenicity. An ultrasound-guided fine needle cell aspirate revealed cells that could have been neoplastic (*diagnosis of possible hepatoma/ hepatocarcinoma*). The neoplastic cells were obtained from several sites suggesting widespread involvement of the liver. Ultrasonography of the kidneys revealed bilateral dilation of the renal pelvices and hyperechoic renal cortices. Unfortunately, the animal was euthanased but a necropsy not done.

Possible reasons for changes: there are marked elevations in ALP and ALT indicating significant cholestasis and hepatocellular damage. This combination might suggest hepatic disease. Another possibility is that the ALP elevation is unrelated to cholestasis and due to corticosteroid induction (eg hyperadrenocorticism). However, clinical signs are not highly supportive of HyperA (we do not have any information about the animal has been medicated with corticosteroids). The elevation of blood urea in combination with hyperphosphataemia and a specific gravity close to isosthenuria (in a dog with possible dehydration indicated by an elevated TPP) might suggest a degree of renal disease. The mildly increased AMS could be related to renal disease (increased GFR perhaps increasing globulin bound amylase [macroamylase]) , but may be related to pancreatic/intestinal disease (chronic diarrhoea). The elevated bicarbonate is difficult to assess. Respiratory, intestinal, renal and hepatic disease could all be contributing to electrolyte and acid/base levels. The low anion gap is difficult to explain. Could the bicarbonate be falsely high? The dog has a marginal non-regenerative anaemia which could be simply related to chronic disease. Leukocyte changes are minimal (marginal neutrophilia), but do not rule out ongoing mild inflammatory disease. The toxic neutrophils could be due to inflammation or metabolic disturbances. The hypersegmented neutrophils are often due to corticosteroid effects. The high platelets (with some macroplatelets) might be related to inflammatory disease. Intestinal disease could be contributing? The urinalysis shows a 1+ bilirubin, which at this sp gr could be significant and reflect the cholestasis. The trace of blood is related to mild haematuria. The pyuria is significant and probably related to the bacturia (a fresh cystocentesis sample should be free of bacteria) and suggests urinary tract infection. Considering the large bladder and likely neurological defects, the dog is likely to have cystitis. The ratio of WBC to RBC numbers is roughly 2:1 and suggests that the haematuria and pyuria are related to inflammation rather than haemorrhage.

Likely conclusions: the dog certainly has strong indications of multisystemic disease. Many could be due to old age (eg degenerative joint/spinal disease). The likely conclusions from the clinical pathology results are hepatic disease, renal disease and cystitis. Pancreatic/gut disease has not been assessed effectively. The possibility of respiratory disease has not been assessed.

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A 13 YO, male neutered cross Persian cat was presented for prolonged inappetence. The cat appeared depressed, was in poor condition, icteric and assessed as 3-5% clinically dehydrated. On abdominal palpation the kidneys felt small, whilst there was an increased temperature of 39.2 ºC.

TEST	SAMPLE	REF VALUES
ALP u/L	44	<50
ALT u/L	**660**	<60
CK u/L	199	<200
Serum protein (biuret) g/L	53.0	53-76
Albumin (BCG) g/L	25.0	19-38
Globulins g/L	28	26-51
Bilirubin total µmol/L	**75**	2.5-3.5
Total cholesterol mmol/L	2.4	1.9-3.9
Glucose mmol/L	**8.1**	3.6-6.6
Urea mmol/L	**13.0**	7.2-10.7
Creatinine µmol/L	**75**	90-180
Calcium mmol/L	2.09	1.75-2.6
Inorganic phosphate mmol/L	1.3	1.3-2.3
Sodium mmol/L	151.1	147-156
Potassium mmol/L	**3.5**	4.0-4.6
Chloride mmol/L	121.0	115-130
Bicarbonate mmol/L	17	17-21

TEST	SAMPLE	REF VALUES
Plasma appearance	**Icteric**	Clear
PCV L/L	**0.08**	.30-.45
Plasma protein g/L (refractometer)	59	59-78
Haemoglobin g/L	**27**	80-140
Erythrocytes $x10^{12}$/L	**1.6**	6-10
MCV fL	**54**	40-45
MCHC g/L	318	310-350
MCH pg	17.0	13-17
Leukocytes $x10^9$/L	**2.1***	8-14
Neutrophils (seg.) $x10^9$/L	**0.4**	3.76-10.8
Neutrophils (band) $x10^9$/L	0.0	0-0.42
Lymphocytes $x10^9$/L	**1.5**	1.6-7.0
Monocytes $x10^9$/L	0.1	0.08-0.56
Eosinophils $x10^9$/L	**0.1**	0.16-1.4
Basophils $x10^9$/L	0.0	0-0.14
Platelets $x10^9$/L	**124**	300-700
Reticulocyte % (uncorrected)	**1.8**	0-1

Blood film: *Erythrocytes* – **3+ macrocytosis, 1+ polychromasia, some Howell-Jolly bodies. Nucleated erythroid cells 29 per 100 leukocytes.** *Platelets* – **moderate numbers (2-3+) of macroplatelets**. *leukocyte value has been corrected for larger number of nucleated erythroid cells.

Urine (cystocentesis): clear and yellow	Glucose: -ve
Specific gravity: 1.020	Ketones: -ve
Protein (SSA): **1+**	Blood: -ve
pH: 6.0	Bilirubin**: 1+**
Microscopic findings (centrifuged): some lipid, 1-2 white blood cells per high power field, 1-2 erythrocytes per HPF, scattered transitional epithelial cells.	

Likely conclusions: the cat appears to have multi-organ disease (haematopoietic [spleen/bone marrow], liver [possibly post-liver], and kidney).
(**postscript:** a necropsy 3 weeks later revealed that the cat had evidence for *chronic ongoing cholangitis, mild renal fibrosis and lymphosarcoma affecting liver and abdominal lymph nodes (and possibly spleen).* In addition, there was evidence for widespread thrombotic disease. In hindsight, the cat had chronic liver and kidney disease, but developing lymphosarcoma was a major issue. The anaemia and other penias could have been related to immune-mediated destruction as a paraneoplastic phenomenon? It is also possible that the terminal thrombotic condition was another paraneoplastic phenomenon.)

Possible reasons for changes (related to this case): since the cat is clinically mildly dehydrated (derived osmolality is around 300 mosm/l and normal and the clinical dehydration is normonatraemic), this could cause false slight increases in PCV, the protein values (both albumin and globulins are increased) and possibly urea. We would also expect the urine specific gravity (USG) to reflect the mild dehydration. Unfortunately, the USG does not indicate adequate concentrating ability (should be over 1.035 for a moderately dehydrated animal), and with the 1+ protein (significant at this USG) might suggest some renal disease. This is supported by the palpation of small kidneys. The 1+ bilirubin could be related to the hyperbilirubinaemia if that is conjugated in form (bilirubinurua in the cat is due to increased conjugated form). The non- regenerative anaemia (corrected reticulocyte count of about 0.4%; absolute reticulocytes of 0.029 x 10^{12}/l) is significant. Considering the sudden drop in PCV (0.23 down to 0.08) one might expect an acute blood loss or haemolysis, with little time for a regenerative response. An acute intravascular haemolysis should give rise to red plasma, but that is not always consistent. Moreover, this anaemia seems a little more complicated than that as it is accompanied by large numbers of circulating nucleated erythroid cells (this could suggest bone marrow or splenic disease), and it is macrocytic (increased MCV and evidence for moderate macrocytosis on the blood film). Moreover, there are penias related to platelets and neutrophils that may or may be associated with the anaemia (a marked leukopenia due to marked neutropenia, mild lymphopenia, and mild eosinopenia). Bone marrow disease may be a possibility for these penias (although macroplatelets suggest a bone marrow response to the thrombocxytpenia), as well as considering destruction or increased utilization of the platelets and neutrophils. The whole leukogram perhaps suggests a stress component (lymphocytopenia and eosinopenia), but this cannot explain the significant neutropenia. The elevated ALT suggests moderate hepatocellular damage, which could be secondary to the dramatic drop in PCV (hypoxic damage) or be due to a primary hepatic disease. The jaundice could be related to either haemolysis or liver disease. The normal ALP might suggest that there is little cholestasis, but bilirubinuria seems to contradict that. The plasma protein value is likely to be influenced by dehydration (nb the difference between the serum protein and plasma protein readings may not be due to just fibrinogen, but may be partly due to the discrepancy between the biuret and refractometer methods of analysis). So, the lowish protein could follow a sudden internal bleed, but we cannot ignore that the cat has chronic illness , which may be contributing to hypoproteinaemia. Remember, despite the drop in PCV from 0.23 to 0.08, the protein level actually went up (another clue to the possibility of haemolysis?)The mildly elevated urea could be both pre-renal and renal in origin (dehydration, poor condition, evidence for renal disease). The low creatinine is a little unusual but could be partly due to decrease muscle mass (poor condition). Depending on the method of analyzing blood creatinine, the low value could be affected by hyperbilirubinaemia. The increased glucose could be related to stress of the sudden drop in PCV. The hypokalaemia may be related to inappetence and/or renal disease. The finding of borderline metabolic acidosis without increased anion gap (in fact AG is 13.6 and slightly low) can occur with diarrhea (secretion acidosis), but there is no clinical evidence for that. Another perplexing issue is that metabolic acidosis often causes hyperkalaemia, not hypokalaemia. Because of these conflicting findings it might be wise to consider that perhaps the low bicarbonate reading is due to delays in processing the sample or perhaps laboratory error.

Likely conclusions and further investigation: the issue of acute clinical signs on top of chronic illness could indicate a chronic disease that has developed into a crisis point or could be because of two separate disease processes (always an issue in an older animal). For these reasons it is probably best to focus on the key problems in your further investigation rather than try to pattern recognize. However, we can state that the cat appears to have multi-organ disease (haematopoietic [spleen/bone marrow], liver [possibly post-liver], and kidney).A key finding is the significant anemia and the neutropenia, which need to be explored further – as does the jaundice and possible liver disease .A Coomb's test might be useful if you are considering immune-mediated reasons for the anaemia (eg secondary to neoplasia?) However, monitoring may be required to ensure that this becomes a regenerative anaemia. If it does not then, splenic or bone marrow disease should be considered and samples taken if necessary. Considering the unexplained neutropenia, bone marrow examination may be useful from the start. Also, some of you may be concerned by the presence of macrocytosis and nucleated erythroid cells in a non-regenerative anaemia and might want to check FIV and FeLV status? Since some clinical pathology results suggest **renal disease**, this could be further investigated by ultrasonography (and FNA if warranted on kidney appearance). A urinary protein to creatinine ratio could also be performed to assess the significance of the proteinuria. Monitoring of PCV and TPP during further investigation and management is also warranted. The prognosis is probably poor because of the age and condition of the animal and the fact that there appears to be multi-organ disease.

(**postscript:** the cat appeared to respond to a blood transfusion, prophylactic antibiotics and supportive nutritional therapy, so little further investigation was done (especially since the owner had little funding). However, 2-3 weeks later there was further deterioration in demeanour and the jaundice and anemia redeveloped. The owners decided to euthanase the cat and at necropsy and subsequent histopathological examination it was revealed that the cat had evidence for chronic on going cholangitis, mild renal fibrosis and lymphosarcoma affecting liver and abdominal lymph nodes (and possibly spleen). In addition, there was evidence for widespread thrombotic disease. In hindsight, the cat had chronic liver and kidney disease, but developing lymphosarcoma was a major issue. Neoplastic infiltration probably contributed to a number of the clinical signs. The anaemia and other penias could have been related to immune-mediated destruction as a paraneoplastic phenomenon? It is also possible that the terminal thrombotic condition was another paraneoplastic phenomenon.)