Session 3 – Haematology - Leukocytes

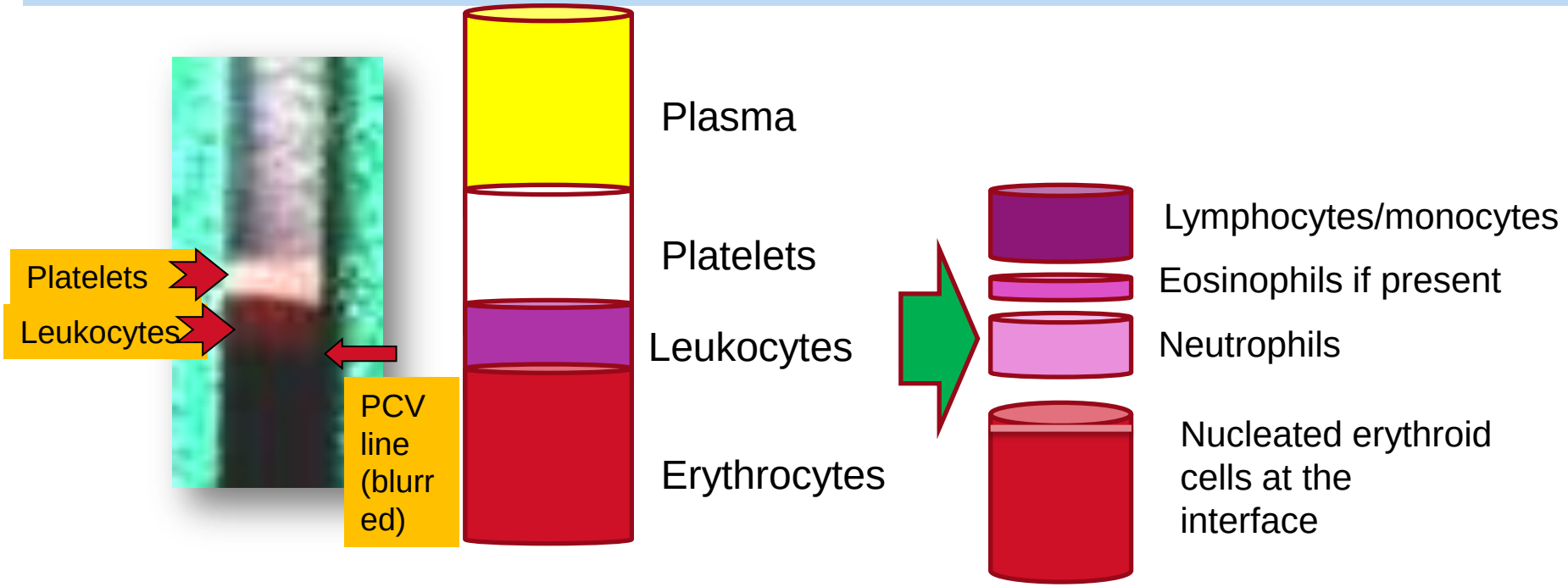
- What is useful for the referring veterinarian?
- What are the difficult or controversial parts for me?
- A good opportunity to integrate the numbers with the morphological findings!

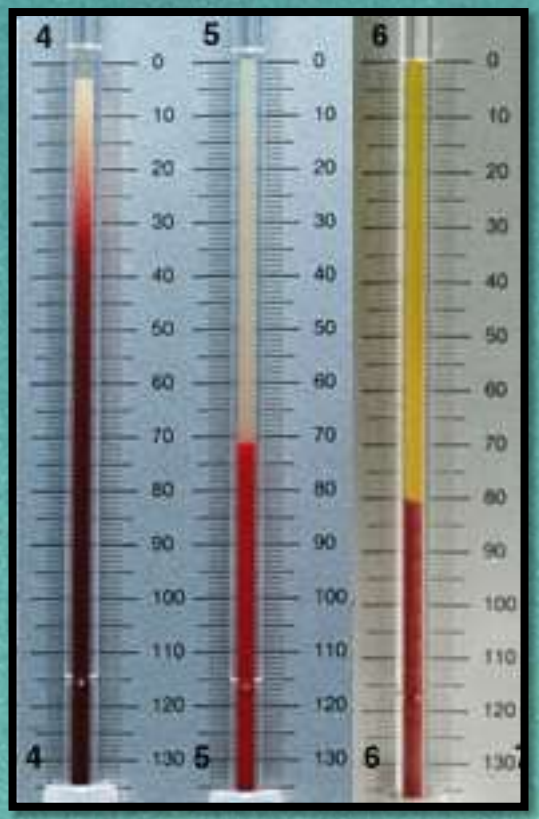
Australian Animal Pathology Standards Program (AAPSP) 2013 Roadshow

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

The buffy coat

- The Wintrobe tube for HCT and ESR – good for looking at layers of the buffy coat
- Microhaematocrit method takes over in veterinary medicine and buffy coat analysis diminishes
- Examination of the buffy coat, 'macroscopically' and microscopically for increases and alterations (*clinicians may ask you about this*)
- The buffy coat **smear** is useful for detecting microbes and abnormal circulating cells
- Beware, **any haematopoetic cell** may be detected in a buffy coat smear



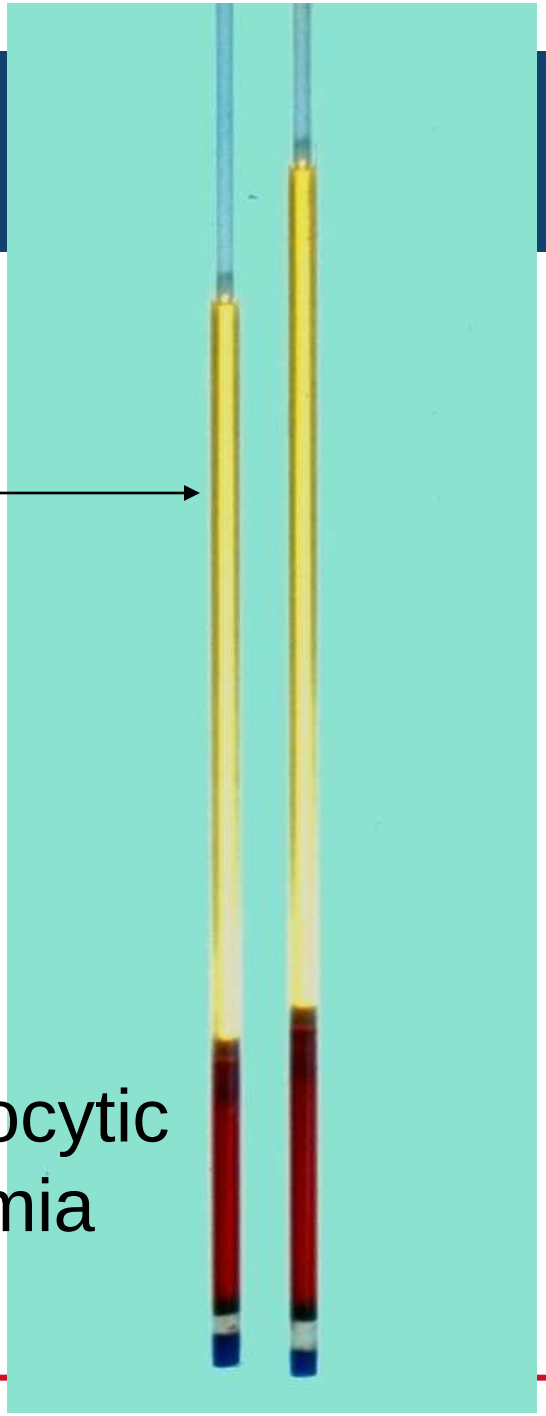


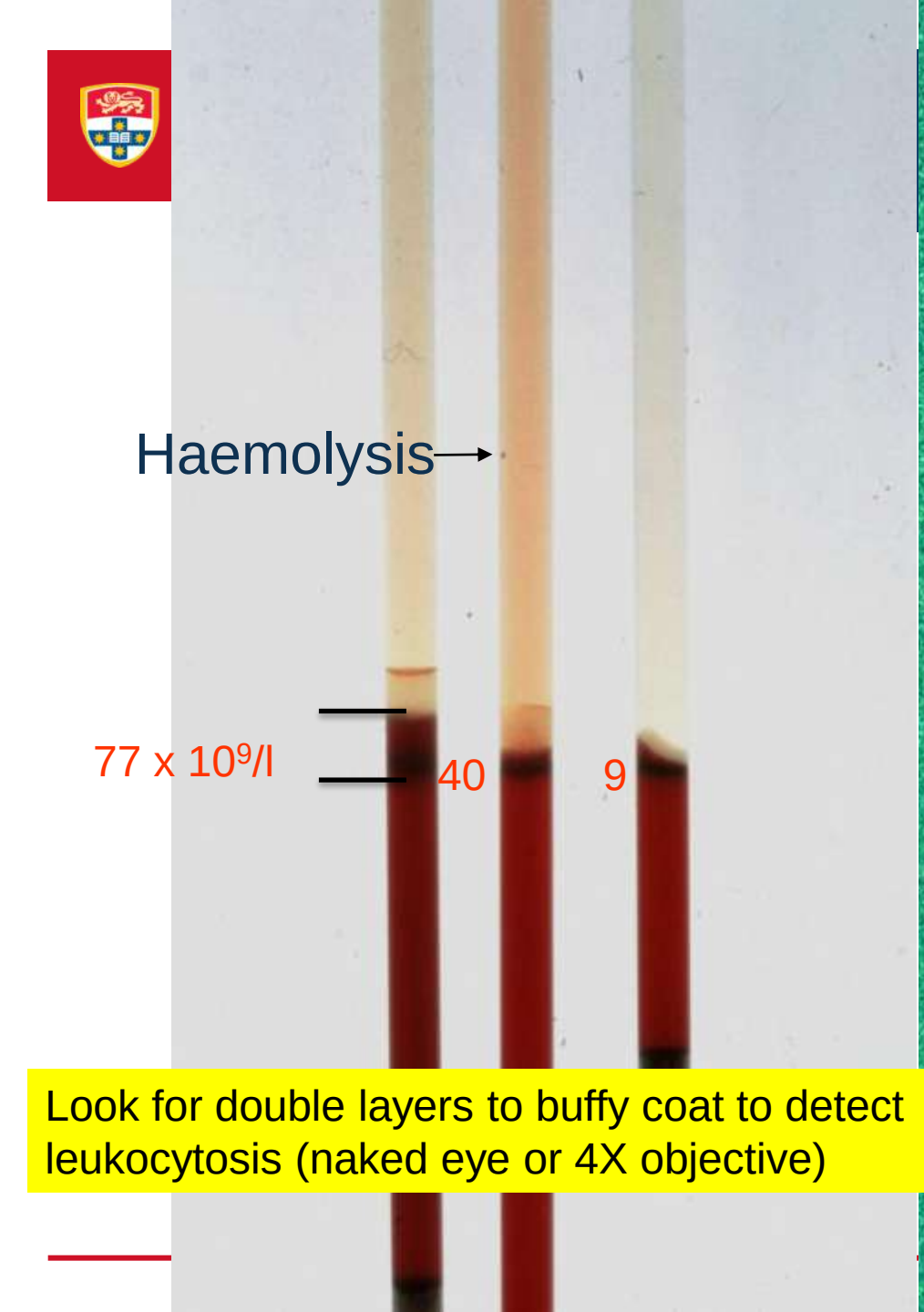
Wintrobe tubes and ESR

Icterus

Chronic lymphocytic leukaemia

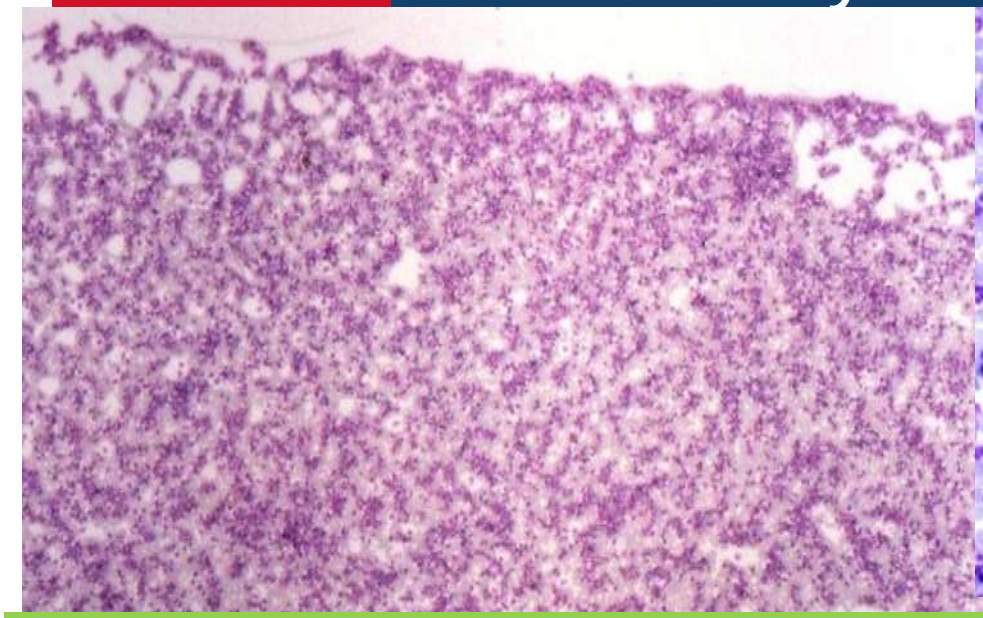
Granulocytic leukaemia

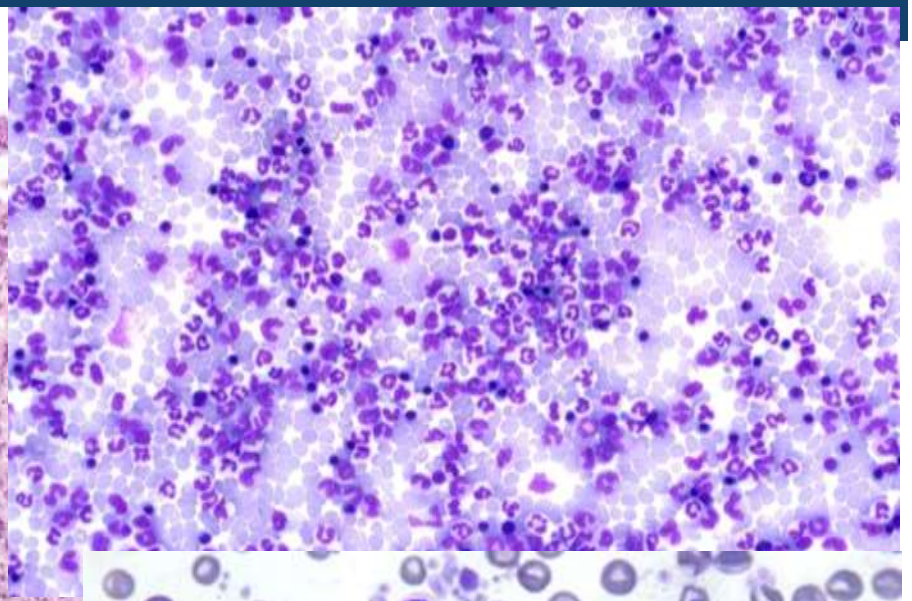




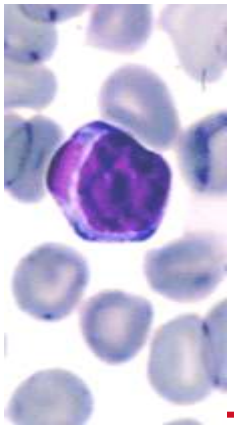
Look for double layers to buffy coat to detect leukocytosis (naked eye or 4X objective)

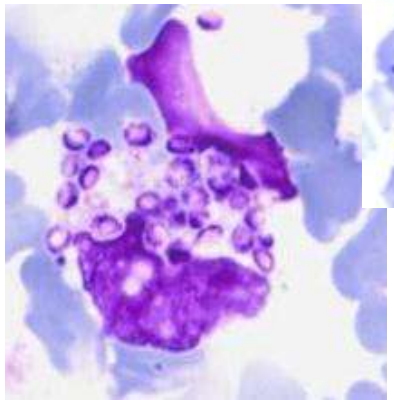
Buffy coat smears

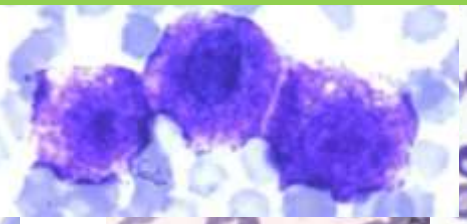


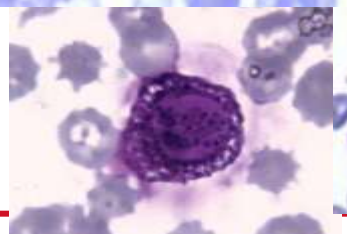


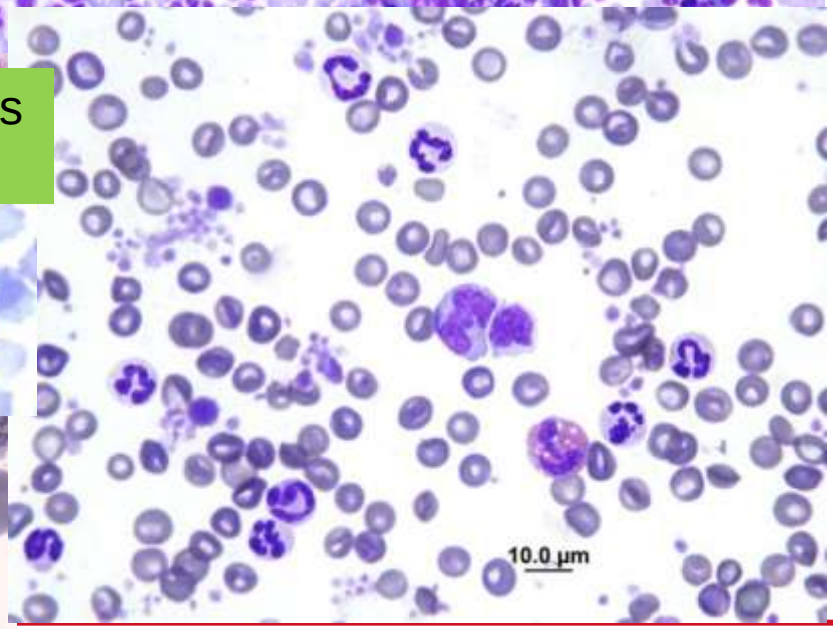
Detection of circulating mast cells and pathogens (histoplasmosis and Distemper inclusion)











The value of interpreting leukocytes - ‘One cell doth not a diagnosis make’ – looking for consistent change (the numbers game as in any fluid analysis)

- Generalisations:
  - Peripheral blood levels of leukocytes (particularly neutrophils) are a reflection of **bone marrow production and storage**, **EM production** (can mislead), **tissue demand** and distribution between the **marginal and circulatory pools**.
  - These are the cells of inflammation (immunity)
    - Responses tell you an awful lot about the **innate and adaptive immune responses** by the host
  - **Leukocytosis/leukopaenia (species variation)**:
    - In dog, cat, horse and pig (?) total leukocyte changes *usually* due to changes in neutrophils.
    - In ruminants, changes in lymphocytes also commonly contribute to total leukocyte changes (hence, greater use of the N:L ratio – also applies to some other species eg the rabbit)
  - *Evaluation of blood leukocyte responses to inflammation is a good starting point–but diagnosis invariably depends on cytopathology or histopathology to elucidate site and cause!*

Leukocyte responses –invariably depends on histopathology to elucidate cause!

› Haemic cytopathology

- **Peripheral blood** and cell type (rarely detect cause)
- **Bone marrow –**rarely done unless decreases

› Histopathology

- **Bone marrow** biopsy for architecture
- **Spleen and liver** for EMH
- **Capillary beds?**
- **Tissues** for utilization and loss

Complementary

Neutrophils

- **Some basic facts:**
  - Production and storage in BM (under the control of granulopoietin and LIF)
  - Concept of marginal (MNP) and circulating (CNP) pools (and rapid movement between)
  - Neutrophils are short lived in the circulation (less than one day) and lost through tissues.
- **Rapid neutrophilia** due to utilisation of cells in MNP and BM storage pool; **sustained neutrophilia** due to increased BM production (after 3+ days)
  1. Physiological causes (including adrenalin release)
  2. Corticosteroids
  3. Regenerative anaemia
  4. Inflammatory demand

Vessels (capillary beds)

Bone Marrow

Marginating blood

Production

Storage

Peripheral Tissues

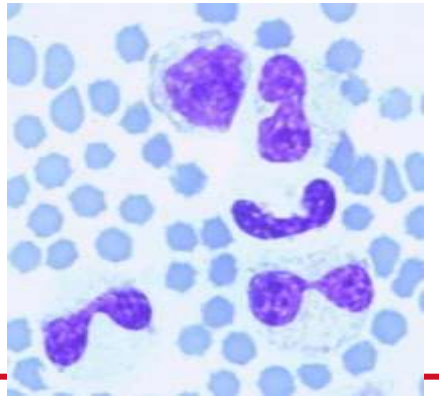
Circulating blood

loss

Extramedullary neutropoiesis

Spleen, liver

Neutrophils – key cells for innate immunity - microbiocidal

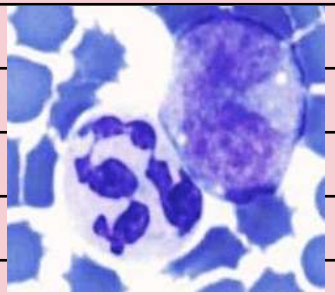


What levels of neutrophils can be expected (expressed in total leukocyte numbers)?

- **Physiological neutrophilia**: rapid and transient (MNP involved). *Most important cause* is adrenalin, especially seen in frightened young horses and cats (*total leukocyte* values can reach 20-25 x 10^9/L). Lymphocytes often increase due to adrenalin (rarely in the dog), and eosinophils may decrease in cow.
- **Corticosteroid induced neutrophilia**: after a few hours (MNP and BM storage involved), due to intense illness, more common in the dog (*total leukocytes* can reach 40 x 10^9/L or greater in the dog [disputed], 30 in the cat, 20 in the horse and about 18 in cattle – depends on stored levels and degree of lymphocytopenia in cattle). Get increases in M (mainly dog) and N, and decreases in L and E. *May co-exist with inflammatory demand neutrophilia*.
- **Neutrophilia related to regenerative anaemia –** unknown mechanisms. More common in haemolytic forms, which in the dog can have a left shift. Numbers vary depending on the degree and type of regenerative anaemia. *Corticosteroid influences may co-exist.*

A 2 years old female greyhound with muscle pain and reluctance to move after race. Suspected paralytic rhabdomyolysis

HEMATOLOGY	SAMPLE	REFERENCE INTERVAL
Plasma appearance	Clear	Clear
PCV L/L	**0.67**	0.42-0.63
Plasma protein g/L	**77**	55-75
Hemoglobin g/L	**274**	110-220
Erythrocytes $x10^{12}$/L	**11.7**	6.1-9.6
MCV fL	66	62-76
MCHC g/L	356	310-380
Leukocytes $x10^{9}$/L	**18.7**	3.4-9.5
Neutrophils (seg.) $x10^{9}$/L	**14.6**	2.0 -6.1
Neutrophils (band) $x10^{9}$/L	0	0-0.24
Lymphocytes $x10^{9}$/L	**0.75**	0.9-3.6
Monocytes $x10^{9}$/L	**3.3**	0.2-1.0
Eosinophils $x10^{9}$/L	**0.1**	0.14-1.2
Basophils $x10^{9}$/L	0	0-0.4
Platelets $x10^{9}$/L	130	110- 300
Blood film: **hypersegmentation of the neutrophils**		



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- May not apply to greyhounds less than 6 months of age
- Whether racing or retired has a particular impact on erythron values
- Neutrophil levels different for most greyhounds
- Most biochem values not significantly different from other breeds of dogs (base T4 and fT4 may be an issue)

Table 1. Analytes and features characteristic of Greyhound dogs compared with those of other breeds.

Higher Values	Lower Values	Unique Features
PCV/HCT	WBC count	Non-staining eosinophil granules
RBC count	Neutrophil count	
Hemoglobin concentration	Platelet count	
MCV*	Fibrinogen	Higher frequency of DEA 1.1-negative dogs
MCHC	TEG values: K-time, angle, MA, and G	
Hemoglobin affinity for O_2		
Creatinine	Potassium	
Glomerular filtration rate	Phosphate	
Alanine aminotransferase	Calcium, ionized	
Aspartate aminotransferase	Magnesium, ionized	
Sodium	Serum total protein	
Chloride	Total globulins	
Total CO_2	α- and β-globulins	
Bicarbonate	IgA and IgM	
Cardiac troponin I	Haptoglobin	
	Total T4 and free T4	

*Reported in only one study.

Applies to most sighthounds

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INVITED REVIEW

Clinical pathology of Greyhounds and other sighthounds

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Inflammatory demand neutrophilia

- Levels in the CNP depend on **supply** (BM, MNP, sometimes extramedullary neutropoiesis) and **demand** (loss in tissues)
- Situations in tissue inflammation:

  1. No or little increase N (circulating cells cope)

  2. Increased N but no need for left shift

  3. Increased N with left shift (may get initial neutropenia if overwhelming demand – *this is common in ruminants in the early stages of inflammation – lower reserve levels in BM and MNP*)
- Left shift due to BM storage pool depletion leading to release of granulopoietin

Inflammatory demand neutrophilia – what levels of leukocytosis can be expected?

- Any level of **leukocytosis** can occur (stress can contribute)
  - **Dogs:** 15- 50 x 10^9/L common, but local purulent infections can cause higher levels (eg leukaemoid response: greater than 50 x 10^9/L and extreme left shift). Left shifts common in local purulent conditions
  - **Cats:** up to 35 x 10^9/L common – rarely get leukaemoid responses. Left shifts common
  - **Horses:** up to 35 x 10^9/L , BUT left shifts rare (except in purulent conditions in foals)
  - **Pigs:** levels can be similar to the dog and cat. Left shifts may occur in purulent infections *NB problem of wide reference interval for leukocytes (*up to 30 x 10^9/L may occur in normal pigs!) .

A 6-years-old female desexed cat was presented with respiratory distress and an occasional mild cough of at least two weeks duration. The cat had a mild fever (39.4°C) and was inappetant. Pneumonia and/or pyothorax were considered possibilities.

TEST	SAMPLE	REFERENCE VALUES
Plasma appearance	Clear	Clear
PCV L/L	0.40	0.30-0.45
Plasma protein g/L	**85**	59-78
Haemoglobin g/L	141	80-140
Erythrocytes $x10^{12}$/L	9.6	6-10
MCV fL	42	40-45
MCHC g/L	352	310-360
MCH pg	15	13-17
Leukocytes $x10^9$/L	**31.1**	8-14
Neutrophils (seg.) $x10^9$/L	**22.4**	3.8-10.1
Neutrophils (band) $x10^9$/L	0.3	0-0.4
Lymphocytes $x10^9$/L	5.6	1.6-7.0
Monocytes $x10^9$/L	**1.6**	0.1-0.6
Eosinophils $x10^9$/L	1.2	0.2-1.4
Basophils $x10^9$/L	0	0-0.2
Blood film: **slight polychromasia and anisocytosis**. One nucleated erythroid cell per 100 leukocytes. **Hyperbasophilic lymphocytes** (immunocytes or reactive lymphocytes).		

Hyperproteinaemia could be due to haemoconcetration and/or increased globulins. The leukocytosis due to neutrophilia and monocytosis is likely due to ongoing inflammation.

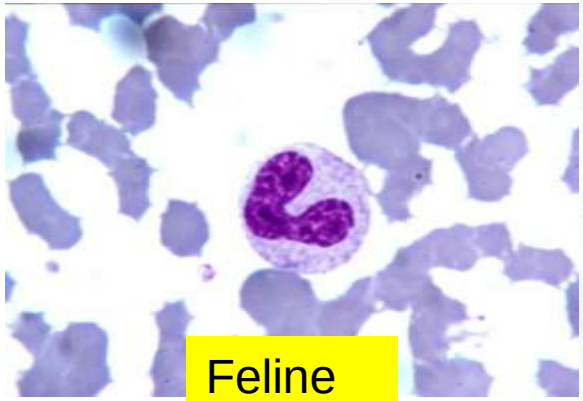
Inflammatory demand neutrophilia - ruminants

- The level of **leukocytosis** is tempered in inflammatory demand because of poor reserves of neutrophils and the higher levels of lymphocytes (these are commonly reduced in inflammatory demand due to corticosteroid release). Consequently, the N:L ratio is often used in ruminants to supplement interpretation of leukocytosis
- In cattle, early neutropenia is common in purulent conditions (and endotoxaemia due to Gram –ve bacteria) due to low storage pools. Low L contribute to leukopenia. About 20-30 x 10^9/L for total leukocytes after a few days.
- Young calves respond as for dogs and cats
- Fibrinous inflammations may lead to little neutrophilia (hence the use of fibrinogen to total protein ratios in pigs and ruminants). Increased fibrinogen directly influences ESR!

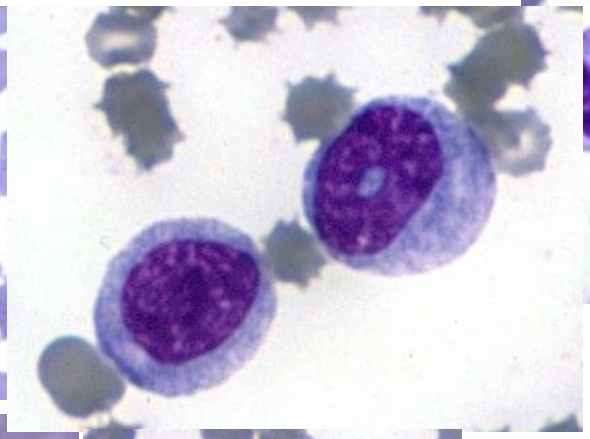
Inflammatory demand neutrophilia and left shift

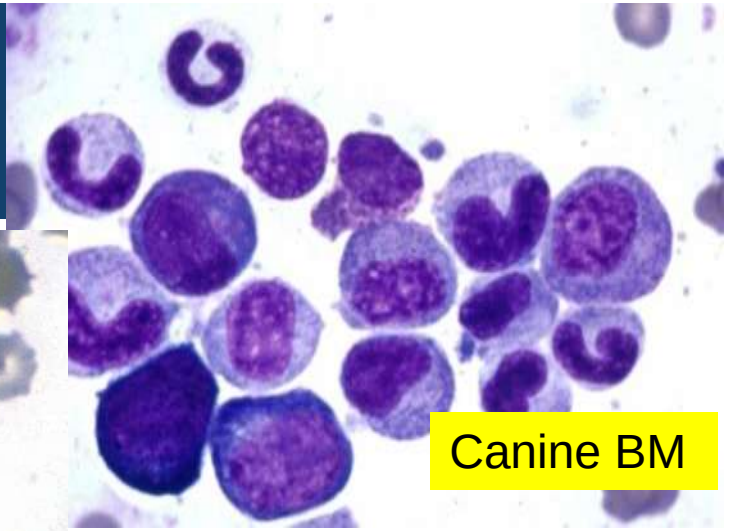
- Left shift determined from ratio of segmented (mature) to immature circulating N (proportions usually less than 1:16-18 for dog, 1:10-12 for cat and 1: 12-16 for horse [ie >10% for most species]; **OR total bands greater than 1 x 10^9/L in dog or cat and 0.3 x 10^9/L in horse – is this an urban myth?)**
  - Nb ruminants: since rarely immature neutrophils circulate in health then **any increase is a left shift** (seems to hold for sheep and goats, but healthy cattle may have 0-0.1 x 10^9/L bands?) **.** Healthy adult pigs can have between 1-4% circulating band neutrophils.
  - The young of any species behave similarly in terms of producing left shifts in inflammation and sometimes different from their adult counterpart
  - **What values do you use for left shift?**
  - **Do you use the term 'degenerative left shift'?**

Left Shift (band and earlier neutrophils)

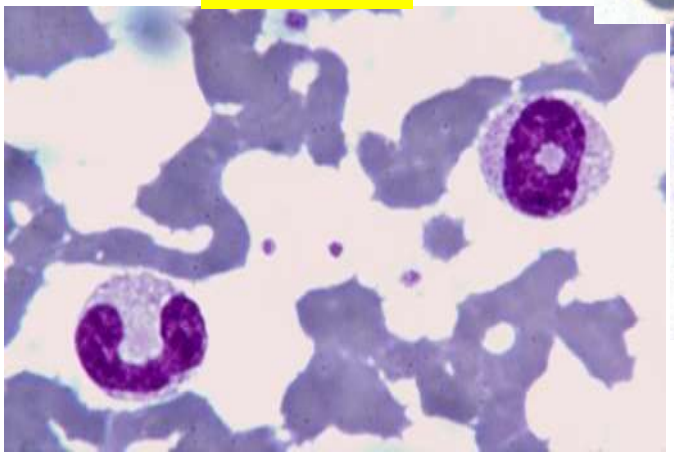


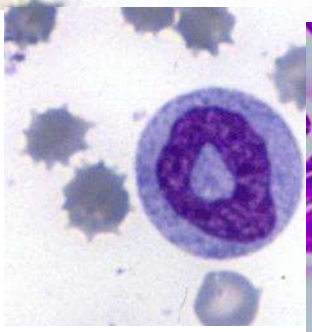
Feline

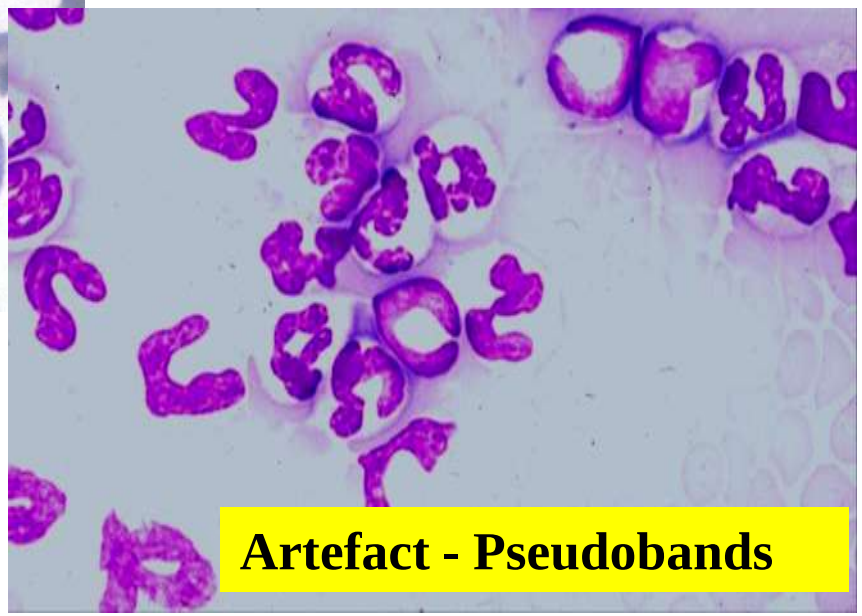




Canine BM







Artefact - Pseudobands

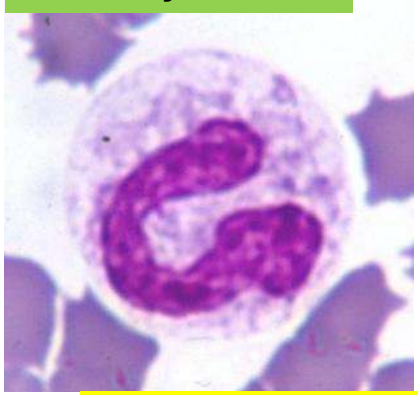
Giant neutrophils common in cats with inflammatory left shift? Sometimes called a 'toxic' change

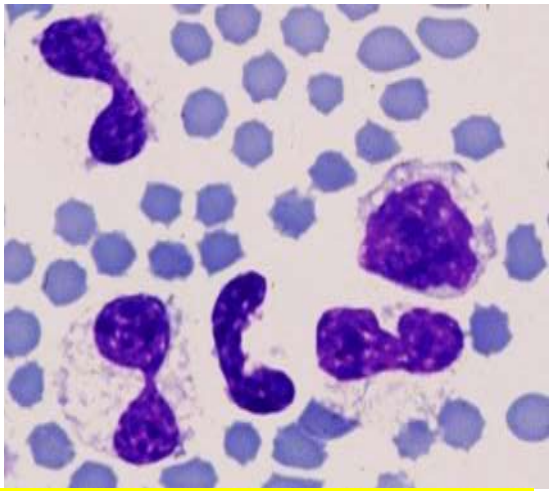
Inflammatory demand neutrophilia and toxic changes

- 'Toxic' changes in N (can only be done on fresh smears):
  1. Cytoplasmic Döhle bodies
  2. Cytoplasmic basophilia (mild, moderate, marked)
  3. Cytoplasmic vacuolation (mild, moderate, marked)
  4. Coarse or prominent cytoplasmic azurophilic granulation (uncommonly seen and mainly in horse, ruminants and camelids – are remnant primary granules]).
  5. Nuclear membrane indistinct (final stage before complete lysis and accompanies marked cytoplasmic vacuolation [basophilia variable])
- Some toxic changes are due to **accelerated maturation** (eg Döhle bodies, basophilia and primary granules), some due to **storage artefacts** (Döhle and vacuolation) some due to **toxaemia or drugs** (eg Döhle bodies and vacuolation) – a problem to differentiate, but no doubt toxic changes are more common in toxaemia!
- The most common cause of toxic changes is the most common cause of left shift: no coincidence - *localised purulent infections* BUT not exclusively!

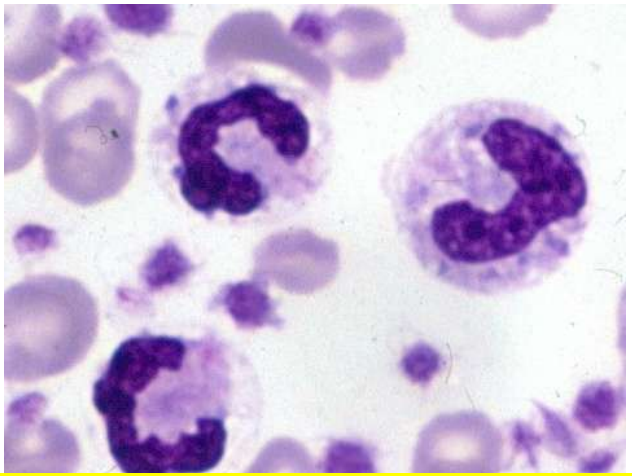
Toxic changes to neutrophils

Healthy cat

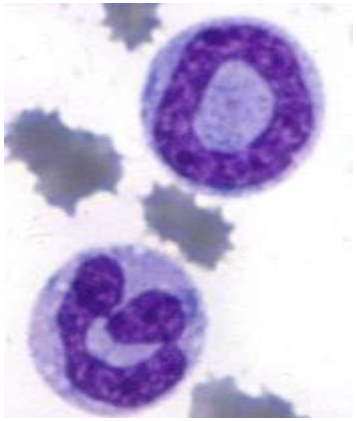


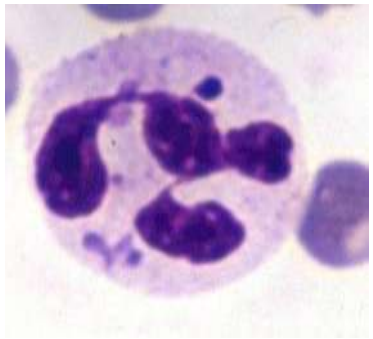


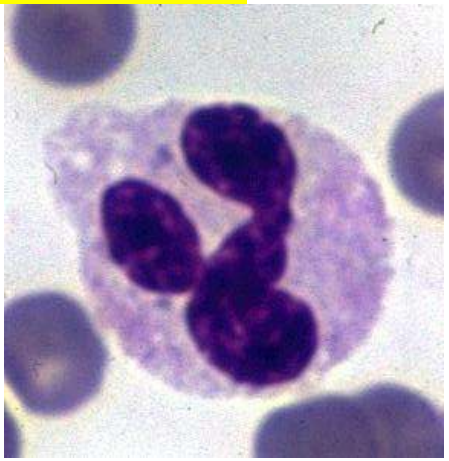
Vacuolation, basophilia and Doehle bodies in the feline

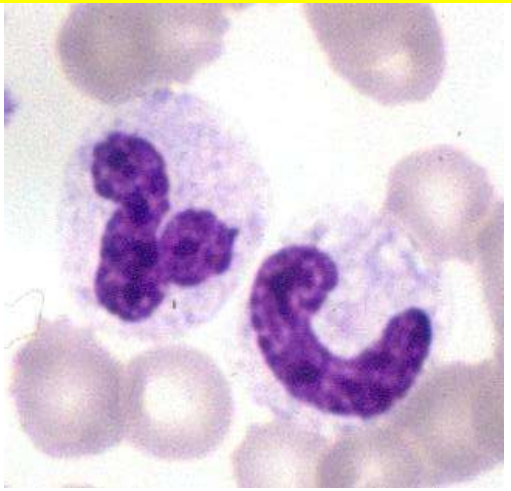


Vacuolation, basophilia and Doehle bodies in the canine









Neil Horadagoda Camden, USYD: 6-12 months Friesian heifer with diarrhoea and salmonellosis – left shift and toxic neutrophils

Haematology	Results	
RBC x 10^{12}/L (5-10)	**13.1**	
Haemoglobin g/L (80-150)	144	
PCV L/L (0.24-0.46)	0.46	
MCV fl (40-60)	**35**	
MCH pg (11-17)	11	
MCHC g/L (300-360)	313	
WBC x 10^9/L (4.0-12.0)	**22.8**	
	%	X 10^9/L
Band Neutrophils (0-0.24)	5	**1.14**
Neutrophils (0.6-4.0)	83	**18.92**
Lymphocytes (2.5-7.5)	10	**2.28**
Monocytes (0-0.8)	2	0.46
Eosinophils (0-2.5)	0	0
Basophils (0-0.3)	0	0
Plasma Protein (60-85)	**56**	
Fibrinogen (3-7)	**8.6**	
Reticulocyte %corr. (0)	ND	
Platelets x 10^9/L (100-800)	**2612**	
Comments: Mild to moderate degenerative changes to neutrophils (1-2+ vacuolation and basophilia). Moderate crenation of RBC (causing decreased MCV?) and moderate poikilocytosis.		

Inflammatory demand neutrophilia – when do the numbers indicate a poor prognosis?

- **Neutropenia** in the early stages of overwhelming demand cannot always be regarded as a poor prognostic sign (especially in ruminants or in endotoxaemia [pseudoneutropenia] in horses and ruminants – *can be transient in nature*)
- A **poor prognosis** may be indicated when, especially after treatment, there is a continuing neutropenia (especially without left shift) with persistence of neutrophilic toxic changes. *This indicates bone marrow failure and a severe compromise of an important part of innate immunity*.

Gribbles Adelaide (Daren Hanshaw): Hx: 1-3 wk old calves, scouring, depression, death - Salmonellosis

RBC	5.01	x 10 12 /L	(5.00 - 10.00)
Hb	81	g/L	(80 - 150)
Hct	0.24	L/L	(0.24 - 0.46)
MCV	**37**	fL	(40 - 60)
MCH	13.0	pg	(11 - 17)
MCHC	318	g/L	(300 - 360)
Reticulocytes	0 %	0 x 10 ^9/L	

WBC	**3.6**	x 10 ^9 /L	(4.0 - 12.0)
Neutrophils	6 %	**0.2** x10 ^9 /L	(0.6 - 4.0)
Band Forms	50 %	**1.8** x10 ^9 /L	(< 0.2)
Lymphocytes	17 %	**0.6** x10 ^9 /L	(2.5 - 7.5)
Monocytes	22 %	0.8 x10 ^9 /L	(< 0.9)
Eosinophils	5 %	0.2 x10 ^9 /L	(< 2.5)
Platelets	**82**	x10 ^9 /L	(100 - 800)
Fibrinogen	**10.9**	g/L	(3.0 - 7.0)

FILM MORPHOLOGY: RBC: **1+ anisocytosis, 1+ microcytosis**. WBC: **3+ toxic change**. Platelets: appear mildly reduced.

Sodium	**127**	mmol/L	(132 - 152)
Potassium	**6.1**	mmol/L	(3.9 - 5.8)
Chloride	**89**	mmol/L	(95 - 110)
Bicarbonate	**10**	mmol/L	(20 - 30)
Anion gap	**34.1**	mmol/L	(<20)
Na/K	**20.8**		
Urea	6.5	mmol/L	(2.1 - 9.6)
Creatinine	**43**	umol/L	(55 - 130)
Calcium	2.12	mmol/L	(2.00 - 3.00)
Phosphate	**2.50**	mmol/L	(1.29 - 2.26)
Magnesium	1.2	mmol/L	(0.5 - 1.5)

GLDH	**26**	U/L	(< 20)
B-OH Butyrate	0.3	mmol/L	(< 0.9)
Protein	**47**	g/L	(58 - 80)
Albumin	**38**	g/L	(22 - 36)
Globulin	**9**	g/L	(24 - 40)
T. Bilirubin	14	umol/L	(2 - 18)
Alk Phos	**372**	U/L	(35 - 350)
GGT	**55**	U/L	(< 36)
AST	66	U/L	(60 - 150)
CK	155	U/L	(50 - 400)
Cholesterol	**0.8**	mmol/L	(2.1 – 6.5)

SERUM INDICES (Clear/+/++/+++/++++)	
Icterus index	Clear
Lipaemia index	Clear
Haemolysis index	**1+**

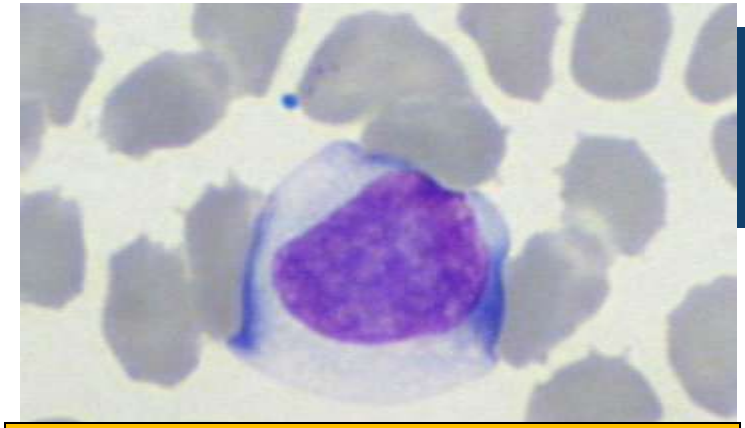
most of the changes can be attributed to the age of the animal, dehydration, hypersecretory diarrhoea and possibly Gram negative sepsis (enterotoxins [exotoxin with effect on gut lining] and systemic endotoxin). The lack of globulin might suggest a lack of colostrum (what about the high GGT). What about the high anion gap?

Th e increased anion gap could be related to production of D-Lactate (and perhaps D-L). L lactate is produced in the body due to anaerobic glycolysis and is not usually increased in calf diarrhoea due to Gram negatives.
(*J Vet Intern Med 2003;17:940–942* **Anion Gap Correlates with Serum D- and DL-Lactate Concentration in Diarrheic Neonatal Calves.** Julia B. Ewaschuk, Jonathan M. Naylor, and Gordon A. Zello).

More useful references - *Irish Veterinary Journal 62(1): 58-61 (2009).* **An update on calf diarrhoea Part 1: Pathophysiology and treatment.** Ingrid Lorenz.
New Zealand Veterinary Journal, 29:12, 223-226 (1981). **Colostral transfer of gamma glutamyl transpeptidase in calves**. J. C. Thompson & J. V. Pauli.

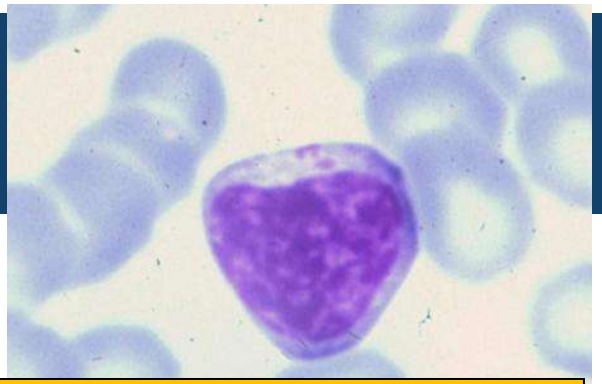
Changes in other leukocytes

- **Lymphocytes**:
  - long-living; BM retains the capacity for production. Circulating forms are mainly T (species variation). Different morphological forms in blood
  - **Lymphocytosis -** indicates antigenic stimulation, but could be due to excitement or leukaemia
  - **Lymphocytopaenia** could be due to corticosteroids, acute infection, T cell deficiency or loss of lymph.
- **Monocytes**:
  - produced in BM (bipotential cell), exist in marginal and circulatory pools
  - **Monocytosis** is due to demand for macromolecular phagocytosis (usually long term, but in lower orders may be rapid). Can occur with neutropaenia, corticosteroid release (dog), rarely leukaemia
  - **Monocytopaenia** – difficult to assess in main domestic species

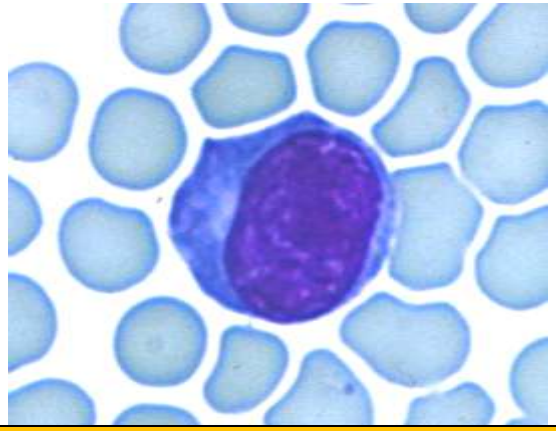


Canine lymphocyte with expanded cytoplasm (artefact?)

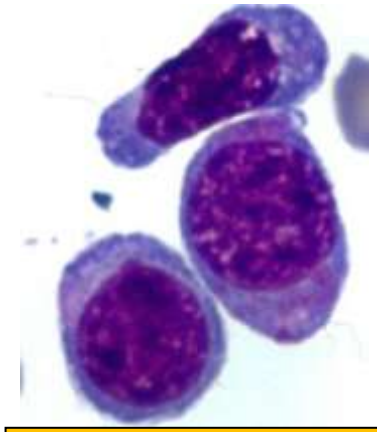
Do plasma cells circulate?



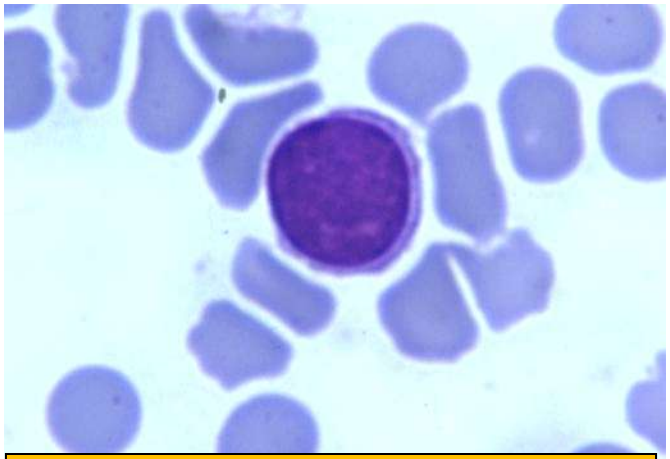
Canine lymphocyte with azurophilic granules in the cytoplasm. Lots of granules think NK or T lymphocyte



Feline lymphocyte with basophilic cytoplasm (‘reactive’ or ‘stimulated’). Could this be neoplastic?



Feline lymphocytes with hyperbasophilic and pink granulated cytoplasm



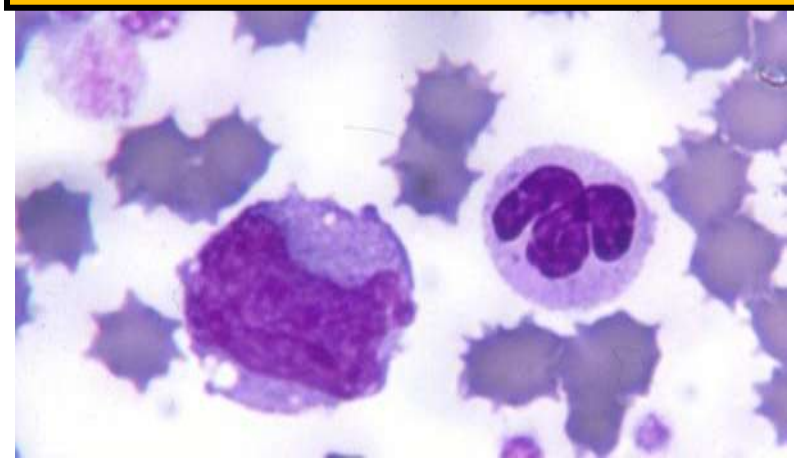
Feline small lymphocyte with limited cytoplasm

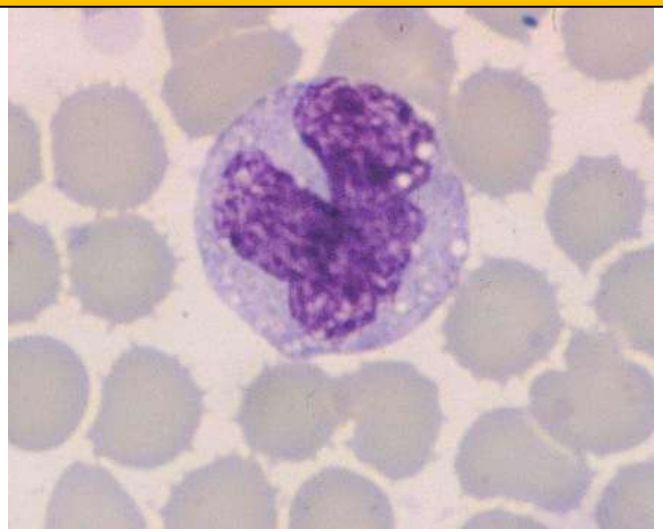
Changes in other leukocytes

- **Eosinophils**: long-living in tissues. Not well retained in BM unless corticosteroid release
  - **Eosinophilia** often related to histamine release or prolonged antigenic stimulation (via sensitized T lymphocytes) in skin, gut, resp and reprod tracts. Can be due to leukaemia (rare), paraneoplasia (cytokine [IL5 mainly] release by a variety of tumours) or be idiopathic
  - **Eosinopaenia** may be due to corticosteroids or acute infection, but there is the problem of detection in some species because of zero for low end of RI
- **Basophils**:
  - **basophilia** may be due to Ag-Ab complexes or go along with eosinophilia; **basopaenia** of no consequence and difficult to detect.

Feline basophils and eosinophils – spot the grey basophil!

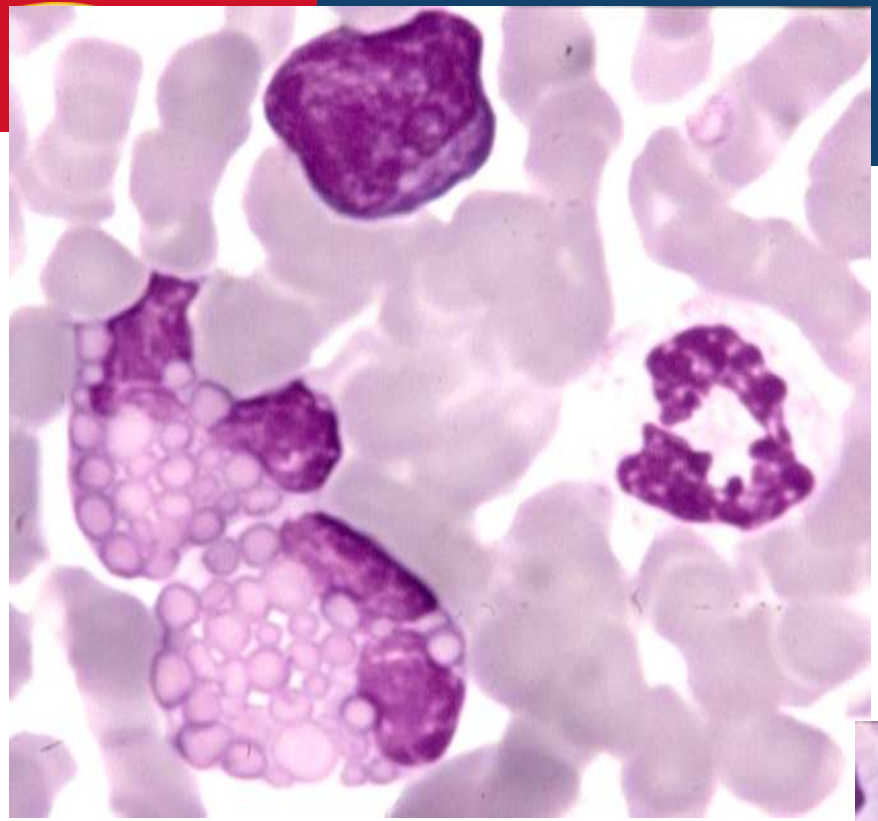
Canine basophil and eosinophils – spot the Greyhound eosinophil!



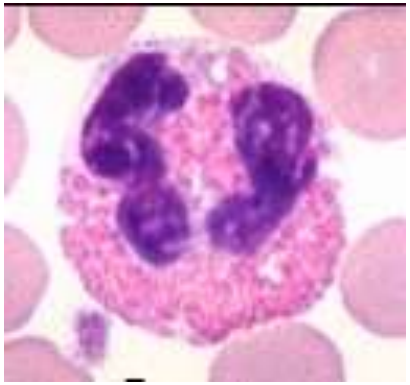


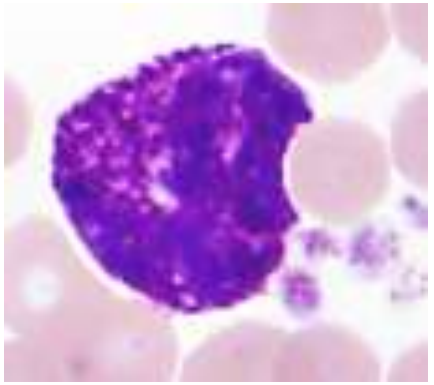
Feline monocyte and neutrophil. Note the comparative size

Canine monocyte

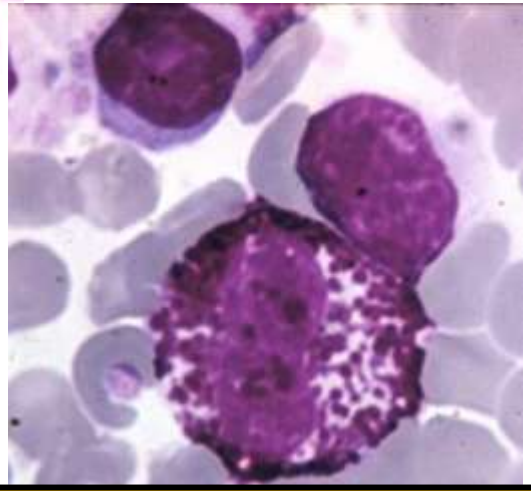


Equine eosinophils, small lymphocyte and neutrophil





Bovine eosinophil and basophil



Equine basophil and lymphocytes

A 9 years old male Schnauzer dog with a long history of spasmodic inappetence, polydipsia and polyuria. For the last four days has been collapsed and vomiting. Now presented jaundiced and dehydrated.

HAEMATOLOGY	SAMPLE	REFERENCE INTERVAL
Plasma appearance	**Yellow-pink**	Clear
PCV L/L	**0.34**	0.37-0.50
Plasma protein g/L	62	55-75
Haemoglobin g/L	127	100-150
Erythrocytes $x10^{12}$/L	5.3	5-7
MCV fL	64	60-75
MCHC g/L	**373**	300-360
Leukocytes $x10^9$/L	**89**	7-12
Neutrophils (seg.) $x10^9$/L	**70.2**	4.1-9.4
Neutrophils (band) $x10^9$/L	**0.4**	0-0.24
Lymphocytes $x10^9$/L	3.3	0.9-3.6
Monocytes $x10^9$/L	**14.7**	0.2-1.0
Eosinophils $x10^9$/L	0.4	0.14-1.2
Basophils $x10^9$/L	0	0-0.4
Blood film: normal		
Reticulocyte % (uncorrected)	1	0-1.5
Reticulocytes (absolute) $x10^9$/L	53	0-75

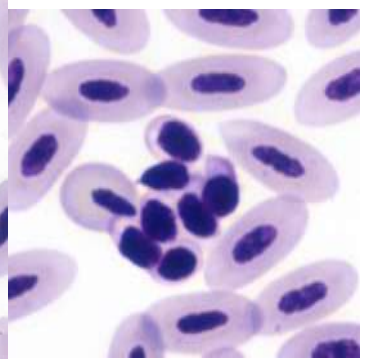
Non- regenerative anaemia, marked leukocytosis due to neutrophilia and monocytosis.
PM: Extensive cholangiohepatitis and a large hepatic abscess. Acute nephrosis also present

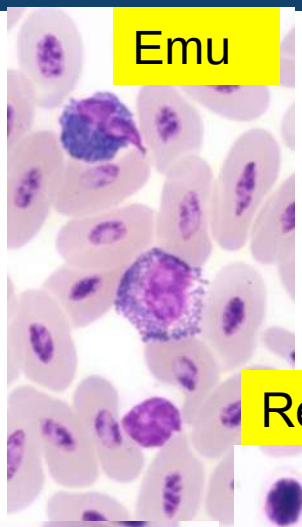
Comparative leukocyte morphology (lower Orders) – the five cell 'rule' for identification in all species

- **Heterophil (equivalent to neutrophil), eosinophil, basophil, lymphocyte and monocyte are the five main leukocytes BUT variations may exist within the one smear** eg many reptiles have a monocytic variant called the azurophil (in snakes this is thought to be more aligned with the mammalian neutrophil based on cytochemistry)
- Similar reasons for leukocytosis and leukopenia occur
- Leukocytosis/leukopenia primarily due to heterophil or lymphocytes changes (in those species where lymphocytes predominate)
  - Rodents and birds vary in whether the heterophil (neutrophil) or lymphocyte predominates
  - Reptiles and fish: monocytes may contribute significantly (major phagocytic cell in the fish and important in many reptiles). Can you get a left shift for monocytes? Granulocytes commonly mononuclear but not always!
- Heterophilic left shifts and toxic changes occur in inflammation in birds and reptiles, but sometimes the cells are a little harder to recognize!
- Fish – the main granulocyte is called neutrophil and stress and inflammation can cause increases
- Thrombocytes (reptiles, birds and fish) may be confused with small lymphocytes in some species

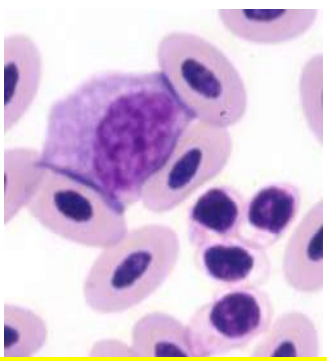
Bird Leukocytes and thrombocytes – lobed heterophils and eosinophils!

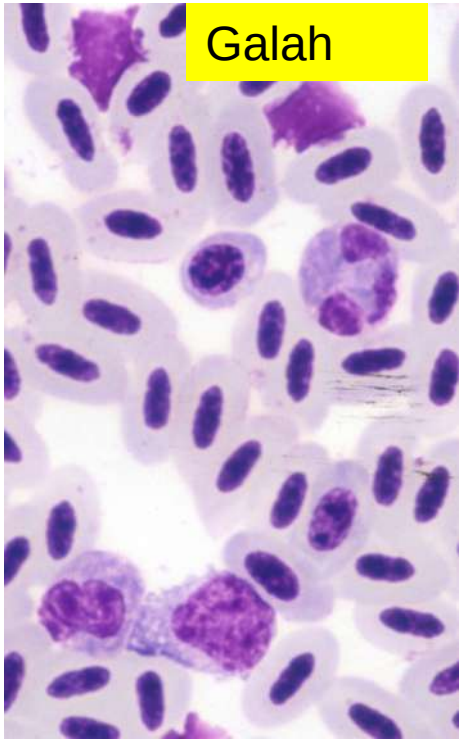
African Grey Parrot



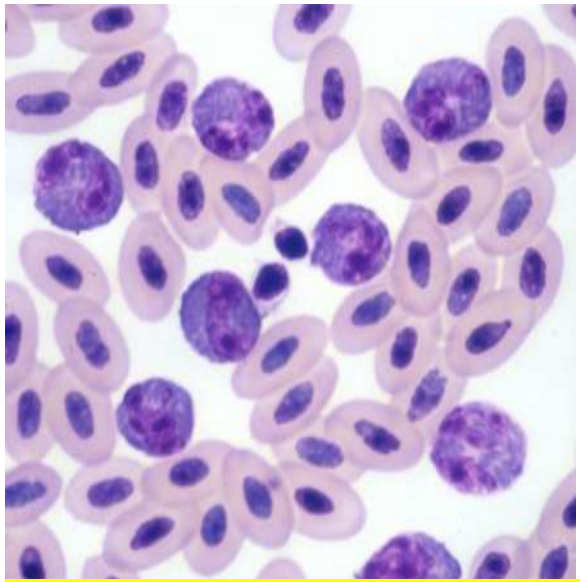


Emu



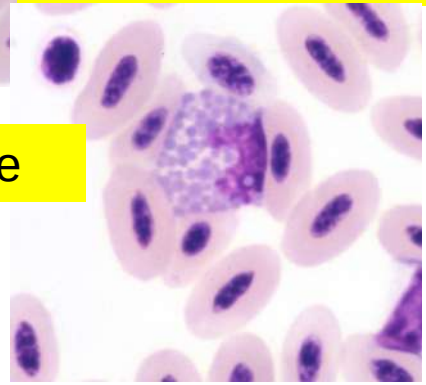


Galah

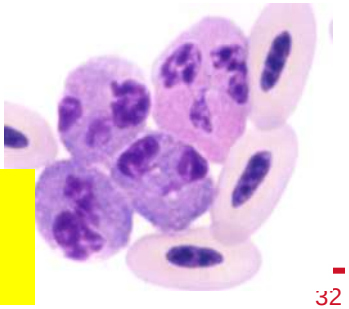


Palm Cockatoo – embolic myocarditis

Kite

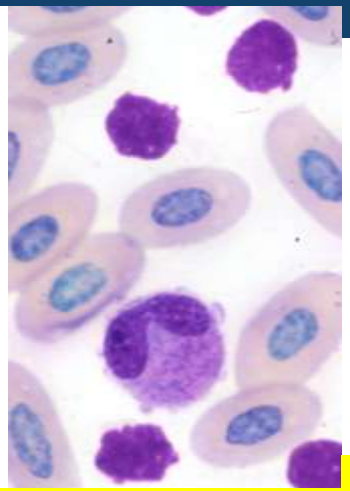


Reeve's Pheasant

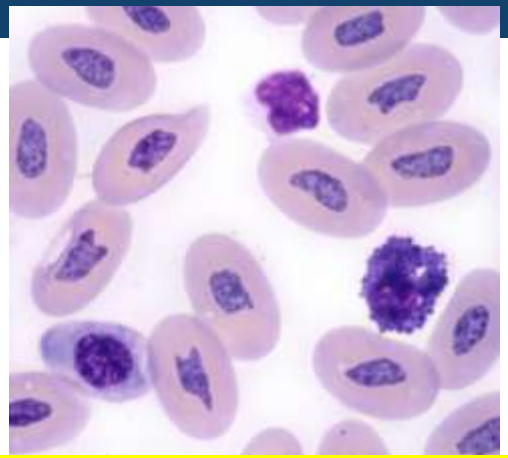


Swan: lobed granulocytes

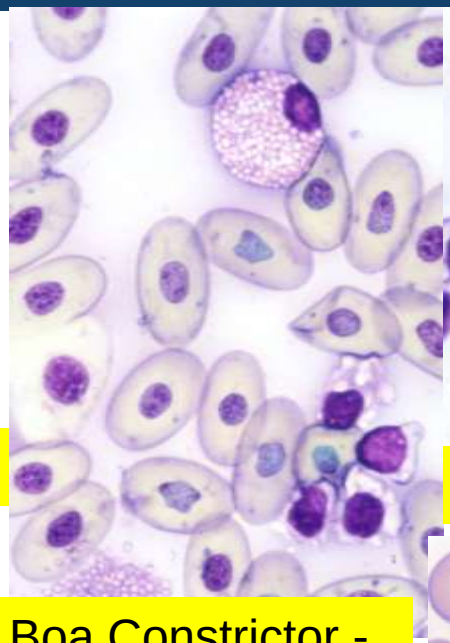
Reptile leukocytes and thrombocytes – everything is nucleated but lobing (mainly two) of heterophils and eosinophils is variable depending on whether the species is crocodilian, chelonian (turtles) or squamatan (lizards and snakes)

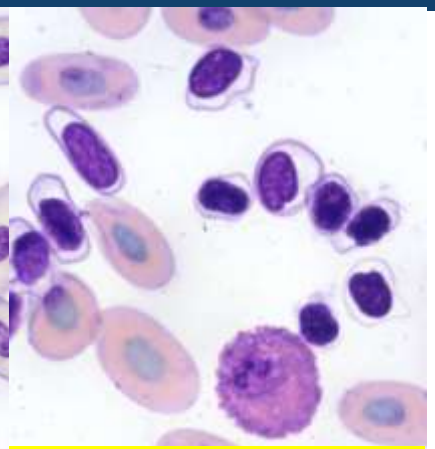


Bearded dragon



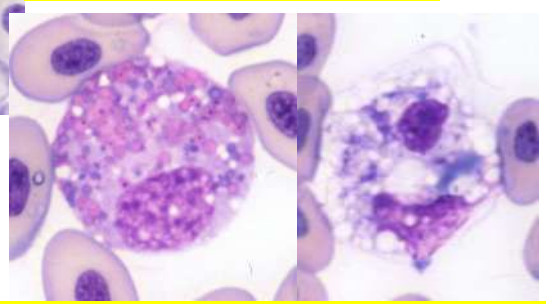
Blue-bellied Black Snake



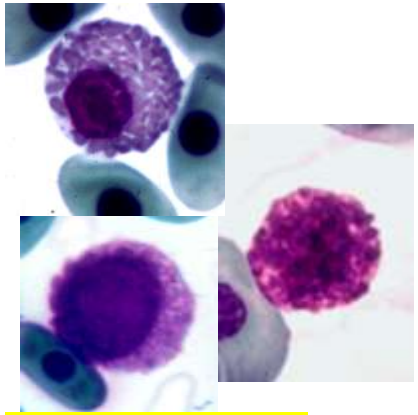


Green Python

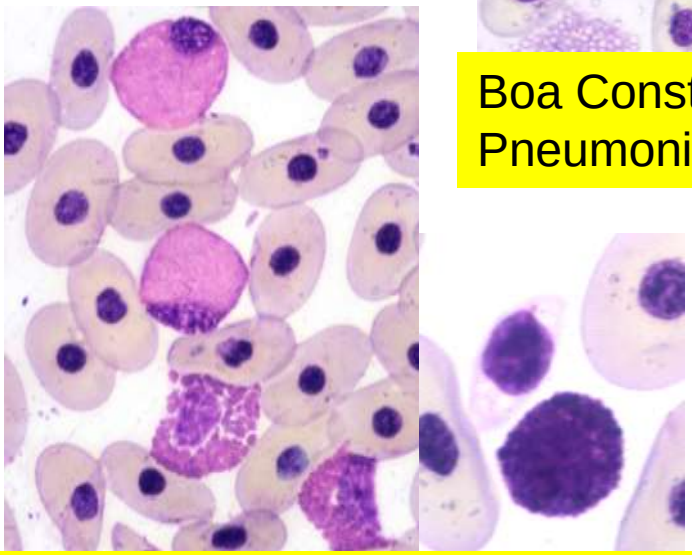
Boa Constrictor - Pneumonia



Diamond Python – septicaemia: toxic heterophil and macrophage



Saltwater Crocodile

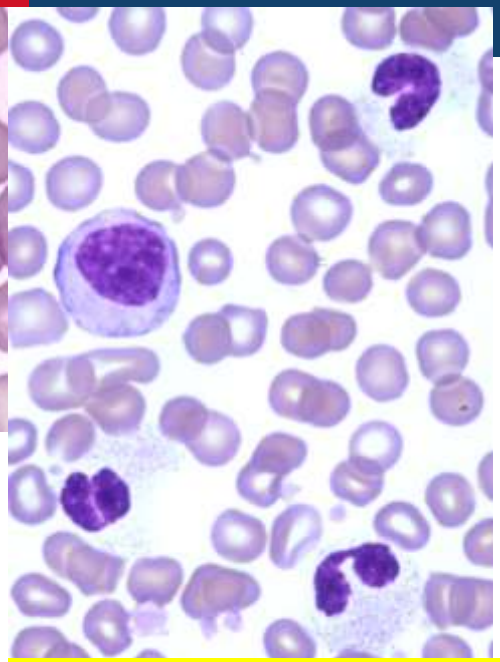


Short-necked Turtle – acidophils and basophil

Miscellaneous species leukocytes

Snapper fish have ‘neutrophils’ rather than ‘heterophils’ because the granules are less prominent and fine

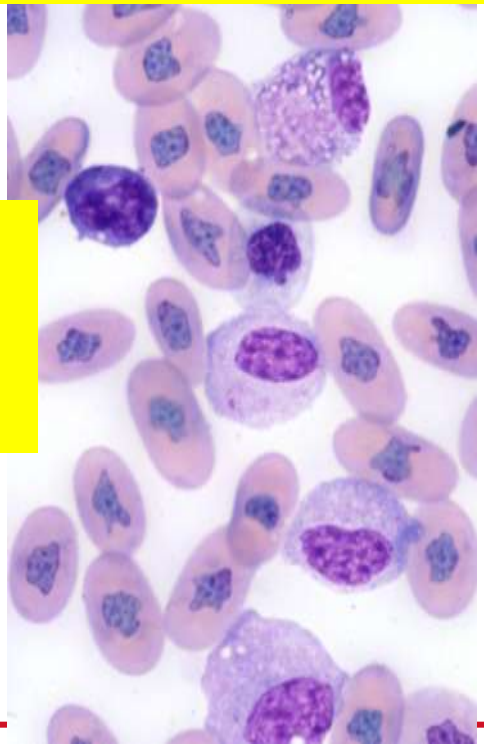
Owen’s Civet

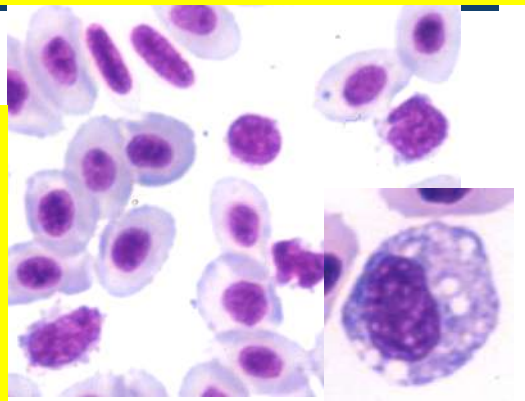


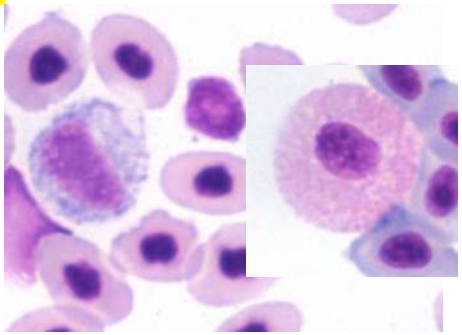
Koala – neutrophilia with left shift. Do the neutrophilic granules mean anything?

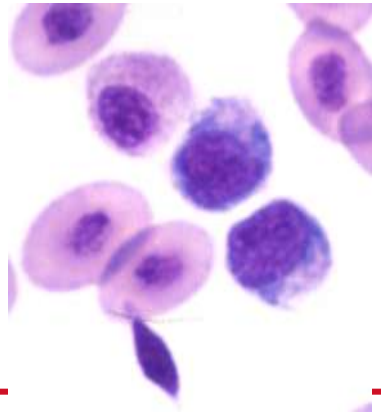
Tas Devil

Taipan – cloacal abscess – note monocytes plus irregular erythrocyte nuclear contour





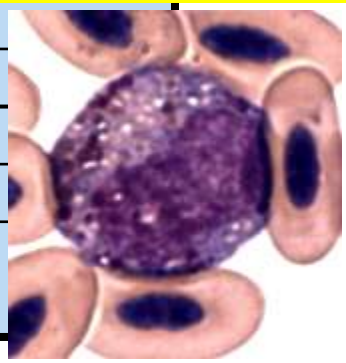




Taronga Wildlife Hospital: Female adult Metallic Starling (*Aplonis metallica*). It was in poor condition and dehydrated, with elbow and keel swellings. It was euthanased and found to have mycobacteriosis

Haematology	Results
RBC x 10^{12}/L (2.87-7.20)	4.01
Haemoglobin g/L (125-192)	ND
PCV L/L (0.39-0.64)	0.49
MCV fl (61-174)	147
MCH pg (31.7-43.6)	ND
MCHC g/L (250-339)	ND
WBC x 10^{9}/L (2.5-27.2)	**74.6**
Band Heterophils (0)	**1.5**
Heterophils (0.08-11.80)	**35.8**
Lymphocytes (0.32-18.2)	9.7
Monocytes (0.03-2.7)	**26.9**
Eosinophils (0.3-2.7)	0.7
Basophils (0.15-3.07)	**0**
Plasma Protein (refract-29-42)	**44**
Thombocytes x 10^{9}/L (15-30)	**11.88**
Plasma colour: *clear*	
Smear: *+anisocytosis, ++polychromasia; ++ toxic heterophils*	

Likely conclusions: the bird has a marked inflammatory process, which is most likely to be of infectious origin. Note low thrombocytes – can you trust the number?



Reference intervals from ISIS (International Species Information System)

Female adult Metallic Starling (*Aplonis metallica*).

Possible reasons for clinical pathology changes: the leukocytosis is due to marked heterophilia (left shift and toxic changes) and monocytosis related to the ongoing inflammation. The basopenia is of little consequence to this case. The anisocytosis and polychromasia is likely normal as the bird is not anaemic, but some compensation due to blood loss or destruction cannot be completely discounted (mycobacteriosis would more likely cause a non-regenerative anaemia) The hyperproteinaemia is due to dehydration and possible globulin increases related to the inflammation. The mild thrombocytopenia is likely to be due to increased utilisation related to the inflammation and tissue destruction (unless it is false due to clumping).

Likely conclusions: the bird has a marked inflammatory process, which is most likely to be of infectious origin.

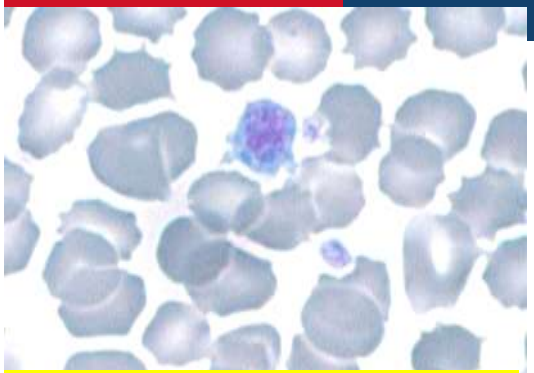
Platelets numbers and appearance – may be useful in assessing bleeding disorders, but can be confusing in other situations

- Platelet mass is the key to functional capacity (total nos x MPV)
  - look at buffy coat – the thrombocrit!
  - Platelet numbers measured by machine, manually or indirectly on blood film (a problem with clumping in cat and sometimes horse).
  - The blood film for morphology (eg mega/macroplatelets [shift platelets]).
- **Serious thrombocytopaenia** (assuming normal size and activity
  - Dog and cat less than 100 x 10^9/L (nb breed exceptions), Horse probably less than 50 x 10^9/L .
  - Causes include BM disease, splenic sequestration, increased utilisation or destruction (latter 2 lead to macroplatelets)
- Thrombocytopathy and thrombocytosis

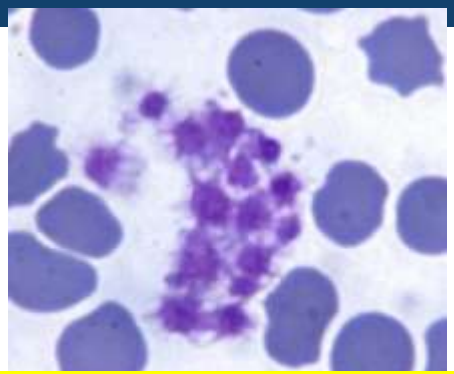
Platelets numbers and appearance – may be useful in assessing bleeding disorders, but can be confusing in other situations

- **Thrombocytopathy** – common, inherited or acquired. Numbers mean nothing.
- **Thrombocytosis –secondary (reactive)** far outweighs **primary (myeloproliferative**) , **physiological (splenic contraction**) and **pseudo-(false counting)** thrombocytosis. Causes can include inflammation (eg IL6 can stimulate TPO), neoplasia (through paraneoplasia and inflammatory cytokines), iron deficiency, drugs (eg corticosteroids) and loss of spleen (stores and destroys)

Platelets

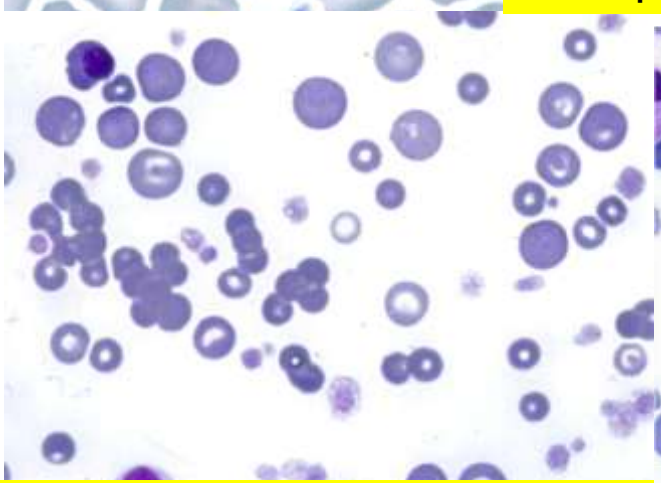


Macroplatelet in a dog

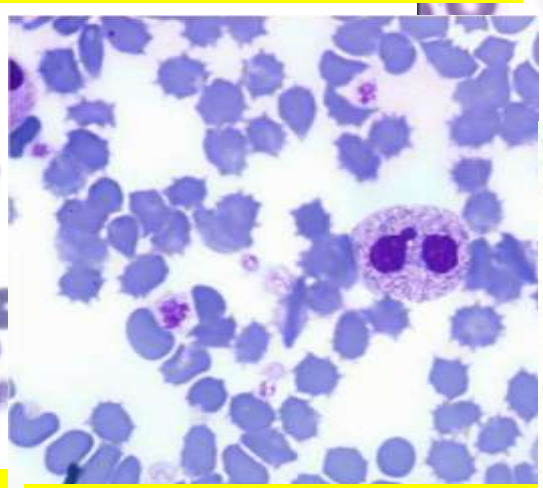


Clumping in a cat

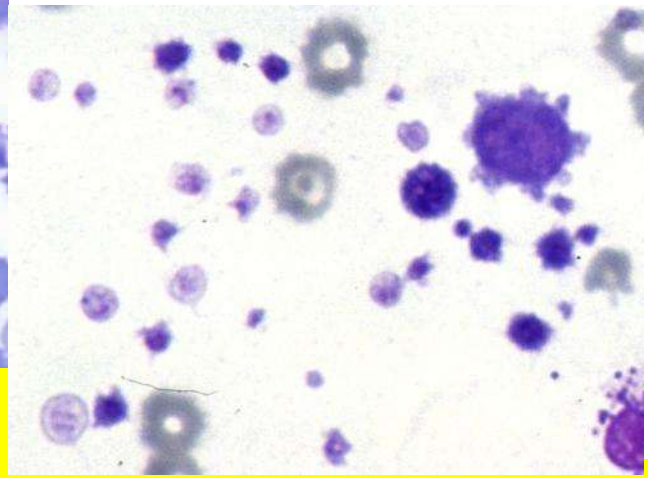
Micromegakaryocyte in a dog withAML-M7



AIHA plus ITP in a dog



Macroplatelet in a cat – significance?



Hypogranular platelets in a case of AML- M7

Useful references?

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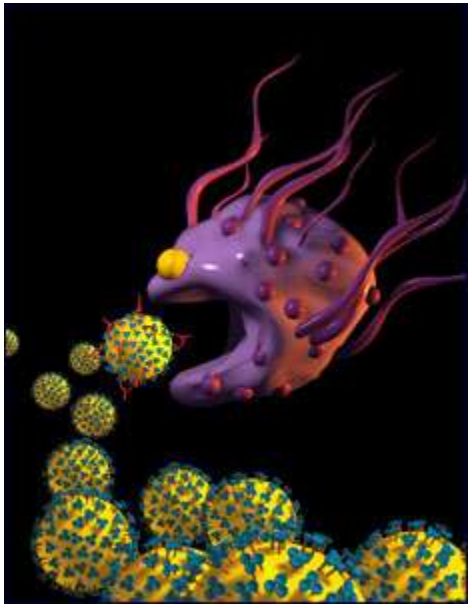
Avian Hematology and Related Disorders (2008). Elizabeth B. Mitchell, Jennifer Johns. *Vet Clin Exot Anim* 11:501–522.

Diagnostic Hematology of Reptiles (2011). Nicole I. Stacy, A. Rick Alleman, Katherine A. Sayler. *Clin Lab Med* 31:87–108.

Clinical Hematology in Reptilian Species (2013). Giordano Nardini, Stefania Leopardi, & Mattia Bielli. *Vet Clin Exot Anim* 16:1–30.

Cases for discussion



Cases for Discussion

Veterinary Clinical Pathology

Each case will have reasons for selection, for example:

- **pecularities of a species**
- **breed, age, sex or activity related effects on RI's**
- **biochemical and haematological disturbances related to organ and/or specific aetiologies that may be of interest or controversial**

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

What is acceptable about approach?

Everything!

- 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)?

Neil Horadagoda USYD Camden: 27 YO pony mare with lethargy, inappetence , weakness, possible ataxia and blood tinged nasal discharge

TEST	SAMPLE	REFERENCE VALUES
GGT IU/L	**394**	<36
AST IU/L	321	<400
ALP IU/L	**838**	<260
CK IU/L	278	<400
GD (GLDH) IU/L	3.5	0.9-4.7
Serum protein (biuret) g/L	**91**	60-76
Albumin (BCG) g/L	**24**	29-38
Globulins g/L	**67**	26-40
A:G ratio	**0.36**	0.62-1.46
Bilirubin total µmol/L	**55.9**	<50
TBA µmol/L	**70.2**	<20
Triglycerides mmol/L	**1.05**	<0.61
Glucose mmol/L	6.3	4.5-6.3
Urea mmol/L	**3.4**	3.7-8.2
Creatinine µmol/L	97	87-149
Calcium mmol/L (uncorrect)	3.1	2.8-3.3
Inorganic phosphate mmol/L	1.04	0.80-1.77
Magnesium mmol/L	0.58	0.73-0.91
Sodium mmol/L	135	132-150
Potassium mmol/L	3.3	2.8-5.0
Chloride mmol/L	107	99-110

TEST	SAMPLE	REFERENCE VALUES
Plasma appearance	Sl icteric	Clear
PCV L/L	0.45	0.32-0.52
Haemoglobin g/L	162	110-190
RBC $x10^{12}$/L	9.87	6.5-12.5
MCV fL	46	34-58
MCH pg	16	12-18
MCHC g/L	360	300-390
Plasma protein g/L (refract)	**86**	55-75
Leukocytes $x10^9$/L	**13.8**	6.0-13.0
Neutrophils (seg.) $x10^9$/L	**12.1**	2.5-6.9
Neutrophils (band) $x10^9$/L	0	0-0.24
Lymphocytes $x10^9$/L	**1.5**	1.6-3.4
Monocytes $x10^9$/L	0.2	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	248	80-300
Fibrinogen g/L	**4.5**	2-4
PT secs	**17** (C: 14)	10-15
PTT secs	**70** (C: 50)	37-54
Blood film: Neutrophils have slight cytoplasmic vacuolation. Mild RBC anisocytosis		

ABDOMINAL FLUID	SAMPLE	REFERENCE VALUES
Appearance	Yellow and slightly turbid	Clear and light yellow
Total protein g/L	**44**	<25
Erythrocytes x 10^6/L	None	None
Nucleated cells x 10^6/L	2500	<10,000
Smear	64% non-lytic (non-degenerate) neutrophils, 2% small lymphocytes, 34% monocytes/macrophages and mesothelial cells.	Scattered mix of mononuclear cells and non-lytic (non-degenerate) neutrophils

Likely conclusion: overall, most results can be explained by *chronic (primarily cholestatic) liver disease*

Ultrasound-guided needle liver biopsies were collected for bacterial culture (no growth) and histopathology.

Histopathologic changes (megalocytosis, biliary hyperplasia, periportal fibrosis) were consistent with **pyrrolizidine alkaloid toxicosis.**

Possible reasons for changes: the animal has elevated ALP and GGT suggesting marked cholestasis; although inappetence may be contributing to the hyperbilirubinaemia so may potential cholestatic liver disease, increased TBA support liver disease and possibly reduced hepatic function (low urea and albumin in the face of mild dehydration support reduced hepatic function – maybe end stage?; the potential for hepatic encephalopathy here with failure to convert ammonia to urea amongst other toxic elements?), the high globulins could be related to chronic liver disease. The hypertriglyceridaemia could be caused by cholestasis. Both the plasma protein and serum proteins are high due to increased globulins (dehydration may be contributing some, but then the albumin would be even lower!). The increased fibrinogen is possibly related to ongoing inflammation (mild leukocytosis due to neutrophilia with some toxic change), but its value could be subdued if there is increased liver production. The eosinopenia, marginal lymphocytopenia (and perhaps some of the neutrophilia) could be due to release of endogenous corticosteroid. The increased PT and PTT could fit in with decreased liver production of clotting factors. The abdominal fluid is a modified transudate.

Likely conclusions: overall, most results can be explained by *chronic (primarily cholestatic) liver disease*

Further testing: abdominal ultrasound and perhaps FNA/biopsy of the liver? Further questioning of the owner about exposure to plant poisons or chemicals?

Postscript: The mare was initially treated with intravenous fluids with added glucose, and parenteral B vitamins. A mild transient improvement in mentation and appetite occurred over the first 48 hours, however signs of Hepatic Encephalopathy persisted. Liver ultrasound examination was performed 48 hours after admission; a mild increase in echogenicity was recorded, however no evidence of cholelithiasis or a space occupying lesion was detected. Needle liver biopsies were collected for bacterial culture (no growth) and histopathology. *Histopathological changes (megalocytosis, biliary hyperplasia, periportal fibrosis) were consistent with pyrrolizidine alkaloid toxicosis.*

The prognosis for pyrrolizidine alkaloid toxicosis is poor given the anti-mitotic nature of the toxin and therefore the inability of the liver to regenerate. Most horses die or are euthanased within a few months of diagnosis. The mare has been discharged with recommendations of supportive care (offering palatable feeds, access to shade and water), and a poor to grave long term prognosis.

Common bleeding disorders

	PC	APTT	OSPT	Fib	FDP
Thrombocytopenia	↓	N	N	N	N
Vit K antagonism	N	↑	↑	N	N
Heritable factor deficiency	N	Variable	Variable		N
Hepatic insufficiency	N	↑	↑	↓	N
Acute DIC	↓	↑	↑	↓	↑

Thrombocytopathy or vascular endothelial disorder will not be detected by these tests

12-years-old entire male xbred dog with severe respiratory distress for a period of 2-3 weeks. The dog had a normal temp, was cyanotic and had a marked expiratory effort. Biochemistry showed mild increases in ALP, glucose, cholesterol, IP and bicarbonate (metabolic alkalosis)

Plasma appearance	Clear	Clear
PCV L/L	0.47	0.37-0.50
Plasma protein g/L (refractometer)	**85**	55-75
Haemoglobin g/L	**154**	100-150
Erythrocytes $x10^{12}$/L	6.64	5-7
MCV fl	71	60-75
MCHC g/L	328	300-350
MCH pg	23	20-25
Leukocytes $x10^9$/L	**59.1***	7-12
Neutrophils (seg.) $x10^9$/L	**52.17**	4.1-9.4
Neutrophils (band) $x10^9$/L	**1.24**	0-0.24
Lymphocytes $x10^9$/L	**0.89**	0.91-3.6
Monocytes $x10^9$/L	**4.85**	0.2-0.96
Eosinophils $x10^9$/L	**0**	0.14-1.2
Basophils $x10^9$/L	0	0-0.36
Platelets $x10^9$/L	250	200-600
Reticulocyte % (uncorrected)	**6.8**	0-1.5
Absolute reticulocytes $x10^9$/L	**451**	0-75
Blood film: **8 NRBCs per 100 WBCs. Moderate anisocytosis and polychromasia**		

*total leukocyte count has been corrected for the number of circulating nucleated erythroid cells

Likely conclusions: some of the changes (enhanced erythropoiesis and inflammatory demand) could be due to *cardio-respiratory disease*. Little else can be deduced and there are some confusing results.

Postscript: this dog had primary pulmonary carcinoma that was detected on diagnostic imaging, suspected on bronchoalveolar lavage and, later, confirmed on biopsy and at necropsy. The dog died and it was suspected that the dog had neoplastic complications, possibly pulmonary thromboembolic disease, but this was not confirmed at necropsy. In hindsight, some of the haematological changes could have been related to paraneoplastic phenomena (eg granulopoietin production by tumour cells).

Possible Reasons for changes: In light of the history, the animal has a respiratory problem, which could be due to cardiac or primary airway/Lung disease. With this in mind, the results of the laboratory tests may suggest the following – the increased proteins could be due to haemoconcentration (ie was the animal dehydrated?) since albumin is high end of normal. The increased ALP could be due to mild cholestasis (secondary effect on liver), or due to prolonged stress inducing an isoenzyme (corticosteroid responsive). Mild elevations are often seen in association with cardio-respiratory disease and it is difficult to determine the cause. A reason for the increased inorganic phosphate is not apparent from the history. The increased cholesterol could be related to liver disease or an endocrinopathy (no other evidence of the latter). The mild metabolic alkalosis could be related to the suspected cardio-respiratory disease affecting acid/base balance. The increased regeneration of erythrocytes (increased reticulocytes, nucleated erythroid cells and polychromasia and anisocytosis of erythrocytes on blood film; absolute reticulocytes are 0.451 x 10^{12}/L [RR 0-0.08]) **without** anaemia (compensating anaemia/increased stimulation?) could be related to cardio-respiratory disease (ie the hypoxia causing reduced supply of oxygen to the kidneys and stimulating erythropoietin production?). Increased turnover of erythrocytes through mild destruction cannot be discounted (compensated anaemia). The leukocyte changes could be partly due to stress (neutrophilia, lymphocytopenia, monocytosis and eosinopenia) but the very high levels of neutrophils and the left shift (>1.0 x 10^{9}/L) suggest inflammatory demand as well. This could be in response to cardio-respiratory disease (infection, neoplasia etc).

Likely conclusions, further investigation and implications for management and prognosis?: Some of the changes (enhanced erythropoiesis and inflammatory demand) could be due to cardio-respiratory disease. Little else can be deduced and there are some confusing results. Therefore, further investigation is mandatory. Radiographs, and possibly ultrasonographs, should be undertaken to assess cardio-respiratory disease. Blood gas analysis, if available, might be useful to assess respiratory function. If lung disease is detected on diagnostic imaging, a transtracheal aspirate or bronchoalveolar lavage might be useful. Fine needle cell aspirate (ultrasound guided) might be employed if distinct masses are detected. Some of you might want to investigate liver disease further because of the increased ALP and cholesterol. Some might want to put the dog on antibiotics because of the inflammatory demand. That is acceptable while further investigation is undertaken. (**Postscript**: this dog had primary pulmonary carcinoma that was detected on diagnostic imaging, suspected on bronchoalveolar lavage and, later, confirmed on biopsy and at necropsy. The dog died and it was suspected that the dog had neoplastic complications, possibly pulmonary thromboembolic disease, but this was not confirmed at necropsy.). In hind sight, the leukocytosis and left shift could have also been partly due to paraneoplasia (production of granulopoietin-like substance)?

Mechanisms associated with increased circulating nucleated erythroid cells

1. **Reduced splenic function** (eg newborn of many species, normal in some species, splenic infiltration, damage or removal)
2. **Compensatory erythropoiesis due to anaemia** (eg regenerative [haemolytic and blood loss], iron deficiency, extreme anaemia of any sort)
3. **Hypoxia** (eg CHF and severe pulmonary disease)
4. **Myelophthisis** (eg marrow infiltration: neoplasia or granulomatous inflammation; or marrow damage/fibrosis)
5. **Extramedullary haematopoiesis**
6. **Miscellaneous** (eg uraemia, sepsis, liver disease, DKA, forms of chemotherapy)

Benie T. Constantino, Bessie Cogionis (2000) Nucleated RBCs—Significance in the Peripheral Blood Film, Laboratory Medicine, **31:**4, 223-229

A 14 yr, male neutered, domestic short hair cat was presented with a prolonged history of polyuria/polydipsia, inappetence and occasional vomiting. On examination, the animal had pale mucous membranes and mouth ulcers. It was dehydrated, depressed and now oliguric.

TEST	SAMPLE	REFERENCE VALUES
Plasma appearance	Clear	Clear
PCV L/L	**0.12**	0.30-0.45
Plasma protein g/L	**92**	59-78
Haemoglobin g/L	**42**	80-140
Erythrocytes $x10^{12}$/L	**2.33**	6-10
MCV fL	**52**	40-45
MCHC g/L	350	310-360
MCH pg	**18**	13-17
Leukocytes $x10^9$/L	**17.5**	8-14
Neutrophils (seg.) $x10^9$/L	**17**	3.8-10.1
Neutrophils (band) $x10^9$/L	0	0-0.4
Lymphocytes $x10^9$/L	**0.2**	1.6-7.0
Monocytes $x10^9$/L	0.2	0.1-0.6
Eosinophils $x10^9$/L	0.1	0.2-1.4
Blood film: moderate numbers of burr cells, elliptocytes, schistocytes. Some ghost cells. Neutrophils have Doehle bodies.		
Reticulocyte % (uncorrected)	0.8	0-1

TEST	SAMPLE	REFERENCE VALUES
Amylase IU/L	**1720**	<1400
ALP IU/L	15	<50
ALT IU/L	48	<60
Serum protein (biuret) g/L	**77**	54-73
Albumin (BCG) g/L	28	19-38
Globulins g/L	48	25-50
Total cholesterol mmol/L	**5.3**	1.9-3.9
Glucose mmol/L	3.8	3.6-6.6
Urea mmol/L	**47**	7.2-10.7
Creatinine µmol/L	**924**	98-180
Calcium mmol/L	2.1	1.7-2.6
Inorganic phosphate mmol/L	**6.7**	1.3-2.3
Sodium mmol/L	153	147-156
Potassium mmol/L	**5.1**	4-4.6
Chloride mmol/L	**109**	115-130
Bicarbonate (TCO_2) mmol/L	**15**	17-24

Urinalysis (cystocentesis)	
Appearance: clear	pH: 6
Colour: light yellow	Glucose: -ve
Specific gravity: 1.013	Blood: -ve
Protein: **1+**	Bilirubin: -ve
Microscopic findings: much lipid, one leukocyte per HPF.	

Likely diagnosis: marked azotaemia and close to isosthenuric urine in a dehydrated animal usually means renal failure. Considering the history, the biochemical and haematological changes this is likely to be end stage renal disease.

(**Postscript:** the owners were given a grave prognosis and the option of supportive therapy. Euthanasia was agreed upon. A necropsy was not permitted).

Probable reasons for changes: marked azotaemia (both urea and creatinine) and close to isosthenuric urine in a dehydrated animal usually means renal failure. Considering the history this is likely to be end stage renal disease. This is supported by the hyperkalaemia and hyperphosphataemia and the metabolic acidosis (decreased bicarbonate) with increased anion gap (value 34.1 – RI 7-17). The hypochloridaemia is probably related to the vomiting or the polyuria. The increased osmolality (derived value is >310), The increased cholesterol can sometimes occur in certain types of renal disease. Hyperamylasaemia can occur in renal failure in the cat most likely related to decreased GFR (but may also be related to the vomiting in this case). The non-regenerative anaemia (no need to correct percentage as below 1%; absolute reticulocytes are 0.019 x 10^{12}/L [RI 0-0.06]) is probably related to renal failure and is due to lack of erythropoietin and increased turnover (some of the abnormal erythrocyte shapes indicate this). Burr cells can be seen in renal failure and are due to the toxaemia. The altered erythrocyte indices are possibly due to laboratory error (MCHC is normal). The leukocyte changes (leukocytosis due to neutrophilia, lymphocytopenia and eosinopenia) are probably due to terminal stress although some inflammatory component to the renal disease cannot be ruled out completely. Doehle bodies in neutrophils may indicate mild toxaemia (eg due to the azotaemia and other metabolic disturbances present), but normal cats may have low numbers. The 1+ proteinuria in unconcentrated urine is significant and is probably due to tubular or glomerular damage as there is no evidence for cystitis. It is probably the reason why the urine specific gravity is just outside the isosthenuric range (it can falsely elevate the value registered on the refractometer).

Likely conclusions and further investigation: overall, the results are consistent with *chronic renal failure*. Further investigation could involve palpation of the kidneys or diagnostic imaging. If they were large and irregular, a fine needle cell aspirate might be considered to detect inflammatory or neoplastic disease.

(**Postscript:** the owners were given a grave prognosis and the option of supportive therapy. Euthanasia was agreed upon. A necropsy was not permitted).

Steven Kopp University of Queensland – Friesian dairy cow with mastitis

TEST	Cow	REF VALUES
CK IU/L	**475**	<228
AST IU/L	**513**	<150
GGT IU/L	46	<60
LDH IU/L	**1726**	<800
Serum protein g/L	75	59-86
Albumin g/L	33	25-42
Globulins g/L	**42**	25-41
A:G ratio	0.79	0.7-1.2
Tot bilirubin µmol/L	**11.50**	<10
Glucose mmol/L	2.8	2.5-5.00
Urea mmol/L	**15.1**	2.1-10.7
Creatinine µmol/L	**234**	20-177
Cholesterol mmol/L	1.9	2.1-6.6
Calcium mmol/L	**1.97**	2.0-3.10
In phosphate mmol/L	**3.11**	1.5-2.9
Triglycerides mmol/L	**0.80**	2.1-3.1
Magnesium mmol/L	1.09	0.5-1.75
Sodium mmol/L	140	132-152
Potassium mmol/L	**6.2**	3.9-5.8
Chloride mmol/L	99	95-111
Bicarbonate mmol/L	**14**	19-36
Anion gap mmol/L	**33.2**	6-14

HAEMATOLOGY	Cow	REF VALUES
Plasma appearance	Clear	Clear
PCV L/L	0.32	0.24-0.46
Haemoglobin g/L	113	80-150
Erythrocytes $x10^{12}$/L	6.46	5.0-10.0
MCV fl	50	40-60
MCHC g/L	350	300-360
Leukocytes $x10^9$/L	**1.6**	4-12
Neutrophils (seg.) $x10^9$/L	**0.07**	0.6-4.0
Neutrophils (band) $x10^9$/L	**0.7**	0-0.2
Lymphocytes $x10^9$/L	**1.35**	2.5-7.5
Monocytes $x10^9$/L	**0.11**	0.25-0.84
Eosinophils $x10^9$/L	0	0.0-2.4
Basophils $x10^9$/L	0	0-0.2
Platelets $x10^9$/L	127	100-800
Blood film: sample had platelet clumping, slight anisocytosis and polychromasia		

Two weeks previously: mixed organisms cultured from milk, but most consistent isolate was *Staphylococcus aureus.*

Possible reasons for changes: The leukogram changes (significant leukopaenia due to marked mature neutropaenia with left shift, lymphocytopaenia and monocytopaenia) could be due to early high demand inflammation and corticosteroid release. There is also the possibility that bacterial toxin release (eg endotoxaemia or exotoxins) may be influencing the values (although no toxic changes are present in neutrophils). The mildly elevated LDH, CK and AST could be related to muscle/tissue damage, especially if the cow had become recumbent or is in shock. The borderline increase in globulins may be partly due to increased acute phase proteins, but some degree of dehydration cannot be completely excluded (despite the total protein being in the reference interval). The mild hyperbilirubinaemia could be due to anorexia. The mild azotaemia could be prerenal if the animal is dehydrated, undergoing protein catabolism, and possibly in shock (decreased GFR). However, a renal component to the azotaemia cannot be excluded without urinalysis. The borderline decrease in calcium and increased inorganic phosphate and potassium are difficult to explain, but if some renal disease is present that may be contributing. The low triglycerides may be related to the current body condition of the animal. The metabolic acidosis with increased anion gap could be related to renal disease, but if the animal is in toxaemic shock then lactic acidosis (anaerobic glycolysis and bacterial production?) might be the cause?

Likely conclusions: the animal appears to have overwhelming inflammation with the possibility of dehydration or even shock. Renal failure cannot be excluded without urinalysis, but endotoxaemic shock (or even exotoxin effects associated with staphylococcal gangrenous mastitis) may be responsible for many of the biochemical changes. Unfortunately, no other history is available for this animal, so one can only *speculate* as to whether this animal developed staphylocccal gangrenous mastitis (related to previous bacterial isolates) or perhaps even developed a peracute coliform mastitis with endotoxaemia at the time of bleeding? Irrespective of the likely organism involved, the prognosis is probably poor because of the systemic impact.