Session 2 Haematology - Erythrocytes

- 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

Blood – the oldest of the investigative sciences?

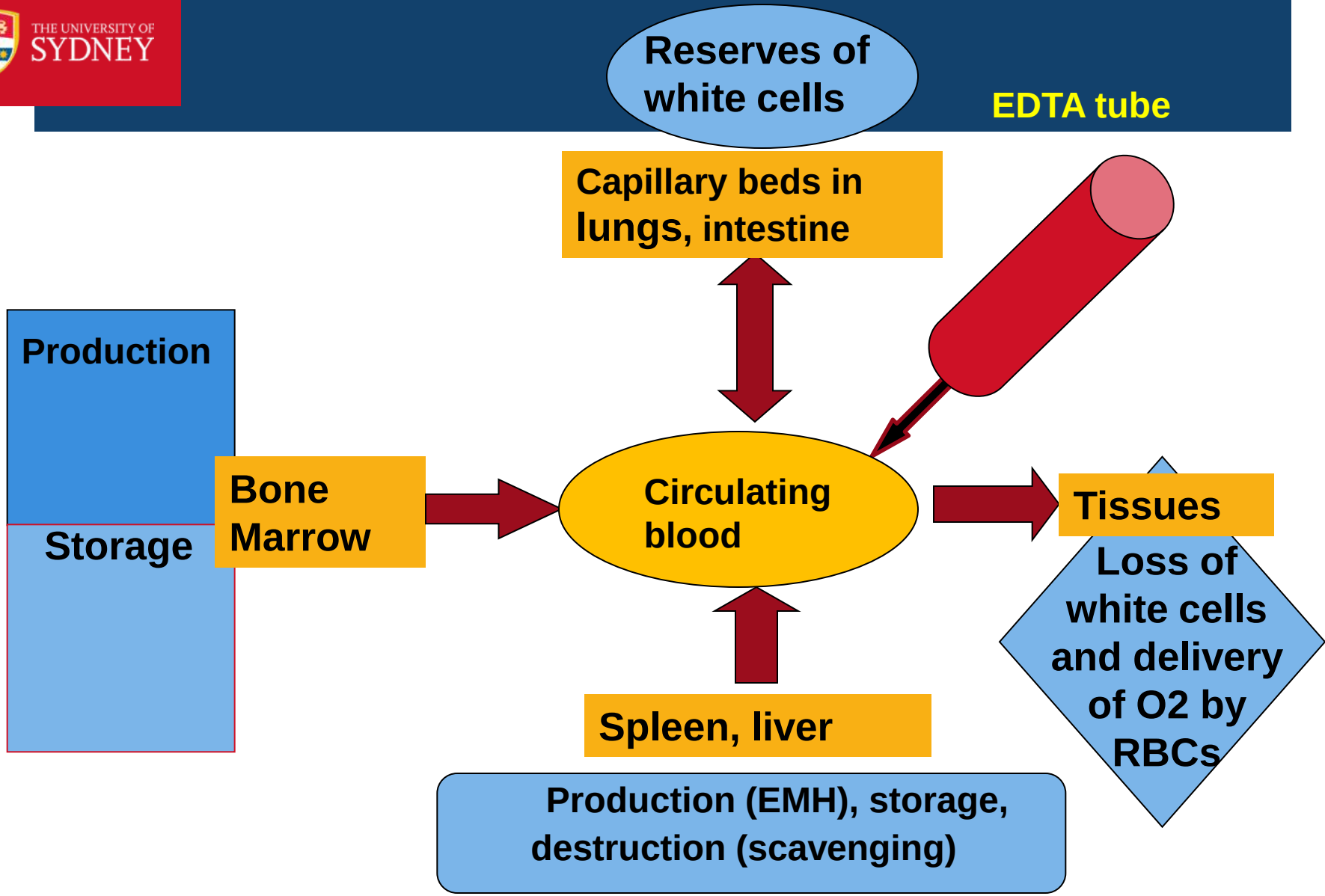
- The Egyptians and ancient Greeks use blood letting to treat ‘imbalance of the humours’
- Jan Swammerdam (1658)and Anton van Leeuwenhoek (1674) use microscopes to describe erythrocytes
- The study of blood occurred much before the development of histopathology and laboratory haematology (mid to late 1800’s). Consequently, medicine rather than pathology has had a greater influence on approach and interpretation (eg clinical problems: anaemia, leukaemia, and bleeding disorders).

So if haematological investigation is viewed by most practising veterinarians as a clinical problem, how can the pathologist help help?

- The interconnection of haematology with other laboratory results (using more of a pathological rather than clinical approach)
- Looking down the microscope!
- Understanding the complexity of haematological results and looking further at the haematopoietic system

What do I think is a major limitation of the FBC?

- It is done on **peripheral (circulating) blood** which reflects the *net result* of what is happening in all of the haematopoietic tissues
  - I need to keep reminding myself that components of the haematopoietic system also include: **bone marrow** (Production, Storage), **spleen** (Prod, Stor, Destruction), **liver** (Prod, Destr), **capillary beds** (leukocytes) and **tissues** (Utilisation)


Reserves of white cells
EDTA tube
Capillary beds in lungs, intestine
Production
Storage
Bone Marrow
Circulating blood
Tissues
Loss of white cells and delivery of O2 by RBCs
Spleen, liver
Production (EMH), storage, destruction (scavenging)

Anaemia – FBC the starting point in the laboratory!

› Haemic cytopathology

- **Peripheral blood** - erythrocyte morphology and cause
- **Bone marrow –** regeneration and cause

› Histopathology

- **Bone marrow** biopsy for architecture
- **Spleen and liver** for EMH and destruction
- **Tissues** for utilisation

I need to keep reminding myself of the complementary nature of the two disciplines!

Aspects of anaemia of use in diagnosis: The regenerative response and its variation amongst species

- **The similarities in haemorrhagic or haemolytic (regenerative) anaemia:**
  - *Response is rapid* (a few days - 2-3+ for most species) and involves increased circulating levels of reticulocytes (nucleated in non-mammals) and early forms (nucleated in all) for MOST species
  - The *greater availability of iron*, and the more extreme the drop in PCV, influences the intensity of the regenerative response
  - There is probably as much *variation* in response amongst mammalian species (I'm including marsupials as well as eutherians) as there is across Orders of birds, reptiles and fish
  - Detecting *cytoplasmic polychromasia* useful for aggregate reticulocytes, but not so much for punctate (later maturation) forms
  - Anisocytosis (macrocytes) useful for aggregate retics in mammals; but polychromatophilic (immature) cells can be smaller than mature cells in non-mammals (ie *use MCV values with care in lower orders of animals*!)

Who uses the indices MCHC and MCV in regenerative anaemia in mammals? MCH R.I.P.?

I know regenerative anaemia is supposed to be (pseudo – transient?)macrocytic and hypochromic – but what about primarily intravascular haemolytic anaemias with hyperchromic change and red cell changes that can alter the MCV and MCHC?

Direct measurement of Hb in erythrocytes using laser technology (called CHCM) means that haemolysis and lipaemia minimizes the impact on MCHC but Heinz bodies may still give false highs (optically dense)

What about horses and the use of MCV (and RDW) to indicate regenerative anaemia?

(Jim Taylor DPIPWE Launceston) – one year old Merino sheep Hx: Illness in flock for about 10 days. There were now 12 plus dead and about 12 ill in a flock of 1500. This sheep killed (moribund) and had brown mucous membranes and blood. Fat was also brown . Cut surface of liver was rather red/gold and the kidneys were very dark and haemorrhagic.

RBC	**3.13**	x 10 12 /L	(9.00 - 15.00)
Hb	**49**	g/L	(90 - 150)
Hct	**0.11**	L/L	(0.27 - 0.45)
MCV	35.1	fL	(28 - 40)
MCH	**15.7**	pg	(8 - 12)
MCHC	**445**	g/L	(310 - 340)

WBC	**18.1**	x 10 ^9 /L	(4.0 - 12.0)
Neutrophils	54 %	**9.77** x10 ^9 /L	(0.7 - 6.0)
Band Forms	0 %	0.0 x10 ^9 /L	(< 0.4)
Lymphocytes	27 %	4.89 x10 ^9 /L	(2.0 – 9.0)
Monocytes	3 %	0.54 x10 ^9 /L	(< 0.9)
Eosinophils	2%	0.36 x10 ^9 /L	(< 0.2)
NRBCs	14%	**2.53** x 10 9 /L	(0)

FILM MORPHOLOGY: RBC morphology: **anisocytosis +++, poikilocytosis +++, hypochromasia, polychromasia +, Heinz Bodies ++** ;WBC morphology: okay; **macroplatelets present**

Sodium	**129**	mmol/L	(139 - 152)
Chloride	95	mmol/L	(95 - 103)
Urea	**51.76**	mmol/L	(2.8 – 7.2)
Creatinine	**800**	umol/L	(70 - 97)
Calcium	2.89	mmol/L	(2.40 - 3.20)
Phosphate	**3.66**	mmol/L	(1.61 - 2.35)

Protein	76.4	g/L	(60 - 82)
Albumin	28.5	g/L	(25 - 40)
Globulin	**47.9**	g/L	(30 - 42)
A:G ratio	**0.59**	L/L	(0.6-1.3)
T. Bilirubin	**118.5**	umol/L	(< 9)
GLDH	**80**	U/L	(0.3-33)
GGT	**133**	U/L	(30 - 66)
CK	**4556**	U/L	(69 - 182)

SERUM INDICES
(Clear/+/++/+++/++++)

Icterus index	uncertain
Lipaemia index	Clear
Haemolysis index	**brown-red**

PM Report: liver necrosis, cholestasis and pigment accumulation in hepatocytes and Kupffer cells (positive on rubeanic acid). Kidney had haemoglobinuric nephrosis. Serum copper 45 µmol/L (RI 7.5-20.0 µmol/L).
Diagnosis: subacute/chronic copper poisoning

A 5 years old female pony with weakness, rapid respiration, dark urine and jaundice. Noticed after a 3-day period when the horse was left in a paddock.

HAEMATOLOGY	SAMPLE	REFERENCE INTERVAL
Plasma appearance	Red-yellow	Variable
PCV L/L	**0.10**	0.32-0.52
Plasma protein g/L	**82**	58-78
Haemoglobin g/L	**47**	130-190
Erythrocytes $x10^{12}$/L	**2.1**	6.5-12.5
MCV fl	47	41-49
MCHC g/L	**470**	300-390
Leukocytes $x10^9$/L	**17.1**	6.0-13
Neutrophils (seg.) $x10^9$/L	**14.6**	2.5-7
Neutrophils (band) $x10^9$/L	0.2	0-0.2
Lymphocytes $x10^9$/L	**1.4**	1.6-5.4
Monocytes $x10^9$/L	**0.9**	0-0.7
Eosinophils $x10^9$/L	**0**	0.2-1
Basophils $x10^9$/L	0	0-0.4
Blood film: **many ghost erythrocytes, some eccentrocytes and Heinz bodies**		

BIOCHEMISTRY	SAMPLE	REFERENCE INTERVAL
Total bilirubin µmol/L	**160**	0-50
Unconjugated bilirubin µmol/L	**154**	3.4-50
Conjugated bilirubin µmol/L	6	0-6.8
Urea mmol/L	**16**	3.7-8.2

SERUM INDICES Clear/+/++/+++/++++)

Icterus index	**++**
Lipaemia index	Clear
Haemolysis index	**+++**

URINALYSIS (voided)			
Appearance	Cloudy	PH	6.0
Colour	Red	Glucose	-ve
Specific gravity	1.021	Ketones	-ve
Protein	**4+**	Blood	**4+**
		Bilirubin	-ve
Microscopic findings: 0-2 erythrocytes per HPF			

phenothiazine (anthelmintic) poisoning – an old case!

Likely interpretation and possible conclusions: The results (the low PCV, high MCHC [through the presence of free Hb and the Heinz bodies falsely elevating the Hb reading through increasing optical density], haemoglobinuria and high unconjugated bilirubin) suggest that the pony has had a recent intravascular hemolytic crisis (**conclusion)**. Some of the increase in unconjugated bilirubin is probably due to anorexia. Ghost cells (ie ruptured erythrocytes) and Heinz bodies (denatured Hb) suggest haemolysis through Hb denaturation mechanisms (sulphydryl groups on Hb can be oxidised to give sulphhaemoglobin (methaemoglobin formation may or may not accompany oxidative damage) nb cat has more sulphydryl groups than most species and this is one reason why they are more susceptible to Heinz body formation – one of the spleen's macrophage role is to remove Heinz bodies). Oxidative chemicals (Drugs and plants) could be causes (**conclusion)**.

The hyperproteinaemia is possibly partly spurious, perhaps related to free Hb elevating the reading on the refractometer (at least giving a fuzzy line). However, some haemoconcentration can't be ruled out. The leukocyte changes can be explained by corticosteroid release (monocytosis rarely occurs in horses). The mild azoatemia is probably related to protein catabolism and reduced renal blood flow through any haemoconcentration (pre-renal azotaemia). The positive urinary blood strip is assumed to be due to free Hb as there is no indication of muscle damage and intact erythrocytes are few in the sediment. The 4+ protein is probably related to the 4+ blood reading. No bilirubin is being passed as it is almost all unconjugated in the blood (only conjugated is passed in the urine of most domestic species - the dog is an exception).

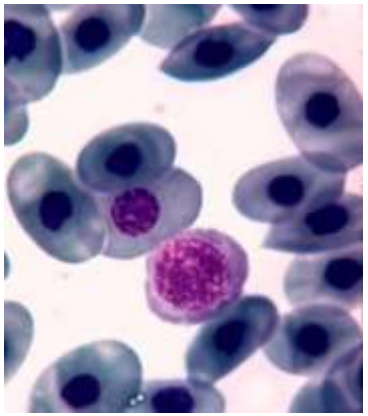
Further investigation would involve obtaining more history from the owner about the environment and the past history for the horse. Did it have access to poisonous plants? Had it access to chemicals?

Diagnosis and postscript: Heinz body hemolytic anemia. On further questioning of the owner, it was found that the horse had been given phenothiazine (anthelmintic) prior to being released in the paddock. If high enough dose it could have caused the haemolysis. The horse died a day later.

Those reticulocytes and other indicators of regeneration

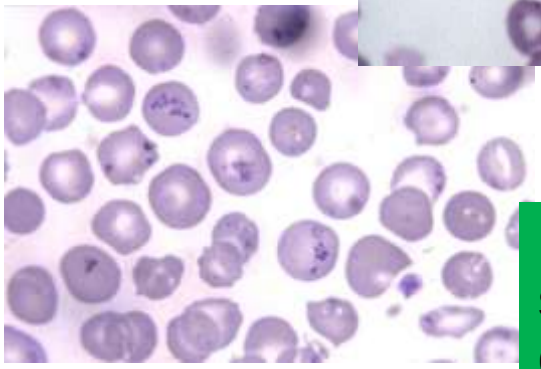
Dog - IMHA

Ibis - regeneration

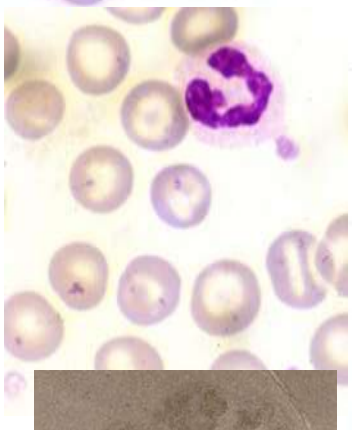


Crocodile - regeneration

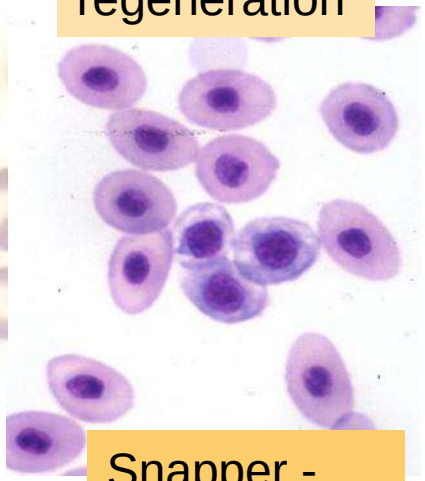
Cat – basophilic stippling in regeneration



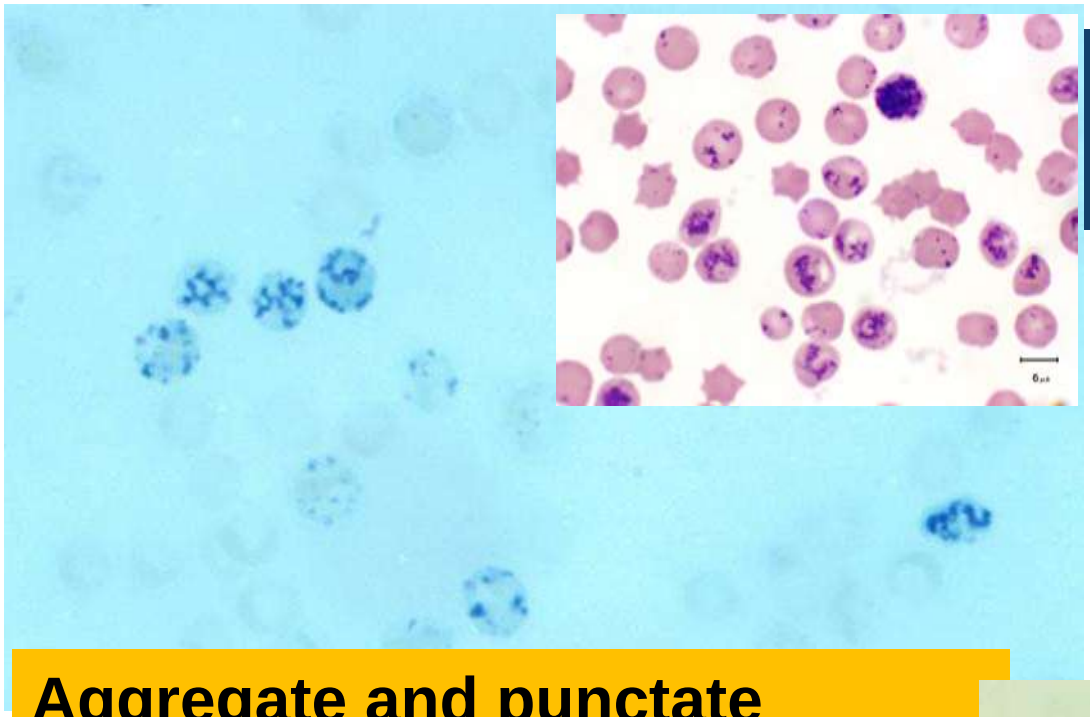
Dog- basophilic stippling (familiar defect)



Koala tick anaemia



Snapper - regeneration

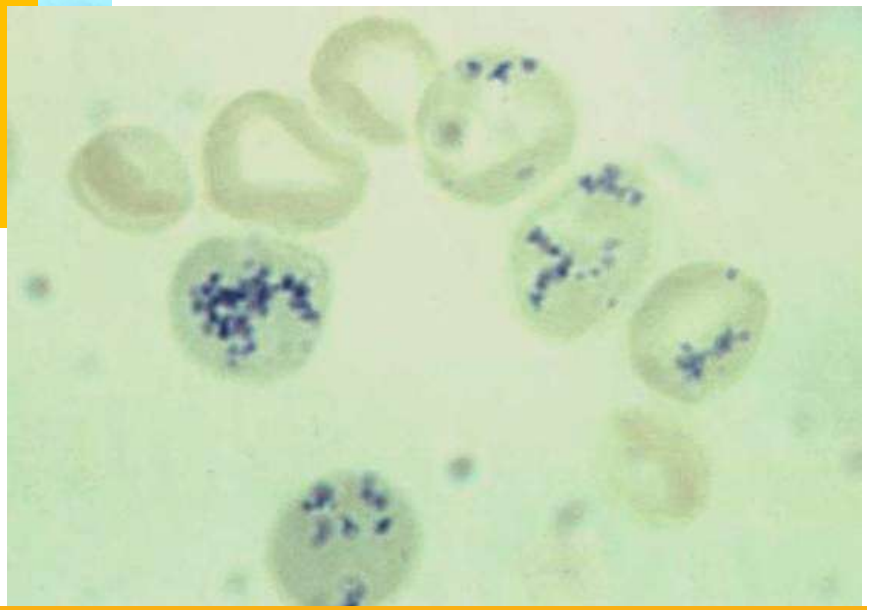


Need to do reticulocyte counts in the cat to be certain of regeneration. Can you get away with polychromasia on the blood film in the dog?

Reticulocyte smears made from blood mixed with a supravital stain eg BCB

Aggregate and punctate reticulocytes in a cat – what does your lab measure?

Punctate reticulocytes in the cat are not usually counted (can be as many 10% present in health), but can be useful at times if markedly increased – past regeneration?

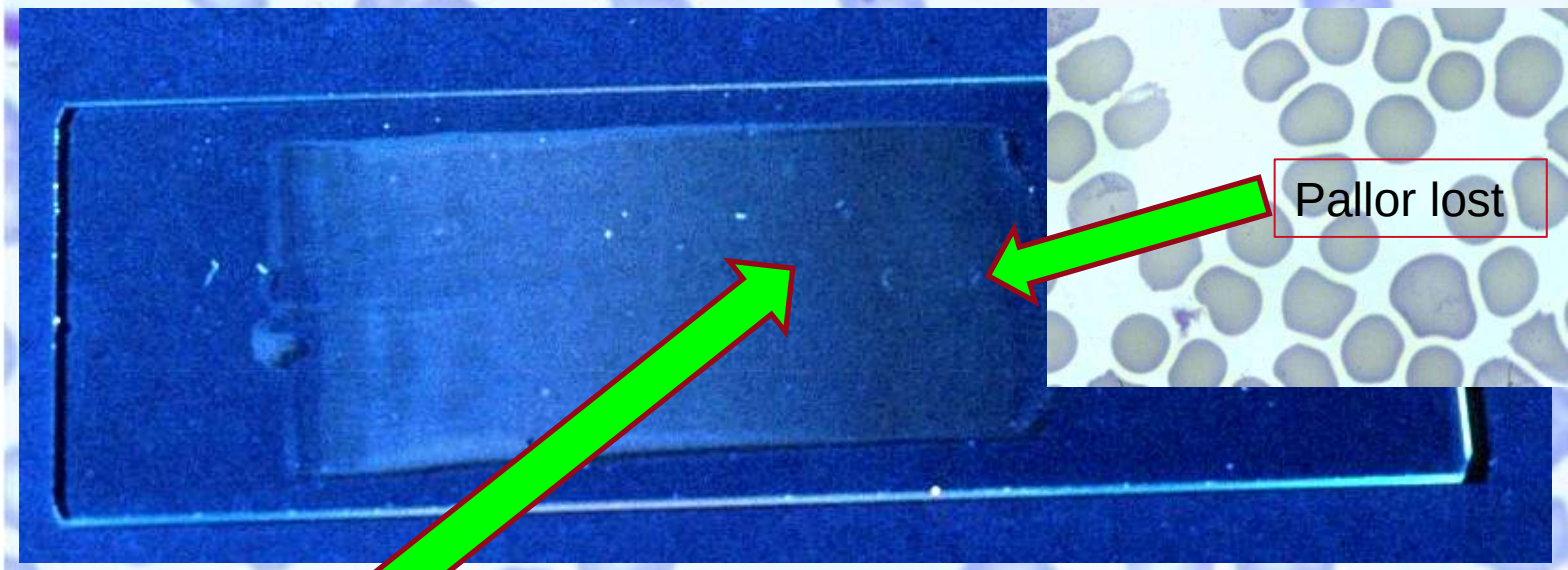


Aggregate reticulocytes in a dog

Splenic erythroid scavenging – why is there such species variation?

- Erythrocytes are **recycled** at the end of life span through **intravascular lysis** or removal by **splenic** (and hepatic) **phagocytes** (haemolytic anaemia uses both mechanisms)
- **Splenic macrophages** (also 'pit' circulating NRBCs and remove abnormal cells – and parasites!). A back up system for those bone marrow macrophages ('nurse cells'). The effectiveness of the spleen in this activity seems to depend on its circulatory pattern and relationship to littoral cells
- *In other words, what you see in normal circulating blood and any form of regenerative anaemia, at least in mammals, depends on the leakiness of bone marrow and the scavenging potential of macrophages in bone marrow, spleen (and perhaps liver)*

‘Normal’ cat film –who has trouble picking up central pallor?



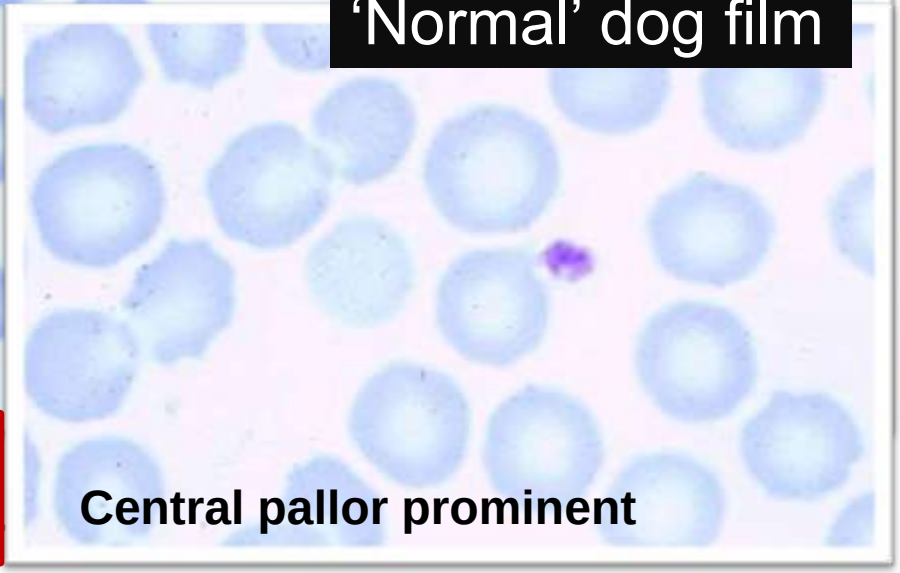
Crenation

Pallor lost

Pallor and erythrocyte concavity retained

HJ body

‘Normal’ dog film



Central pallor prominent

‘you have to know the normal before you can detect the abnormal’

Non-regenerative anaemia – how can you help the referring veterinarian?

- Chronic illnesses because erythrocytes have finite life span
  - Mammals – great variability: cattle 160 days, sheep 150 d, horses 145 d, goats 125, dogs 110-120 d, pigs 85 d, cats 70 d, rabbits 45-68, mice 19-25). May be shorter in the very young of the species!
  - Birds – generally shorter than eutherian mammals, but still variability (eg pigeons 35-45 d, chickens 28-35 d)
  - Reptiles – often longer than birds and mammals (eg Turtles > 500 d ; some can be up to 800 d)
  - Fish – 80-500 d

Why the variation? – cell lifespan influenced by body mass, cell mass and temperature (metabolic rate determinants), whilst replicative capacity is primarily affected by body mass!

Erythrocyte morphology – a minefield?
The change has to be in a significant number of erythrocytes

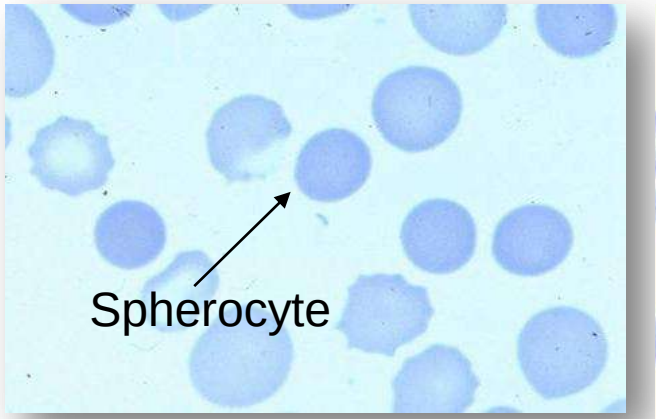
Erythrocyte Morphology - number of cells per oil field of 200 - 250 erythrocytes – adapted from Weiss DJ 1984 VetClinPath 13:27-31 and Reagan WJ et al 2008 Veterinary Hematology 2nd Ed Wiley Blackwell

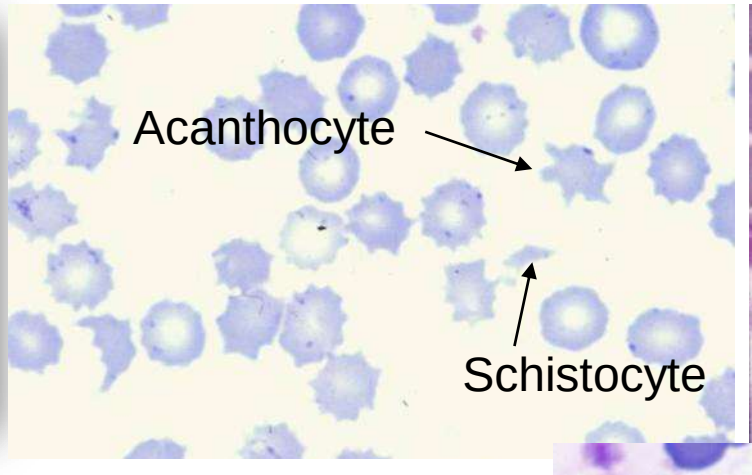
Is any cell morphological change pathognomonic for a specific disease? In my view rarely, but it does indicate the patho-mechanism. What is your view?

ABNORMALITY	SLIGHT (1+)	MODERATE (2-3+)	MARKED (4+)
Anisocytosis			
Dog	7-15	16-29	>29
Cat	5-8	9-20	>20
Horse	1-3	4-10	>10
Cow	10-20	21-40	>40
Polychromasia			
Dog	2-7	8-29	>29
Cat	1-2	3-15	>15
Horse	rarely observed (except in foals)		
Cow	2-5	6-20	>20
Poikilocytosis *(only used when there is a variety of abnormal shapes)*			
All species (except pig, and neonatal calves and kids, where may be normal))	3-10	11-50	>50
Hypochromasia			
All species	1-10	11-50	>50
Codocytes			
Dogs only	3-5	6-30	>30
Spherocytes			
All species*	1-5	5-50	>50
Echinocytes			
All species	1-2	3-20	>20
Acanthocytes, schizocytes, keratocytes, elliptocytes, dacryocytes, depranocytes, stomatocytes			
All species	1-2	3-20	>20

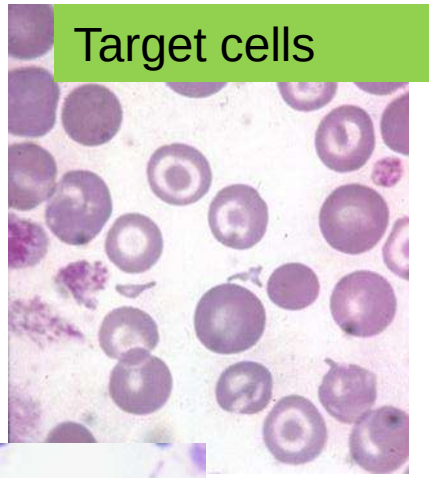
* spherocytes are not so easily identified in species with small erythrocytes (eg cats, ruminants)

Erythrocyte morphological changes in anaemia

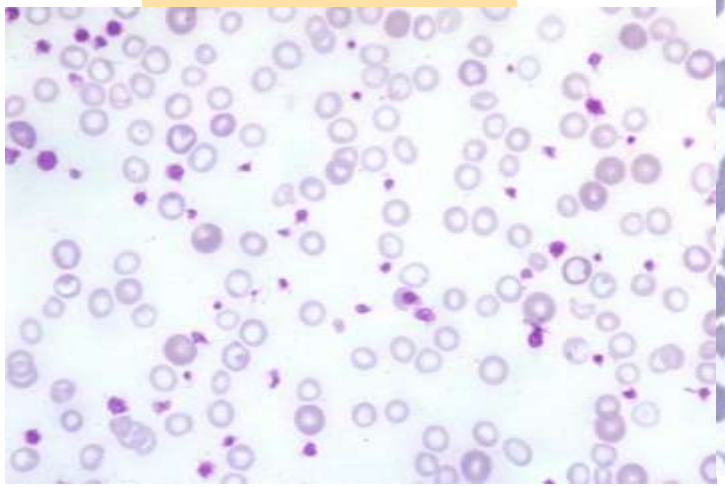




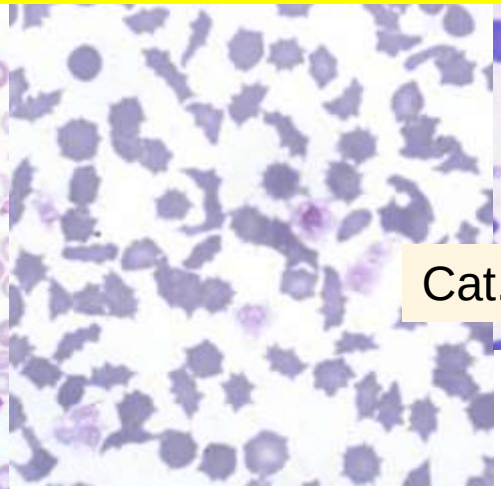
Target cells

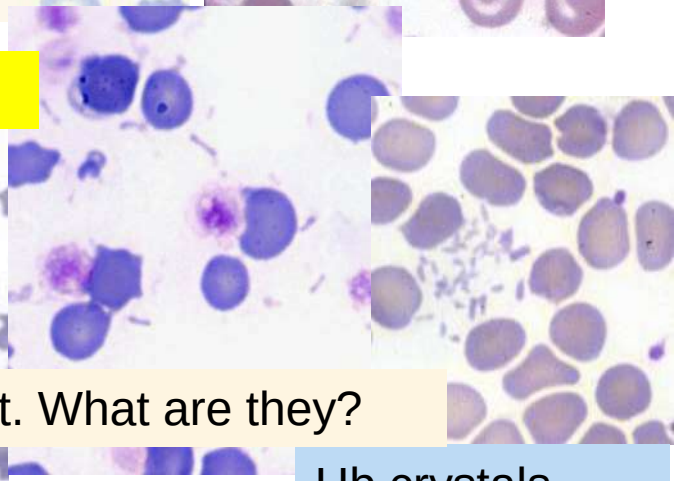


Hypochromasia



Poikilocytosis in a cat





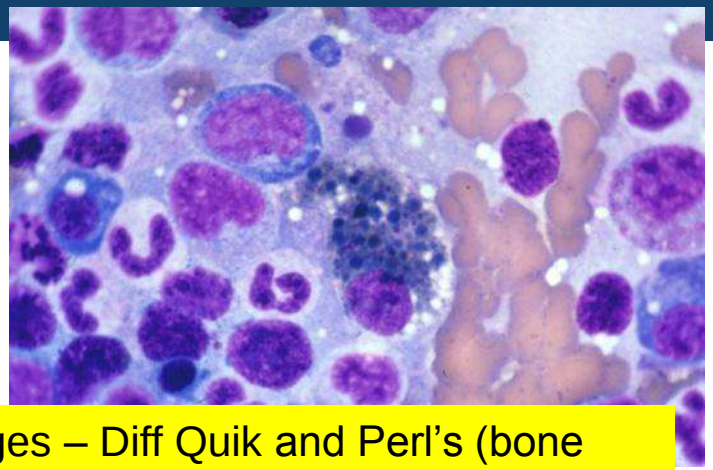
Cat. What are they?

Hb crystals – agouti and dog

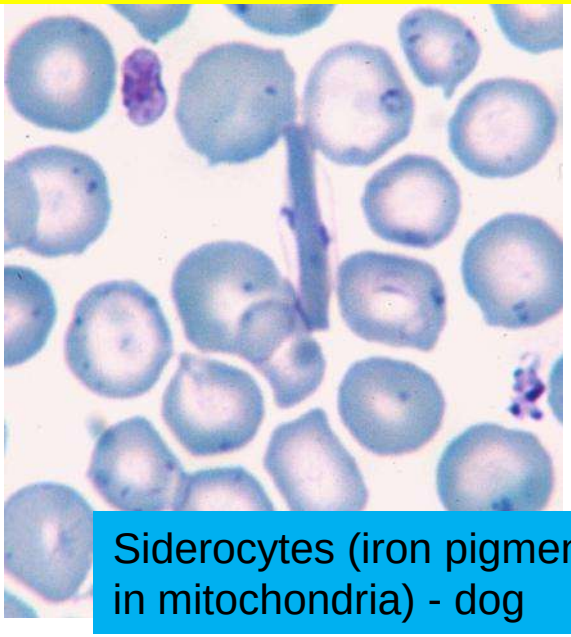
Disorders of Iron - Iron constipation versus iron deficiency

- I will raise this again in the Bone Marrow session in relation to haemosiderin assessment (iron storage in the insoluble form)
- What else is there to measure to assess iron metabolism?
  - Serum ferritin (species specific measurement), Serum iron and TIBC (indirect measure of serum transferrin)
- **Sideroblastic anaemia** (disorders of heme synthesis with iron accumulation in mitochondria) - inflammatory disease, some drugs and chemicals in dogs (other causes?). Pyridoxine deficiency in pigs (does it ever occur?)
- **Cu deficiency in ruminants** is a form of functional iron deficiency (hephaestin and ceruloplasmin affected)
- **Iron overload** – chronic haemolytic anaemias and secondary haemochromatosis
- **Iron deficiency and functional iron deficiency (inflammatory) anaemias**

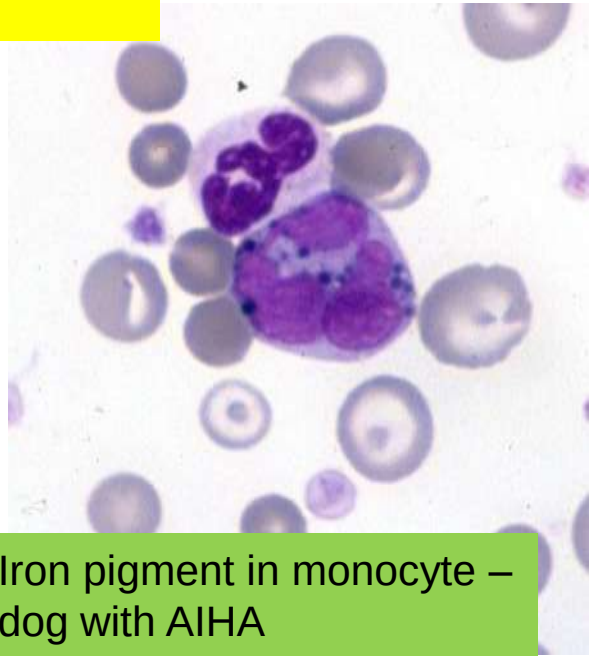
Disorders of Iron



Iron pigment in macrophages – Diff Quik and Perl's (bone marrow)



Siderocytes (iron pigment in mitochondria) - dog



Iron pigment in monocyte – dog with AIHA

Measurement	Iron deficiency anaemia (microcytic hypochromic – hypochromasia mainly seen in dogs and ruminants)	Functional iron deficiency (iron constipation) Anaemia of chronic (inflammatory) disease (normocytic normochromic)
Serum iron	Low (slight to marked)	Low (slight to moderate)
TIBC(transferrin)	Normal to Increased (more likely to be Low in the dog?)	Normal to Low
Serum ferritin (species specific)	Low	Normal to Increased
BM haemosiderin	Low (nb healthy cats and some healthy cattle may lack)	Normal to Increased

Anaemia of inflammatory disease – hepcidin increases and inhibits cell release of iron; inflammatory cytokines decrease erythropoiesis through many mechanisms; in some species have decreased cell life span

Jeremy Allen DAFWA: anaemia in lambs in haemonchosis vaccine trial (chronic haemonchosis)

HEMATOLOGY	SAMPLE 1	S2	S3	S4	REFERENCE INTERVAL
Plasma protein g/L	64	68	66	**58**	60-75
Albumin g/L	**27**	30	29	**22**	28-34
Globulins g/L	37	38	37	36	30-42
Haptoglobin mg/mL	0.5	**0.7**	**0.8**	**0.6**	<0.6
Plasma appearance	Clear	clear	clear	clear	Clear
PCV L/L	**0.20**	**0.26**	**0.23**	**0.21**	0.27-0.45
Hemoglobin g/L	**53**	**71**	**64**	**52**	90-150
Erythrocytes $x10^{12}$/L	**6.18**	**8.5**	**7.7**	**6.5**	9-15
RDW %	17.7	19.9	19.1	21.4	12-27
MCV fl	33	31	29	32	28-40
MCHC g/L	**264**	**272**	**283**	**252**	310-340
MCH pg	8.6	8.4	8.3	8.0	8-12
Leukocytes $x10^9$/L	5.6	8.6	8.3	5.9	4-12
Neutrophils (seg.) $x10^9$/L	2.0	2.9	3.9	2.3	0.7-6.0
Neutrophils (band) $x10^9$/L	0	0	0	0	rare
Lymphocytes $x10^9$/L	3.3	5.2	3.8	3.1	2.0-9.0
Monocytes $x10^9$/L	0.2	0.3	0.5	0.3	0-0.8
Eosinophils $x10^9$/L	0.1	0.2	0.1	0.1	0-1.0
Basophils $x10^9$/L	0	0	0	0	0-0.3
Blood film: RBC and WBC morphology normal?					
Platelets $x10^9$/L	360	443	419	546	250-750
Iron umol/L	45	**19**	**18**	**6**	33-36 (*24-33?*)
Total iron binding capacity Umol/L	61	**55**	62	**44**	56-63

Expected findings in chronic haemonchosis*: Moderate normochromic, normocytic anaemia with no or mild reductions in albumin and serum iron. **Are Jeremy's a mixture of anaemia of chronic inflammatory disease and iron deficiency anaemia?**

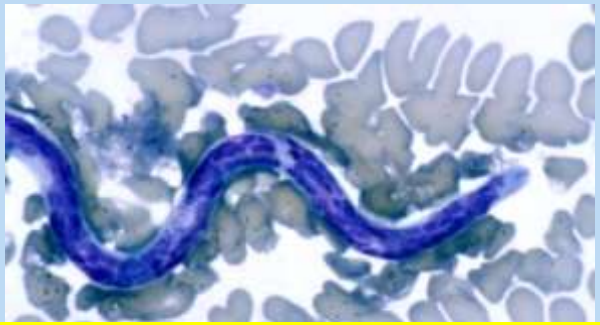
The value of multiple samples to see trends!

***ABBOTT et al (1984) Studies on the pathophysiology of chronic ovine haemonchosis in Merino and Scottish Blackface lambs. Parasitology 89:585-596**

Identifying those haemoparasites

- Traditionally the term haemoparasite refers to infectious agents that are protozoa or metazoa, but some bacteria are included because they are principally found in, or have their effect on, blood cells (**a tropism and/or trophism for blood**)
- Many of those infectious agents are transmitted by biting or blood sucking insects, but not exclusively
- Broad classification:

1. **Protozoa (haemotropic)**
   - **Haemoflagellates**
   - **Apicomplexans**
2. **Bacteria**
   - **Haemotrophic mycoplasmas**
   - ***Ehrlichia* and *Anaplasma* genera**
3. **Filarial nematodes**



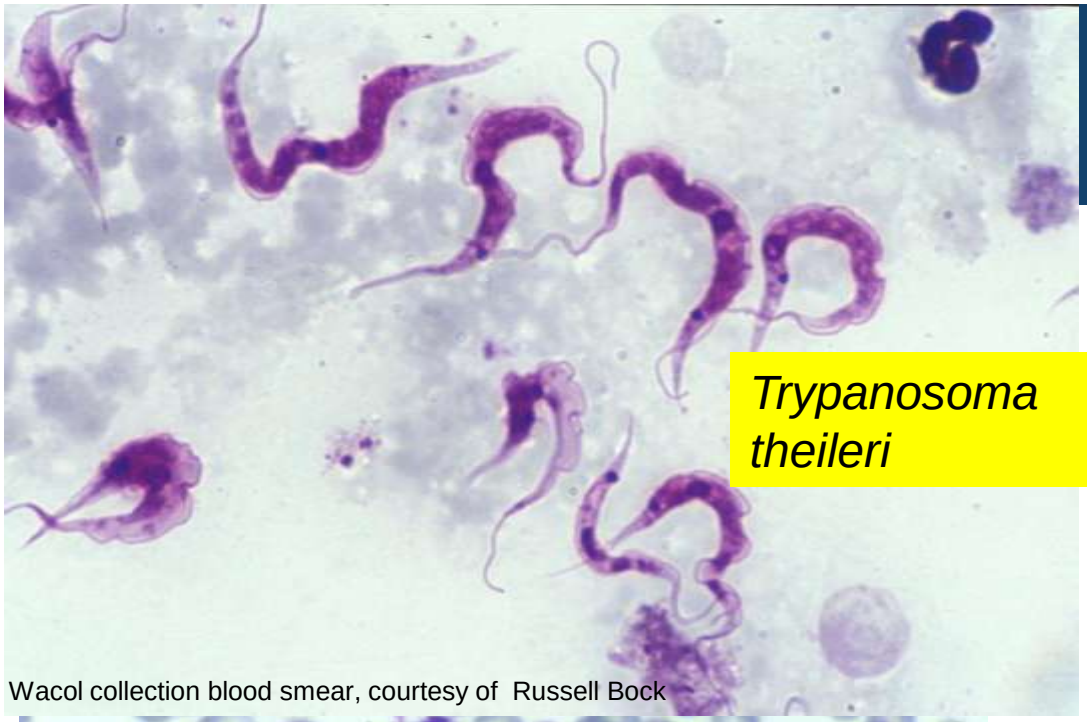
Microfilarial form of *D. immitis*

How do haemoparasites cause disease and how do you detect them?

- Many seem to have little **effect** at all, unless the animal is compromised in some way **ie they commonly act as opportunistic pathogens!**
  - Those that do have an effect, may do it by direct destruction of erythrocytes, leukocytes or platelets BUT erythrocytic destruction is often due to immune-mediated mechanisms (ie the adaptive immune response against the parasite leads to death of the cell)
- **Detection**:
  - Host specificity and parasite morphology – still a good starting point
  - Serology – specific antibody detection -ELISA; IFA
  - Molecular biological techniques – Nucleic acid detection - PCR!

Let's get back to classification

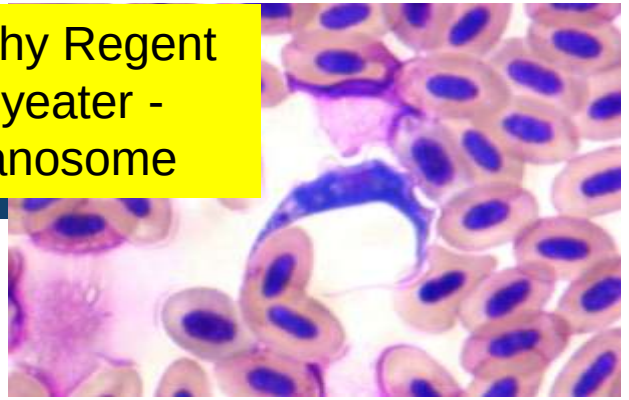
- Protozoa – not all protozoologists agree on the classification!
  - **All haemotropic protozoa** have an **asexual phase** of multiplication characterized by **trophozoites** (Greek for "animal that feeds"): this is the form that commonly causes most effect on the host (directly or indirectly)
  - Most are transmitted by biting/blood sucking insects, many of whom contribute to the **sexual phase** of the life cycle. Aquatic animals may have oral transmission.
  - **HAEMOFLAGELLATES** – have flagella
    - **Trypanosomes –** single flagellum
      - Wide range of vertebrates
      - May cause anaemia
      - Trypomastigote common form in blood (kinetoplast lies immediately anterior to the nucleus))
    - **Leishmania** – is this a true haemoparasite?
    - ***Trypanoplasma*** in fish – two flagella



Trypanosoma theileri

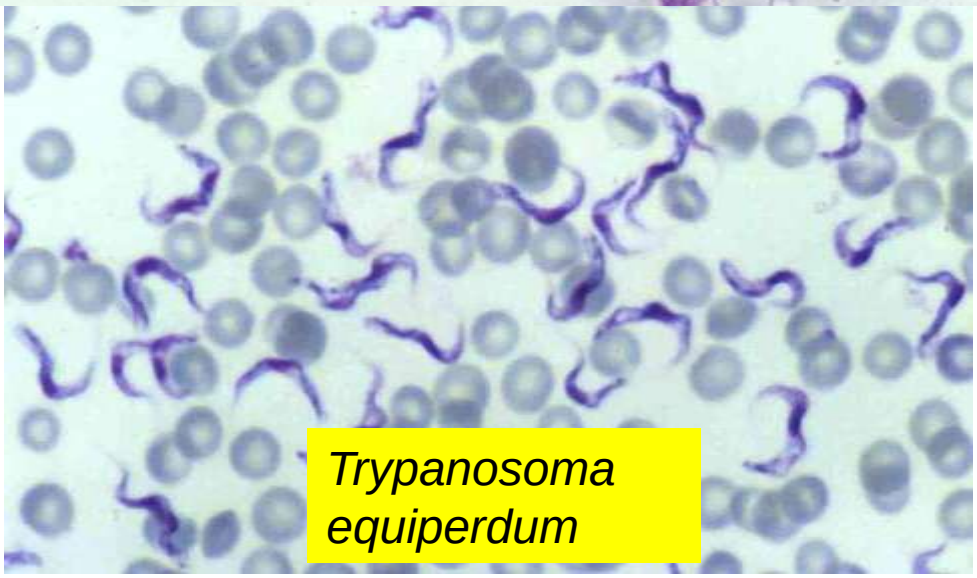
Wacol collection blood smear, courtesy of Russell Bock

Healthy Regent Honeyeater - Trypanosome



Leishmania sp amastigotes

Picture courtesy of K Papasouliotis



Trypanosoma equiperdum

Haemotropic protozoa: Apicomplexans (sporozoa)

- **Typical coccidial life cycle for these obligate intracellular parasites:**
  - ***Merogeny (Schizogony)*** – *asexual.* S**porozoite** (from sporulated oocyst) infects a host cell and becomes **trophozoite.** Trophozoite develops into **schizont (meront).** Can be multiple stages of schizogany
  - ***Gamogony(Gametogony)*** – *sexual.* Merozoite produced by final stage of schizogony enters host cell and becomes a **gamont,** either a female gametocyte (**macrogamont** or **macrogametocyte**) or a male gametocyte (**microgamont** or **microgametocyte).** The zygote formed from fertilization develops a wall and is called an **oocyst.**

important apicomplexans operating as haemoparasites

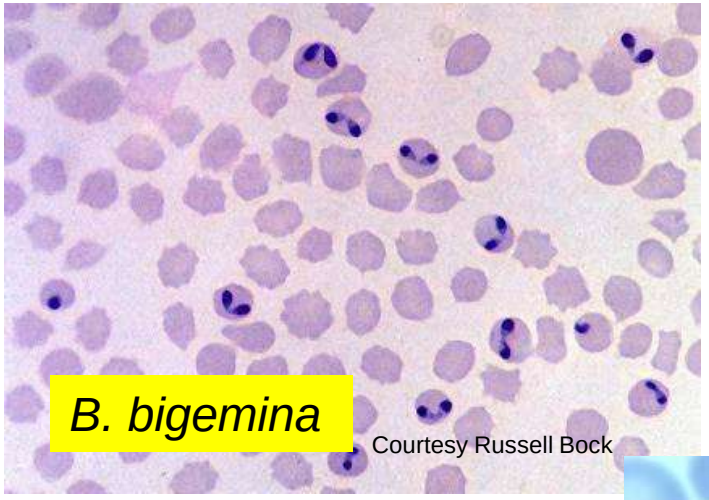
Apicomplexa (syn. Sporozoa)

- class **Coccidia**
  - order **Adeleida** — ‘haemogregarines’
    - ***Hepatozoon*** *(many orders)* ★★
    - ***Babesioma*** *(fish)*
    - ***Haemogregarina*** *(reptiles)*
    - ***Karyolysus*** *(reptiles)*
  - order **Eimeriida** — ‘true coccidia’
    - ***Toxoplasma / Neospora*** *(NOT haemoparasites)*
    - ***Sarcocystis*** *(NOT haemoparasites)*
    - ***Schellackia*** (syn. *Lainsonia*) **(lizards and snakes)**
    - ***Lankesterella*** *(amphibians)*
    - ***Isospora*** (syn. *Atoxoplasma*) **(canaries)** ★
- class **Hematozoa**
  - order **Haemosporidia** — ‘malarias’
    - ***Plasmodium*** *(many orders)* ★★★
    - ***Haemoproteus*** *(birds, reptiles)* ★★
    - ***Leukocytozoon*** *(birds)* ★★
    - ***Fallisia*** *(lizards and turtles)*
    - ***Saurocytozoon*** *(lizards)*
    - ***Hepatocystis*** *(mammals)*
  - order **Piroplasmida** — ‘piroplasms’
    - ***Babesia*** *(mammals and reptiles)* ★★★
    - ***Theileria*** *(ungulates)* ★★★
    - ***Cytauxzoon*** *(cat)* ★
    - ***Sauroplasma*** *(reptiles)*
    - ***Mesnilium*** *(fish)*

★ Importance rating as haemoparasite

Babesia – mammals, some reptiles; tick borne

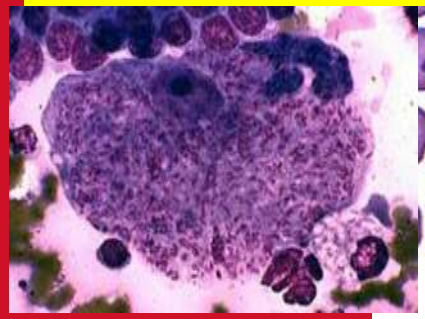
- Schizogony in erythrocytes. They occur as single, variably shaped trophozoites, paired pyriform merozoites or tetrad cruciform merozoites
- May cause IMHA

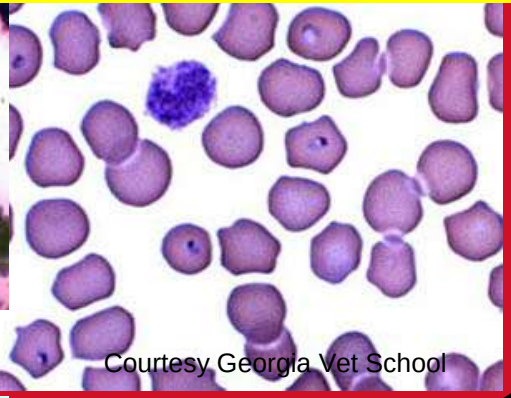


B. bigemina

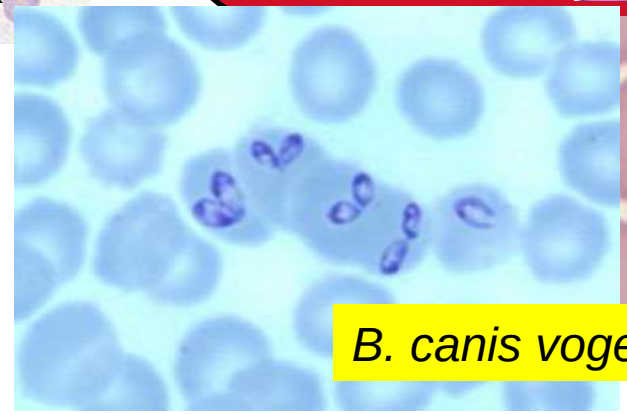
Courtesy Russell Bock

C. Felis – intra-erythrocytic signet ring trophozoites; schizont in macrophage

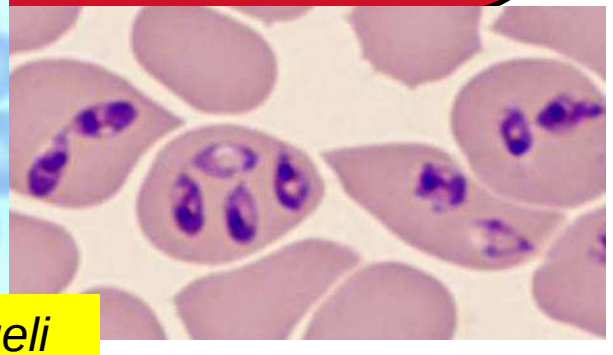




Courtesy Georgia Vet School

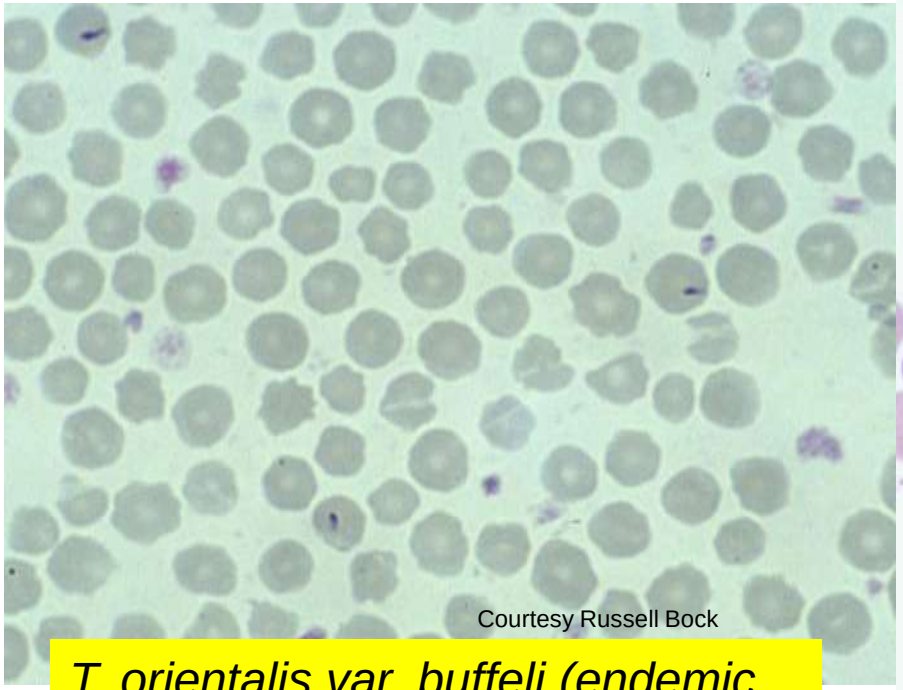


B. canis vogeli



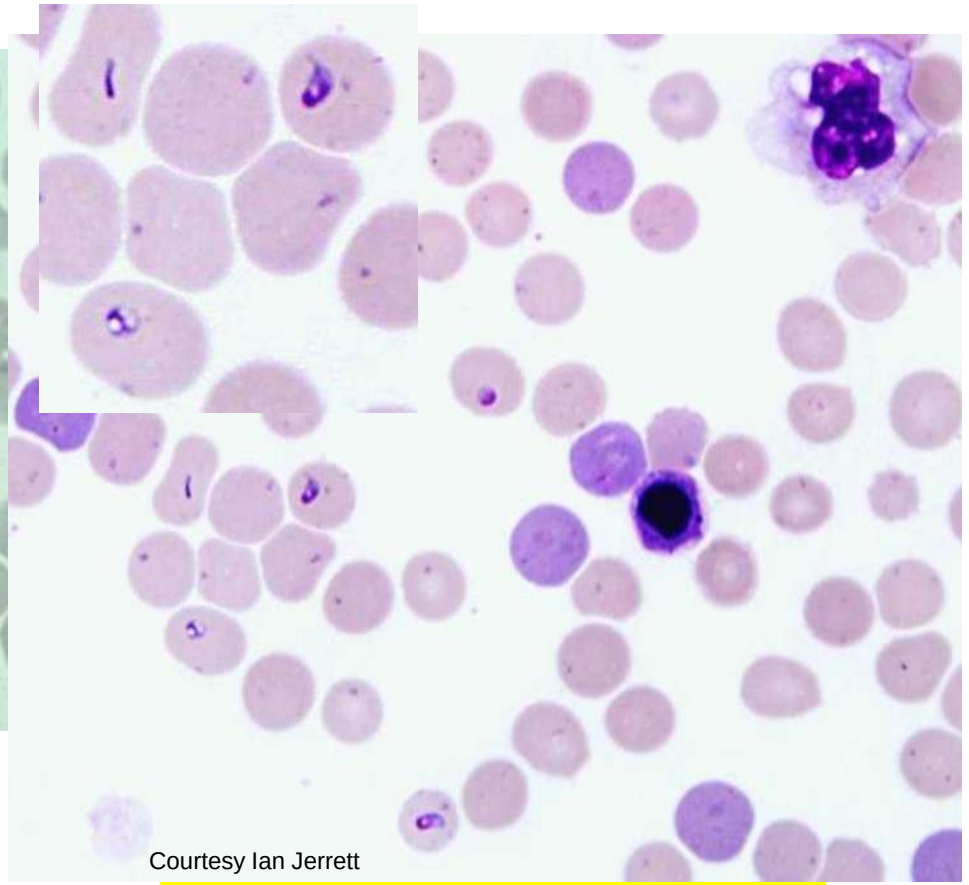
Theileria – ungulates; tick borne

- Sporozoites infect lymphocytes and forming macroschizonts. Merozoites released from these infect erythrocytes and appears as signet ring-form trophozoites



Courtesy Russell Bock

T. orientalis var. buffeli (endemic in Qld, with or without anaemia). NSW, Vic, WA: T orientalis var ikeda more important as a cause of haemolytic anaemia



Courtesy Ian Jerrett

T orientalis var ikeda

Jeremy Allen DAFWA: Clinical signs of anaemia were present on two properties, and included pyrexia, weakness, haemoglobinuria, jaundice, pallor and death. Late-stage abortions and premature births were seen on both properties.

Animal #	Cholesterol	CK	Alb	Creatinine	ALT	Ca	Cu	Total bilirubin	conjug bilirubin	GGT	GLDH	Haptoglobin	Iron	BHB	Mg	Alb/Glob	Phosphorus	Total protein	urea
A-19*	**1.63**	**903**	37.4	151	45	**2.66**	1.12	**61**	**13**	**45**	**451**	0.32	**66.8**	0.38	**0.61**	0.9	**1.11**	78.7	9.5
A-2E	3.03	133	38.2	78	31	2.32		3	0	5	12	0.26	**42**	**0.87**	0.96	0.9	2	**82.3**	8.4
A-4C	**1.74**	161	35.2	100	27	2.29		7	2	13	28	0.15	**33.5**	**0.87**	**0.65**	0.8	1.69	78.3	4.7
A-16	2.71	162	35.7	71	33	2.21		5	1	14	9	0.16	**33.1**	**0.71**	0.89	0.8	1.46	**80.5**	7.7
A-17	3.19	142	35.4	73	30	2.24		5	0	21	18	0.19	**29.8**	**0.73**	0.86	0.7	1.71	**84.2**	9
A-30	**1.51**	133	32.1	91	**63**	2.08		**42**	**9**	35	22	0.18	**36.4**	0.51	0.73	0.8	**1.19**	73.6	3.4
A-41	2.2	**482**	31.9	70	33	2.13		4	0	13	4	0.19	**50.5**	**0.81**	0.76	0.7	2.16	79.1	8.9
A-3	**1.61**	**756**	34.2	112	38	2.28		**51**	**13**	15	19	0.5	**63.4**	0.26	0.69	0.7	1.43	**80.1**	7
RI	1.8 - 5.6	20 - 450	27 - 39	60 - 190	< 40	2.0 - 2.5	0.6 - 1.1	0 - 10	0 - 8	0 - 35	0 - 40	< 0.6	12.0-23.0	< 0.7	0.7 - 1.0	0.6 - 1.2	1.2 - 2.3	60 - 80	1.0-10

*=sick	WBC	NE#	LY#	MO#	EO#	BA#	RBC	HB	HCT	MCV	MCH	MCHC	RDW	PLT	MPV
	K/uL	K/uL	K/uL	K/uL	K/uL	K/uL	M/uL	g/dL	%	fL	Pg	g/dL	%	K/uL	fL
A-2*	**12.72**	2.7	**8.87**	0.71	0.37	0.07	**1.38**	**3.6**	**13.4**	**97.4**	**26.1**	**26.9**	**39**	**173**	10.4
A-4C	5.04	1.48	3.03	0.45	0.07	0.01	**2.85**	**6.6**	25.5	**89.4**	**23.2**	**25.9**	20	**133**	9.4
A-16	4.6	1.65	2.04	0.44	0.43	0.03	**3.14**	**6.9**	25	**79.5**	**22**	**27.9**	28	**82**	7.3
A-17	8.68	3.88	3.01	0.58	1.11	0.1	**4.88**	11.2	37.5	**76.9**	**23**	29.9	27	**125**	8.5
A-30	7.68	3.02	4.29	0.26	0.08	0.03	**1.68**	**3.8**	**16.6**	**98.7**	**22.6**	**22.9**	26	**64**	9.6
A41	4.78	2.04	2.2	0.34	0.17	0.03	**2.73**	**7**	26.4	**96.6**	**25.6**	**26.5**	18	**103**	7.4
A-3* later died	6.2	2.03	3.56	0.38	0.16	0.07	**0.72**	**2.1**	**7.7**	**107.3**	**29.2**	**27.3**	20	**14**	4.6
RI	4.0-12	0.6-4.1	2.5-7.5	0-1.2	0-2.4	0-0.4	5.0-10	8.0-15	24-46	40-60	11.0-17	28.2-36	12.0-27	200-800	5.0-20

Macrocytic , hypochromic (only MCHC) regeneration effect – mainly extravascular haemolysis?

Animal nb*sick	anisocytosis	polychromasia	basophiilic stippling	Howell-Jolly bodies	Theileria like bodies
A-2*	1+	1+	1+	1+	1+(<1%)
A-2E	+	+/-	+	rare	+
A-4C	+/-	+/-	+	+	Rare
A-16	1+ acanthocytes	+/-	rare	rare	rare
A-17	+	+/-	-	rare	-

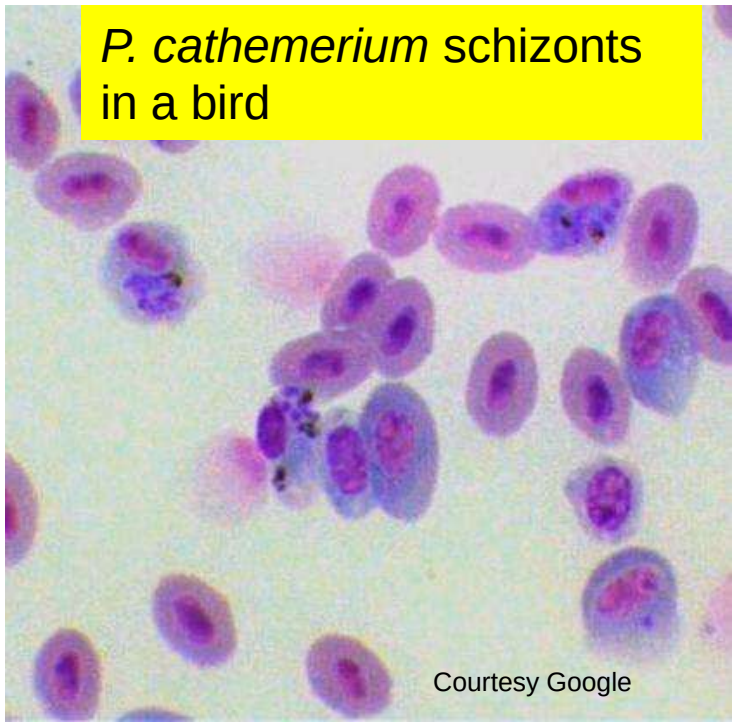
The combination of PCV below 15%, evidence of regenerative anaemia, and PCR evidence of the organism in two of the affected cattle constitutes the case definition of BATOG (Bovine anaemia due to T.orientalis Group). **T. orientalis var. ikeda**

T. orientalis has eight variants, of which four are found in Australia (through PCR testing **– ikeda, chitose, buffeli and type 4 or c type)**. Only the ikeda variant has been clearly associated with severe disease in Australia, but mixed infections with chitose and buffeli have been noted to cause some disease. Infection with the buffeli variant alone does not appear to cause any more disease in Australia than it has historically. There is often up to a 6 months lag between introduction of the parasite and development of clinical signs. Affected herds in Victoria in 2011 had an average of 2.5% of cattle clinically affected, with an associated mortality up to 32%.

Host factors – Under normal circumstances, cattle in endemic areas develop immunity to T. orientalis by approximately 6 months of age. Disease is typically seen in naive animals introduced into endemic areas, or on farms in 'ticky' areas that import stock from endemic regions. Young cattle (2 – 3 months), late pregnant and recently calved cows are most likely to be affected. Animals remain infected for life and disease can re-emerge during periods of stress, particularly around late pregnancy/early calving.

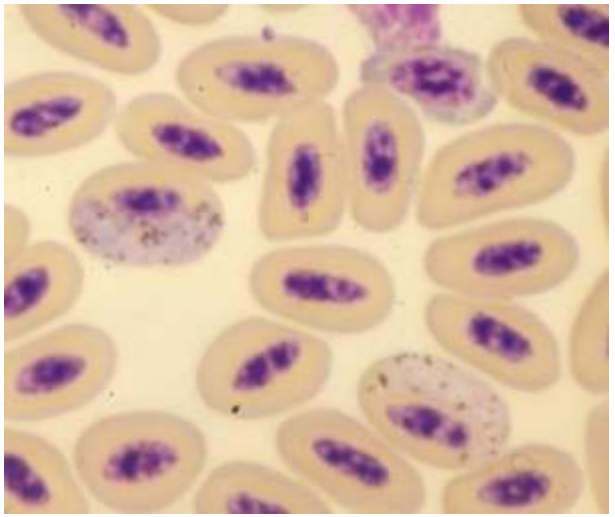
- ***Plasmodium*** – (primates, rodents, birds, reptiles, amphibians; mosquitoes transmit in mammals and birds)
- Haemolytic anaemia, primarily in mammals
- Ring form trophozoites, iron pigment containing schizonts and gamonts in erythrocytes and other blood cells

P. cathemerium schizonts in a bird



Haemoproteus (birds, turtles and lizards; insects)

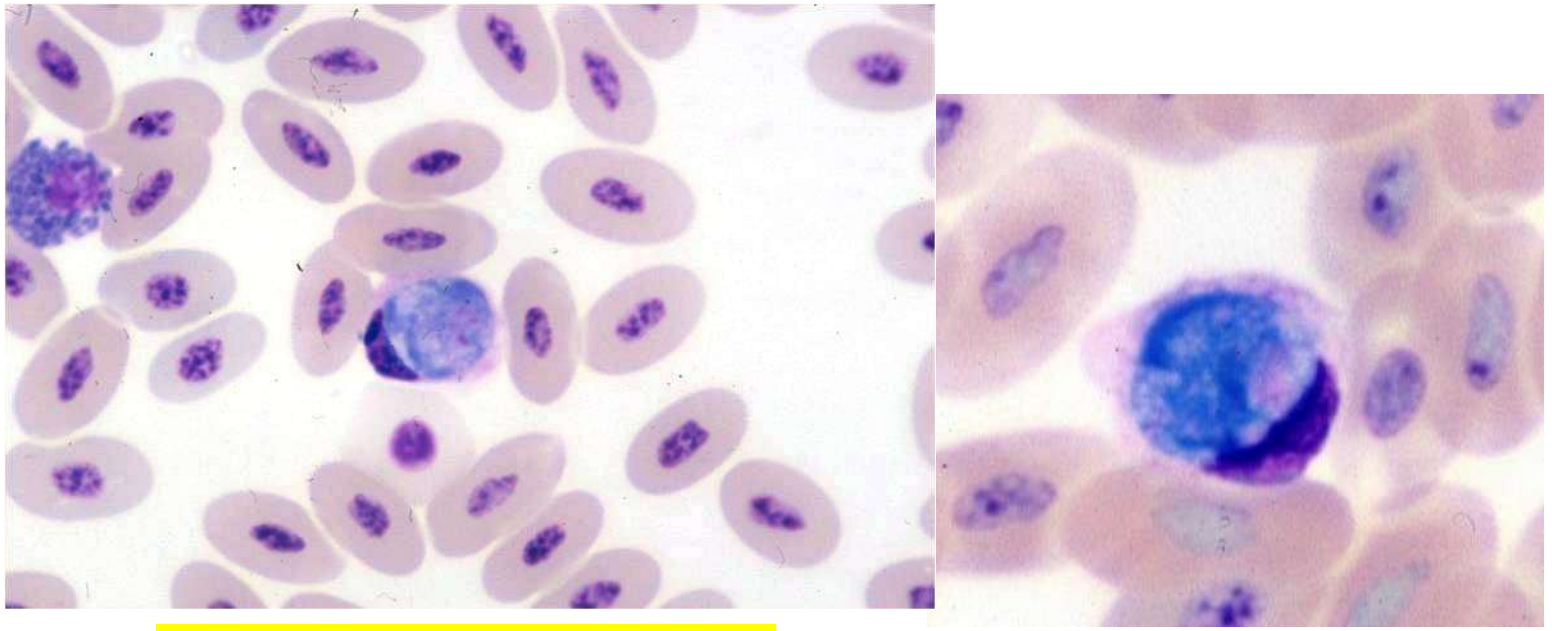
- May cause anaemia in compromised host
- Intraerythrocytic, iron containing gamonts



Haemoproteus sp in Indian koel

Leukocytozoon – birds; flies and biting midges

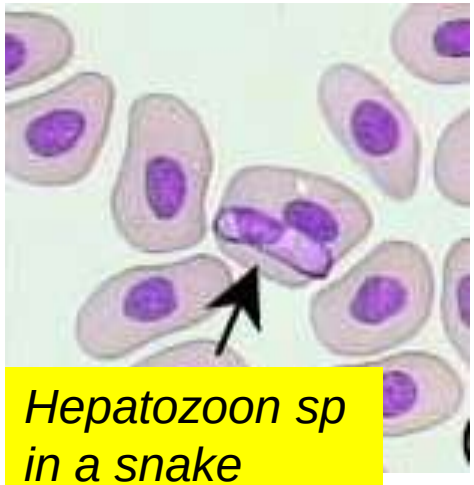
- Pathogenicity low, but may cause anaemia in young waterfowl and turkeys
- Elongate, iron-free large gamonts in erythrocytes, lymphocytes and monocytes



Leukocytozoon sp - emu

Hepatozoon - Mammalian carnivores, rodents, birds, snakes, amphibians; arthropods, leeches

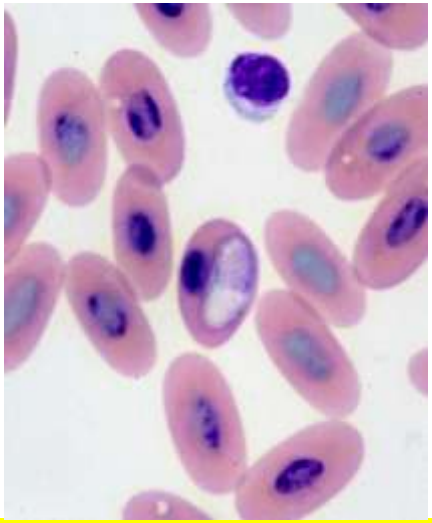
- May cause anaemia/leukocytosis in dogs and cats
- Intraerythrocytic (mainly snakes, amphibians, birds) and intraleukocytic (mainly neutrophils in eutherian and marsupial mammals) ellipsoid gamonts



Hepatozoon sp in a snake

H. tachyglossi - echidna

Courtesy Richard Ploeg



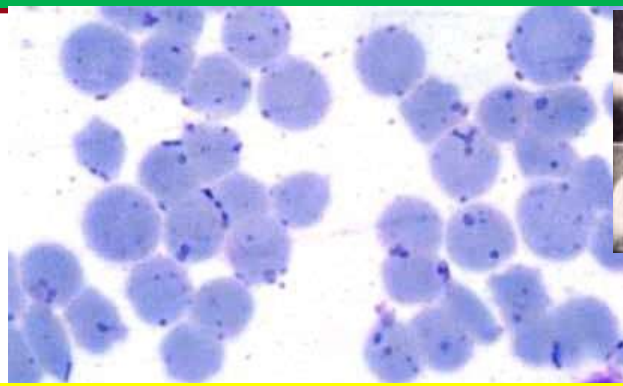
Haemogregarina sp in scrub python? Rarely pathogenic

Bacterial haemoparasites

1. Protozoa ✓
   - Haemoflagellates ✓
   - Apicomplexans ✓
2. **Bacteria**
   - **Haemotrophic mycoplasmas**
   - ***Ehrlichia* and *Anaplasma* genera**
3. Filarial nematodes

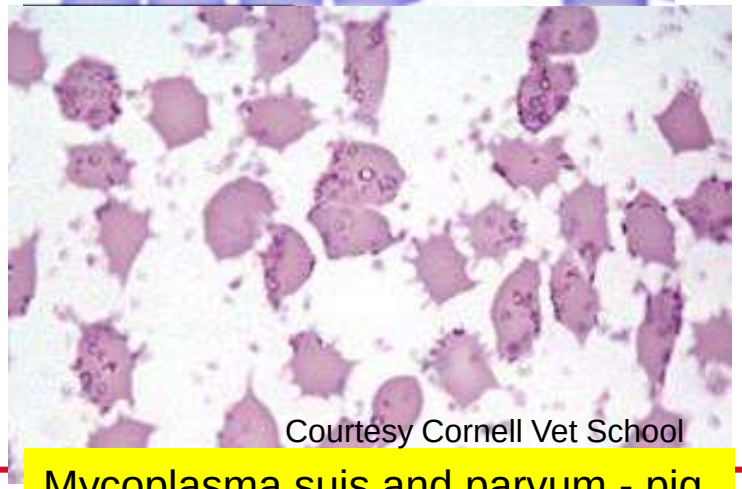
Bacteria – haemotrophic mycoplasmas – mammals; ticks, fleas, in utero?

M. Haemocanis (a second one found!)



Courtesy Sue Jaensch

M. haemofelis and haemominutum? A third type discovered!

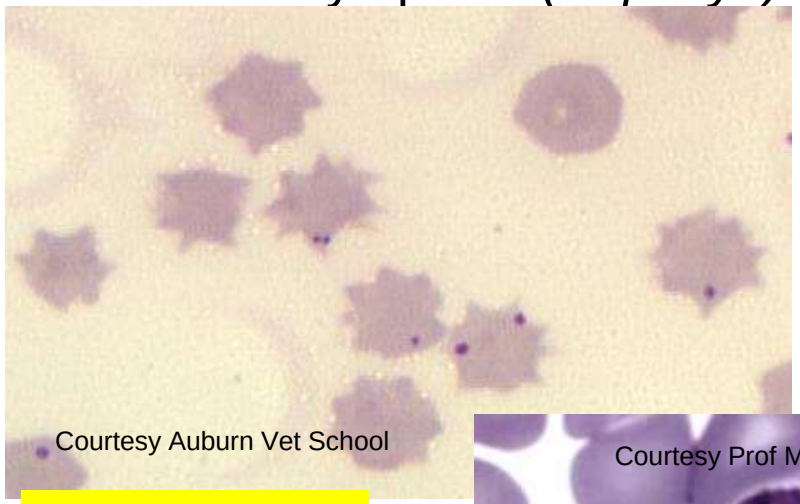


Courtesy Cornell Vet School

Mycoplasma suis and parvum - pig

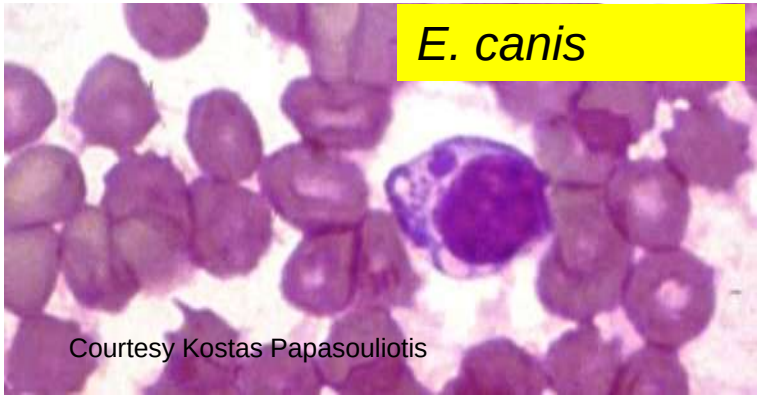
Ehrlichia and *Anaplasma* – mammals; ticks likely vectors

- ***Ehrlichia*** Membrane-bound clusters of coccoid to ellipsoid, Gram negative bacteria **(Morulae**) in leukocytes. ***Anaplasma*** in erythrocytes (most spp) and platelets *(A. platys)*
- May cause penias (***Ehrlichia***), IMHA (***Anaplasma)*** and thrombocytopenia *(A. platys)*



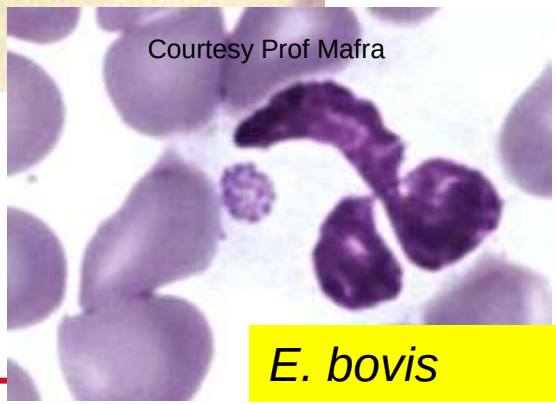
Courtesy Auburn Vet School

A. bovis



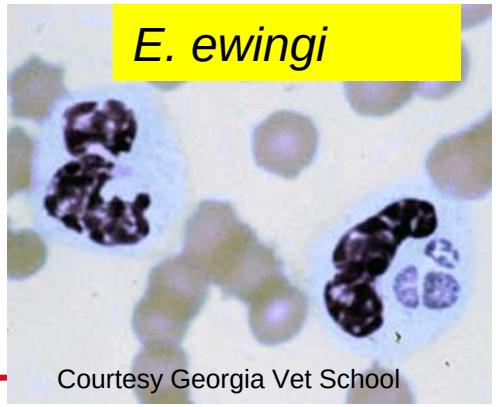
E. canis

Courtesy Kostas Papasouliotis



Courtesy Prof Mafra

E. bovis



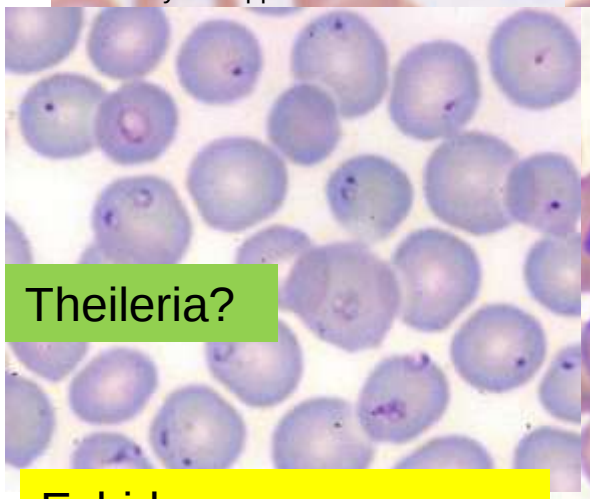
E. ewingi

Courtesy Georgia Vet School

What is this parasite?

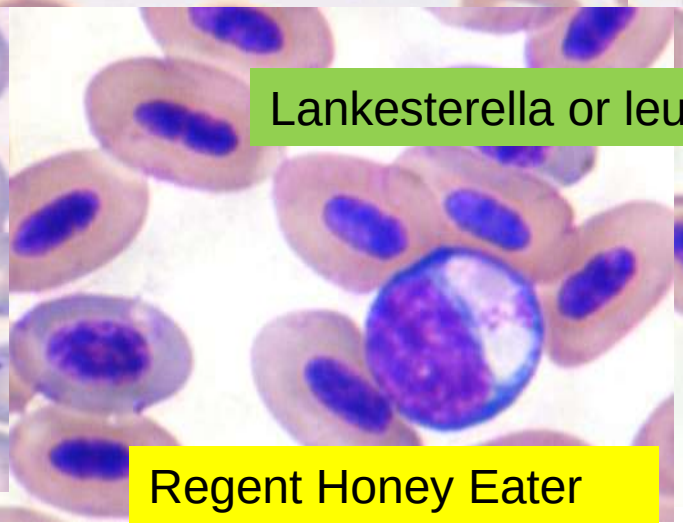
Courtesy G Reppas

Yellow Bellied Glider – erythrocytic parasite?



Theileria?

Echidna



Lankesterella or leukocytozoon?

Regent Honey Eater

Noisy Pitta Bird

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)?

A 3 years old male Cocker Spaniel with depression and weakness started 5 days earlier. Now jaundiced.

HAEMATOLOGY	SAMPLE	REFERENCE INTERVAL
Plasma appearance	**Yellow**	Clear
PCV L/L	**0.21**	0.37-0.50
Plasma protein g/L	**77**	55-75
Haemoglobin g/L	80	100-150
Erythrocytes $x10^{12}$/L	**2.5**	5-7
MCV fl	**84**	60-75
MCHC g/L	**380**	300-360
Leukocytes $x10^9$/L (corrected for NRBC)	**25.1**	7-12
Neutrophils (seg.) $x10^9$/L	**17.5**	4.1-9.4
Neutrophils (band) $x10^9$/L	**2.6**	0-0.24
Lymphocytes $x10^9$/L	**0.7**	0.9-3.6
Monocytes $x10^9$/L	**4.0**	0.2-1.0
Eosinophils $x10^9$/L	0.3	0.14-1.2
Basophils $x10^9$/L	0	0-0.4
Blood film: **marked anisocytosis and polychromasia, 30 nucleated erythroid cells (NRBC) per 100 leukocytes, many spherocytes**		
Reticulocyte % (uncorrected)	**10.2**	0-1.5
Absolute reticulocytes $x10^9$/L	**255**	0-75

BIOCHEMISTRY	SAMPLE	REFERENCE INTERVAL
ALP IU/L	**409**	<110
ALT IU/L	**238**	<60
Total bilirubin µmol/L	**203**	1.2-5.1
Unconjugated bilirubin µmol/L	**19**	1.2-5.1
Conjugated bilirubin µmol/L	**184**	1.2-5.1

***Other tests*: Coomb's test was positive (end point 1/32 ie highest dilution of serum showing agglutination).**

Diagnosis: AIHA

Direct Coombs' Test for AIHA (detects IgG, IgM and complement attached to the surface of erythrocytes)

- **Used mainly in dog, cat, horse (also for neonatal isoerythrolysis) and cow (also used for assessing antibody titres after brucellosis vaccine)**
- **Is it an urban myth to state that titres help decide if it is primary or secondary AIHA?**
- **Do you need to do the test at 4 degrees C as well as 37 degrees C to detect cold agglutinins (some IgM) ?**

Likely reasons for changes and possible conclusions: the anaemia is regenerative on the basis of a high reticulocyte count. Correction for the level of anemia gives a value of 4.8% (.21/.45 [as average PCV] x 10.2), correction for both level of anaemia and erythroid maturation time (taken as 2 days for this PCV) gives a value of 2.4 (>2 is regarded as truly regenerative, a value of 1-2 indicates less than optimum regeneration while a value less than 1 is considered non-regenerative. The absolute reticulocyte count is 0.255 x 1012/L (RI less than 0.75 x 1012/L). The polychromasia and anisocytosis are a reflection of the high numbers of circulating reticulocytes. These large cells, because of their significant numbers, have raised the MCV above reference interval, and this will be maintained until reticulocytosis subsides. Normally in intense regenerative anaemia the MCHC will be depressed due to the fact that the reticulocytes are immature and have too little Hb for their size (the so called pseudomacrocytic, pseudohypochromic response in intense regenerative anaemia). However, in this case the MCHC is actually increased above reference interval. A high MCHC is not possible as erythrocytes cannot be oversaturated with Hb. Therefore, the value is due to laboratory error or due to the presence of free Hb (as in haemolytic states and most likely here).

The large number of circulating nucleated erythroid cells (NRBC) is appropriate in regenerative anaemia when reticulocytosis is marked. This is more likely to occur in haemolytic rather than blood loss regenerative anaemias as iron is more easily re-utilized (**conclusion)**. The presence of spherocytes (dense cells without a central pallor which occur because of the pinching off of damaged or antibody-coated surface membrane by macrophages) and the positive Coomb's test suggest that the haemolytic anaemia is immune mediated (**conclusion**). In this case, because of the high titre in the Coomb's test and the lack of other disease processes that may give rise to antibody coating of erythrocytes (eg erythrocyte parasites, drugs etc), it is likely that the problem is primary immune-mediated (auto-immune) (**conclusion and final diagnosis)**.

The total leukocyte count has been corrected for a falsely high count created by nucleated erythroid cells being included in counting by the automatic cell counter. Normally, the total leukocyte count is not corrected unless the circulating nucleated erythroid cells reach 5 per 100 leukocytes (total leukocyte count x 100/100 + no. of circulating nucleated erythroid cells per 100 leukocytes). The neutrophilia, lymphocytopaenia and monocytosis (eosinophils are within reference interval but at the lower end) could be interpreted as stress induced (the animal was still intensely ill). However, there is a left shift to the neutrophilia, which suggests true inflammatory demand for part of the neutrophilia. This is probably in response to the hemolytic anemia. Intense haemolytic anaemias commonly cause left shifts, especially in the dog, but the exact mechanisms are poorly understood. Non-specific stimulation of the macrophage system may be involved as may be released products from cell breakdown. Part of the monocytosis in this case may be directly related to the haemolytic anaemia.

ALP elevation suggests mild cholestasis, ALT elevation suggests mild hepatocellular damage (fatty change for example). Both of these could be related to hepatic hypoxia created by acute onset of anaemia. The markedly high total serum bilirubin is to be expected in haemolytic anaemias that are predominantly intravascular. Free Hb produced by intravascular erythrocytic destruction is quickly converted to bilirubin. In the early stages most of the bilirubin is unconjugated but as time goes on an increase in conjugated may occur. In this case the conjugated levels are marked. This may be partly due to some cholestasis caused by hepatocyte swelling but is most likely to be due to simple regurgitation related to enhanced production.

A 4 years old male crossbred dog with weight loss, inappetence and depression for a period of three weeks.

HAEMATOLOGY	SAMPLE	REFERENCE INTERVAL
Plasma appearance	Clear	Clear
PCV L/L	0.46	0.37-0.50
Plasma protein g/L	**87**	55-75
Haemoglobin g/L	150	100-150
Erythrocytes $x10^{12}$/L	6.7	5-7
MCV fl	69	60-75
MCHC g/L	326	300-360
Leukocytes $x10^{9}$/L (corrected for NRBC)	**13.4**	7-12
Neutrophils (seg.) $x10^{9}$/L	8.7	4.1-9.4
Neutrophils (band) $x10^{9}$/L	0	0-0.24
Lymphocytes $x10^{9}$/L	1.9	0.9-3.6
Monocytes $x10^{9}$/L	**2.5**	0.2-1.0
Eosinophils $x10^{9}$/L	0.3	0.14-1.2
Basophils $x10^{9}$/L	0	0-0.4
Blood film: **8 nucleated erythroid cells (NRBC) per 100 leukocytes, moderate numbers of target cells, basophilic stippling of erythrocytes**		
Reticulocyte % (uncorrected)	**2**	0-1.5
Absolute reticulocytes $x10^{9}$/L	**134**	0-75

Other tests:
Urinary delta aminolevulinic acid (ALA) analysis: 190 (reference interval <38) at a urinary specific gravity of 1.030 (ie moderately concentrated urine)

Diagnosis : lead poisoning

Chronic lead poisoning and haematological changes:

- Mild or no anaemia
- Dyserythropoiesis leading to a variety of effects
  - Basophilic stippling (aggregation of ribosomal RNA?) – can occur in man and other primates, dog, horse, cow, pig, rabbit, rat, guinea pig, mongolian gerbil and chicken)
  - Normoblastosis (many species) – considered to be due to leaky bone marrow vasculature, but what mechanism?
  - Altered erythrocyte shapes (poikilocytosis) – direct membrane effects of lead – quite variable amongst domestic species – may lead to shortened life span and could be antibody-mediated (at least in the dog, where the Coombs Test may be positive)

Likely reasons for changes and possible conclusions: The moderately elevated urinary delta ALA is suggestive of lead poisoning (**conclusion and main diagnosis)**. Delta ALA elevates in urine when there is interference with porphyrin metabolism (as in heme synthesis for erythrocytes). Lead is capable of doing this as it interferes with several of the enzymatic steps (causes dyserythropoiesis). Blood lead levels would have given an indication of recent exposure to lead but would not have indicated past exposure (lead is quickly stored in tissues and blood levels will drop). Delta ALA is an indication of the **effect** of increased body lead, whether obtained now or in the past.

Basophilic stippling of erythrocytes, the presence of target cells and the occurrence of circulating nucleated erythroid cells in ***the absence of anaemia*** **support the diagnosis of lead poisoning.** These are the viewed effects of altered erythropoiesis which lead produces through several mechanisms. These changes are not always present in lead poisoning and moreover, may occur in other derangements of erythropoiesis.

Basophilic stippling can occur in intense regenerative anaemias, especially in ruminants and pigs. In dogs and cats it rarely occurs and its appearance is slightly different to that in lead poisoning. The presence of circulating nucleated erythroid cells with a low reticulocyte count and/or in the absence of the appropriate level of anaemia indicates inappropriate (defective or disorderly) erythropoiesis and can occur in bone marrow or splenic disorders (eg myelofibrosis, leukaemia, haemangiosarcoma). Target cells are a form of leptocyte that have a large surface area for their volume. They may develop through membrane lipid derangements such as in certain types of hepatic disease or endocrinopathies (eg hypothyroidism). The mild monocytosis in this case is difficult to explain. It does not seem to be of importance in the diagnosis.

Diagnosis and postscript: Lead poisoning. On further questioning, the owner admitted that he had been renovating an old home and there may have been the possibility of access to lead-based paint. The dog responded well to treatment.

A 17 years-old, male neutered crossbred Terrier who was presented for investigation of lethargy, weight loss, periods of inappetence and decreased exercise tolerance over the past six months. The dog was very depressed, dehydrated (assessed as 8-10%), flea ridden, had raspy breathing, a grade 4/6 systolic cardiac murmur, poor body condition (1.5/5), jaundice and marked abdominal pain on palpation (acute onset).

TEST	SAMPLE	REF VALUES
AMYLASE u/L	874	<1400
ALP u/L	**5063**	<110
ALT u/L	**714**	<60
CK u/L	**230**	<200
Serum protein (biuret) g/L	**72.7**	50-70
Albumin (BCG) g/L	**22.8**	23-43
Globulins g/L	**49.9**	27-44
Total cholesterol mmol/L	**16.11**	1.4-7.5
Total Bilirubin µmol/L	**178.0**	1.2-8.1
Glucose mmol/L	4.3	3.3-6.4
Urea mmol/L	**11**	3.0-10
Creatinine µmol/L	**151**	40-120
Calcium mmol/L (uncorrected)	2.55	2.1-2.9
Inorganic phosphate mmol/L	**1.77**	0.8-1.6
Sodium mmol/L	**151.1**	137-150
Potassium mmol/L	4.1	3.3-4.8
Chloride mmol/L	114.3	105-120

TEST	SAMPLE	REF VALUES
Plasma appearance	Yellow	Clear
PCV L/L	0.41	.37-.50
Plasma protein g/L (refract)	**85**	55-75
Haemoglobin g/L	144	100-150
Erythrocytes $x10^{12}$/L	6.12	5-7
MCV fL	67	60-75
MCHC g/L	**351**	300-350
MCH pg	23.5	20-25
Leukocytes $x10^9$/L	**23.1**	7-12
Neutrophils (seg.) $x10^9$/L	**20.8**	4.1-9.4
Neutrophils (band) $x10^9$/L	0.00	0-.24
Lymphocytes $x10^9$/L	**0.46**	.91-3.6
Monocytes $x10^9$/L	**1.62**	.2-.96
Eosinophils $x10^9$/L	**0.0**	.14-1.2
Basophils $x10^9$/L	0.0	0-.36
Platelets $x10^9$/L	318	200-600
Reticulocyte % (uncorrected)	0.8	0-1.5
Blood film: 2+ target cells, 1+ poikilocytosis. Occasional hypersegmented neutrophil.		

Urine (voided) colour & appearance: slightly cloudy and dark yellow	Glucose: -ve
Specific gravity: 1.018	Ketones: -ve
Protein (SSA): **trace**	Blood: -ve
pH: 5.0	Bilirubin: **3+**
Microscopic findings: occasional bacteria, some squamous cells, 4-5 White blood cells (leukocytes) per HPF, 2-3 Erythrocytes per HPF.	

Likely conclusions: Multi-organ disease (liver, kidney, cardiac). All the findings suggest that **liver disease** is a distinct possibility and is likely to be the reason for jaundice and abdominal pain. Although post-hepatic obstruction cannot be ruled out, intra-hepatic cholestasis is more likely because of the markedly elevated ALT. The low-ish albumin and high cholesterol can also be explained by liver disease. Some clinical pathology results suggest **renal disease.**
Postscript: On ultrasound, this dog had a large liver mass in the middle lobe. It also had enlarged mesenteric lymph nodes. Kidneys were unremarkable. Fine needle cell aspiration of liver mass and mesenteric lymph nodes revealed a **hepatic carcinoma that had metastasized to lymph nodes.** The renal and likely cardiac diseases were not investigated further because of the poor prognosis. The owners elected to have the dog euthanased, but did not wish to have a necropsy performed.

Possible reasons for changes : the elevated ALP and ALT indicate marked cholestasis and moderate to marked hepatocellular damage. The mildly elevated CK is more difficult to explain, but could be due to handling of the animal during examination . The elevated blood protein values 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). The borderline hypoalbuminaemia is probably worse than it seems because of dehydration and, likewise, the high globulins could be lower (and closer to normality). One issue is that these changes cannot be simply explained by dehydration and one would have to consider the possibility of decreased intake, production and increased loss of albumin; and causes of hyperglobulinaemia (eg acute phase reactants, increased immunoglobulins in chronic inflammation/illness). The increased cholesterol could be related to liver disease or due to other diseases that may affect lipid metabolism. The hyperbilirubinaemia, in the absence of haemolytic anaemia would suggest liver (or post liver cholestatic) disease. The marginal azotaemia could be partly due to pre-renal factors but a renal factor may be involved because of the dilute urine and trace protein in a dehydrated animal. The increased inorganic phosphate could be related to renal disease. The increased sodium could be related to dehydration.
The leukocyte changes could be explained by stress related to acute abdominal pain (corticosteroid release effects on leukocytes – neutrophilia, lymphocytopenia, monocytosis, eosinopenia and hypersegmented neutrophils in the blood film), but some degree of inflammatory demand for neutrophils cannot be completely ruled out. The moderate (2+) target cells could be related to liver disease or other diseases that affect the lipid structure of erythrocyte membranes. Poikilocytosis is a non-specific change to intense or prolonged illness, but could be related to the liver disease. Although the animal is not anaemic, the PCV may be lower than what it seems because of dehydration (8-10%). At the most, however, it would be a borderline anaemia (and probably non-regenerative because of the reticulocyte percentage – 0.048 x 1012/L).
The major urinary change is the presence of dilute urine in an animal that is significantly dehydrated, which indicates renal tubular dysfunction. The hyperbilirubinuria is related to the marked hyperbilirubinaemia. The trace protein at a specific gravity of 1.018 is probably significant and could be related to the renal disease.
Likely conclusions: 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. 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 dog has multi-organ disease (liver, kidney, cardiac). All the findings suggest that **liver disease** is a distinct possibility and is likely to be the reason for jaundice and abdominal pain. Although post-hepatic obstruction cannot be ruled out, intra-hepatic cholestasis is more likely because of the markedly elevated ALT. The low-ish albumin and high cholesterol can also be explained by liver disease. Some clinical pathology results suggest **renal disease**
(**postscript:** On ultrasound, this dog had a large liver mass in the middle lobe. It also had enlarged mesenteric lymph nodes. Kidneys were unremarkable. Fine needle cell aspiration of liver mass and mesenteric lymph nodes revealed a hepatic carcinoma that had metastasized to lymph nodes. The renal and likely cardiac diseases were not investigated further because of the poor prognosis. The owners elected to have the dog euthanased, but did not wish to have a necropsy performed. In hindsight, it is still not possible to determine why this relatively chronic condition developed into acute abdominal pain, however, neoplasia is notorious for producing the unexpected in relation to disease expression. What can be stated is that the suspected renal and cardiac diseases were probably unrelated to the hepatic neoplasia, although without a necropsy one cannot be definite.)

One year old male Burmese cat with respiratory distress, coughing and elevated temperature for 3 weeks.

BIOCHEMISTRY	SAMPLE	REFERENCE INTERVAL
Serum protein (refract.) g/L	**78**	54-73
Albumin (EPG) g/L	**30.9**	24-30
α globulins (EPG) g/L	17.7	9-21
β globulins (EPG) g/L	9.8	8-15
γ globulins (EPG) g/L	19.6	9-23

HAEMATOLOGY	SAMPLE	REFERENCE INTERVAL
Plasma appearance	Clear	Clear
PCV L/L	**0.29**	0.30-0.45
Plasma protein g/L	**84**	59-78
Haemoglobin g/L	95	80-140
Erythrocytes $x10^{12}$/L	6.7	6-10
MCV fl	44	40-45
MCHC g/L	328	310-360
Leukocytes $x10^{9}$/L	**29.3**	8-14
Neutrophils (seg.) $x10^{9}$/L	**23.6**	3.8-10.1
Neutrophils (band) $x10^{9}$/L	**3.1**	0-0.4
Lymphocytes $x10^{9}$/L	2.3	1.6-7.0
Monocytes $x10^{9}$/L	0.1	0.1-0.6
Eosinophils $x10^{9}$/L	0.2	0.2-1.4
Basophils $x10^{9}$/L	0	0-0.2
Blood film: **toxic granulation and Doehle bodies in neutrophils**		

***Other tests*: Trans-tracheal aspirate: Cytology revealed numerous alveolar macrophages and clusters of lytic (degenerate) neutrophils. A pure growth of *Staphylococcus intermedius* was obtained from the fluid.**
The animal was positive for Feline Leukemia Virus (antibody test on serum).

The haematology and trans-tracheal aspirate findings support a diagnosis of septic pneumonia**.** Alveolar macrophages admixed with lytic neutrophils likely suggest that the inflammation has reached the level of the pulmonary alveoli and is not just a simple tracheitis or bronchitis. The isolation of *S. intermedius* is unusual as this part of the skin's normal flora. The finding of an opportunistic (conditional) pathogen and the fact that the cat is FeLV positive could well indicate immune-compromisation. Once treatment is initiated, repeat haematology may be useful to ensure that the inflammatory demand and toxic changes diminish (ie good prognostic signs develop).

Diagnosis and postscript: Bacterial pneumonia. The owner was warned of the possibility that the cat might be predisposed to unusual infections in the future. The cat recovered from antibiotic treatment and supportive therapy.

Possible reasons for changes and likely conclusions: the trans-tracheal aspirate findings support a diagnosis of septic pneumonia (**conclusion and diagnosis).** Alveolar macrophages admixed with lytic neutrophils likely suggest that the inflammation has reached the level of the pulmonary alveoli and is not just a simple tracheitis or bronchitis. The isolation of *S. intermedius* is unusual as this part of the skin's normal flora. It is obviously acting as an opportunistic pathogen but how it got into the respiratory tract is difficult to answer. The animal is probably haemoconcentrated (elevated total protein and albumin), which could mean that the cat probably has a true mild anaemia (although a one year old cat might not be expected to have a PCV greater than 0.35 L/L?). The anaemia could go along with inflammatory disease (bacterial utilisation of iron or, in the long term, iron sequestration by macrophages), but since the cat is FeLV positive, the possibility of direct bone marrow depression by the virus cannot be excluded. The neutrophilia with left shift is consistent with an inflammatory process of 3 weeks duration. The neutrophil response is not entirely satisfactory as the bacteria are obviously having an effect on neutrophil function (toxic granulation and Doehle bodies), but at least the bone marrow is responding well. If the changes in the neutrophils persist with treatment this may be a poor prognostic sign but they are to be expected in severe bacterial disease in the early stages. The Doehle bodies are an indication of altered neutrophil maturation and suggest toxemia or the effects of certain drugs (ie they are not specific for toxemia). It should be remembered that in the cat small 'Doehle' bodies may occur in a small number of neutrophils in health. Therefore only large Doehle bodies in a significant number of cells (say 30% plus) are considered indication of altered neutrophil function.

Both the plasma and serum proteins would have been measured by refractometer. Consequently, the values can be directly compared and the difference (indirect measurement) is usually fibrinogen. In this case, it is elevated (6 g/L – normally 2-4 g/L). Fibrinogen is an acute phase reactant and elevations may be seen in inflammatory or, less so, acute degenerative disease. This occurs more consistently in ruminants and horses than in dogs and cats. Other acute phase reactant proteins are being developed as tests for inflammatory disease in the dog and cat (see notes under Session 1). If the serum protein is measured by the chemical method (Biuret) then it is not directly comparable to plasma protein measured by refractometer and fibrinogen must be determined by a different method. The cat's pneumonia may well be a result of immune- compromisation. The finding of an opportunistic (conditional) pathogen and the fact that the cat is FeLV positive could well indicate this fact (**conclusion**). **Further investigation** is probably not required once appropriate antibiotic therapy is initiated, but repeat haematology may be useful to ensure that the inflammatory demand and toxic changes are diminishing (ie good prognostic signs). Presumably, diagnostic imaging had been performed at the same time as the pulmonary wash was undertaken.

Diagnosis and postscript: Bacterial pneumonia. The owner was warned of the possibility that the cat might be predisposed to unusual infections in the future. The cat recovered from antibiotic treatment.