Veterinary Clinical Pathology

- Are Clinical Pathologists still relevant? If so, how do they value add for the referring veterinarian?
- What do I think are the difficult or controversial aspects of practising as a Clinical Pathologist?
- What have I learnt over the years which has proved useful in practising clinical pathology (ie how should I think and what do I need to understand and remember in reaching a diagnosis or a way forward to a diagnosis)?

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

A tortuous journey as a clinical pathologist

Acknowledgements – successful outcomes are always dependent on the assistance and support from colleagues, friends and family

- This Roadshow 2013 would not have been possible without the support and assistance from Animal Health Australia and many individuals:
  - I wish to thank all the State Co-ordinators for AAPSP (Liz McInnes, Jeremy Allen, Ian Jerrett, Jim Taylor, Mel Gabor and Helen Owen) for their generosity of time (including travel assistance) and in organising farm animal clinical pathology cases. Taronga Wildlife Hospital, Daren Hanshaw (Gribbles) , Neil Horadagoda (University of Sydney Veterinary Teaching Hospital Camden) and Steven Kopp (University of Queensland) provided some excellent case material for discussion.
  - I wish to thank Tony Ross for cajoling me into undertaking this Roadshow (you were right Tony – it was fun!), advising and supporting me through this whole process and in caring for me whilst I visited Launceston.
  - I wish to thank all the participants who made it so pleasant and interesting. I certainly learnt a lot through those robust discussions of the case material!
  - Finally, I wish to acknowledge the significant input of my colleagues at the University of Sydney in supporting this process. In particular, I would like to acknowledge the professionalism and knowledge of Patricia Martin who contributed to many of the cytological images and their interpretation, and my friend Richard Malik who over many years of discussion convinced me of the need to use all your mental capacities to reach a diagnosis (even pattern recognition!).

What you need to know about me: firstly, I started life as an anatomical pathologist

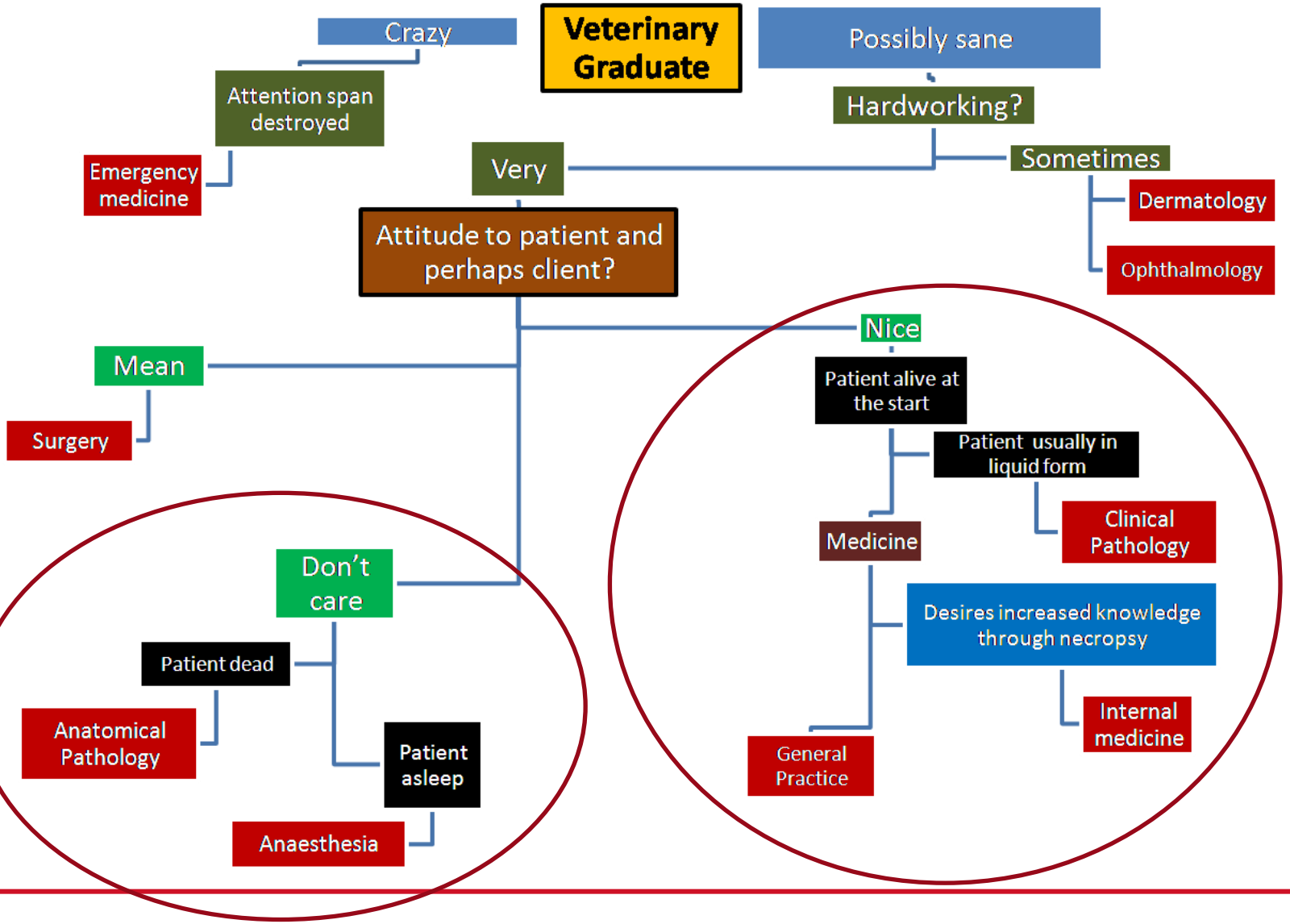
Perez' view of the influences on the Anatomical Pathologist

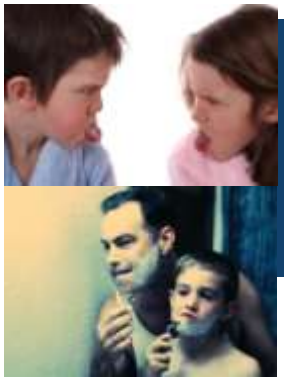
So the way I approach clinical pathology is heavily influenced by my beginnings

You will also need to indulge an old man with his fair share of eccentricities and peccadilloes !

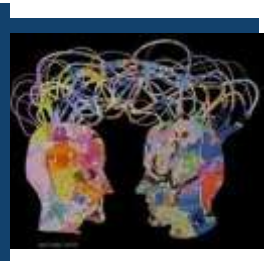
So, perhaps I should let you know my approach to the practise of veterinary clinical pathology – your first indulgence, getting to know how I think!

A very personal algorithm of where clinical pathologists fit into the veterinary profession





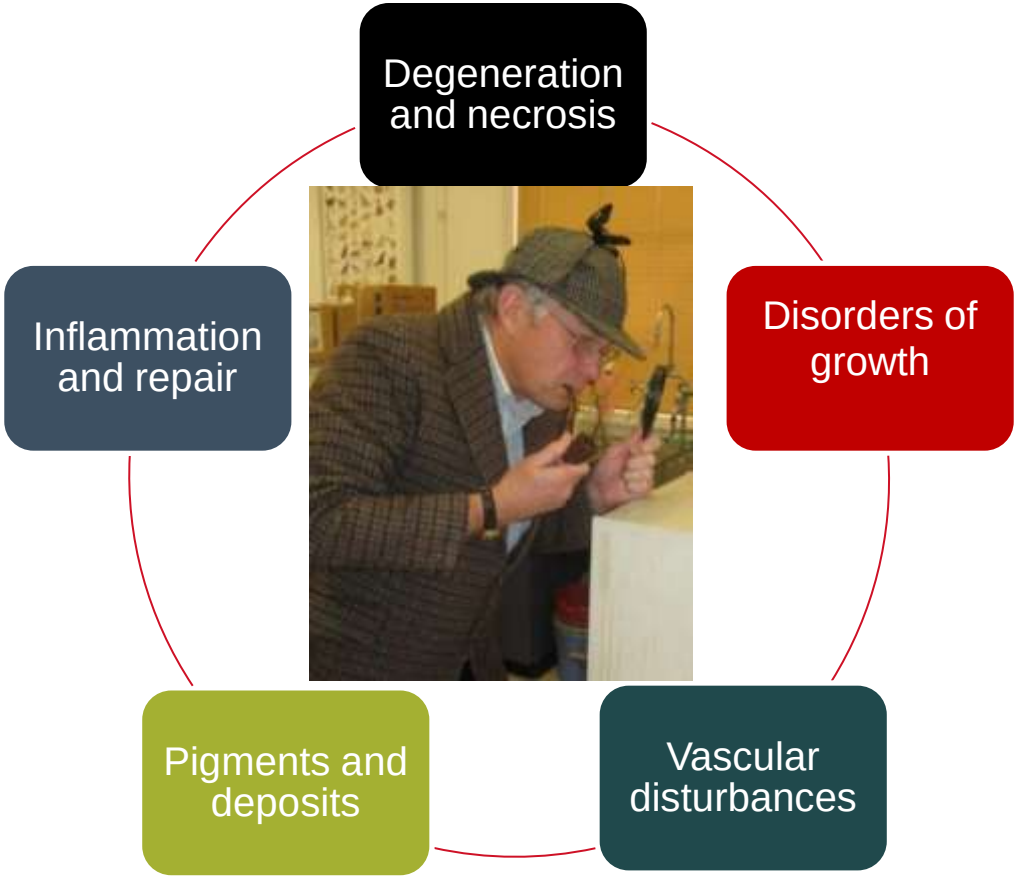
My approach to the practise of veterinary clinical pathology (in a nutshell) – get your mirror neurons into gear!



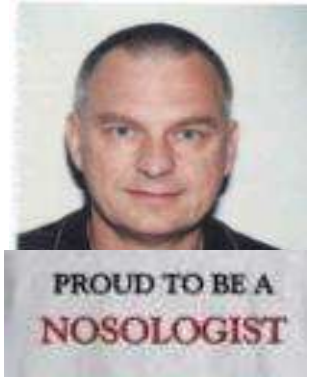
- **The anatomical pathologist's 'five and five' approach to diagnosis**
- **The three 'P's' influence – and sometimes mislead – my approach to diagnosis**
  - I now know how my brain, both reasoning and emotion, influences my decision-making in diagnosis
  - I now know why I love reading detective stories and how it fits in with diagnosis!
- **Sir Francis Bacon and Father Ockham's approach to science and decision-making influence me greatly**
  - Cognitive bias in decision making
  - The value and limitations of the use of heuristics
  - OOSA (objectivity, open-mindedness, scepticism and accuracy) and the three'D's' for diagnosis (detect, describe, deduce) greatly impact on my methodologies

The anatomical pathologist's 'five and five' approach to diagnosis (a 'Mantra'!)

Five Pathological processes: I Diagnose **D**istinct **V**ital **P**rocesses



Pathologists love lists – scratch the surface of a pathologist and you will detect a nosologist

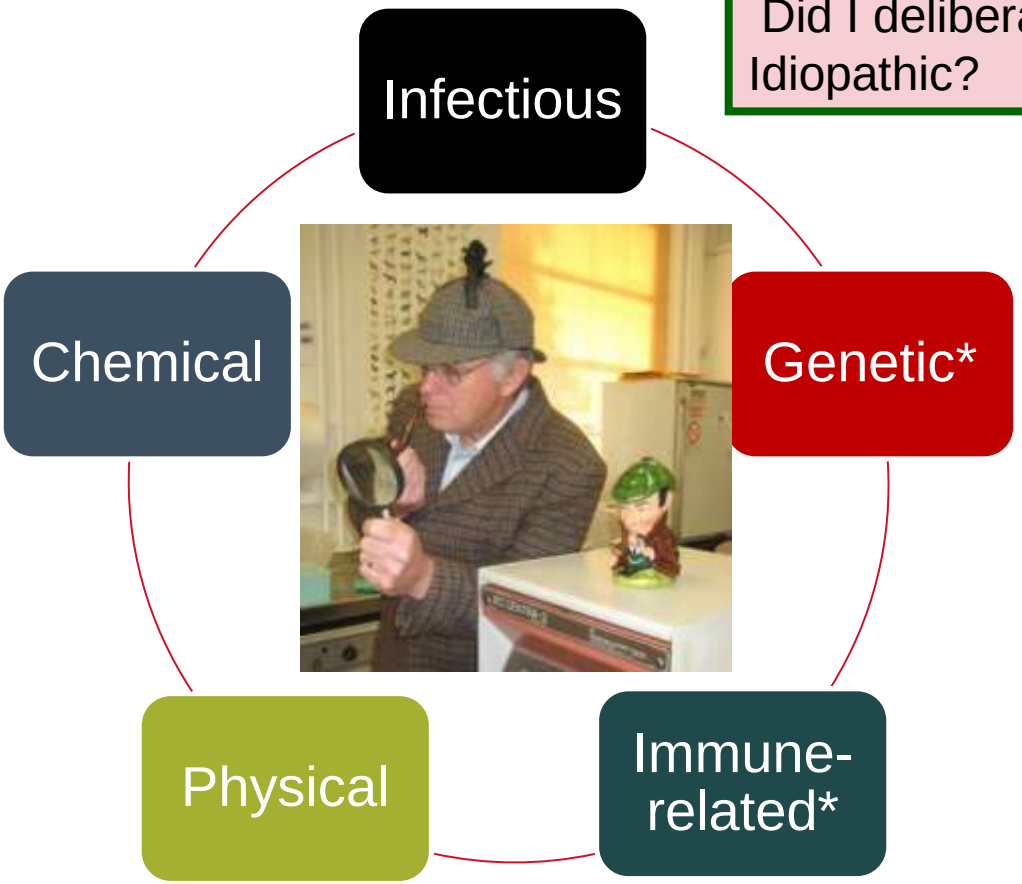


Basis for **morphological diagnosis**

The second five!

Five Causes: I Gather Important Pathological Causes

Did I deliberately forgot Idiopathic?



*often act through or with other agents of disease

Basis for **aetiological diagnosis**

The three ‘P’s’ influence – and sometimes mislead – my approach to diagnosis

- **Personality** – I’m an introvert that enjoys order, logic and lists. **The 3 D’s for Diagnosis – detect, describe and deduce rule my professional life!**
- **Past Experiences and beliefs** – my past mistakes and triumphs influence my present decision-making – sometimes too much!
- **Present Mood** – I’m a morning person! My decision-making capacity is greatly diminished when I am distracted, depressed or distressed.

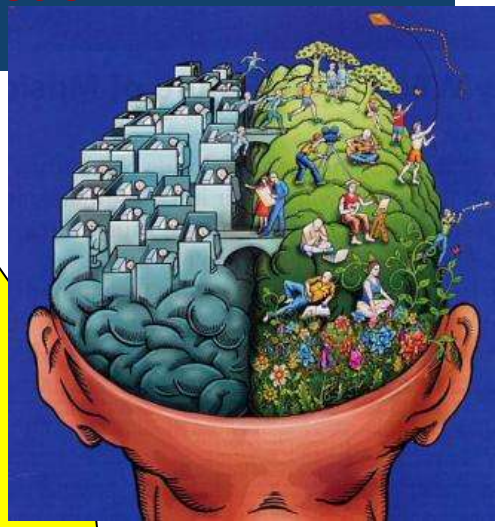
I know the way I use my brain heavily influences the way I detect and interpret disease. I feel more comfortable with System 2 Thinking (the logical stepwise approach of hypothetico-deductive reasoning [the problem-oriented approach]) rather than with System 1 (pattern recognizing). However, I have learnt to trust my intuition and combine the two!

Is pattern recognition really that evil?

Whole brain model for thinking (cognition)

Cerebral (prefrontal lobe) mode

Where do you gravitate?



PROPOSITIONAL KNOWLEDGE

(gaining facts, rules, definitions through logic and analysis)

MEANING UNDERSTANDING

(grasping the pattern, insight and intuition, linking, holistic)

Left mode

Right mode

PROCEDURAL KNOWLEDGE

(being able to do, detailed, apply, plan, sequential)

PERSONAL & EMOTIONAL KNOWLEDGE

(feelings, senses emotions)

Limbic mode (temporal lobe)

Adapted from 'The Creative Brain' 1989 - Herrman N, McGraw-Hill

Is this the reason why some of us naturally take to the problem-oriented approach for diagnosis (lefties) and why others gravitate to pattern-recognition? But all of us can use both sides of the brain! Are 'lefties' all anally-retentive about lists?

Whole brain model for thinking (cognition)

Cerebral mode

Another way of looking at it

Left mode

GOOD AT PUB TRIVIA NIGHTS

HOUSE FULL OF COLLECTIONS

IDEAS PERSON, EVEN IF 9 OUT OF 10 ARE 'CRAP'

GETS BORED EASILY

STRESS OVER SURPRISE PARTIES

APT TO RECHECK MORE THAN ONCE IF THE CAR IS LOCKED

GOOD AT PARTIES.

INTO GROUP HUGS

CRIES AT OLD MOVIES

Limbic mode

Right mode

Remember, the brain is 'plastic'. It can adapt to different usage (but this gets harder as you get older). The best 'laboratory vetectives' use both sides of the brain! **'Use it or lose it' principle.** Keep those hippocampi active and feed the homunculus!

Sir Francis Bacon and Father Ockham's approach to science and decision making influence me greatly

Ockham's Razor: ``*Pluralitas non est ponenda sine neccesitate*", which translates as ``entities should not be multiplied unnecessarily". This is often paraphrased as "**"All other things being equal, the simplest solution is the best**." The forerunner of the KISS principle?

However, Father Ockham also argued that one cannot reach any knowledge of the world purely through logical argument or speculation. Instead we have to look and see how things actually are before we can reason (ie emotion affects perception).

Sir Francis Bacon – the advocate for a new scientific method (OOSA).

One of my heroes

- He advocated observation of fact and recording of observations through collectives rather than individuals (**accuracy**)
- At this stage, one has to be careful not to impose ideas on the facts – but let them speak for themselves (**objectivity)**
- When there are sufficient facts, then regularities and *patterns* will emerge. Causal connections will reveal themselves. However, it is important to keep our eyes open for contrary instances (**open-mindedness)** and not to leap to conclusions

Where is the **scepticism**?

Beware the idols (deceptive beliefs) when engaging in diagnostic reasoning! Maintain healthy scepticism and open-mindedness when reading or listening

1. Idols of the Tribe – deceptive reasoning inherent to the human species **(hardware defects)**
2. Idols of the Cave – deceptive reasoning particular to an individual based on past experiences and prejudices **(software problem)**
3. Idols of the Marketplace – misunderstandings about words – flaws in the written (and spoken?) language when explaining scientific concepts –could they be done on purpose? **(don't accept everything you read without questioning)**
4. Idols of the Theatre - false or misguided philosophies cultivated by experts – is this where I come in? Do these relate to perpetuation of 'urban myths'? **(be sceptical of 'paradigm protectors')**

In essence, he was presenting the reasons for **cognitive bias** that particularly operate in System 1 thinking (pattern recognition), but also in System 2 (problem-oriented or hypothetico-deductive reasoning)

Cognitive bias (flawed thinking) is at its peak when employing intuition/instinct and engaging in pattern recognition because of the 3 'P's'

- **Heuristics (mental short cuts)** are thought to play a greater role in pattern recognition (unconscious, rapid decision making) – and I use them! Three common ones I use are:
  - **Availability heuristic**: what comes to mind first? Is intuition involved ie that unconscious thought? Is this partly about 'common things occurring commonly'?
  - **Representativeness heuristic**: what does this remind me of? The mind tries to link the new to a past experience.
  - **Anchoring and adjustment heuristic**: start off with a premise or 'anchor' and then adjust. Influences the way people 'intuitively' assess probabilities.

"As a rule, when I have heard some slight indication of the course of events, I am able to guide myself by the thousands of other similar cases which occur to my memory. " Sherlock Holmes in *The Red-Headed League* referring to a heuristic. Even he used some 'pattern' recognition' – but he always followed it up with solid evidence and never lost sight of alternatives!

Do you now think you have a better idea of how my mind works as a clinical pathologist?

- **Is it scary?**
- **Do you feel sorry for me?**
- **Do you want to leave the room now?**

It partly explains why I'm naturally interested in the morphological aspects of clinical pathology – although the numbers intrigue me as a devotee of murder-mysteries and criminal investigation (a 'laboratory vetective' who is looking for the right clues!)

And I try to be empathic with of the referring veterinarian. If I don't know the diagnosis will they be happy with a way forward to a diagnosis? Should I console them over the telephone about a difficult case?

More mundane things you need to know about me

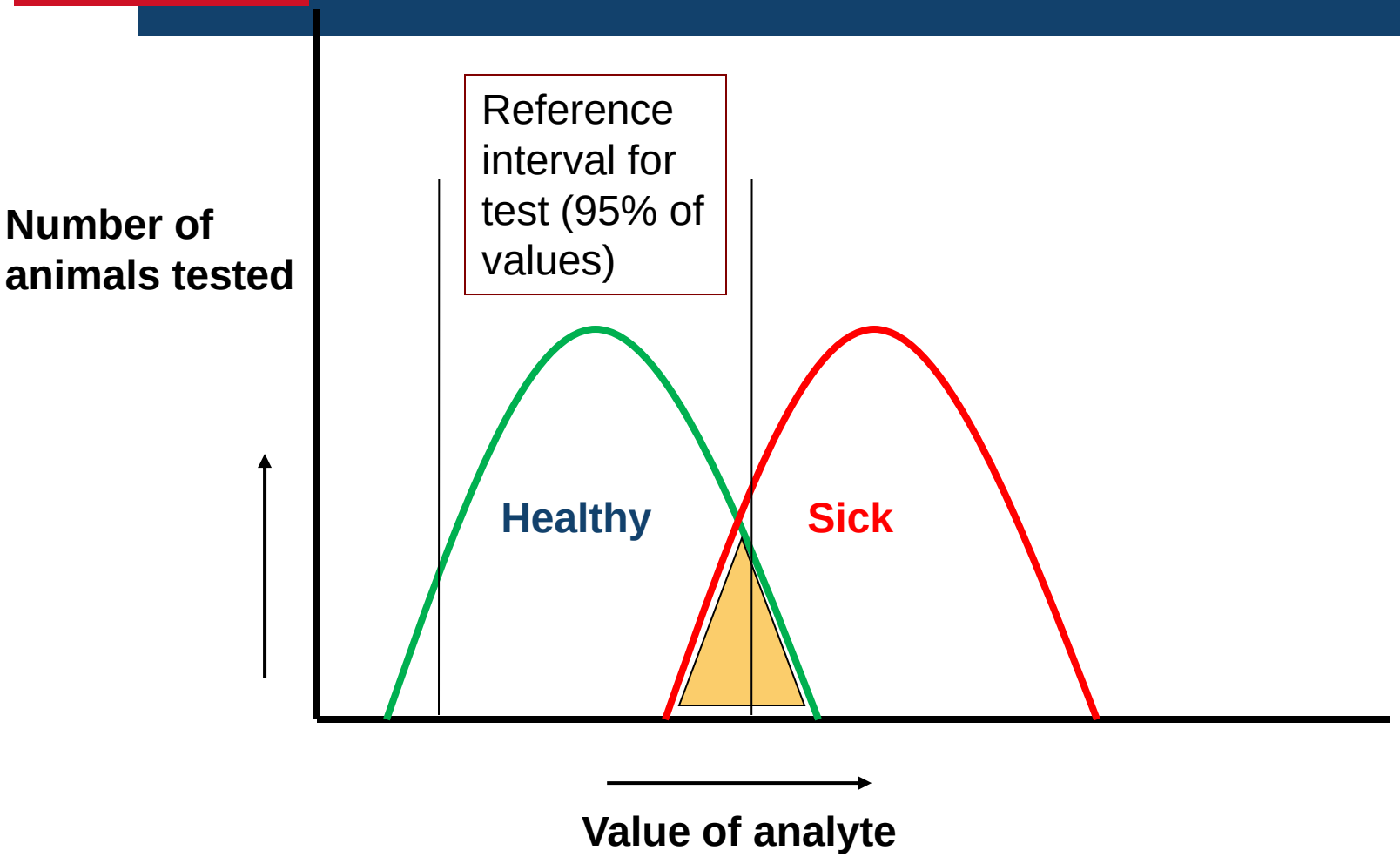
- Least experience with laboratory microbiology, parasitology and toxicology
- My major species focus have been companion and wildlife, but I did teach farm animal clinical pathology and engage in research of sheep and cattle diseases.
- I loath talking about topics related to accuracy, precision, machine calibration, measurement traceability and uncertainty, and quality control and assurance programs (even though NATA expects me to understand it all!)
- But I do accept that having the most appropriate reference values and intervals is vital for interpreting the numbers (even though I abhor statistics!)

Reference Values and intervals

- I started off with using 6-10 'normal' individuals and working out actual ranges of values (real intervals). I threw out any unusual results! Still useful for new wildlife species when investigating a group disease problem (clinically healthy versus sick)
- But if you really want to know all about outliers, parametric and non-parametric statistics in determining values and intervals, and confidence intervals:
  - **Reference values: a review (2009)** - Anne Geffre´ , Kristen Friedrichs, Kendal Harr, Didier Concordet, Catherine Trumel, Jean-Pierre Braun; *Vet Clin Pathol* 38/3 288–298.
  - **American Society for Veterinary Clinical Pathology (ASVCP) Quality Assurance and Laboratory Standards Committee (QALS) - Guidelines for the Determination of Reference Intervals in Veterinary Species and other related topics: SCOPE (November 2011 - *33 pages of stats! – under 'Publications' and 'Quality Assurance Guidelines')***

Reference intervals ('normal values') for tests – this is what I was brought up on

- Determined from clinically normal group but often does not take into consideration variation in age, sex or activity
- Usually cover about 95% of the population
- The reference interval usually overlaps with the disease group (**grey zone**)
- The best tests are those that produce few false negatives and positives (ie high specificity and sensitivity)



This depends on having a bell-shaped distribution of values that lends itself to parametric statistics (RI = mean ± 2SD). If not, then either transform data or use non-parametric methods (2.5 and 97.5% confidence intervals)

If Clin Path is all about interpreting the numbers and the images how does this help the referring veterinarian?

- For the general practitioner, they like to be given at least a way forward, if not diagnosis, from interpreting both the numbers and the images
- The medical specialists interpret the numbers themselves – but many are reluctant to look down the microscope. Even if they look down the microscope, they usually struggle with neoplasia (don't we all?)
- The specialist surgeon wants a definite answer or at least one that helps him make the decision whether to cut or not to cut!
- The production animal veterinary officer or practitioner needs help to determine the cause of group disease and to prevent further disease
- The researcher needs accurate values so statistics can be done. Group results are more important than individual animal results
- The wildlife vet or carer needs all the help they can get!
- **Guess which group I find most frustrating to deal with**?

Individual animal disease – the dilemma for clinical pathologists

- The results for clinical biochemistry (and for everything else for that matter) for an individual animal don't always follow the 'rules'. We can put this down to biological vagary or natural variation, or we can accept that there are limitations for the hypotheses or 'anchors' employed to interpret
  - serial samples and test combinations (especially overlap testing) help
  - Complete confidence in your numbers (and machinery) rules out this as a contributor to the variation
  - Of course, getting some help from the submitting vet through appropriate information is really useful!

How does group animal disease affect the way we interpret the numbers and images?

- Anatomical pathology rules!
  - But clinical pathology may be asked to follow up from necropsy and look for a cause
- If necropsy is not possible then clin path comes to the forefront and there is the luxury of having multiple samples from animals variably affected by the disease process (mild, moderate, marked) – **averages and percentages can be used (looking for trends), so it diminishes the effect of biological vagary**!
  - My experience is that infectious agents or chemicals (eg plant poisons, metabolic disorders) are most likely to cause group illness in production animals. Management practices are a key factor in pathogenesis
  - Laboratory animals and captive wildlife may experience group problems because of infectious agents, but most are predisposed by management practices
  - Free-living wildlife are difficult to catch for sample collection if healthy. Most group problems are going to be due to *habitat alteration* predisposing to infectious disease

What am I going to attempt to cover over the next two days?

- **Clinical biochemistry (enzymes, proteins, electrolytes etc)**
- **Haematology**
- Microbiology
- Parasitology
- Serology
- Toxicology
- **Cytology (cytopathology) (fluid analysis and solid tissue cytology)**
- Pathology (biopsy, necropsy)

Today, the sessions will be primarily on comparative haematology, but I will start on cytopathology

Tomorrow, I will take the cytopathology further, but will also introduce aspects of bone marrow examination, leukaemia and fluid analysis

I also want this to be interactive rather than completely didactic

Did you know that 60% of the responders to Tony's email on this course said they did not want interactive material. That does tell me that there will be resistance to involvement!

So, does that mean I shouldn't try and engage with you?

I'm reminded of the old Chinese proverb: **'Tell me and I will forget, show me and I might remember, involve me and I will understand'**

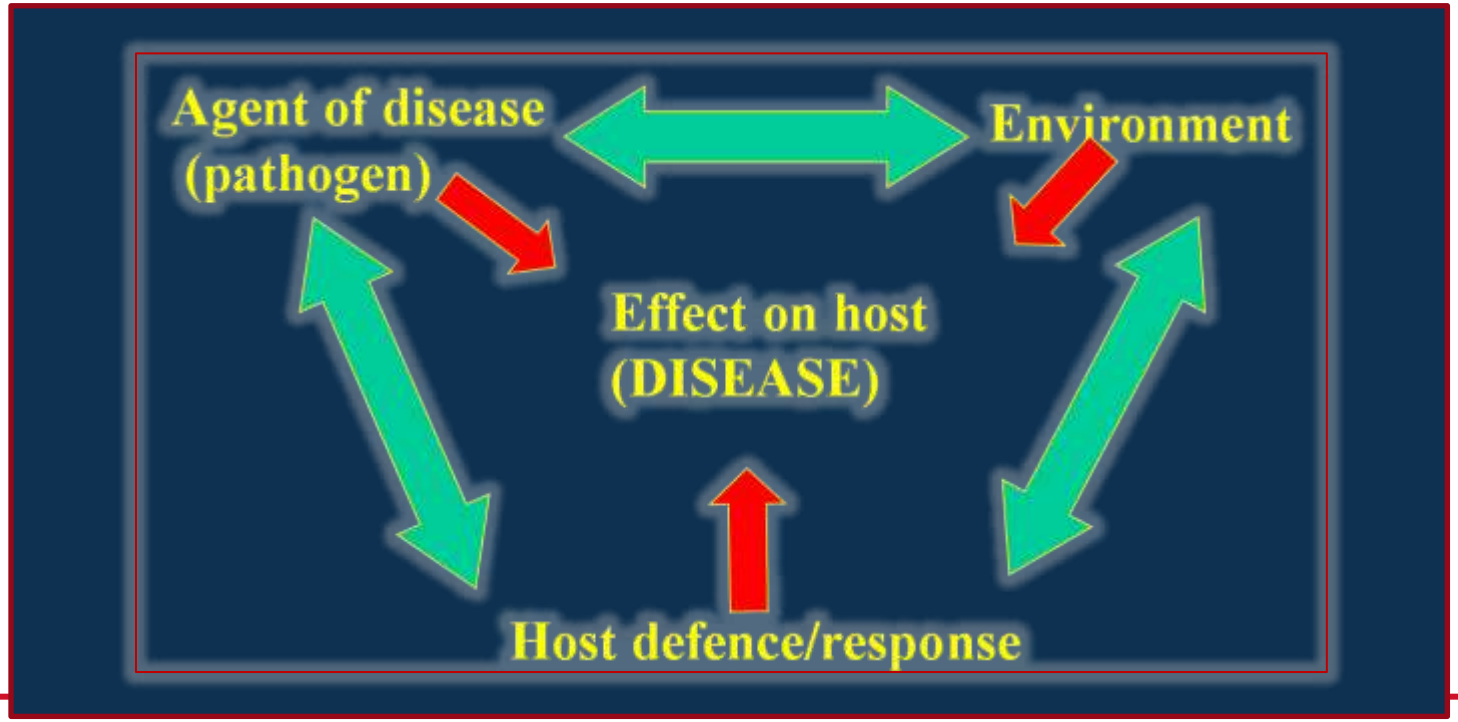
I think you know what is coming: yes, I will attempt to involve you through –

1. Asking for your views and experiences
2. Asking some questions (maybe straw polls to be non-threatening)
3. Working through perplexing cases (group involvement – your choice?)
4. Any other way I can keep you awake and interested!

No apologies, but I am mindful that some of you are highly experienced, so I would like to call on your assistance in some of these exercises

Some fundamental thoughts on the laboratory tests. What do they actually tell you?

- Tests will detect **tissue dysfunction and damage (effect on host – generally degeneration and necrosis), host response (immunity – the fifth pathological process)** and help in understanding **aetiopathogenesis (cause and development)**



Why did I show that diagram? Just to remind you of the complexity of disease?

- **General Blood Biochemistry** – trying to detect tissue damage or indications of dysfunction (**effect on host** - may detect which tissue) but may indicate **host response**
- **Haematology** - trying to detect:
  - Diseases that *primarily* affect blood eg AIHA, leukaemia (**aetiology**)
  - Blood responses/effects *secondary* to disease elsewhere eg inflammatory demand neutrophilia to an abscess (**host immunity**), anaemia of chronic renal failure (**effect on host**)
- **Cytology –** may detect **aetiology**, will show cell damage (**effect on host**) and **host response** (inflammation)

A reminder for us all!

- Clinical biochemical and haematological tests are usually of low specificity (why you use them in **combination**)
- Others are more specific for disease determination (eg cytological and microbiological examination)
- Tests will need to be varied depending on species

Issues for me in clinical biochemistry (and haematology) interpretation

- Trying to stay with the *big picture* for the referring veterinarian and animals' sake
- Trying to remember *all the reasons* why the numbers go up and down
- Forgetting the *limitations* of individual test analytes
- Forgetting which analytes are affected by *time delays, haemolysis and lipaemia*
- **Making sure I'm not distracted when interpreting**

Specific issues for me in clinical biochemistry (and haematology) interpretation

- Reminding myself most ‘damage’ enzyme values are only increased significantly if most of the damage is occurring at that point in time
- Reminding myself that protein analysis is primitive in the first instance
- Reminding myself that electrolyte variations are always complex to interpret because of interactive intracellular and extracellular influences
- Reminding myself that groups of tests may give me a **pattern** for a disease process (tissue or pathological process)
- Reminding myself that cancer can lead to inflammation and necrosis which contribute to clin path changes

What are the major issues for you when interpreting clinical biochemistry?

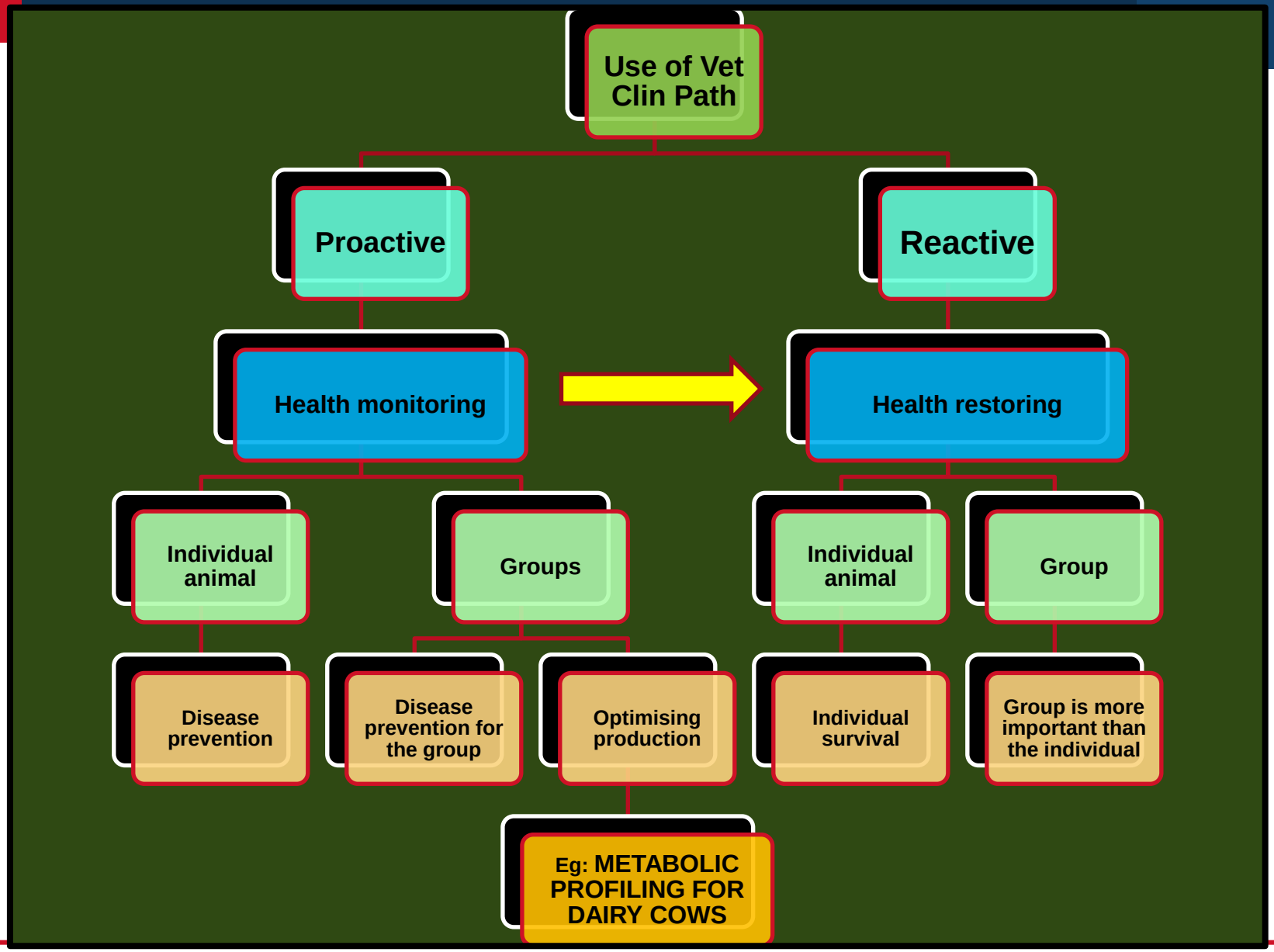
Patterns in the numbers?

- I look for *mini-patterns* –some biochemical and haematological tests are linked/inter-related (eg organ biochemical tests; leukogram; electrolyte relationships).
- The *final disease pattern* can vary from likely organ or tissue involvement to a pathological process (or combination), but actual causation is the Holy Grail (and trickier).
- My approach to the numbers is very much detect, describe and deduce. I focus on the **general** in deduction before I move to the **specific**, but sometimes a specific disease pattern hits me (those nasty heuristics!). If this happens then I force myself to checklist the evidence and think of alternatives, just to make sure cognitive bias is not misleading my pattern recognition
- If I can't see a pattern, I momentarily panic (I have a hypersensitive amygdala) but then fall back on the logical, sequential approach to at least come up with a list of possibilities and provide a way forward for the referring veterinarian

I want to ‘Cherry Pick’ a couple of issues for discussion

The Proactive Versus the Reactive Approach to Animal Care

Proactive versus reactive – how clinical pathologists can value add!



How many of you are involved in metabolic profiling for Dairy Cows?

1. Aim: to recognise *subclinical metabolic disorders* in a dairy herd that might be contributing to deficiencies and negative energy balance and less than optimum production or reproduction
   - If possible, all groups should be represented (fresh cows, peak lactation, middle lactation, dry – say between 6-12 animals per group)
   - Clinically unwell cows should be excluded (although their investigation may provide leads to any subclinical issue within the herd)
   - Preferably, results should be compared with previous results for the herd as well as to established reference values for the breed of dairy cow
   - Means for the group are of little value compared to determining the proportion of the group falling outside a reference value (an indication of the level of risk). Pooled results are cheaper, but will only give a mean, and individual animals cannot be identified for further investigation.

Classic Compton Metabolic Profile – 13 tests (Payne et al. [1970]. Vet. Rec.)

1. Glucose
2. Urea
3. Phosphate
4. Calcium
5. Magnesium
6. Potassium and sodium
7. Albumin
8. Globulin
9. Haemoglobin and PCV
10. Copper
11. Iron

Three groups of 7 cows

- **Dry cows**
- **Middle yielding**
- **High yielding**

This profile can be altered to suit local issues that might be affecting milk production eg cobalt deficiency, Vitamin E or selenium deficiency, BHB for ketosis

Assessed potential or real disease problems and the nutritional status of the herd.

But where is assessment of negative energy balance?

A later approach, focusing on assessment of Negative Energy Balance added to profile

Blood:
Ketone (BHB)
Non-esterified fatty acids (NEFA)
Glucose
Urine (can be done additionally and at the farm):
pH, net acid-base excretion (NABE)
Ketones

This profile is added to other tests in order to suit local issues that might be affecting milk production eg **protein assessment** (total protein, albumin, globulin and urea); **minerals and trace elements** (calcium, copper, cobalt, selenium, magnesium, phosphate); **vitamins**(Vit E, beta-carotene); **liver enzymes**

Test *Blood from tail stick.*	Eligible Group	Test Cut Points	Alarm Level portion
NEFA	Pre-Fresh cows, ideally 2 to 14 days from calving	>0.400 mEq/L	> = 10%
NEFA	Dry cows >2 weeks from calving	<0.32 mEq/L	> = 10%
BHBA	Lactating cows, about 5 to 50 days in milk.	> 14.4 mg/dL	> = 10%

NEFA is sensitive but not specific for production-induced NEB before calving

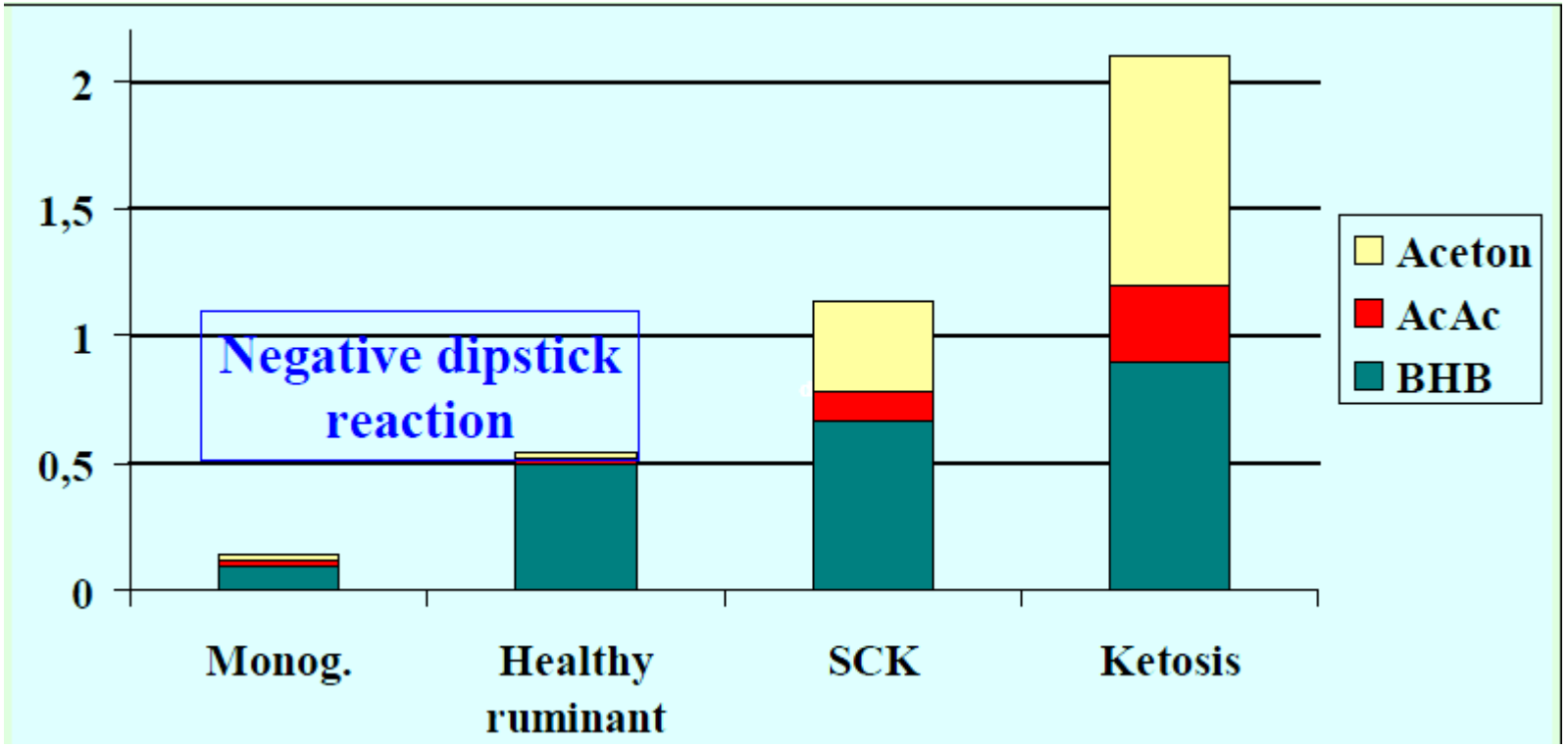
Assessment of NEB has become important because of the focus on subclinical leading to clinical metabolic disorders in the periparturient or transition period

Periparturient or "Transition"Period (3 wk before to 3 wk after calving) is the most critical 6 wk of lactation cycle

Most common disorders of transition period are:

1.Fatty liver (ketosis type 1, due to NEB pre-partum)
2."Classic"ketosis type 2, due to NEB post-partum)
3.Milk fever (+ often downer cow)
4.Subacute ruminal acidosis (SARA)

Ketone bodies in blood plasma – subclinical and clinical Ketosis



Nitroprusside-Na tests react with acetoacetate, partially with acetone, but **DO NOT REACT** with BHB (usually first ketone body to rise as AcAc is degraded to BHB quickly, but SCK and clinical K have good rises in the others as BHB reaches saturation) *nb POC instrument for blood BHB available as is dipstix for milk BHB*

Blood beta-hydroxybutyrate

”Gold standard”method for evaluation of NEB post-partum

- CUT-OFF point is 1.4 mmol/L in early lactation (for subclinical ketosis [>2.0 mmol/L for clinical])
- Individual higher concentrations are common (may give mean above cut-off point) **but** the ALARM LEVEL PROPORTION is when ≥10% of sampled fresh cows are above cut-off point!
- Increased by poor quality silage

- **Clinical ketosis rates are of limited value in assessing true ketosis status of a herd (Oetzel, 2004)**
- **The measured prevalence of subclinical ketosis(SCK) is more important for herd management**

Subacute ruminal acidosis (SARA)

- Increasing problem in well-managed, high yielding dairy herds (11-18%)
- Australia: pasture-based acidosis
- May get ruminal parakeratosis(→hyperkeratosis →liver abscesses), thiamine deficiency (→cerebrocortical necrosis) and bone demineralisation (→osteomalacia and urolithiasis)
- Milk fat content reflecting the metabolic acidosis caused by SARA is a promising tool (but not in fresh cows – first week of milking)

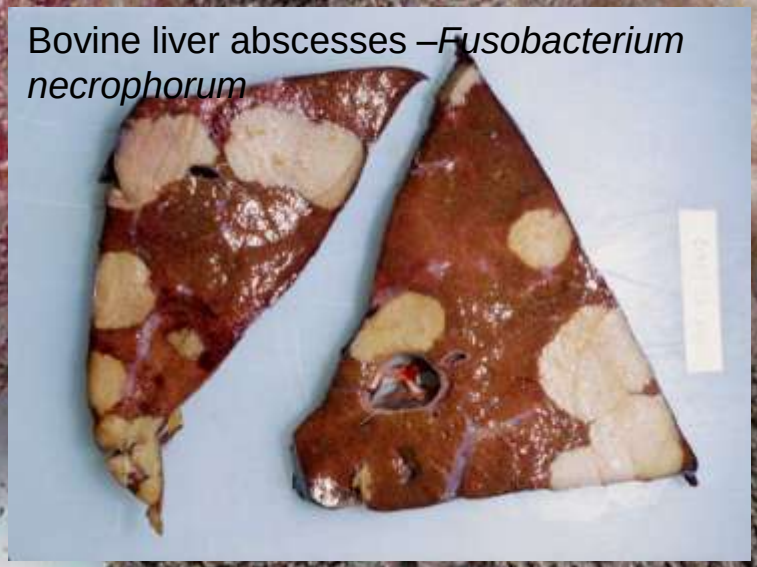
Ruminal pH cut-off point for SARA: pH ≤5.5 considered as SARA positive; pH ≥5.8 negative

Urine pH, NABE and SARA:
Urine pH often decreases with SARA as renal excretion of H+ is increased, but assessment of renal net acid-base excretion(NABE)is more accurate

SB (UNE) Experimental bovine ruminal acidosis, rolled barley

ruminal acidosis: predominantly affects ventral sac

normal rumen



Look at AAPSP Website for Diseases of Production Animals - Barbara Vanselow

Metabolic profiling in ewes and goats – has anyone had experience with this?

Mainly for metabolic disorders in later pregnancy or parturition (peripartum) – looking for NEB, deficiencies and ketonaemia

E.G. PREGNANT (PERIPARTUM) EWES AND DOES ('NANNY GOATS')

Energy assessment

total cholesterol; triglycerides; nonesterified fatty acids (NEFA); Beta-hydroxybutyrate (BHB=BOHB); Glucose

Protein Assessment

Creatinine; Urea; total protein, albumin

Liver assessment

total bilirubin; AST; GGT

Electrolytes

Cl; K; Na; Ca; IP

May vary depending on local conditions and breed under investigation

Ruminal acidosis in sheep

•Probably similar pathophysiology to cattle. Does SARA exist?

•A common cause of severe clinical acidosis outbreaks is when sheep have had accidental access to large amounts of grain.

Extensive management outbreaks:

- Large amounts of grain are spilled in harvested paddocks or during grazing in paddocks that contain grain silos.
- Sheep given access to grain stubble after harvest (cropping areas).
- Introduction onto crops, particularly brassicas such as rapes, kales or turnips, that are highly digestible

Intensive management outbreaks (feedlots for live export and 'opportunity lots' for fattening lambs for slaughter):

- Still accidental access to large amounts of grain or any soluble source of carbohydrate

For dairy cattle information: with thanks to Dr Tibor Gaál DVM PhD Dipl ECVCP, Clinical Pathology, College of Veterinary and Life Sciences, Murdoch University, 90 South Street, Murdoch, 6150 WESTERN AUSTRALIA

Useful References:

1. University of Guelph AHL LabNote: Number 4, 4 January, 2013. **Nutritional and metabolic profile testing of dairy cows.** Brent Hoff, DVM, DVSc, DipTox, Animal Health Laboratory; Todd Duffield, DVM, DVSc, Department of Population Medicine, Ontario Veterinary College
2. *The Veterinary Journal* (2013) (on line). **Review: elevated nonesterified fatty acids and β-hydroxybutyrate and their association with transition dairy cow performance.** A. McArt, J.A., Nydam, D.V., Oetzel, G.R., Overton, T.R., Ospina, P.A.
3. *Journal of Animal Science* (1998) **76**:323-327. **Application of research findings and summary of research needs: Bud Britton Memorial Symposium on Metabolic Disorders of Feedlot Cattle.** M. L. Galyean and K. S. Eng.
4. *Animal Science Journal* (2010) **81,** 722–730. **Preliminary study on metabolic profile of pregnant and non-pregnant ewes with high or low degree of behavioural lateralization**. Massimo Morgante, Matteo Gianesella,elisabetta Versace, Laura Contalbrigo, Stefania Casella, Chiara Cannizzo, Giuseppe Piccione and Calogero Stelletta.
5. *Australian Journal of Experimental Agriculture,* (2008), **48**, 1004—1008**. Metabolic profile and oxidative status in goats during the peripartum period**. Pietro Celi, Adriana Di Trana and Angelo Quaranta.

Another cherry picking topic! Enzymes

- Most tested are **plasma non-specific** (ie no role in plasma) and related to either *cell metabolism* (both **organ specific** and **non-specific**) or *secretion*
- Isoenzymes make organ non-specific enzymes more specific
- Factors affecting levels **not completely understood**, and include:
  1. Alterations in cell permeability or internal organization (eg CK release from exercised muscle)
  2. Cell death
  3. Altered enzyme synthesis (eg steroid induced production of ALP)
  4. Interference with removal (eg AMS in the cat [and dog?])
  5. Laboratory issues (eg technique of assay, storage – enzymes are proteins!)
  6. Time of collection during a disease process

ENZYME USAGE IN DOMESTIC ANIMALS – some species variation

	Dog/Cat	Horse	Sheep/Cattle	Pig
GENERAL DETECTION OF CELL DAMAGE	AST (mainly muscle/Liver), LD (present in most cells) for all species			
HEPATIC DISEASE - HEPATOCYTE DAMAGE	ALT (ARG - can be used for all species but rarely done)	ID (SDH) or GLDH - (ARG)	GLDH or ID (SDH) or OCT - (ARG)	OCT - (ARG)
HEPATIC DISEASE – CHOLESTASIS	ALP, GGT	GGT, ALP (less commonly used)	GGT	GGT
CARDIAC/ SKELETAL MUSCLE CELL DAMAGE	CK, AST, LD (especially isoenzyme analysis) for all species. NB it has been reported that certain skeletal muscle diseases in some dogs and cats may cause elevations of ALT. ALT elevations in horse, ruminants, pigs and birds are mostly due to muscle damage, but are not usually marked			
PANCREATIC ACINAR CELL DAMAGE	AMS, LPS (other laboratory aids may be more useful)	AMS	not done	not done
GUT DAMAGE	AMS?, ALP? other laboratory aids are of greater use for all species			
CNS DAMAGE	CK (in CSF) for all species, other laboratory aids of greater use			
REPRODUCTIVE TRACT DAMAGE	other laboratory aids of greater use			
JOINT DISEASE	Other laboratory aids of greater use (AST, ICD, LD can be measured in the synovial fluid, especially in the horse)			
RENAL DAMAGE	other laboratory aids of greater use (GGT has been measured in urine in dogs and cats to detect renal cell damage)			
ENDOCRINE DISORDERS	laboratory confirmation usually requires hormonal assays - enzymes may detect secondary organ dysfunction (eg diabetes mellitus may cause elevation in liver enzymes) NBs hyperadrenocorticism in the dog can induce a specific isoenzyme of ALP; hypothyroidism in the dog can be characterised by increases in CK			

Birds: LD, AST, CK, (GLDH and SDH, GGT for liver, but rarely used)
Reptiles (only a few species tested): LD, AST, CK. No specific liver enzymes?

Plasma or serum proteins

- **Serum proteins = plasma proteins - fibrinogen and factors V+ VII**
- **Total proteins = albumin +globulins (+pre-albumin if present)**

- **Acute phase proteins (reactants)** *modulate immunity*
  - Major negatives are **pre-albumin, albumin and transferrin (**transferrin may increase in acute liver disease)
  - Major positives are alpha or beta globulins and vary amongst species (and diseases within a species)
    - **Fibrinogen** (and some other **non-enzymic clotting proteins**) for all mammalian species (limited use in dog and cat), birds and probably reptiles and fish (*but usually only 1-10 fold increases*)

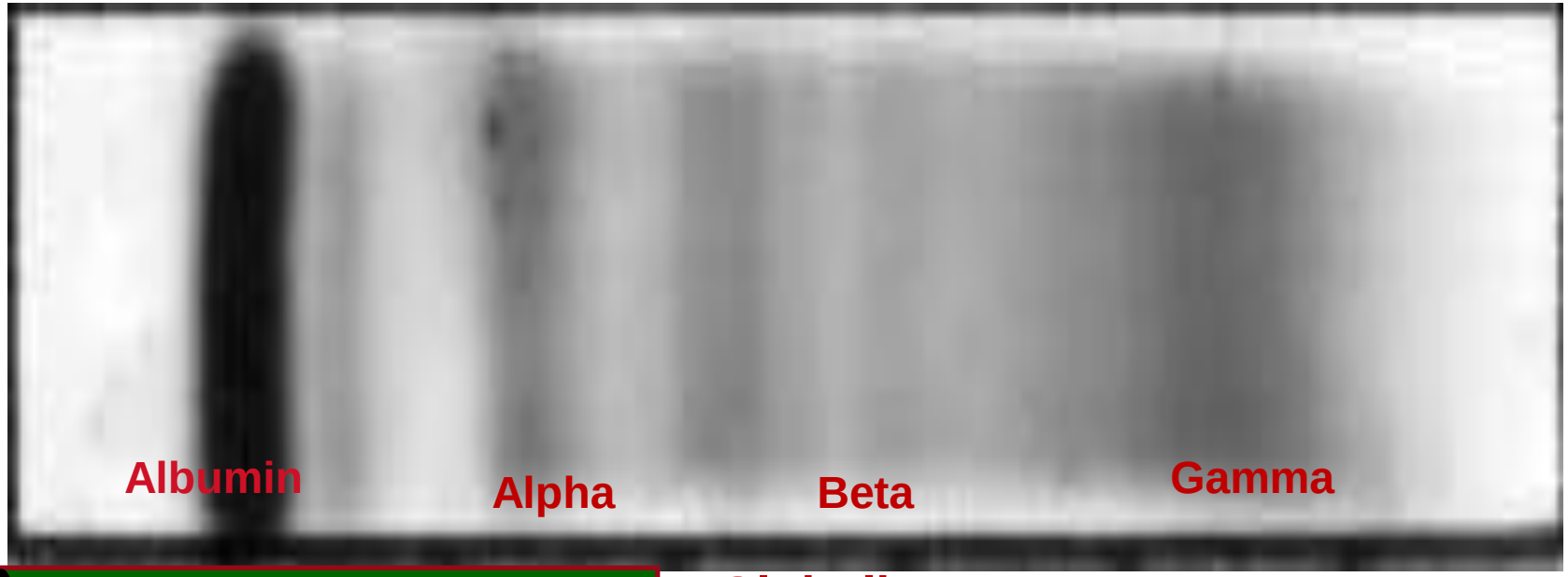
By how much do they push up total protein and globulin values?

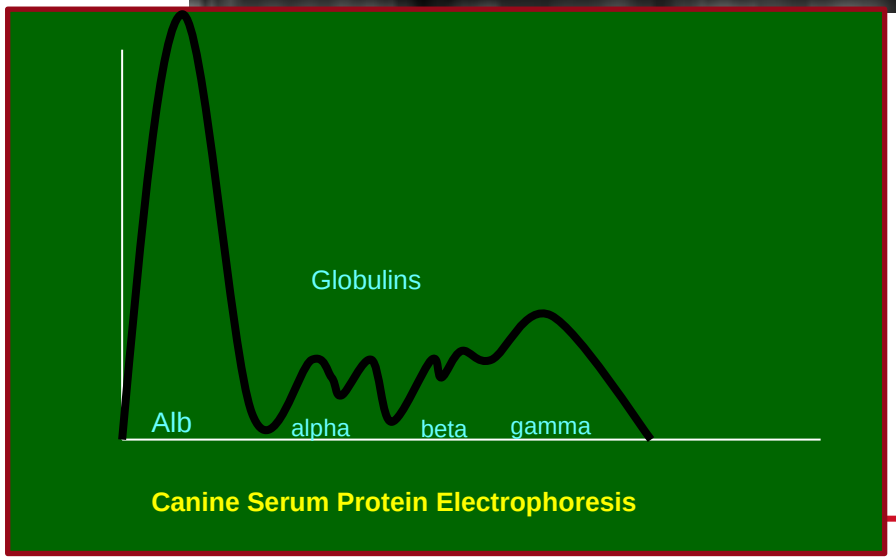
Other positive Acute Phase Protein usage

Species	Moderate to Major APP (1-100 fold increases)
Dog	CRP, SAA, Hapt, a1-AGP
Cat	SAA, Hapt, a1-AGP, TNF-a?
Horse	SAA, Hapt
Cow	SAA, Hapt, a1-AGP, a2-Macroglobulin?
Sheep	SAA, Hapt
Pig	CRP, SAA, Porcine Major AP, Hapt
Rat, mouse, rabbit	a2-Macroglobulin (R), SAA , Hapt, a1-AGP
Birds and reptiles	SAA, Hapt, CRP?
Fish (only a few species reported)	SAA, Hapt, CRP

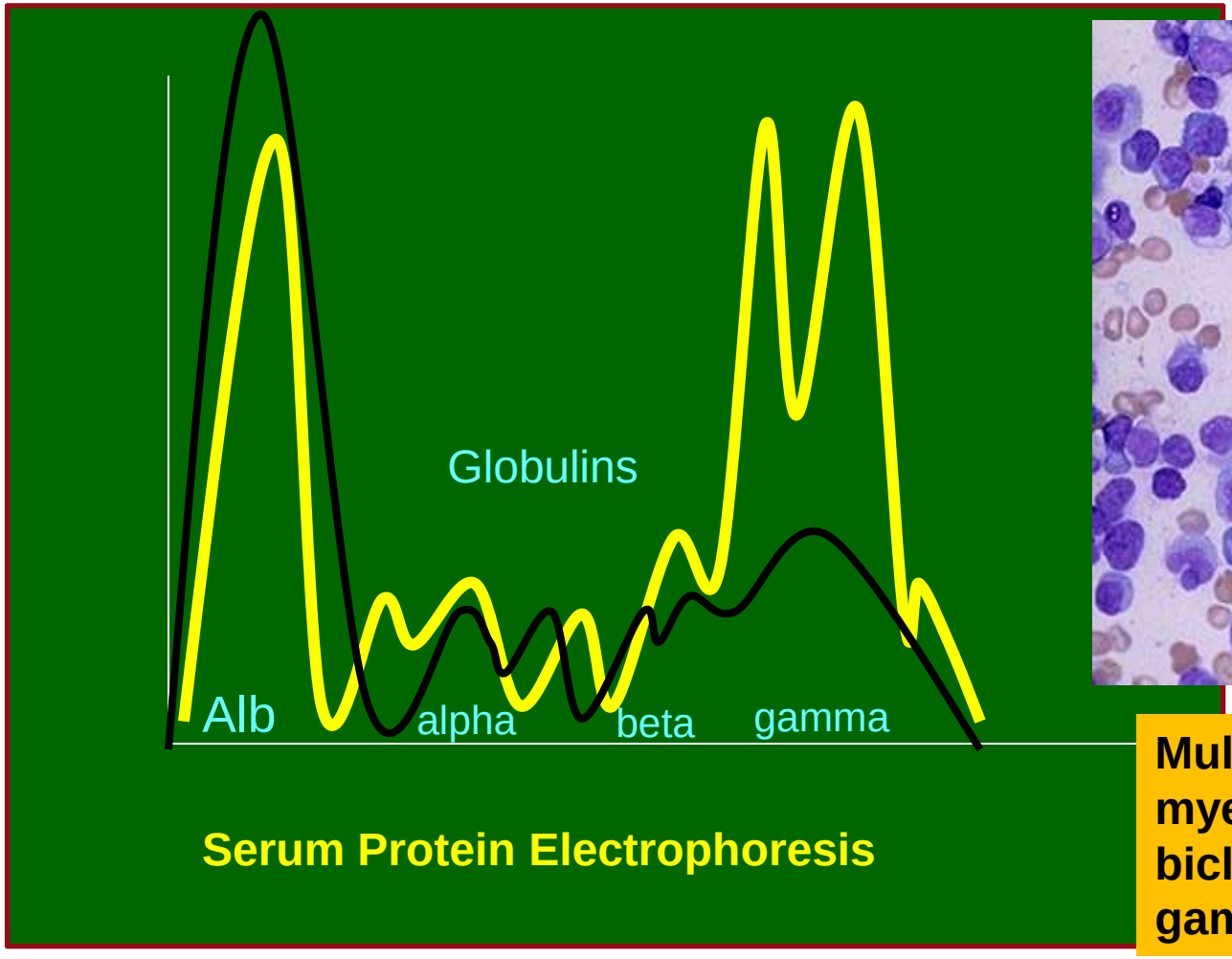
- Many others reported (eg coglutinin, beta 2-Macroglobulin, ceruloplasmin)
- **What are you using? Should you use them in combination, as they can rise and fall at different rates and times and are variable depending on the disease process affecting the species?**

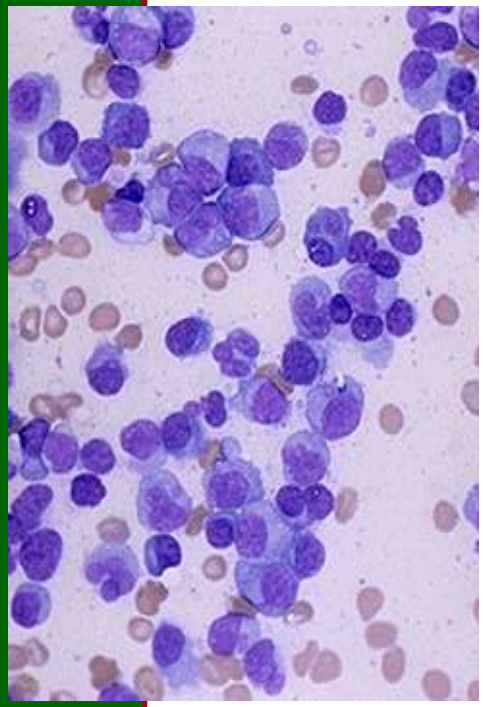
How often are you being asked for Serum Protein Electrophoresis for increases or decreases? Or is it all A:G ratios?





An old dog with exercise intolerance and lassitude





Multiple myeloma with biclonal gammopathy

The relationship between total calcium and protein levels

- How many are doing ionised calcium?
- How many of your referring veterinarians realise that total calcium need to be corrected for hyper or hypoproteinaemia (nb formulae apparently only work if you have normal acid-base balance)
  - What formula are you using for correction (eg corrected calcium = measured calcium + [average albumin - measured albumin, divided by 40]; where average albumin is taken as 33 g/L for dog, 29 for cat and 30 for horse)? Ruminants (30-32)? Birds?

Jeremy Allen DAFWA: **Hypocalcaemia in Sheep Based on Ocular Fluid**

Spec No.	Spec ID	Magnesium in Vitreous Humor	BHB in Vitreous Humor	Urea in Vitreous Humor	Inorganic Phosphate in Vitreous Humor	Calcium in Vitreous Humor	Creatinine in Vitreous Humor
		mmol/L	mmo/L	mmol/L	mmol/L	mmol/L	umol/L
8	Sheep 1	**0.96**	0.92	**10.4**	1.68	**0.82**	80
9	Sheet 2	**1.30**	0.91	5.7	3.42	**0.89**	36
Ref values:		0.4 - 0.8		<7.0		1.5 - 2.1	

RESULTS OF EYE FLUID ANALYSIS THAT ARE SUGGESTIVE OF CLINICAL DISEASE IN CATTLE AND SHEEP

	Aqueous humour	*Vitreous humour*
Calcium	<1 mmol/litre	<1 mmol/litre
Magnesium		
Cattle	<0·25 mmol/litre	<0·55 mmol/litre
Sheep	<0·33 mmol/litre	<0·65 mmol/litre
Urea	>30 mmol/litre	>6·6 mmol/litre (sheep) >7·3 mmol/litre (cattle)
Beta-hydroxybutyrate* (sheep)	>2·5 mmol/litre	No verified data

*No verified data are available for anything other than aqueous humour in sheep. Values for cattle may be extrapolated from those in sheep as both species have a similar reference range in plasma (the VLA uses 0 to 1·2 mmol/litre, as a guide to interpretation)

Use of ocular fluids to aid postmortem diagnosis in cattle and sheep

Gareth Edwards and Aiden Foster

In Practice 2009 31: 22-25

Ocular fluid analysis of calcium in cattle and sheep

- As ocular fluid calcium is in equilibrium with ionised calcium, not total plasma calcium, ocular fluids usually contain approximately 50 per cent less calcium than blood
- Calcium levels of <1 mmol/litre are suggestive of terminal hypocalcaemia. The plasma reference range for calcium in cattle and sheep is 2 to 3 mmol/litre
- AH may more accurately reflect recent rapid changes in serum calcium than VH
- Ocular fluid biochemistry should not be used as a diagnostic criterion but more as an adjunct to other diagnostic information regarding the animal sampled. Results must be considered in relation to clinical history, gross pathology and the estimated time of death

Interpretation of magnesium concentrations in cattle and sheep

- AH magnesium levels of <0·25 mmol/litre in cattle and <0·33 mmol/litre in sheep, and VH magnesium levels of <0·55 mmol/litre in cattle and <0·65 mmol/litre in sheep are consistent with hypomagnesaemia
- Low magnesium concentrations in ocular fluids are usually clinically significant, unless bacterial growth has caused the decrease
- Normal or elevated levels of magnesium in AH or VH do not rule out the possibility of hypomagnesaemia, as magnesium contamination of the sample may have occurred as a result of autolysis, contamination with cells or cellular debris or the use of contaminated containers

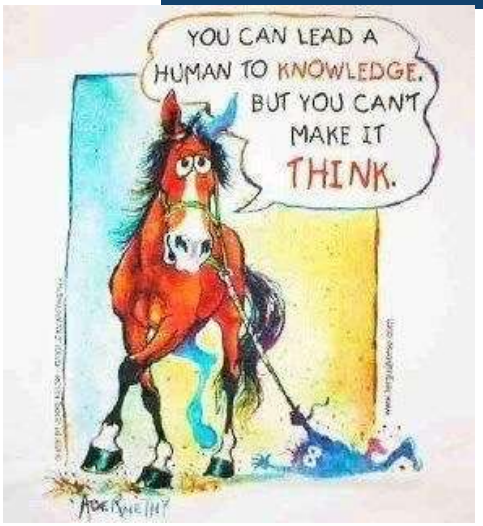
A reminder that biochemical analysis can be done on fluids

Early Reference: Postmortem Eye Fluid Analysis in Dogs, Cats and Cattle as an Estimate of Antemortem Serum Chemistry Profiles. Paul E. Hanna, James E.C. Bellamy and Alan Donald. Can J Vet Res 1990; 54: 487-494

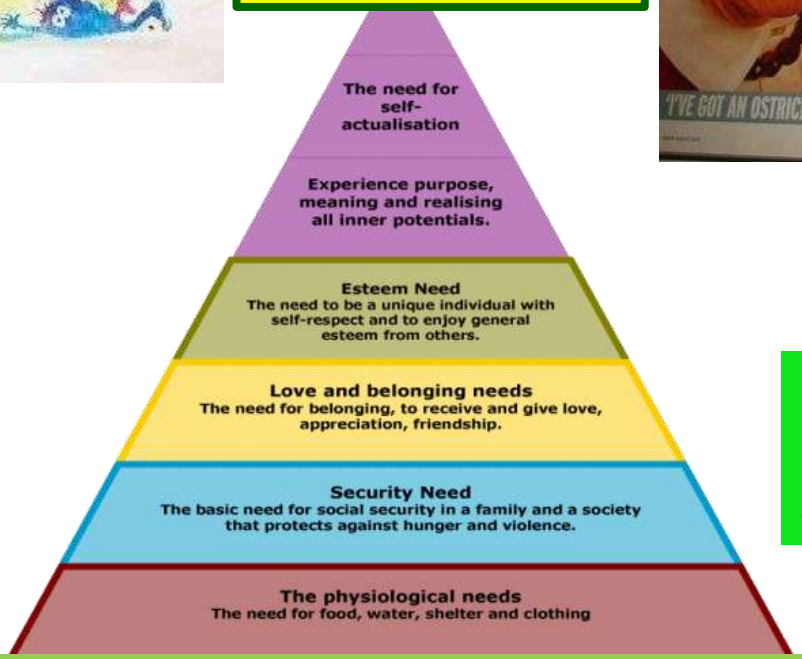
Useful References:

1. Vet Clin Food Anim (2007) 23: 403–426. **Evaluation of the ruminant serum chemistry profile**. Karen E. Russell, Allen J. Roussel.
2. Martina Klinkon and Jožica Ježek (2012). **Values of Blood Variables in Calves, A Bird's-Eye View of Veterinary Medicine,** Dr. Carlos C. Perez-Marin (Ed.), ISBN: 978-953-51-0031-7, InTech, Available from: http://www.intechopen.com/books/a-bird-s-eye-view-of-veterinary-medicine/values-of-blood-variables-in-calves
3. The Veterinary Journal (2010) 185: 23–27. **Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine** P.D. Eckersall, R. Bell.
4. In Practice (2009) 31: 22-25. **Use of ocular fluids to aid postmortem diagnosis in cattle and sheep**. Gareth Edwards, Aiden Foster And Chris Livesey.

Images that remind me of what I can and cannot do during this Roadshow!



fulfilling one's individual potential. The Philosopher's stone! Only comes after the basics are met



Maslow's hierarchy of needs and desires

Herbert engaging in metacognition – even a frog can do it!

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)?

Ian Jerrett DPI Victoria: Acute outbreak of staggering, lethargy, dropped milk production and bleeding from nose after dairy herd rested in a new paddock for 2-3 days. Some cows went down quickly and died. Others (now 1 wk later) still sick.

TEST	Cow 1	Cow 2	Cow 3	REF VALUES
CK IU/L	**519**	**519**	**507**	<300
AST IU/L	**5653**	**7112**	**1084**	<120
GGT IU/L	**276**	**161**	**108**	<35
GD (GLDH) IU/L	**4641**	**6311**	**1673**	<30
Total serum protein (biuret) g/L	74.6	76.6	**57.0**	60-85
Albumin g/L	34.5	**38.5**	29.1	25-38
Globulins g/L	40.1	38.1	**27.9**	30-45
A:G ratio	0.9	1.0	1.0	0.7-1.1
Total bilirubin µmol/L	**37.8**	**41.0**	11.1	<24
Conjugated bilirubin µmol/L	**24.3**	**30.2**	**8.5**	<8.0
Urea mmol/L	6.5	7.0	4.4	2.1-10.7
Creatinine µmol/L	115	155	94	0-186
Urea/Creat ratio	0.06	0.05	0.05	0.00-0.70
Calcium mmol/L	**1.72**	2.50	2.15	2.0-2.75
Inorganic phosphate mmol/L	1.70	1.95	1.31	0.8 – 2.8
Beta hydroxybutyrate mmol/L	0.34	0.18	0.30	<0.8
Magnesium mmol/L	1.29	**1.47**	0.91	0.74-1.44

HAEMATOLOGY	Cow 1	Cow 2	Cow 3	REF VALUES
Plasma appearance	Clear	Clear	Clear	Clear
PCV L/L	0.26	0.29	0.31	0.23-0.44
Haemoglobin g/L	89	96	100	80-150
Erythrocytes $x10^{12}$/L	**4.51**	5.14	5.81	5.0-8.0
MCV fl	58	56	53	44-62
MCHC g/L	340	330	320	300-350
Leukocytes $x10^9$/L	6.1	6.4	7.9	4-12
Neutrophils (seg.) $x10^9$/L	2.5	1.54	2.37	0.6-4.0
Neutrophils (band) $x10^9$/L	0	0	0	0-0.2
Lymphocytes $x10^9$/L	3.29	4.67	4.9	2.5-7.5
Monocytes $x10^9$/L	0.12	0.19	0.40	0.03-0.84
Eosinophils $x10^9$/L	0.18	0.00	0.24	0.0-2.4
Basophils $x10^9$/L	0	0	0	0-0.2
Blood film: all samples had platelet and WBC clumping				
Fibrinogen g/L	4	4	5	3.0-7.0

Necropsy: enlarged red swollen liver, gall bladder thickened and bloody, yellowing of carcase, possible nephrosis (pale and swollen), abomasal and small intestinal haemorrhage, subcutaneous petechiation, epicardial and endocardial haemorrhage

Diagnosis: *acute bovine liver disease (ABLD)*

A disease that affects grazing cattle in SE Australia. Sudden deaths are due to widespead periportal necrosis. Surviving cattle may develop photodynamic dermatitis. Associated with warm weather. Rough dog's tail grass (*Cynosurus echinatus*) is often growing, but does not appear to be directly involved (a sentinel for the right conditions?). A mycotoxin is suspected.

Possible reasons for changes (my interpretation): the usefulness of multiple samples is to look for trends in changes (the percentages game). All three cows have marked elevations of GD suggesting significant hepatocellular damage. In light of the mild increases in CK (terminal muscle damage or activity?), the marked AST increases are more likely related to the hepatocellular damage than to muscle damage. The moderate GGT elevations often go along with marked hepatocellular damage in the cow, but still could indicate a degree of intrahepatic cholestasis. Total bilirubin is mildly increased in two cows, but all three have an increased conjugated form suggesting that cholestasis is present (from the hepatocyte [failure to export conjugated form to the canaliculi because of lack of energy] to the bile duct). Protein changes are inconsistent. One cow is hypoproteinaemic due to a lack of globulins (cause unclear), but another has increased albumin (likely due to dehydration). The hypocalcaemia in one cow and hypermagnesaemia in another are probably of little significance to the major disease process. The haematology is unremarkable except for one cow having a marginally low erythrocyte count (not supported by PCV or Hb). However, all three cows do have erythroid values towards the lower end of the Ris.
Diagnosis: Acute bovine liver disease (ABLD)

Neil Horadagoda USYD: Female dog presented because of continual vomiting after a family BBQ. The sample of blood had haemolysis (2+) and lipaemia.

Haematology		Results	
RBC x 10^{12}/L	(5.5-8.5)	7.8	
Haemoglobin g/L	(120-180)	**196**	
PCV L/L	(0.37-0.55)	0.49	
MCV fl	(60-77)	63	
MCH pg	(19-25)	25	
MCHC g/L	(320-360)	**400**	
WBC x 10^9/L	(6.0-17.0)	**17.6**	
Band Neutrophils	(0-0.24)	5 %	**0.88**
Neutrophils	(3.6-13.1)	81	**14.26**
Lymphocytes	(0.72-2.21)	10	1.76
Monocytes	(0-1.7)	4	0.70
Eosinophils	(0-1.7)	0	0.0
Basophils	(0-0.36)	0	0.0
Plasma Protein	(58-84)	**110**	
Platelets x 10^9/L	(200-500)	460	
Smear: mild to moderate toxic changes to neutrophils.			

Biochemistry		Results
CK U/L	(<400)	339
AST U/L	(<80)	75
ALT U/L	(<80)	**134**
ALP U/L	(<120)	**521**
Total Bilirubin µmol/L	(<10)	**15.2**
Cholesterol mmol/L	(3.49-7.18)	**14.3**
Creatinine µmol/L	(46-96)	**137**
Urea mmol/L	(4.3-7.1)	**13.6**
Glucose mmol/L	(3.9-6.9)	**7.2**
Amylase U/L	(500-1500)	**3428**
Phosphate mmol/L	(0.84-2.00)	1.62
Calcium mmol/L	(1.9-2.8)	**2.87**
Serum Protein g/L	(54-78)	**85**
Albumin g/L	(23-40)	28
Globulin g/L	(27-44)	**57**
A:G ratio	(0.59-1.11)	**0.49**
Sodium mmol/L	(144-160)	146
Potassium mmol/L	(3.6-5.8)	4.2
Chloride mmol/L	(110-125)	**100**

Snap CPL: Strong Positive.

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Likely conclusions: fits in with dehydration, Acute Pancreatic Necrosis and secondary metabolic effects on liver of a few days duration. The lipaemia and haemolysis are common complications of APN and are affecting some laboratory results.

Possible reasons for changes: elevated Hb, MCHC, TPP, TSP likely be due to dehydration and haemolysis (and perhaps lipaemia). The effect of haemolysis (due to the lipaemia or because of increased fragility related to metabolic disturbances?) and lipaemia on other biochemical values depends on species (whether values in erythrocytes and higher than plasma values eg K higher in RBC than plasma for the horse and pig but not for dog [except Akita and Shiba Inu] or cat) and method of analysis employed. The elevated bilirubin, ALT and ALP are probably related to hepatic cell swelling and cholestasis (can mild elevations be due to haemolysis?), The hypercholesterolaemia is likely related to liver and perhaps pancreatic lipase release. The mild azotaemia is likely to be pre-renal in origin. The amylase and snap CPL are likely to be due to pancreatic cell damage, although sustained vomiting may be partially contributing (as well as the dehydration causing reduced GFR). Hyperglycaemia could be due to corticosteroid release and endocrine pancreatic shutdown. The increased Ca could be due interference with the mode of measurement (if colourimetric endpoint then marked lipaemia can cause mild increases) as albumin correction leads to increased values (2.94). The elevated globulins could be partially due to dehydration and the lowish albumin, but the value might also be related to present (APP) and perhaps ongoing inflammation? The albumin could be lower than expected because it is a negative APP? The hypochloridaemia is likely related to vomiting (HCl loss). The leukocytosis due to neutrophilia and borderline left shift (and toxic changes) are probably due to metabolic disturbances and inflammatory demand.

Likely conclusions: fits in with *dehydration, Acute Pancreatic Necrosis and secondary metabolic effects on liver of a few days duration. The lipaemia and haemolysis are common complications of APN and are affecting some results.*

Interference factors for chemical laboratory results (depends on the species [and sometimes breed], type of analyzer, methodology, and amount of interfering substance)

- **Haemolysis:** *in vitro* due to poor collection, lipaemia, freezing of whole blood samples, delayed separation of serum/plasma from RBCs. Can interfere with chemistry tests by:
  - *Increased absorbance* - in the Hb spectral range
  - *Inhibition of reactions* – Hb can directly inhibit chemical reactions
  - *Analyte release*: **when levels in RBCs are higher than plasma levels (eg K in horses, cattle, pigs and cat, and some breeds of dog)**
  - *Enzyme release*: may participate in chemical reactions
  - *Water release*: RBC water may dilute measured analyte
- **Lipaemia (visible):** due to **i**ncreased triglycerides (chylomicrons or VLDLs). Can interfere by:
  - *Light scattering* - falsely increased readings for some analytes, e.g. total bilirubin, Hb
  - *Volume displacement/solvent exclusion* - falsely decreases values of some analytes, e.g. Electrolytes (Na, Cl and to a lesser extent K) depending on the methodology employed
  - *Causes haemolysis*
- **Icterus:** bilirubin may reduce creatinine concentrations when measured by certain methods
- **Paraproteins (high concentrations)**: may interfere by:
  - *Hyperviscosity*: dependent on the class of immunoglobulin
  - *Binding to analytes*: Immunoglobulin binding to some analytes producing increased or decreased analyte values, e.g. hyperphosphataemia in the dog
  - *Volume displacement*: as for lipaemia
- **Drugs:** variable depending on the drug and species

A 24 month old Friesian bull was presented recumbent with sunken eyes, anorexia and lethargy of 2-3 days duration. Physical examination revealed a distended abdomen, normal temperature, absence of ruminal contractions and lack of faeces upon rectal palpation. No other abnormalities were detected upon rectal palpation.

HAEMATOLOGY	SAMPLE	REFERENCE VALUES
Plasma appearance	Clear	Clear
PCV L/L	0.33	0.24-0.45
Haemoglobin g/L	123	80-150
Erythrocytes $x10^{12}$/L	7.44	5-10
MCV fL	44	40-60
MCHC g/L	375	310-360
Leukocytes $x10^9$/L	**20.1**	4-12
Neutrophils (seg.) $x10^9$/L	**15.3**	0.6-4.0
Neutrophils (band) $x10^9$/L	**0.4**	0-0.2
Lymphocytes $x10^9$/L	3.8	2.5-7.5
Monocytes $x10^9$/L	0.6	0.1-0.8
Eosinophils $x10^9$/L	0.2	0.2-2.4
Basophils $x10^9$/L	0	0-0.2
Blood film: normal		
Fibrinogen g/L	6.0	3.0-7.0

TEST	SAMPLE	REFERENCE VALUES
AST IU/L	**407**	<120
GGT IU/L	29	<60
GLDH IU/L	39	<45
CK IU/L	**4374**	<400
Beta HOB IU/L	0.2	<0.5
Bilirubin µmol/L	4	1-8
Serum protein (biuret) g/L	80	60-85
Albumin (BCG) g/L	33	21-40
Globulins g/L	47	25-52
A:G ratio	0.7	0.6-0.9
Urea mmol/L	**33.5**	2.1-10.7
Creatinine µmol/L	**583**	50-180
Calcium mmol/L	**1.8**	1.9-2.6
Inorganic phosphate mmol/L	**4.5**	1.2-2.8
Magnesium mmol/L	**1.36**	0.6-1.1
Sodium mmol/L	139	132-152
Potassium mmol/L	4.5	2.4-4.7
Chloride mmol/L	**69**	92-109
Bicarbonate (TCO_2) mmol/L	**48**	20-30
Anion Gap mmol/L	**26**	6-14

Likely conclusions: the underlying problem could be *renal dysfunction and possibly nephritis or pyelonephritis. The other changes can be explained by recumbency and GIT stasis.*

Possible reasons for changes: AST and CK due to recumbency? The azotaemia may be partly due to pre-renal causes but since it is marked and accompanied by hypocalcaemia, hyperphosphataemia and hypermagnesaemia there is a possibility of renal dysfunction. The hypochloridaemia, and metabolic alkalosis could be due to stasis of the abomasum and forestomachs. The high AG could be due to renal dysfunction and retention of acids (could the animal also have increased lactic acid?). The moderate neutrophilia with left shift (even though mild) might suggest inflammation somewhere (even though fibrinogen is normal)
Likely conclusions: the underlying problem could be *renal dysfunction and possibly nephritis or pyelonephritis. The other changes can be explained by recumbency and GIT stasis.*
Further testing? Urinalysis is a must to confirm renal disease. Monitoring electrolytes and azotaemia may be useful

Jeremy Allen DAFWA: Acute death in 8 month old Merino weaners with 60 dead and 150 ill from a flock of 1600. The flock was treated with Maximin and vitamin E in November and turned into a wheat stubble, possibly with access to a dump. Weaners have been found dead without evidence of struggling, with largest sheep affected. Necropsy of two weaners noted a body condition score of 1 with little abdominal fat. Pale skeletal muscle and pale myocardium with focal white areas was noted.

TEST	SAMPLE	REFERENCE VALUES
CK IU/L	**1418**	<500
ALT IU/L	**94**	<30
GGT IU/L	47	<67
GD (GLDH) IU/L	11	<20
Total serum protein (biuret) g/L	73.4	60-75
Albumin g/L	30.8	28-34
Globulins g/L	**42.6**	30-42
A:G ratio	0.70	0.6-1.1
Total bilirubin µmol/L	0	<15.0
Total conjugated bilirubin µmol/L	2	<5.0
Cholesterol mmol/L	1.60	1.2-2.6
Glycerol mmol/L	**0.21**	0-0.1
Beta hydroxybutyrate (BHB)	**0.72**	<0.7
Urea mmol/L	7.4	3.3-8.0
Creatinine µmol/L	66	50-150
Calcium mmol/L	2.02	2.2-3.0
Inorganic phosphate mmol/L	**2.82**	0.9 – 2.5
Magnesium mmol/L	0.95	0.8 - 1.44
Iron µmol/L	34.4	33-36
GSHPx in red blood cells U/g Hb	**21**	>50
Vitamin E mg/L	**0.14**	>1.0

Possible reasons for changes (my view): an elevated CK and ALT are likely due to muscle damage (no evidence of terminal struggling) Elevated ALT in ruminants is invariably due to muscle damage. The mildly elevated globulins could be related to ongoing disease or increases in acute phase proteins (no evidence for dehydration). Mild elevations of BHB and glycerol in indicate increased fat metabolism secondary to decreased nutrient intake (**ie Negative Energy Balance and lipolysis**). The mild hyperphosphataemia may be related to myolysis (intracellular release). Low vitamin E and selenium (indirectly indicated by reduced glutathione peroxidase levels in erythrocytes – useful in ruminants and horses but not pigs) could suggest a nutritional base to a myopathy.

Likely diagnosis: nutritional myopathy

Necropsy findings: nutritional myopathy and rumenitis (two sheep necropsied)