Veterinary Bioscience: Digestive System

Learning objectives:

Understand how clinical pathology can help in defining and diagnosing gastrointestinal disease
Describe how haematology and biochemistry values may change in response to some common gastrointestinal diseases
Explain the diagnostic tests for exocrine pancreatic insufficiency
Describe what disease mechanisms may result in changes to peritoneal fluid

Lecture overview

1. Introduction to clinical pathology
2. Laboratory evaluation of gastrointestinal disease
    - Case studies:
        - Vomiting
        - Diarrhoea
        - Protein-losing enteropathy (PLE)
        - Exocrine pancreatic insufficiency (EPI)
            - Evaluating data and considering further tests

What is clinical pathology?

Utilisation of laboratory tests to:

- Diagnose disease
- Narrow down differential diagnoses (DDx)
- Direct further investigations
- Monitor disease progression or response to therapy
- Screen for underlying disease in clinically healthy animals

How to use clinical pathology

Appropriate laboratory tests are selected after a full history and complete physical examination

- Identify a problem list first, to ensure selection of appropriate and cost-effective tests

Laboratory tests are frequently used in conjunction with other diagnostic procedures, as part of an integrated diagnostic approach:

- e.g. imaging, endoscopy, laparotomy, biopsy, treatment trial

Learning the lingo

Common clinical pathology acronyms (CCPA just joking!)

- Haematology
  - PCV: packed cell volume
  - Hct: haematocrit
  - Hb (or Hgb): haemoglobin concentration
  - RCC: red cell count
  - MCV: mean corpuscular volume
  - MCHC: mean corpuscular Hb concentration
  - RDW: red cell distribution width
  - WCC: white cell count (aka leukocyte count)
  - TNCC: total nucleated cell count
  - Neut: neutrophil (aka seg or polymorph)
  - Lym (or Lymph): lymphocyte
  - Mono: monocyte
  - Eo: eosinophil
  - Baso: basophil
  - PLT: platelets (platelet count)
  - PDW: platelet distribution width

Biochemistry: Electrolytes

Na: sodium
K: potassium
Cl: chloride
Mg: magnesium
Ca: calcium (may also be “Total calcium”)
Ca^{++}: ionised calcium
PHOS: Phosphate
Anion Gap: calculated value: (Na+K) – (Cl+n)

Biochemistry

Urea: measured as BUN (blood urea nitrogen) in USA
Cr (or CREAT): creatinine
Gluc: glucose
Chol: cholesterol
Bili: bilirubin, usually “total bilirubin”
Bili Total ~ Unconjugated + Conjugated
BA: (serum total) bile acids

Biochemistry: Enzymes

ALT: alanine aminotransferase (previously SGPT)
AST: aspartate aminotransferase (previously SGOT)
AP: alkaline phosphatase (aka ALP, AlkPhos)
GGT: gamma glutamyltransferase (aka gamma GT, gGT)
SDH: sorbitol dehydrogenase

GLDH: glutamate dehydrogenase
CK: creatine kinase (previously CPK)
TLI: (serum) trypsin-like immunoreactivity
Amylase: sometimes AMYL
Lipase: sometimes LIP
cPL: canine pancreatic-specific lipase
cPLI: canine pancreatic lipase immunoreactivity

Biochemistry: Proteins

TP: total protein (measured biochemically)
TS: total solids (refractometer total protein value)
Alb: albumin
Glob: total globulins
A:G (ratio): Alb/Glob
Fib: fibrinogen
SPE: serum protein electrophoresis

Urinalysis (UA):

USG: urine specific gravity ('concentration')
RBC: red blood cells (erythrocytes)
WBC: white blood cells (leukocytes)
#/LPF: number per low power field (x100 magnification)
#/HPF: number per high power field (x400 magnification)
TNTC: too numerous to count (lots and lots!)

Miscellaneous:

Ag: antigen
Ab: antibody
EDTA: ethylene diamine tetraacetic acid
Ig: immunoglobulin
SDMA: symmetric dimethyl arginine
T_4: thyroxine
T_3: triiodothyronine
TPR: temperature, pulse (rate) and respiration (rate)
MN or MC: desexed male
FS: spayed female
NAD: no abnormalities detected
NS: not stated
WRI: within reference interval
WNL: within 'normal' limits
(AbN: abnormal; N: normal)

Diagnoses:

EPI: exocrine pancreatic insufficiency
DM: diabetes mellitus
DKA: diabetic ketoacidosis

DI: diabetes insipidus
HyperA: hyperadrenocorticism
HypoT: hypothyroidism
etc, etc, etc!!

Choosin' the tube

Haematology

PCV and TS
Complete blood count (CBC)
- Erythrocytes
  - Hct (PCV), Hb, RCC, MCV, MCHC
- Leukocytes
  - Total and differential cell counts
- Platelets

Blood smear examination
- Cell morphology and validation of analyser results

Reticulocyte count

Coagulation tests

ACT: activated coagulation time
APTT: activated partial thromboplastin time
PT: prothrombin time
TT: thrombin time
Fibrinogen
vWf (VWF): von Willebrand factor
FVIII: Factor VIII (similar for other Factors)

Transfusion medicine

Blood typing
- Performed when reagents (Ab) are available
  - DEA 1.1 in dogs
  - A, B and AB in cats

Cross-matching for compatibility
- Major crossmatch
  - Donor's RBC tested against recipient's serum
- Minor crossmatch
  - Donor's serum tested against recipient's RBC

Diagnostic 'groupings'

- Renal disease: urea, Cr, USG, SDMA
- Electrolytes: Na, K, Cl
- Acid Base: pH, bicarbonate, anion gap
- Minerals: Ca, Phos, Mg
- Muscle injury: CK, AST
- Liver: ALT, GLDH, AST, ALP, GGT, bilirubin, bile acids

- Pancreatic function: amylase, lipase, TLI
- Energy metabolism: glucose, cholesterol
- Proteins: TP, albumin (globulins)
-

Biochemistry ‘profiles’
Larger groupings of biochemistry tests
    Will vary between laboratories

Urinalysis

Testing the physical and chemical characteristics of fresh urine
Collection may be free-catch, catheter or cystocentesis
Assessment of:
    Gross appearance and odour
    Urine specific gravity (USG)
    Urine ‘Dipstick’ chemistry
    Urine sediment (microscopy)

Cytology

The study of cells:

Fine needle aspirate (FNA) or impression smear

    Skin masses, lymph node (LN) or tissue

Body fluids

    Effusions: peritoneal, pleural, pericardial, synovial

    Cerebrospinal fluid (CSF)

Washes

    Tracheal, prostatic, BAL

Swabs

    Vaginal, skin lesions, ears, conjunctiva

Sample collection

Avoid artifactual changes
    Good venepuncture technique
    Correct blood tube
    Correct ratio of blood to anticoagulant
    Harvest plasma or serum asap
    Make ‘fresh’ blood smears
    Gentle preparation techniques for tissue cytology

The ideal patient

Is fasted and rested
Post-prandial samples may have:
- Lipaemia
- Hyperglycaemia
- Mild azotaemia

Excited animals may have:
- Polycythaemia
- Physiological leukocytosis
- Hyperglycaemia

In-house Laboratory

Simple
In-house Laboratory
More sophisticated

Referral Laboratory

'Out-sourcing'
- Quality control (QC)
- still begins with YOU!

Larger range of tests
Provides interpretative support

Interpretation of data

Providing a pertinent history is important!
- The more information given to the laboratory, the better the interpretation and comments

Ultimate responsibility for interpretation of lab results rests with you, the practitioner
You must understand the units, reference intervals and significance of abnormalities

Units of measurement

Australia, Canada, Europe use SI units
USA still use 'conventional' units

For example:
- PCV in SI of 0.45 L/L = 45% in USA
- Total cell count 10 x 10^9/L = 10,000/ml
- Glucose of 5.5 mmol/L = 100 mg/dL
- TP of 75 g/L = 75 g/dL
- Urea of 2.1 mmol/L = Urea nitrogen of 6 mg/dL

Reference values

Reference values (or reference intervals) are results derived from a specified population of clinically healthy animals
They may vary according to:

- Species
- Breed
- Age
- Sex
- Method of collection
- Method of analysis

Reference intervals (values)
Reference intervals usually represent the test results from 95% of the reference ('healthy') population
Therefore ~ 2.5% of 'healthy' animals have values above, and ~ 2.5% below, the reference interval

Warnings!

Always interpret lab results in conjunction with history, clinical findings and other tests
Anticipate expected findings, so that you can:

- Question unexpected results
- Identify pathological processes when lab results are within reference limits
  - e.g. an anaemic animal that is also dehydrated may have a 'normal' PCV

More Warnings!

Do NOT over interpret mildly increased or decreased test results (especially if they are 'unexpected' or do not fit the clinical diagnosis)

Final advice

- The more information that you have steering you in the one diagnostic direction, the more certain you can be of the diagnosis
- However, remember that common things occur commonly and do not succumb to the paralysis of over-analysis
  If you hear hoof-beats…..
  Think horses not zebras!
  You'll be right 99% of the time (unless you're on safari!)

Laboratory evaluation of gut disease

Laboratory investigations are an important component of the diagnostic approach to gastrointestinal disease by:

- Narrowing the list of differential diagnoses (DDx)
- Providing a diagnosis in some cases
- Directing further investigations

Baseline data:

Routine haematology, biochemistry, urinalysis

Laboratory evaluation of GI disease

- Possible other tests/procedures:
  - Imaging (radiographs, ultrasonography, etc.)
  - Effusion analysis (TNCC/TP, cytology)
  - Endocrine (hyperthyroidism, hypoadrenocorticism)
  - Pancreas (pancreatitis, EPI)
  - Folate/Cobalamin (intestinal function)
  - Mass lesions: FNA for cytology
    or surgical biopsy for histopathology
  - Faecal tests

V & D

How do vomiting and diarrhoea affect haematology and biochemistry values?
Case 1: Vomiting
Sandy; 3-year-old, MN, Labrador retriever

History:

Vomiting for the past 3 days, especially after meals

Clinical examination:

Moderate depression. Anorexia. Slight skin tenting.
Abdominal guarding on palpation, with possible mass in the anterior abdomen.
HR 260 beats/min
RR 46 breathes/min

Case 1: Vomiting

DDx include:

- Gastritis/Enteritis
- GI obstruction (e.g. foreign body, GDV)
- Diseases of other systems:
  - Pancreatic, hepatic, renal, endocrine
  - Drugs/toxicity

CBC		Patient	Ref. Values	
PCV	L/L	0.56	0.37-0.55	SI ↑
White cell count	x10^9/L	41.2	6.0-17.0	↑↑↑
Neutrophils	x10^9/L	37.5	3.0-11.5	↑↑↑
Lymphocytes	x10^9/L	0.4	1.0-4.8	↓↓
Monocytes	x10^9/L	3.3	0.2-1.4	↑
Eosinophils	x10^9/L	0	0.1-1.3	↓
Basophils	x10^9/L	0	Rare	WRI
Platelets	x10^9/L	Adequate	200-500	WRI
Total solids	g/L	76	60-80	WRI

CBC:

Marginal erythrocytosis
- A 'relative' increase in RBC is most common
  - Likely secondary to fluid loss (dehydration) and pain (splenic contraction)

Inflammatory leukogram, characterized by:
- Marked mature neutrophilia
- Mild monocytosis
- Moderate lymphopenia ('stress')

Biochemistry		Patient	Ref. Values	
Na	mmol/L	123	144-160	↓↓
K	mmol/L	2.8	3.5-5.8	↓↓
Cl	mmol/L	70	109-122	↓↓↓
Urea	mmol/L	10.3	3.0-7.5	↑
Creatinine	mmol/L	0.15	0.08-0.17	WRI
Amylase	U/L	1700	<2000	WRI
Lipase	U/L	20	<200	WRI
Total protein	g/L	73	54-74	WRI
Albumin	g/L	37	29-37	WRI

Serum biochemistry:

- Increased urea (normal creatinine)
  - Consistent with dehydration (pre-renal azotaemia)
    - Increased urea absorption in renal tubules

        - Decreased glomerular filtration rate (hypoperfusion)
    - Increased urea may also occur with GI bleeding
        - Less likely in this case, as erythrocytosis and 'high normal' albumin support dehydration rather than anaemia
        - Decreased electrolytes - Na, K, Cl
    - Electrolyte loss via persistent/severe vomiting
    - Note that there appears to be a more marked decrease in **chloride** compared to sodium
        - Suggests vomiting of gastric contents
        - Pyloric obstruction or gastritis?

Evaluating Na and Cl

- Chloride and sodium should move together when changes only relate to water
- Determining if there is a selective loss or gain of chloride, helps refine DDx and also suggests an acid base disturbance

  Calculate "corrected Cl"

  $Cl_{(cor)}$= Cl (measured) x (normal Na ÷ measured Na)

  * normal Na is midpoint of Na ref interval
  * Cl_{cor} should be within the Cl ref interval
- Corrected Cl = 70 x (152/123)
  = 86.5
- The corrected Cl value is not within the Cl reference interval – thus **selective** Cl loss is confirmed
    - Gastric vomiting, concern for pyloric obstruction
    - Metabolic alkalosis very likely (need blood gas to confirm)

Case 1: Outcome

Placed on intravenous fluids to correct electrolyte balance and restore hydration
Imaging revealed a likely foreign body
Exploratory laparotomy was performed
A foreign body was found to be obstructing the pylorus
Sandy made a full recovery!

Case 2: Diarrhoea

Bailey; 6-year-old, Arabian gelding

History: Participated in endurance ride yesterday. Today off feed, abdominal discomfort, loose faeces.

Clinical examination:

Mild to moderate depression. Anorexia. Increased gut sounds. Moderate skin tenting.

Tacky mucous membranes.

CRT 1.5 sec. HR 55/minute. RR 14/minute.

Case 2: Diarrhoea

In a horse, DDx include:

- Infectious agent
- Antimicrobial induced colitis
- NSAID toxicity
- Sand enteropathy
- Ingestion of toxicant
- Exertional rhabdomyolysis

CBC		Patient	Ref. Values	
PCV	L/L	0.57	0.32-0.53	↑
Leukocytes	$x10^9$/L	2.5	5.4-14.0	↓↓↓
Bands	$x10^9$/L	1.5	0-0.1	↑↑
Neutrophils	$x10^9$/L	0.5	2.3-8.6	↓↓↓
Lymphocytes	$x10^9$/L	0.5	1.5-7.7	↓↓
Monocytes	$x10^9$/L	0	0-1.0	WRI
Eosinophils	$x10^9$/L	0	0-1.3	WRI
Platelets	$x10^9$/L	320	100-350	WRI
Total solids	g/L	52	60-80	↓

<u>CBC:</u>

Mild erythrocytosis

Likely secondary to dehydration

Supported by physical exam and increased urea with 'normal' creatinine (pre-renal azotaemia)

Decreased TS

Gastrointestinal protein loss most likely

Need TP + Albumin to further characterize

Leukopenia due to neutropenia with degenerative left shift and lymphopenia

Indicates overwhelming inflammatory demand, exceeding the bone marrow's production capacity

Immature neutrophils (bands) released (left shift)

Bands exceed mature neutrophils (degenerative)

A common feature of severe enteritis caused by an infectious agent (e.g. salmonellosis)

Lymphopenia: ‘stress’ response

Biochemistry		Patient	Ref. Values	
Na	mmol/L	128	137-148	↓↓
K	mmol/L	5.7	2.8-5.1	↑
Cl	mmol/L	90	99-109	↓↓
Urea	mmol/L	10.9	4.0-8.0	↑
Creatinine	mmol/L	0.15	0.10-0.17	WRI
CK	U/L	540	25-270	↑
AST	U/L	605	90-450	↑
Total protein	g/L	50	54-74	↓
Albumin	g/L	26	29-37	↓

<u>Biochemistry:</u>

Hyponatraemia and hypochloraemia
- Both are moderately decreased
- Proportionate loss, likely from GIT
  - Diarrhoea can be explosive event in horses!
  - Massive fluid and electrolyte losses in a short time

Hyperkalaemia
- Likely secondary to metabolic acidosis
  - Titrational: lactic acid production secondary to hypovolaemia or ischaemia (anaerobic metabolism)
  - Secretory: bicarbonate loss with diarrhoea

Mildly increased CK (and AST) indicate muscle damage
- Consistent with prior strenuous exercise and/or ischaemia from recumbency
- Unlikely to be due to exertional rhabdomyolysis
  - Values would be much greater (CK likely > 10,000 U/L)

<u>Presumptive Dx: Salmonellosis</u>

Supportive management: IV fluids
Made a full recovery after 3 days of treatment

Case 3: Protein-losing enteropathy

Princess; 5-year-old, FS, Poodle

History: Losing weight for last few months, despite a reasonably good appetite

Clinical examination: TPR OK.
Abdominal distension.
A fluid wave was palpable.

CBC		Patient	Ref. Values	
PCV	L/L	0.46	0.37-0.55	WRI
Leukocytes	$x10^9$/L	12.1	6.0-17.0	WRI
Neutrophils	$x10^9$/L	10.0	3.0-11.5	WRI
Lymphocytes	$x10^9$/L	0.9	1.0-4.8	Marg ↓
Monocytes	$x10^9$/L	1.1	0.2-1.4	WRI
Eosinophils	$x10^9$/L	0.2	0.1-1.3	WRI
Basophils	$x10^9$/L	0	Rare	WRI
Platelets	$x10^9$/L	440	200-500	WRI
Total solids	g/L	34	60-80	↓↓

Hypoproteinaemia: DDx

Haemorrhage
Renal loss (protein-losing nephropathy)
Hepatic 'failure'
Maldigestion/Malabsorption
Severe cutaneous exudation
Starvation
Marked effusion (usually with 'drainage')
Gut loss (protein-losing enteropathy; PLE)

Biochemistry		Patient	Ref. Values	
Na	mmol/L	153	144-160	WRI
K	mmol/L	3.8	3.5-5.8	WRI
Cl	mmol/L	113	109-122	WRI
Urea	mmol/L	6.8	3.0-7.5	WRI
Creatinine	mmol/L	0.09	0.08-0.17	WRI
Cholesterol	mmol/L	2.6	3.6-9.0	↓
Glucose	mmol/L	6.6	3.0-7.0	WRI
Total protein	g/L	32	54-74	↓↓
Albumin	g/L	12	29-37	↓↓↓

Hypoproteinaemia – can we rule anything out?

Haemorrhage
Expect panhypoproteinaemia plus anaemia
Liver failure
Expect decreased alb, chol, glu, urea
Protein-losing nephropathy
Expect hypoalb, hyperchol, plus proteinuria
So this might be a PLE!
Expect weight loss, panhypoproteinaemia, hypocholesterolemia

Peritoneal fluid

- Reference values:
  Volume, colour and turbidity:
  Very low volume, colourless and clear
  TNCC:
  < 3.0 x 10^9/L in small animals
  < 5.0 x 10^9/L in horses
  Types of cells:
  Mostly mononuclear cells (macrophages, lymphocytes, mesothelial cells), with rare neutrophils in small animals
  Mostly neutrophils in horses
  Protein content:
  < 25 g/L

Effusion

Increased volume of fluid in a body cavity:
- Peritoneal, pleural, pericardial, synovial

Classified according to appearance, TNCC, TS, cytology (and possibly other biochemistry tests)
- Low protein transudate
- High protein transudate (modified transudate)
- Exudate
- Other
  - Haemorrhagic effusion
  - Chylous effusion
  - Uroperitoneum
  - Neoplastic effusion

Effusions

Transudate
- 'Normal' gross appearance
- 'Low' cell count, 'low' protein
  - Hypoalbuminaemia (<15 g/L)
  - Local and systemic venous hypertension,
  - e.g. chronic hepatic disease,
  - such as portosystemic shunt or cirrhosis
- High protein transudate (modified transudate)
  - Mild increase in protein, 'normal' TNCC
  - Congestive heart failure, thrombosis of caudal vena cava, 'early' cases of organ torsion

Exudate
- Usually discoloured and turbid
- Increased TNCC and TS
  - Inflammation
  - (increased vascular permeability at serosal surfaces)
  - Neutrophils usually predominate
  - Macrophages may be numerous and phagocytic
  - If cause is bacterial infection *may* see degeneration of neutrophils and presence of organisms
  - Bile leakage, chylous effusion, uroperitoneum and neoplasia may cause an exudate

Haemorrhage
- Haemorrhage or diapedesis of RBC
- can be part of an exudate or other effusions
  - 'A little bit of blood goes a long way'
  - DDx blood contamination at collection
    - Platelets indicate haemorrhage at collection
    - Phagocytosis of RBC and/or haemosiderinophages indicate prior haemorrhage

Haemorrhagic effusion may occur in its 'own right'
- Usually in a coagulopathy,
- e.g. vitamin K antagonist poisoning

Other effusions

Bile peritonitis
- Fluid may have a 'gritty' texture
- Bile is irritant; provokes a good inflammatory response
- [Total bilirubin] in fluid > blood

Chyloabdomen
- Fluid often opalescent
- (likely more so in chylothorax, due to chylomicrons)
- Initially cells are almost all small lymphocytes
- With time, increase of neutrophils and macrophages

Uroperitoneum
- Urine is irritant; provokes an inflammatory response
- Initially [Urea] in fluid >>> blood; ditto [Creatinine]
- With time, these substances equilibrate across the peritoneum; urea equilibrates more rapidly
- In uroperitoneum, fluid [Urea] and [creatinine] will always be greater than the corresponding blood value
- Cases develop a marked hyperkalaemia (with clinical consequences)

Neoplasia
- Effusion may appear as a transudate, modified transudate or exudate
- Neoplastic cells may or may not be visible
- Causes include: lymphosarcoma, metastatic tumours (especially carcinomas), haemangiosarcoma, mast cell tumour, mesothelioma
- Mesothelial cells
- Haemorrhagic effusion

Case 3: Peritoneal fluid analysis

	Patient	Ref. Values	
TNCC $x10^9/L$	1.4	< 1.5	'N'
Total solids g/L	12	< 25	'Low N'

Gross appearance: 20 ml of clear and colourless fluid easily collected

Cytology: Good cell morphology: mostly lymphocytes and large mononuclear cells, little phagocytic activity

Interpretation: Transudate

Outcome

Primary DDx = Protein-losing enteropathy
Associated with a number of GI disorders
    eg IBD, lymphangiectasia, lymphoma, parasitism…
Need further diagnostics!
Princess underwent an exploratory laparotomy for intestinal biopsy
Histopathology report: **Lymphangiectasia**

Lymphangiectasia
'Lacteal dilation'
Most commonly reported cause of PLE in dogs
Most cases are idiopathic
    Some are congenital or secondary to obstruction

Exocrine Pancreatic Insufficiency

Loss of pancreatic enzymes:
    Lipase, Amylase and Proteases (eg trypsin)
Leads to maldigestion
Causes include:
    Pancreatic acinar atrophy
        …which breed is predisposed?
    Secondary to chronic pancreatitis
Extensive loss of pancreatic mass required before signs of EPI evident (~ 90%)
Clinical signs:
    Chronic diarrhoea
        Voluminous, pale, greasy, rancid faeces
    Weight loss, good appetite (coprophagia)
Routine clinical pathology diagnostics:
    Haematology and serum biochemistry usually unremarkable
        Interestingly amylase and lipase are WRI … why?
    Panhypoproteinaemia usually not a feature

OK, so how do we confirm EPI?
    Faecal tests for maldigestion have poor Sn/Sp and are no longer used routinely
        (Faecal fat/starch/mm + faecal proteolytic activity)

Serum TLI
    Trypsin-like Immunoreactivity test
        High Sn/Sp, species specific

EPI is confirmed by subnormal **fasting** sTLI

< 2.5 ng/mL (RI healthy dogs: 5.2 – 35 ng/mL)

Persistent low normal may be subclinical EPI

False negatives:

Non-fasted, decreased GFR,

active pancreatitis

Intestinal function tests

Serum folate and serum cobalamin

Used to evaluate intestinal function (absorption)

Vitamin B_{12} abnormalities are common with EPI

82% dogs, 100% cats can have decreased cobalamin

(folate can vary depending on concurrent disease)

Recommended to assess folate & cobalamin with TLI

Supplementation with vit B_{12} may be necessary before an optimal response to enzyme supplementation is achieved

In addition, if TLI is normal, then finding a decreased folate and cobalamin supports a malabsorption disorder

… don't forget the faeces