

**TDDS Founded July 2001
Exeter UK**

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THE GROUP



www.tddslab.co.uk

CTDS Founded April 2003

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EVDS Founded Feb 2006

Jaime MacDonald, John Chitty,
Exotic Species Specialists

Leeds UK

VLSI Founded April 2007

Cork Ireland

Lucy Hooper, Elaine Lawton,
Melanie Unverfehrt

PTDS Founded Jan 2008

Higham Gobion, Hertfordshire
Roger Powell



Group Philosophy

- ◇ To deliver a personalised high quality diagnostic service backed by genuine expertise in all aspects of clinical pathology from small well equipped and approachable laboratories
- ◇ To found laboratories and services around the preferences and personalities of some of the best individual specialists in the UK
- ◇ To train selected residents with strong medical experience in Clinical and Anatomic Pathology in conjunction with the Universities. To achieve the standard of American and European Specialist Board Examinations over a three year residency



Group Philosophy

- ◆ To make this expertise readily available to Veterinary Surgeons in practice through detailed didactic reporting and highly accessible telephone consultation
- ◆ To offer an alternative choice to the corporatisation of pathological expertise and maintain the philosophy of the small business and individual identity.

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RCVS Recognised Specialist in Clinical Pathology
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The Clinical Pathology of Muscles, Bones and Joints

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A Classification of Muscle Diseases

Non-inflammatory Myopathies

- ◇ Deficiency of Dystrophin (X-linked)
 - ◇ Duchenne Muscular dystrophy deficiency of a cytoskeletal protein structural protein
 - ◇ Golden Retrievers and recently DSH cats
- ◇ Deficiency of Type 2 muscle fibres
 - ◇ Labrador retrievers
- ◇ Malignant hyperthermia
 - ◇ Triggered by halothane
- ◇ Exercise induced collapse
- ◇ Myotonia
- ◇ Glycogen Storage disorders
- ◇ Lipid storage myopathies
- ◇ Electrolyte myopathies
 - ◇ Hypokalaemic myopathy
- ◇ Mitochondrial myopathies
- ◇ Endocrine-associated myopathies

A Classification of Muscle Diseases

Inflammatory Myopathies (Shelton -200 cases)

- ◇ Masticatory muscle myositis (22.5 %)
- ◇ Generalised inflammatory myositis (70%)
 - ◇ Immune mediated polymyositis 65%
 - ◇ Infectious polymyositis 28.5%
 - ◇ Preneoplastic polymyositis syndrome 8.5%
 - ◇ Dermatomyositis-like syndrome 1.5%

A Classification of Muscle Diseases

Junctionopathies

◇ Myasthenia gravis

- ◇ Congenital

- ◇ Acquired

Focal, chronic generalised, acute fulminant
generalised

The Clinical Signs of Muscle Diseases

- ◇ Muscle weakness, exercise-induced collapse, plantar stance, neck ventroflexion
- ◇ Generalised or localised atrophy or hypertrophy
- ◇ Fasciculation's, myotonia, tremor
- ◇ Dysphonia, dysphagia, dyspnoea, regurgitation
- ◇ Pain, stiffness, abnormal defensive gait, falling
- ◇ Hyperthermia

Laboratory Diagnostic testing for Muscle Diseases

- ◇ Biochemistry, haematology and urinalysis
 - ◇ CK, AST, ALT, electrolytes, calcium , glucose
- ◇ Troponin I (cardiac muscle affected)
- ◇ T4/TSH and cortisol
- ◇ Plasma lactate, pyruvate and L:P ratio
- ◇ Anti-ACHR antibody titres
- ◇ Anti 2 M antibody titres
- ◇ Serology for infectious agents
 - ◇ Toxo, Neospora, tick born diseases including Borrelia
- ◇ Plasma, urine, muscle carnitine quantification
- ◇ Urinary organic acid analysis, plasma amino acid quantification
- ◇ DNA testing for hereditary myopathies and muscular dystrophies
- ◇ Muscle biopsy

Muscle enzymes, electrolytes, calcium and glucose

- ◇ CK highly specific, sensitive, returns to normal within 24-48 hours
- ◇ AST less specific, sensitive, returns to normal in 1 week
- ◇ ALT in dogs and cats can be elevated by marked generalised muscle injury, persists 10 days to 2 weeks
- ◇ Hypokalaemic myopathy – burmese kittens, adult cats with hyperthyroidism, renal disease or acidifying diets
- ◇ Hyperkalaemic periodic paralysis
- ◇ Hyponatraemia, hypernatraemia, hypoglycaemia, hypocalcaemia, hypercalcaemia

The effects of muscle diseases on serum CK

- ◇ Marked to massive elevations of CK (10-50,000 IU)
 - ◇ Infectious neoplastic and immune mediated polymyositis, Dystrophin deficiency (muscular dystrophy), exertional rhabdomyolysis, malignant hyperthermia
- ◇ Mild to moderate elevations of CK (1-10,000)
 - ◇ Hypokalaemic myopathy, IM injection, Masticatory myositis, endocrine myopathies, dermatomyositis, hyperkalaemic periodic paralysis
- ◇ Minimal elevations of CK (<1000)
 - ◇ exercise induced collapse, mitochondrial myopathies, Type 2 fibre deficiency, Storage diseases, myasthenia gravis

Endocrine Myopathies

- ◇ Type 1 muscle fibres – wt bearing, sustained force, slow twitch, red
- ◇ Type 2 muscle fibres – sudden movements, purposeful motion, fast twitch, white
- ◇ Hypothyroid Myopathy – weakness, muscle wasting, exercise intolerance, Type 2 muscle fibre atrophy
- ◇ Steroid Myopathy – pseudomyotonia, muscle hypertrophy may occur, disorganisation of fibres with abnormal mitochondrial morphology

Lactate, Pyruvate and L:P ratios

- ◇ Used to investigate myopathies associated with derangements of energy metabolism in muscle eg., enzyme deficiencies, mitochondrial myopathies, storage diseases, malignant hyperthermia, rhabdomyolysis and exercise induced collapse
- ◇ These are probably more common than we realise currently.

Lactate, Pyruvate and L:P ratios

Key features of energy metabolism in muscle

- ◇ Glucose is converted to glucose 6-phosphate which can form glycogen or undergo glycolysis to form pyruvate.
- ◇ Pyruvate is converted to lactate under anaerobic conditions or is converted to Acetyl CoA and enters the tricarboxylic acid (Krebs) cycle to form CO₂ and H₂O (releasing ATP) under aerobic conditions. Pyruvate is also used to regenerate glucose by gluconeogenesis

Key features of energy metabolism in muscle

- ◇ The Pyruvate concentration depends upon the rate of glycolysis (aerobic), the rate of conversion to AcetylCoA (aerobic -Pyruvate dehydrogenase), the rate of conversion to lactate (anaerobic), the rate of regeneration (pyruvate carboxylase) and the rate of gluconeogenesis
- ◇ The Lactate concentration depends upon the rate of conversion from pyruvate by anaerobic glycolysis and the rate of removal of lactate by the circulation. Lactate is used in the liver to regenerate glucose via gluconeogenesis.

The diagnostic value of lactate, pyruvate and L:P ratios

- ◇ Elevations of lactate reflect anaerobic muscle activity and occur in strenuous exercise. They can be a marker of enzyme deficiencies in energy metabolism within muscle
- ◇ Elevations of pyruvate occur during exercise and will be exacerbated by deficiencies in Pyruvate dehydrogenase or gluconeogenic enzymes
- ◇ Reductions in pyruvate may occur with excessive conversion to lactate or reduced regeneration associated with eg., pyruvate carboxylase deficiency

The diagnostic value of lactate, pyruvate and L:P ratios

- ◇ Elevations in the L:P ratio may occur in vigorous exercise. They may also indicate derangements in oxidative metabolism and reduced regeneration of pyruvate by eg., pyruvate carboxylase deficiency
- ◇ Normal or reduced L:P ratios reflect appropriate aerobic metabolism but in the presence of lactic acidosis reflect defects in oxidative metabolism which cause increases in pyruvate such as pyruvate dehydrogenase deficiency and defects in gluconeogenic enzymes.

Key facts in the measurement and interpretation of lactate, pyruvate and L:P ratios

- ◇ Human muscle has different fibre composition (less aerobic) than canine and feline muscles. Consequently the striking increase in the L:P ratio seen in exercising humans is not noted in dogs and cats. In these species exercise induces a more modest increase in the L:P ratio. Interpretation developed in the human field cannot be extrapolated to the Veterinary species
- ◇ Concentrations of lactate and pyruvate and the ratios depend upon the level of exertion. Consequently measurement of these factors should be performed on the animal under investigation and a control exercised to the same extent.
- ◇ Lactate can be measured on OXF plasma or deproteinised plasma (treated with eg., acetoacetic acid). Pyruvate requires deproteinised plasma

Key facts in the measurement and interpretation of lactate, pyruvate and L:P ratios

◇ Pre-exercise ranges mmol/L (canine)

- ◇ Lactate – 0.7 – 2.0
- ◇ Pyruvate – 0.06 – 0.1
- ◇ L:P – 8.8 – 25.2

◇ Post –exercise ranges

- ◇ Lactate – 1.35 – 5.8
- ◇ Pyruvate – 0.11 – 0.27
- ◇ L:P – 14.6 – 26.4

Exercise-induced hyperthermia or exercise induced collapse ?

- ◇ Normal dogs can develop temperatures $>42^{\circ}\text{C}$ on vigorous exercise
- ◇ Normal dogs can have lactates as high as 10 on extreme exercise and values as high as 30 have been seen in greyhounds post racing.
- ◇ Dogs with exercise induced collapse have normal L:P ratios

Urinary Organic Acids and Plasma Amino acids

- ◇ This is a relatively simple principle. Animals with enzyme deficiencies and errors of metabolism will excrete a different spectrum of urinary organic acids (products of metabolism) than normal animals
- ◇ The principal is similar for plasma amino acids.
- ◇ Samples are analysed for a large range of organic acids or amino acids and the pattern is compared with normals. Animals with single enzyme deficiencies may just show a massive elevation or reduction in one particular organic acid or amino acid.

Plasma, urine and muscle carnitine quantification

- ◇ Carnitine is essential for allowing the entry of long chain fatty acids into the mitochondria for oxidation and energy production
- ◇ Deficiency of carnitine causes muscle weakness and fatigue.
- ◇ Carnitine is also linked to the production of Pyruvate.
- ◇ Changes in carnitine and pyruvate have been noted in some cases of exercise-induced collapse.
- ◇ Supplementation of carnitine is often helpful in treating muscular diseases associated with energy production in which a specific diagnosis has not been reached.
- ◇ Carnitine deficiency can be primary or secondary to organic acidosis – replacement is beneficial in both situations.

Diagnostic Findings in the Mitochondrial Myopathy of Sussex Spaniels

- ◇ Exercise intolerance and post exercise collapse
- ◇ Pyruvate dehydrogenase deficiency prevents pyruvate for entering the tricarboxylic acid cycle of aerobic metabolism
- ◇ Lactic acidosis (pre and post exercise) with increased pyruvate concentration
- ◇ Normal or reduced L:P ratio
- ◇ Muscle carnitine reduced and urinary carnitine increased
- ◇ Urinary pyruvic acid and lactic acid markedly increased

Availability of DNA testing for metabolic myopathies

- ◇ University of Pennsylvania Genetic disease unit, Animal Health Trust, Vetgen
- ◇ PFK deficiency (Cockers and Springers)
- ◇ Glycogen Branching enzyme deficiency (Horses)
- ◇ Glycogenosis (Norwegian Forest Cat)
- ◇ Mannosidosis, Mucopolysaccharidosis,
- ◇ Myotonia Congenita (Miniature Schnauzer)

Titres to Infectious Agents in myositis

- ◇ At least 30% of cases of myositis are due to infectious agents
- ◇ Toxo IgM, IgG, Neospora serology
 - ◇ Toxo IgG <50, 50 – 200, 400 – 800
 - ◇ Toxo IgM <20, 20 or above. False negatives and positives are possible , interpret with IgG
 - ◇ Neospora IgG <100, 100 – 200, 400 - 1600

Myasthenia Gravis

- ◇ Anti Acetyl Choline Receptor Antibodies (Anti-ACHR) The assay is available for dogs and cats
- ◇ The test is sensitive and specific for acquired (immune mediated) generalised myasthenia gravis. 2% cases are seronegative
- ◇ The test is less sensitive for Focal acquired myasthenia gravis
- ◇ Titres >0.6 nmol/L and >0.3 nmol/l are positive in dogs and cats respectively
- ◇ This test should be considered in all dogs and cats with generalised weakness, dysphagia or megaesophagus
- ◇ Congenital cases are always seronegative
- ◇ $>7-10$ days of immunosuppressive doses of corticosteroids will reduce the anti-ACHR titre

Masticatory Muscle Myositis

- ◇ Masticatory muscles (all innervated by the Trigeminal nerve) contain a unique muscle fibre type known as Type 2 M
- ◇ Anti 2 M muscle fibre antibodies are directed at these muscle fibres and are not present in any generalised immune mediated myositis or polymyopathy
- ◇ Circulating IgG antibodies to 2M fibres are present in 85% of dogs with masticatory myositis. The sensitivity of the test is 85% and specificity is 100%
- ◇ Other findings which may be present in masticatory myositis include mild to moderate Ck elevations, hyperglobulinaemia, mild anaemia and proteinuria. Eosinophilia is an inconsistent finding.
- ◇ Anti 2M antibodies have been found in dog as young as 4 months, particularly CKCS with early onset masticatory muscle atrophy

Muscle Biopsy

- ◇ This is a very specialised histopathological analysis which is difficult to source in the UK. The best service is Diane Shelton's lab in California – this presents difficulties with sample handling
- ◇ First biopsy 0.5 x 1.0 cm from affected but not end-stage muscle kept cold (not frozen) and shipped on ice to arrive at the lab within 24-36 hours
- ◇ Second smaller biopsy placed in formalin.
- ◇ If delays are anticipated, freeze at -80C and ship on dry ice.

A young dog with recurring colitis and back pain

◇ Species Canine

◇ Recurring large bowel signs

◇ Stiffness walking

◇ Lethargic

◇ Breed Westie

◇ Pain on palpation of the back

◇ Age 3 years

Biochemistry and haematology

◇ Total protein	78	Hi	54-77
◇ Albumin	32		25-37
◇ Globulin	46		25-52
◇ Sodium	148		139-154
◇ Potassium	5.4		3.5-6.0
◇ Na:K ratio	27		25-35
◇ Chloride	107		99-110
◇ Total calcium	2.6		2.0-3.0
◇ Phos	2.05	Hi	0.8-1.6
◇ Urea	6.2		2.0-9.0
◇ Creat	82		40-106
◇ Alk Phos	840	Hi	0.0-50.0
◇ ALT	4072	Hi	0.0-25.0
◇ GLDH	97	Hi	0.0-10.0
◇ Total bili	28	Hi	0.0-20.0
◇ Bile acids	139	Hi	0.0-50.0
◇ Glucose	4.8		0.0-5.5
◇ CK	37992	Hi	0.0-190
◇ Cholesterol	4.4		3.8-7.0
◇ Triglycerides	1.6		0.45-1.9
◇ Amylase	548		0.0-1800
◇ Lipase	21		0.0 -250

◇ RBC	7.6		5.0-8.5
◇ Hb	17.2		12-18
◇ HCT	49.7		37-55
◇ MCV	65		60-80
◇ MCH	22		19-23
◇ MCHC	34		31-34
◇ Platelets	See comment		
◇ WBC	9.06		6.0-15.0
◇ Neutrophils	6.25		3.0-11.5
◇ Bands	0.54	Hi	<0.3
◇ Lymphocytes	1.8		1.0-4.8
◇ Monocytes	0.09		0.0-1.3
◇ Eosinophils	0.36		0.0-1.25
◇ Platelet count appears normal in film, Occ target cells			

Toxo IgM - <20

Toxo IgG - <50

Neospora - >600

A young dog with a 5 week assymetrical temporal muscle swelling

◇ Species Canine

◇ Pain on opening jaw

◇ 40% opening only possible

◇ Mild pyrexia

◇ Breed Dalmation

◇ Crying out

◇ Difficulty eating

◇ Protrusion of the left eye

◇ Age 3 years

◇ Pain on palpation of left temporal and masseter muscles

◇ Sex MN

Images are in the transverse plane of the skull at the level of the mandibular ramus.

A: medial pterygoid muscle

B: temporal muscle

C: masseter muscle

Increased signal (inflammation) is present diffusely throughout the left-sided temporal muscle, the masseter muscle and the medial pterygoid muscle. The muscles are slightly swollen compared to the right sided mastication muscles

Anti 2M muscle fibre antibody titre –
Strongly positive at 1:1000

The Clinical Pathology of joints

Joint Fluid Analysis

- ◇ Appearance
- ◇ Viscosity
 - ◇ Mucin Clot test, Protein
- ◇ Nucleated cell count
 - ◇ Overlapping categories
 - ◇ Role in horses
- ◇ Cytological examination (the priority)
 - ◇ Normal
 - ◇ Mononuclear arthropathy/degenerative disease
 - ◇ Inflammatory arthropathy
 - ◇ Infectious
 - ◇ Traumatic
 - ◇ Immune-mediated

Joint fluid analysis in horses

- ◇ Bacteria can be difficult to see in septic joint fluid on cytology. Possible septic joint effusions are considered emergencies in horses and often action must be taken before a clinical pathologist can examine the fluid
- ◇ Cell counts are often used to guide treatment
 - ◇ Normal/DJD - <500 cells/uL
 - ◇ DJD - <5,000 cells/uL
 - ◇ Trauma <10,000 cells/uL
 - ◇ Sepsis >50,000 cells/uL

A dog with swollen painful joints, inflamed skin and mild generalised lymphadenopathy

◇ Species Canine

◇ Lameness

◇ Multiple joint swellings

◇ Crusting inflamed skin

◇ Breed Crossbred

◇ Mild generalised lymphadenopathy

◇ Had travelled abroad

◇ Age 4 years

◇ Sex MN

Biochemistry and haematology

◇ Total protein	74		54-77
◇ Albumin	29		25-37
◇ Globulin	45		25-52
◇ Sodium	149		139-154
◇ Potassium	4.9		3.5-6.0
◇ Na:K ratio	30		25-35
◇ Chloride	116		99-110
◇ Total calcium	2.56		2.0-3.0
◇ Phos	1.27		0.8-1.6
◇ Urea	5.2		2.0-9.0
◇ Creat	99		40-106
◇ Alk Phos	64	Hi	0.0-50.0
◇ ALT	11		0.0-25.0
◇ GLDH	4		0.0-10.0
◇ Total bili	6		0.0-20.0
◇ Bile acids	4		0.0-50.0
◇ Glucose	6.0	Hi	0.0-5.5
◇ CK	120		0.0-190
◇ Cholesterol	3.5	Lo	3.8-7.0
◇ Triglycerides	0.6		0.45-1.9
◇ Amylase	863		0.0-1800
◇ Lipase	31		0.0 -250

◇ RBC	7.24		5.0-8.5
◇ Hb	16.3		12-18
◇ HCT	49		37-55
◇ MCV	68		60-80
◇ MCH	22		19-23
◇ MCHC	33		31-34
◇ Platelets			See comment
◇ WBC	5.4	Lo	6.0-15.0
◇ Neutrophils	3.46		3.0-11.5
◇ Lymphocytes	1.13		1.0-4.8
◇ Monocytes	0.49		0.0-1.3
◇ Eosinophils	0.32		0.0-1.25
◇ Platelet count appears normal in film, Slight polychromasia, Anisocytosis +, Poikilocytes +			

ANA - Negative

Rheumatoid factor – Positive
1:20

Lyme - Negative

The Clinical Pathology of Bone

- ◇ Biochemical markers of bone turnover have been widely developed in human medicine to monitor osteoporosis
- ◇ Their use in veterinary medicine has been very limited but recent developments with the Bisphosphonate drugs for treating osteosarcoma, hypercalcaemia and bone pain may lead to more interest bone markers.
- ◇ The main Clinical Pathological applications in bone currently are cytological methods for the diagnosis of cancer and osteomyelitis
- ◇ Regulators of calcium homeostasis particularly PTH and Vitamin D3 assays are sometimes used in the investigation of bone disorders such as rickets, nutritional and renal hyperparathyroidism

Biochemical Markers of Bone Turnover

The equilibrium between bone formation and resorption

- ◆ Osteocalcin – the major non-collagenous bone protein. A marker for osteoblast activity and bone formation
- ◆ Bone specific Alkaline Phosphatase – a marker of bone formation
- ◆ Carboxyterminal Cross-linked Telopeptide of Type 1 Collagen – a marker of bone formation
- ◆ Tartrate-resistant acid phosphatase – an osteoclast associated enzyme and marker of resorption
- ◆ Urine Deoxypyridinoline – collagen degradation product marker of bone resorption
- ◆ Urine hydroxyproline – marker of very rapid bone resorption

- ◆ Samples must be taken at a consistent time of day (diurnal rhythms)
- ◆ Many variables control these markers including age, sexual maturity, growth, sex steroids, nutritional status, time of day.
- ◆ These markers are best used sequentially in monitoring individual patients or in cohort studies rather than diagnostic tests.

Cytology of Bone Lesions

- ◇ Cytology has been shown to be more effective in the diagnosis of bone neoplasia than small incisional biopsies
- ◇ More sites can be sampled thus overcoming the problem of mosaicism
- ◇ Many bone tumours (primary and metastases) exfoliate relatively easily with normal cytological sampling techniques.
- ◇ Hard proliferative lesions may require core biopsies.