EVALUATION OF LACTATE DEHYDROGENASE (LDH) ACTIVITY IN BODY CAVITY EFFUSIONS FROM DOGS, CATS AND HORSES

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This thesis is presented for the degree of Research Masters with Training, Murdoch University, 2014
DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Lactate dehydrogenase (LDH) activity is often measured in human effusions to help in differentiating between transudates and exudates. Few studies have been performed using samples of effusions from animals. In the present study LDH activity was measured in 107 effusion samples (pleural, abdominal and pericardial) from dogs, cats and horses. LDH activity was found to be significantly increased in exudates compared to transudates in all species tested. Different methods of measuring LDH activity (wet chemistry using a lactate to pyruvate (L-P) and pyruvate to lactate (P-L) reaction, and a dry chemistry (P-L) reaction) resulted in significantly different values. In general the wet chemistry P-L reaction gave results approximately double those of the wet chemistry L-P reaction and the dry chemistry reaction was approximately double that of the wet chemistry P-L reaction. It is therefore important to know the method of measurement and this should be kept constant if cut-off values are to be used.

LDH activity in abdominal fluid may also be useful in determining prognosis in horses with colic, and cut-off values were estimated using the different methods of LDH activity measurement. LDH activity did not correlate significantly with lactate concentration in the abdominal fluids.

LDH activity was also measured in samples from dead animals in an attempt to differentiate between transudates and exudates. LDH activity was increased in effusions in all three species after death, and could not be used to differentiate between transudates and exudates in dead animals.

Effusion LDH: serum LDH activity may help to separate effusions into transudates and exudates, and may also help in differentiating septic from non-septic effusions, with highest ratios present in septic effusions and lowest ratios in transudates, however, further investigation using larger numbers of animals with effusions is necessary.
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INTRODUCTION

Analysis of body cavity effusions from small animals, horses, humans and other species may help to investigate underlying disease associated with their formation. Routine analysis of the fluid usually includes total nucleated cell counts, red cell count, packed red cell volume, total protein measured by refractometry and cytology. Biochemical analysis may also be performed if considered necessary. Complete effusion evaluation is time consuming and is often performed by a referral laboratory. A screening test applicable to every day practice would be useful to provide early diagnostic information about the characteristics of effusions.

One of the biochemistry tests which is commonly used to differentiate between transudative effusions (often associated with hypoalbuminaemia, chronic liver disease or cardiac insufficiency) and exudative effusions in humans is a measurement of LDH activity performed on the effusion fluid, and/or the effusion fluid and concurrently collected serum/plasma (Light, Macgregor et al. 1972; Romero, Candela et al. 1993; Burgess, Reuter et al. 2002). So far limited studies have been performed on feline thoracic effusions (Zoia, Slater et al. 2009), equine peritoneal effusions (Grosche, Schrodl et al. 2006), bovine abdominal effusions (Wittek, Grosche et al. 2010) and canine abdominal effusions (Rush, Host et al. 1972) to investigate the usefulness of LDH in the characterisation of the effusion. Both wet and dry chemistry tests are available for measuring LDH activity, and these have not been compared in effusion analysis.
AIMS

The aims of the present investigation were to determine whether:

1. LDH results were similar between different test methodologies. Results from two wet chemistry tests (Randox LDH lactate-to-pyruvate and the redox reaction pyruvate-to-lactate), and a dry chemistry test, IDEXX LDH (a pyruvate to lactate reaction) were compared to determine whether the results are similar between the different tests.

2. LDH was stable over time and under different storage conditions, including freeze-thawing, and storage for a 3-7 days at 4°C.

3. There was a significant difference in LDH activity between effusions collected into plain (serum) tubes or into potassium-ethylenediamine-tetraacetic acid (K₃-EDTA) anticoagulant.

4. LDH activity provided useful information for differentiating between transudates and exudates from samples collected post mortem.

5. LDH activity could be used to differentiate between transudative and exudative effusions, and if so was there an optimal cut-off value? Was measurement of this analyte a sensitive and specific test to distinguish transudative from exudative effusions? Is the cut off value consistent between different methods of LDH measurement?

6. LDH activity could be used to distinguish between neoplastic, non-septic inflammatory and septic exudates.

7. The ratio between LDH activity in the effusion compared to that in the serum (effusion LDH:serum LDH ratio) could aid in differentiating between transudates and exudates.

8. LDH measurement could be used as an aid in monitoring the progression of disease.
1 LITERATURE REVIEW

1.1 INTRODUCTION

Abdominal and thoracic paracentesis provide a rapid, easy and safe method of diagnosing diseases in which there is an effusion, such as peritonitis, haemoperitoneum, uroabdomen and exfoliative neoplasia. It can be a useful tool for investigation of patients in shock associated with no apparent cause, investigating postoperative gastro-intestinal wound dehiscence, trauma and causes of refractory pain, and can help to determine whether surgical or medical therapy is warranted (Walters 2003). Animals with body cavity effusions often present as an emergency, requiring rapid and accurate diagnosis for effective treatment. The traditional methods used for classification of effusions are often time consuming and need special skills i.e. microscopic/cytology evaluation of smears of the fluid. The present study explores the use of LDH activity in helping to determine the pathophysiology associated with the formation of an effusion, and establish an early aetiological diagnosis if possible.

1.2 PATHOPHYSIOLOGY OF EFFUSION FORMATION

A small amount of fluid is normally present in body cavities, providing mechanical coupling between the chest wall and lungs allowing for respiration (Dempsey and Ewing 2011) and acting as a lubricant and medium of transport for electrolytes and other substances (Currao, Buote et al. 2011). These fluids are normally clear and have a low protein concentration (<25g/L) and low (<3.0 x 10^9/L) nucleated cell count (Currao, Buote et al. 2011). Extravascular fluid is normally formed at the arteriolar end of capillaries and reabsorbed at the venous end of capillaries and by lymphatic uptake.

A body cavity effusion results due to an abnormal or increased accumulation of fluid in body cavities lined by mesothelial cells (thoracic, abdominal or pericardial). An alteration in one or more of Starling's forces – increased hydrostatic pressure, decreased colloidal oncotic pressure, increased vascular permeability or decreased lymphatic uptake due to increased interstitial osmotic pressure – can result in the formation of an effusion (Ettinger and Feldman 2005). Effusions can result from trauma, neoplasia, cardiovascular compromise, metabolic disorders, infectious/inflammatory diseases or other causes of increased vascular permeability (Alleman
Tight junctions are present between both endothelial and mesothelial cells (Zocchi 2002), and the classic theory is that transudative effusions formed from capillaries with intact endothelium contain less protein than the intravascular fluid. Exudative effusions usually involve some kind of localized inflammation and increased vascular permeability, resulting in increased protein in the effusion and a lower serum-effusion albumin ratio than is seen with transudates (Burgess, Maritz et al. 1995).

In horses the composition of abdominal fluid is often evaluated when there is suspected colic, intestinal rupture, uroperitoneum or other causes of abdominal discomfort in order to determine severity, cause of the disease and prognosis. Various criteria, including heart rate, peritoneal fluid protein concentration, blood or effusion lactate concentration and abnormal mucous membrane colour, have been used in horses to predict the outcome of colic, and the likelihood of survival (Furr, Lessard et al. 1995).

### 1.3 TRADITIONAL CLASSIFICATION OF BODY CAVITY EFFUSIONS

Body cavity fluid is normally collected when there is an effusion, with the aim of determining the cause of the effusion. The goal of evaluating any effusion is to obtain a rapid and accurate diagnosis, allowing appropriate therapy as quickly as possible and minimizing patient morbidity and mortality (Connally 2003). The results of fluid analysis are only useful in that they help to elucidate the underlying disease process (Beatty and Barrs 2010).

Because there are many different causes of body cavity effusion, a classification system helps to simplify diagnostic interpretation and guides further investigations. The most useful method of effusion classification is according to the primary pathophysiological mechanism or aetiology leading to effusion formation (Alleman 2003; Dempsey and Ewing 2011).

Samples can be collected into tubes containing EDTA for cytologic evaluation and into sterile tubes with no additives (and culture medium) for biochemical evaluation (and aerobic and anaerobic culture if indicated). Additional samples can be collected for polymerase chain reaction (PCR) testing, flow cytometry, etc.

Classification schemes for body cavity effusions following gross examination generally take three parameters into account: total protein, total nucleated cell count, cell type and cellular characteristics of the fluid. Biochemical analysis can be performed as required. It is also useful to
look for the presence of foreign material including bacteria, food particles or plant material (Alleman 2003).

Protein concentration of body cavity effusions can be measured using a handheld refractometer, automated biochemistry analyser, the Bradford technique (Bradford 1976), biuret technique or urine test strips (Braun, Guelfi et al. 2001).

- Refractometers may give falsely low readings if the protein concentration is less than 20g/L and often have a lower limit of 25g/L, or values may be unreadable if the fluid is discoloured by haemoglobin, bilirubin or lipid (Braun, Guelfi et al. 2001; Alleman 2003).
- The biuret method is more accurate at determining protein concentration of >5g/L, whereas the Bradford method is a more sensitive test and determines protein concentrations of <5g/L more accurately (Braun, Guelfi et al. 2001).
- The Bradford test uses Coomassie blue stain. This method may also be more suitable for measurement of low protein concentrations than refractometry as this stain reacts more intensely with albumin than globulins (Braun, Guelfi et al. 2001).
- Urine test strips allow semi-quantitative measurement of protein concentration in effusions with a protein concentration as low as 0.3g/L, however test strips are not accurate above 20g/L of protein (Braun, Guelfi et al. 2001).

Cell counts can be determined using three methods: estimation from a smear of the fluid, a manual count using a haemocytometer, or an automated cell counter (Alleman 2003). The automated cell count is most accurate due to the large number of cells counted, followed by the manual count in a haemocytometer. Estimation from a smear is variable as only a small number of cells (100-400) are usually counted. Cytological examination can be performed on direct smears (if the fluid is highly cellular), sediment smears or cytocentrifuge preparations.

One method of classification separates effusions into transudates and exudates, which can be further subdivided into pure transudate, modified transudate, exudate, haemorrhage and neoplasia (Alleman 2003). Exudates can be further subdivided into septic and non-septic exudates (Alleman 2003). Stockham and Scott (2008) do not recommend the use of the term “modified transudate” and instead separate fluid types into the following:

1. transudates
   - protein poor or
   - protein rich;
2. exudates
   - non-septic (inflammatory or viral e.g. feline infectious peritonitis) or
   - septic (bacterial, fungal, parasitic);
3. effusions caused by a ruptured organ or vessels – including haemorrhagic, chylous, non-chylous lymphatic, uroabdomen, bile peritonitis, gastric/intestinal contents; and
4. effusions with cell exfoliation – neoplasia.

Amber and clear to slightly turbid fluid are often transudates of low to moderate cellularity and are less likely to be exudates whereas fluid that appears turbid or flocculent suggests a highly cellular effusion (Rizzi, Cowell et al. 2008). Cytological evaluation is very useful and should be part of any effusion investigation. Poorly cellular effusions may require cell concentration using sedimentation or cytopsin centrifugation (Bauer and Moritz 2005).

Biochemical analytes that can be measured in effusions include urea, creatinine, amylase, lipase, glucose, lactate, pH, potassium, cholesterol, triglycerides and bilirubin. These can be useful for investigating disease associated with the liver, urinary tract, spleen, lymphatics or gastro-intestinal tract (Walters 2003).

1.3.1 TRANSUDATES

Transudates are usually clear and colourless, poorly cellular effusions with low protein concentration. The low concentration of total protein and albumin in transudates can be explained by the selective sieving of these macromolecules by normal intact microvasculature (Blunden, Dyson et al. 2006; Stockham and Scott 2008). Low protein transudates usually have a protein content of less than 15g/L (though due to the cut-off point of refractometers a value of <25 g/L protein is usually recorded) and a cell count of <1-1.5 x 10⁹ cells/L.

Conditions associated with transudate formation include hypoproteinaemia (especially hypoalbuminaemia), hypertension or overzealous fluid administration with isotonic solutions causing haemodilution. Hypoproteinaemia may occur due to increased loss, including protein losing enteropathy, protein losing nephropathy, chronic haemorrhage, parasitism, intestinal neoplasia, or decreased protein production, including liver failure, malnutrition, malabsorption, malabsorption (Porcel and Light 2006; Stockham and Scott 2008). Hypertension may be
associated with cirrhosis, lymphatic obstruction and non-cirrhotic portal hypertension (presinusoidal and sinusoidal).

Hypoalbuminaemia per se may cause effusion if plasma albumin falls below 10g/L, though if hypertension is present (e.g. liver disease) transudative effusion can occur despite serum protein values of >10g/L (Alleman 2003). Because the primary cause of protein-poor transudates is hypoalbuminaemia (Currao, Buote et al. 2011), measurement of serum total protein and albumin concentration can be a useful investigative tool to guide further diagnostics (Dempsey and Ewing 2011).

Animal and human studies have shown that an increase in intravascular hydrostatic pressure can increase the protein content in effusions (Hoefs 1983; Broaddus and Araya 1992), which may be partly explained by enlargement of pores in endothelium with elevated hydrostatic pressure. Thus in a patient with pleural effusion from congestive heart failure, it is possible that the amount of protein in the pleural fluid can increase to exudative activity if the degree of pulmonary artery pressure causing the formation of effusion was very high (Porcel and Light 2006). Right-sided heart failure causing portal venous hypertension (post-sinusoidal) or pulmonary congestion results in a protein-rich effusion, whereas effusion due to pre-sinusoidal hypertension caused by hepatic cirrhosis is protein-poor (Currao, Buote et al. 2011).

The development of a transudative effusion indicates that the serosal membranes per se are intact, so that if the underlying problem can be corrected, the effusion will be reabsorbed (Kinasewitz 1997; Porcel and Light 2006). If a patient has a transudative effusion, therapy should therefore be directed toward the underlying heart failure, cirrhosis or cause of the low plasma protein concentration.

1.3.2 EXUDATES

In contrast to transudates, exudates are formed as a consequence of increased mesothelial and endothelial permeability (Porcel and Light 2006). They are usually turbid and classified as having protein values >30 g/L and nucleated cell count >3.5 x 10⁹/L (Alleman 2003; Buchmann, Kempf et al. 2010). Inflammatory cytokines increase capillary hydrostatic pressure, leakage of large plasma proteins such as globulins and fibrinogen as well as induce chemotaxis of inflammatory and phagocytic cells into the effusion (Currao, Buote et al. 2011). Characteristically, septic fluid contains an increased number of neutrophils and macrophages (neutrophils generally
predominate), though there may be no correlation between total nucleated cell count and the severity of disease (Dempsey and Ewing 2011). Presence of plasma cells, multinucleated giant cells and cytophagia are usually indicative of more chronic antigen stimulation. As the inflammatory cells and fibrin increase in the effusion, lymphatic stomata become obstructed, resulting in further impairment of drainage (Dempsey and Ewing 2011). The Rivalta test may be used to detect high protein concentration, and is especially useful in effusion fluid from cats with feline infectious peritonitis (Raskin and Meyer 2010).

Exudates present more of a diagnostic dilemma than transudates (Porcel and Light 2006). If the patient has an exudative effusion, attempts should be made to define the aetiology (Porcel and Light 2006). Patients with exudates require immediate therapy, and if sepsis is suspected, cytologic examination and culture (both aerobic and anaerobic) are priorities (Dempsey and Ewing 2011).

Pyknosis of neutrophil nuclei generally occurs due to slow cell death in a relatively non-toxic environment (apoptosis) and is more typically observed in nonseptic exudates, however neutrophil degeneration may be seen in the absence of bacteria. Non-septic exudates may be associated with feline infectious peritonitis (in cats), steatitis, pancreatitis, bile leakage, foreign body reactions, immune mediated disease, parasite migration or haemorrhage and many other conditions (Dempsey and Ewing 2011).

Karyolysis and karyorrhexis indicate more rapid cell death in a more toxic environment and are therefore more likely to be indicative of sepsis (Alleman 2003). The degree of cellular degeneration depends on the amount and virulence of toxins present within the exudate. Aerobic and anaerobic culture, Gram staining and cytology are required in cases with septic effusions. Malodorous effusions can be indicative of anaerobic infection, e.g. 80% of cats with pyothorax (Barrs and Beatty 2009a). Septic exudates may be misclassified as non-septic if the patient has received antibiotic treatment prior to fluid evaluation, resulting in negative culture results (Davies and Forrester 1996).

Septic exudates of the pleural cavity in cats, horses and humans are most commonly associated with aspiration of obligate and facultative anaerobic oropharyngeal bacteria (Barrs and Beatty 2009a), although direct inoculation of oral flora into the thorax from a bite wound is likely to be the initiating event in some cases of feline pyothorax (Barrs and Beatty 2009a). In horses pleuropneumonia is a common sequel to transportation. Viral upper respiratory tract infections may also temporarily impair the mucociliary escalator in cats, humans and horses, allowing
colonisation of the lower respiratory tract and direct extension of infection occurs from the bronchi and lung (Barrs and Beatty 2009a).

Cytological findings should be compared with culture results. In some cases cytology allows detection of mixed bacterial infections (although only one bacterial species may be isolated) or the presence of bacteria even if bacterial culture is negative (Barrs and Beatty 2009a). Infectious agents causing septic exudates may not be identified cytologically when prior antimicrobial therapy has been administered or non-staining microorganisms (e.g. Mycobacterium spp.) are present (Barrs and Beatty 2009a). Approximately 20% of cases of feline pyothorax, particularly in kittens, are caused by unusual bacterial, fungal or protozoal pathogens, emphasising the need for pleural fluid cytology and culture (Barrs and Beatty 2009a).

Other causes of exudative effusions include exfoliative neoplasia, rupture of visceral organs and leakage of chylous fluid (Connally 2003). Cytology of the fluid is useful to differentiate septic, non-septic and exfoliative neoplastic effusions (Dempsey and Ewing 2011). Pancreatitis can cause a non-septic chemical peritonitis, which can resemble sepsis cytologically, as the chemical peritonitis results in neutrophil karyolysis (in the absence of sepsis). However, the effusion may contain lipid.

1.3.3 MODIFIED TRANSUDATE

Modified transudates are intermediate between transudates and exudates and are a unique classification in veterinary medicine. This classification is not used in human medicine, and the origin of the term is unknown (Light, Macgregor et al. 1972; Stockham and Scott 2008; Zoia, Slater et al. 2009). The modified transudate classification is based on laboratory data rather than pathological process and fills a grey area between protein-poor transudates and exudates (Currao, Buote et al. 2011) to describe effusions that closely resemble exudates based on increased protein and cellularity, but may result from increased hydrostatic pressure, and this category is not considered diagnostically useful (Stockham and Scott 2008; Zoia, Slater et al. 2009). Pure transudates are rare because they are rapidly modified by leakage of fluid from lymphatics or blood vessels and attraction of mixed inflammatory cells (Whitbread 2012). An alternative definition is a ‘long standing transudate’ (Zoia, Slater et al. 2009). High protein transudates may be formed by transudative mechanisms, whereby fluid leaks out of normal, non-inflamed vessels (increased capillary hydrostatic pressure, lymphatic obstruction resulting in fluid leakage from vessels carrying high-protein lymph/blood) (Alleman 2003), is later modified by the addition of protein.
and/or cells (Alleman 2003), and may develop merely due to the chronic presence of a transudate (Pembleton-Corbett, Center et al. 2000). The presence of any fluid in the peritoneal cavity irritates the peritoneum, causing protein loss into the body cavity and increased cellularity, forming a modified transudate. Increased leakage from lymphatics draining the liver also results in a modified transudate with high protein (Ettinger and Feldman 2005).

Modified transudates vary from tan/slightly turbid to pink or opaque (Currao, Buote et al. 2011). Protein concentration is greater than 25g/L, with a nucleated cell count between 1-5 x 10^9/L (Buchmann, Kempf et al. 2010). They are non-inflammatory and contain predominantly mononuclear cells, though modified transudates may be a transitory stage of a non-septic exudate. The most common cause of a modified transudate is congestive heart failure, though other causes, also often associated with increased intravascular hydrostatic pressure, may also result in a modified transudate including pulmonary atelectasis, hernias, acute organ torsion, obstruction of vena cava/hepatic vein due to trauma/neoplasia, restrictive pericarditis and intrahepatic portal hypertension (Alleman 2003). Pseudochylous (see later) effusions may also fall under this classification (Buchmann, Kempf et al. 2010).

The overlapping protein content and cellularity in modified transudates compared with transudates and exudates, and the large and variable number of disorders associated with modified transudates limits the usefulness of the current veterinary classification, although, once the cause for effusion has been established, the nature of the fluid can sometimes be used to determine treatment plans (Davies and Forrester 1996).

1.3.4 RUPTURED VISCERA

Rupture of a hollow organ can occur due to trauma, penetrating wound/foreign body, obstruction, necrosis or neoplastic erosion (Dempsey and Ewing 2011). Although samples may appear grossly clear, visceral perforation generally progresses to become an exudate, depending on the area from which leakage has occurred (Dempsey and Ewing 2011).
1.3.4.1 URINE

In terms of composition, an effusion containing urine can range from a transudate to an exudate, depending on the chronicity and severity of the disease (Buchmann, Kempf et al. 2010; Dempsey and Ewing 2011). Creatinine evaluation is the most useful biochemical test to detect uroabdomen, (effusion creatinine level may be more than double the serum level), though urea and potassium (fluid: serum concentration >1.4:1) measurements can also assist (Connally 2003; Slatter 2003). In cats with uroabdomen the creatinine effusion/serum ratios may vary from 1.1:1 to 4.1:1, and potassium activity from 1.2-2.4:1. These concentrations raise suspicion for uroabdomen, though specific guidelines have not yet been established for the diagnosis of feline uroabdomen based on these ratios (Dempsey and Ewing 2011). Equine uroabdomen is common in foals detected by an abdominal effusion:serum creatinine concentrations of >2 (Hardy 1998).

1.3.4.2 BILE

Leakage of bile into the abdomen starts as a modified transudate/high protein transudate that progresses to an exudate due to the irritant nature of bile. Grossly there may be an opaque greenish to orange-yellow colour to the effusion (Buchmann, Kempf et al. 2010), though this can later be masked by haemorrhage or inflammation. Tan to light brown to blue-green pigment or bilirubin crystals may be seen microscopically in the background or engulfed by macrophages (Connally 2003). Distinguishing between bile pigment and red cell degradation products can be difficult (Dempsey and Ewing 2011). Normally bilirubin should not be present in abdominal fluid, however in bile peritonitis the total bilirubin concentration should be higher in the effusion than in the serum (Connally 2003). Bile is extremely irritating to the mesothelial lining of body cavities, resulting in marked mesothelial reactivity and hyperplasia as well as an inflammatory cell response (Buchmann, Kempf et al. 2010). Because bile impairs local host defense mechanisms against bacterial contamination by decreasing phagocytic activity (Dempsey and Ewing 2011) cytologic evaluation for sepsis should be performed, as concurrent infection greatly affects prognosis (Dempsey and Ewing 2011).

1.3.4.3 HAEMORRHAGE

Haemorrhage into a body cavity may be secondary to vessel rupture (vascular disease, neoplasia, trauma) or haemostatic defects. Although it is difficult to decide on a threshold for classification of haemorrhagic effusions, significant haemorrhage is present when the packed cell volume of the effusion is 3% or greater (Currao, Buote et al. 2011). Pathological haemorrhage and whether the
haemorrhage is ongoing or more chronic may be differentiated from iatrogenic blood contamination by clinical signs of blood loss, absence of platelets, presence of erythrophagia, haemosiderophagia and lack of clotting (Buchmann, Kempf et al. 2010).

1.3.4.4 CHYLOUS EFFUSIONS

Chyloous effusions occur due to leakage of lymph from the lymphatics into either the abdomen or thorax, and may be idiopathic or occur due to rupture of the thoracic duct following trauma (usually resolves within 1-2 weeks following injury), resistance to outflow from the thoracic duct (e.g. due to neoplasia, increased central venous pressure, etc), or due to increased volume of chyle requiring drainage, e.g. due to portal hypertension (Michelsen and Edwards 2012). The fluid is usually opaque and ranges from white to pink, though not all chyloous effusions are opaque and not all white effusions are chylous (Currao, Buote et al. 2011). Chyloous effusions are normally classifiable as modified transudates or exudates. The cellular content of the fluid changes over time from mostly small lymphocytes (and occasional plasma cells) to a mixed population of vacuolated macrophages and neutrophils, secondary to inflammation induced by the presence of chyle and/or repeated thoracocentesis (Rizzi, Cowell et al. 2008).

Causes of chyloous effusions include neoplasia, steatitis, biliary cirrhosis, lymphatic rupture or leakage, postoperative complication, congenital lymphatic abnormalities (Connally 2003), primary cardiac disease, lung lobe torsion, trauma, mass effect, congestive heart failure or idiopathic (Dempsey and Ewing 2011). In cats, most chyloous effusions are due to intra-abdominal neoplasia or congestive heart failure whereas in dogs most are due to lymphatic obstruction and leakage rather than neoplasia (Alleman 2003). Chyloous effusions often carry a poor prognosis because of the often grave underlying aetiologies, though treatment has become more successful over the past 20 years (Dempsey and Ewing 2011).

Biochemical tests comparing serum and effusion triglyceride and cholesterol concentrations are useful for differentiating chylous and pseudochylous effusions, as are the presence of chylomicrons. In chylous effusions the triglyceride level is >1.1 mmol/L (100mg/dl), and are higher in the effusion than in the serum. At the same time the effusion cholesterol concentration is less than effusion triglyceride content. Most milky fluids that do not clear with centrifugation are true chylous effusions (Raskin and Meyer 2010).
The term pseudochylous effusion is considered outdated and is found in older texts. It includes opaque milky effusions that are high in cholesterol and protein-lecithin compounds (not triglycerides). This type of effusion occurs when exudates remain in the pleural cavity for extended periods of time and become enriched with cholesterol, and usually occur together with grossly thickened pleura and fibrosis such as found with chronic tuberculosis in humans (Hillerdal 1997; Raskin and Meyer 2010).

1.3.4.5 INTESTINAL CONTENTS

Microorganisms (often mixed bacterial populations) and plant material within an effusion suggest rupture of an intestinal viscus (Freeman 2007), especially if the animal is showing clinical signs of shock. Protozoa may also be seen in horses. Cases with peracute rupture (<2-4 hours) may initially have minimal inflammation and few clinical signs (Wilkins 2011). Cytology of abdominal fluid with intestinal rupture usually shows neutrophils, bacteria, and bacteria that have been phagocytized by neutrophils, however, bacterial toxins may cause rapid degradation of inflammatory cells, resulting in degenerate leucocytes or low cell count that is often associated with high protein content. Intestinal rupture in horses is often accompanied by clinical signs of shock, depression, pain, poor capillary refill time and evidence of enterotoxaemia (Pratt, Hassel et al. 2003).

If accidental enterocentesis occurs, cytology of the fluid may reveal plant material, bacteria, and debris, but few cells – in this case the horse has reasonably normal circulatory function.

1.3.5 EXPOLIATIVE NEOPLASTIC EFFUSIONS

Neoplastic effusions yield variable protein concentration and nucleated cell counts, and therefore do not readily fit into the transudate/exudate classification system (Cowell, Tyler et al. 2008; Raskin and Meyer 2010). Neoplastic effusions may contain exfoliated cells of round cell or epithelial origin tumours (rarely mesenchymal tumours) and cytological evaluation may help to determine the site of origin of the neoplasm (Cowell, Tyler et al. 2008). One study found that the median survival time of dogs and cats with an effusion related to neoplasia in the thorax was 15 days, whereas those with inflammatory disease often survived for >785 days (Kovak, Ludwig et al. 2002), and differentiating between these conditions would therefore be beneficial.

Because neoplastic disorders can promote body cavity effusion by a number of methods including increased permeability of serosal surfaces, obstruction of lymphatic flow or impaired venous
drainage as well as inducing inflammation and necrosis, tumour cells may be accompanied by varying degrees of inflammation. Effusions of any kind may irritate mesothelium, causing mesothelial hyperplasia and sloughing of these cells into the fluid, either singly or in rafts, and increasing the nucleated cell count in the effusion (Cowell, Tyler et al. 2008). Reactive mesothelial cells and neoplastic epithelial or mesothelial cells can be difficult to differentiate cytologically. Although reactive mesothelial cells may have a characteristic eosinophilic fringe, they may be multinucleate, and have variable shapes and sizes of nucleoli, sometimes making them difficult to distinguish from neoplastic cells (Cowell, Tyler et al. 2008).

Lymphoma is one of the most common causes of neoplastic effusion in companion animals and identification can be based on cytology, though other tests such as PCR for antigen receptor rearrangement (PARR) and flow cytometry are now available for further classification (Dempsey and Ewing 2011). PARR is a clonality assay which is used to amplify cellular DNA from possible neoplastic cells, using PCR primers to amplify the hypervariable regions of T cell or immunoglobulin genes. The presence of a single PCR product indicates that the cells originate from a single clone, whereas multiple PCR products are associated with a reactive process (Avery 2009).

### 1.4 COMMONLY USED CRITERIA FOR CLASSIFICATION OF BODY CAVITY EFFUSIONS

The composition of an effusion may provide evidence for the pathological process which led to an effusion forming, however, in isolation it may not be diagnostic of a particular disorder and other information (clinical examination, imaging studies, etc.) is usually necessary for interpretation (Currao, Buote et al. 2011). In human medicine effusions are separated into transudates and exudates, with most exudates containing >5 x 10⁹ cells/L (5000 cells/µl) most with a cell count of <1 x 10⁹ cells/L (1000 cells/µl) being consistent with a transudate, however there is some overlap (Light, Macgregor et al. 1972). As mentioned above, in veterinary medicine body cavity effusions are commonly classified into transudates, modified transudates and exudates depending of cell count and protein content. Numbers vary depending on the source referenced, but in general transudates contain <1.5 x 10⁹ cells/L and total protein <25g/L, modified transudates contain 1-7 x 10⁹ cells/L and total protein 25-75 g/L and exudative effusions contain >5 x 10⁹ cells/L and total protein >30g/L (Connally 2003).
1.4.1 REASONS FOR CLASSIFICATION OF EFFUSIONS

The main aim of evaluating effusions is to find an aetiology for its formation. The first step in the evaluation of body cavity effusions is separation into transudates and exudates. In human medicine the presence of an exudate necessitates further testing, including invasive diagnostic procedures, in order to reach a definitive diagnosis and institute specific therapy (Porcel and Light 2009). The classification of effusions according to their underlying aetiology is clinically useful and allows the clinician to proceed with more directed diagnostic tests. If a fluid is clearly a transudate, the list of differential diagnoses is smaller and includes hypertension associated with congestive heart failure, or hypoalbuminaemia due to nephrosis or other causes of protein loss, cirrhosis or decreased liver function, and there is less likelihood of pleural/peritoneum pathology (Light, Macgregor et al. 1972).

In feline medicine, serum albumin should be measured in effusions with a low protein concentration (total protein <25g/L). If serum albumin concentration is >15g/L, oncotic causes can be ruled out and congestive heart failure is the major differential diagnosis (Beatty and Barrs 2010). Response to treatment of cats with pleural effusion is variable and depends on the underlying cause of the disease (Davies and Forrester 1996). In the absence of a diagnosis all that can be offered therapeutically may be intermittent thoracocentesis, diuretics and antibiotics. Fortunately the list of common causes of a moderate to large amount of pleural effusion in cats is short, consisting of feline infectious peritonitis, congestive heart failure, pyothorax, neoplasia (mediastinal lymphoma and other types of intrathoracic neoplasia) and idiopathic causes (Davies and Forrester 1996; Beatty and Barrs 2010).

In many cases the cause of an effusion remains idiopathic. In a study of pleural effusions in 82 cats, cytological evaluation of the fluid identified the underlying cause in just over half of the cats, and in the others there was considerable overlap between fluid type and underlying disease (Davies and Forrester 1996). It is important to interpret laboratory findings together with clinical information (Beatty and Barrs 2010). Aggressive medical management of pyothorax in cats has resulted in improved prognosis for this disease (Barrs and Beatty 2009b).
1.4.2 CLOSER LOOK AT METHODS FOR CLASSIFICATION OF EFFUSIONS

Initial diagnosis of an effusion is generally routine, and it is the underlying cause of the effusion that presents the challenge. Body cavity effusions in humans and animals are routinely investigated using total protein measurements, total nucleated cell counts and cytology. In the past transudates have been separated from exudates based on specific gravity (>1.016 suggested infection), cell count and presence or absence of clot formation, however these methods were found to be inaccurate (Dempsey and Ewing 2011).

1.4.2.1 TOTAL PROTEIN AND CELL COUNTS

Although traditional evaluation of effusions according to their total nucleated cell count and total protein can be useful and practical, it often does not classify effusions according to their inciting cause (Dempsey and Ewing 2011). The traditional system of classification of an effusion is hampered by overlap in total protein concentration as well as cell count between transudates and exudative conditions (Pembleton-Corbett, Center et al. 2000). Up to 40% of exudates in human patients can have a leucocyte count in the transudate range (Light, Macgregor et al. 1972). Red cell counts of >100 x 10⁹/L may suggest that a fluid is an exudate, however, as very few exudates have red cell counts that high, this measurement has poor sensitivity, is of limited use (Light, Macgregor et al. 1972; Vives, Porcel et al. 1996) and may also result from trauma or blood contamination.

A pleural fluid protein cut off of 30g/L was chosen for exudates in veterinary medicine (Zoia, Slater et al. 2009) because it was found to be 90% accurate in human patients (Light, Macgregor et al. 1972), however it has led to the misclassification of many transudates and exudates, especially in the case of neoplastic effusions (Light, Macgregor et al. 1972) and those due to congestive heart failure (Haak and Fleuren 1995; Neiger and Simpson 2000). Unfortunately this value fails to distinguish between modified transudates and exudates in cats (Zoia, Slater et al. 2009) and Zoia et al (2009) found no published evidence to support the classification or the cut-off values chosen to classify pleural (or other) effusions in animals, or the sensitivity or specificity of the chosen values.

1.4.2.2 NEOPLASTIC CELLS

Epithelial and round cell neoplasms often exfoliate into the body cavity effusion, however mesenchymal tumours rarely exfoliate and other diagnostic tests are needed for identification of sarcomas. Mesothelial cells in domestic animals can appear reactive and in long-standing effusions or inflammation a conservative approach is warranted to avoid misdiagnosis of mesothelial...
reactivity as malignant neoplasia (Hirschberger, DeNicola et al. 1999). Despite this, in one study
the sensitivity of cytologic evaluation for the detection of malignant tumours in body cavity
effusions was 64% for dogs and 61% for cats and the specificity was 99% for canine and 100% for
feline effusions (Hirschberger, DeNicola et al. 1999).

1.4.3 OTHER CLASSIFICATION SYSTEMS

Four additional classification systems for body cavity effusions in humans have been suggested in
the literature: Light’s criteria (see below), serum-effusion albumin gradient (or ratio), effusion-
serum cholesterol gradient and effusion-serum bilirubin concentration (Burgess, Maritz et al.
1995). Most of the gradient methods used to distinguish transudates from exudates depend on the
fact that pleural exudates resemble plasma more closely than do transudates (Burgess, Maritz et al.
1995). The serum-effusion cholesterol gradient may be useful for differentiating between
tuberculous and malignant ascites in human medicine (Vyakaranam, Nori et al. 2011), however is
not as sensitive or specific as the serum-effusion albumin gradient at differentiating between
transudates and exudates (Burgess, Maritz et al. 1995) and will not be discussed further. The
effusion-serum protein ratio was found to be more accurate than fluid total protein in
differentiating between transudates and exudates in cats (Zoia, Slater et al. 2009), confirming the
classic publications of Light, McGregor et al. (1972). One of the advantages of protein ratios is that
they should not vary between laboratories.

1.4.3.1 SERUM-EFFUSION ALBUMIN GRADIENT AND RATIO

A serum albumin-effusion albumin gradient is calculated by subtracting the effusion albumin
concentration from the serum albumin concentration (Roth, Omeara et al. 1990). In human
patients a serum albumin-effusion albumin gradient of ≤12g/L (1.2g/dl) is specific for
differentiating exudates from transudates (Roth, Omeara et al. 1990). Increased hydrostatic
pressure due to right-sided congestive heart failure (or caval obstruction) causes increased post-
sinusoidal pressure, causing protein-rich fluid to leach out of the liver into the abdominal cavity.
Because vessel integrity is maintained, the cell count remains low, thereby forming a protein-rich
transudate (Currao, Buote et al. 2011; Dempsey and Ewing 2011).

An advantage of the serum albumin-effusion albumin gradient over simple effusion total protein
measurement is that there is minimal influence of altered hydration, as changes in hydration should
affect both the effusion and serum albumin concentration to the same degree (Pembleton-Corbett,
Center et al. 2000). This distinction is clinically relevant as portal hypertension may be treatable by fluid mobilization whereas ascites unrelated to hypertension may be refractory to such treatment (Pembleton-Corbett, Center et al. 2000).

Serum albumin-effusion albumin gradient in dogs was >11g/L (1.1g/dL) in cases with liver disease and portal hypertension compared to other disorders causing transudates, though could not clearly differentiate between the disorders (Pembleton-Corbett, Center et al. 2000). This may be because small increases in portal pressure are likely to go undetected, as portal pressure is influenced by adjustments in the splanchnic circulation, and portal hypertension is likely to be an important contributor to transudative effusions regardless of the underlying aetiology (Pembleton-Corbett, Center et al. 2000). The serum-effusion gradient is useful when patients are receiving concurrent diuretic therapy.

The serum albumin-effusion albumin ratio is calculated by dividing serum albumin concentration by effusion albumin concentrations. Although serum albumin-effusion albumin ratios have been shown to reflect the balance between hydrostatic and oncotic forces in human patients (Roth, Omeara et al. 1990), with higher ratios (>1) expected in portal hypertension and lower ratios associated with vascular leakage and inflammation, the results in veterinary studies have unfortunately not been as clear cut (Pembleton-Corbett, Center et al. 2000).

1.4.3.2 OTHER BIOCHEMICAL METHODS USED TO DIFFERENTIATE BETWEEN TRANSUDATES AND EXUDATES

Further methods of differentiating exudates from transudates include pH and glucose gradients (Bonczynski, Ludwig et al. 2003). Species differences in blood and fluid glucose and lactate concentration occur: although there may be significant differences between septic and non-septic effusion in dogs, these tests were less sensitive in cats, where cytology and total nucleated cell count were more reliable (Bonczynski, Ludwig et al. 2003).

The pH and glucose of body cavity effusions were lower in septic effusions in dogs and cats, however, there was a large amount of overlap between septic and non-septic exudates (Bonczynski, Ludwig et al. 2003), making it difficult to calculate a suitable cut-off value. In dogs, low glucose concentration and high lactate concentration in abdominal effusions may be markers for neoplasia (Nestor, McCullough et al. 2004).
The pH of a body cavity effusion may be a useful test for detecting neoplastic effusions and is used for determining prognosis in human medicine. Fluid for pH measurement needs to be collected anaerobically into heparin and measured in a blood-gas analyser (Porcel and Light 2009), and is critically dependent on sample handling. Samples therefore require immediate analysis and strict collection conditions, and methods have not been validated in veterinary medicine (Beatty and Barrs 2010).

Measurement of effusion concentrations of adenosine deaminase (useful in cases of human tuberculosis), LDH, interferon-γ, C-reactive protein, carcinoembryonic antigen, interleukin-6, tumour necrosis factor-α and vascular endothelial growth factor concentration (Danill, Zintzaras et al. 2007) have also been used to differentiate between transudates and exudates. LDH activity is commonly measured in human body cavity effusions (Light, Macgregor et al. 1972). Adenosine deaminase and C-reactive protein may be useful in distinguishing between malignant, tuberculous and parapneumonic effusions in human patients (Danill, Zintzaras et al. 2007).

### 1.5 LDH in Evaluation of Effusions

#### 1.5.1 Light’s Criteria

Light, MacGregor et al. (1972) found that, using total protein or total nucleated cell count alone, approximately 70% of pleural effusions in human patients could be separated accurately into transudate or exudate using a protein concentration of >30g/L or cell count <2.5 x 10⁹/L (<2500 cells/μl). A pleural effusion/serum protein ratio of <0.5 yielded slightly more accurate results than a protein concentration of >30g/L, however 10% of exudates were still misclassified (Light, Macgregor et al. 1972). The authors found that all the transudates had LDH activity of <200 IU/L whereas most exudates (71%) exceeded this value (Light, Macgregor et al. 1972). In addition an LDH ratio (pleural fluid/serum LDH activity) of >0.6 was present in 86% of pleural exudates and only 2% of pleural transudates (Light, Macgregor et al. 1972). When any two variables (protein concentration, LDH and/or LDH ratio) were used together the misclassification decreased, and when the three criteria were used simultaneously the chance of misclassification was small (Light, Macgregor et al. 1972). Light et al. (1972) proposed that a pleural fluid is an exudate if one or more of the following are present:
1. Pleural fluid/serum protein ratio > 0.5

2. Pleural fluid LDH activity > 200 IU/L or pleural fluid LDH greater than two thirds of the upper reference limit of LDH

3. Pleural fluid/serum LDH ratio > 0.6 (Light, Macgregor et al. 1972)

In addition, if a human patient was diagnosed with an exudative pleural effusion but the clinical signs indicate a transudate, a serum-pleural fluid albumin gradient >12g/L probably supported a transudate (Light 1997).

In a study of large pericardial effusions in human patients Light’s criteria were found to be the most reliable diagnostic tool for identifying pericardial exudates, with a sensitivity of 98%, though the specificity was only 72% (Burgess, Reuter et al. 2002). However, when a patient was receiving concurrent diuretic therapy a major disadvantage of Light’s criteria was classifying transudates as exudates (Light 1997; Burgess, Reuter et al. 2002). Modified Light's criteria for pericardial effusions in humans have been proposed, with the cut off value for LDH activity in pericardial effusions calculated at 300 IU/L (Burgess, Reuter et al. 2002)

Because of their high sensitivity in identifying exudates, Light's criteria have become the standard method for differentiating transudates from exudates in human medicine. Although Light's criteria vary in sensitivity and specificity between different study groups, they appear to be the most accurate in determining the presence of exudates (Romero, Candela et al. 1993). The major disadvantage of Light’s criteria is the misclassification of transudates as exudates (Burgess, Maritz et al. 1995). Because three criteria must be present for an effusion to be considered a transudate and only one to be considered an exudate, one would expect a lower specificity than sensitivity for exudates (Romero, Candela et al. 1993), and it may be worth modifying Light’s criteria for each specific laboratory depending on the needs of the laboratory (Romero, Candela et al. 1993).

Various groups have used Light's criteria for evaluating human pleural effusions, and some have suggested minor modifications. Out of 393 cases of pleural effusions in a hospital in South Africa the use of Light's criteria yielded an accuracy of 93% with a sensitivity of 98% and a specificity of 83% in differentiating exudates from transudates (Burgess, Maritz et al. 1995). In addition it was found that use of a serum-effusion albumin gradient was useful in patients receiving concurrent diuretic therapy (Burgess, Maritz et al. 1995). In a study of almost 300 human patients with pleural effusions, Light’s criteria were found to be more accurate than most other measurement systems.
(95.2% accurate, 98% sensitive, 77% specific), with most of the transudates misclassified as exudates being due to congestive heart failure (Romero, Candela et al. 1993).

Despite originally being described for pleural fluid, Light's criteria have been adapted and applied to peritoneal fluid in human patients (Runyon, Montano et al. 1992) and horses (Grosche, Schrodl et al. 2006). Increased activities of LDH in peritoneal fluid may indicate inflammation and tissue damage but may also be due to ischaemia, as reported in dogs (Rush, Host et al. 1972). In normal cattle the LDH activities were markedly greater than in human beings, with an upper limit of 960 IU/L, and a peritoneal fluid/serum ratio of 0.79 (Wittek, Grosche et al. 2010). Species variation may therefore affect the LDH values used.

1.5.2 TOTAL LDH

LDH is an end enzyme in the glycolytic pathway. It acts as a hydrogen transfer enzyme that catalyses the oxidation of L-lactate to pyruvate using nicotinamide-adenine dinucleotide (NAD+) as a hydrogen acceptor, and is the final step in the metabolic chain of anaerobic glycolysis. The serum half-life of LDH is approximately 50 hours (Kaneko, Harvey et al. 2008). The reaction is reversible and the reaction equilibrium favours the reduction of pyruvate to lactate (reviewed by Drent, Cobben et al. 1996; Panteghini and Bais 2008).

\[
\text{LDH, pH 8.8 – 9.8} \\
\text{L-lactate + NAD}^+ \xleftrightarrow{\text{Pyruvate + NADH + H}^+} \xrightarrow{\text{pH 7.4 – 7.8}} \text{Lactate}
\]

The lactate to pyruvate (L-P) assay in vitro has the following advantages: substrate inhibition by lactate is less than that produced by pyruvate; and the reaction linearity is more prolonged than that of the pyruvate to lactate (P-L) assay.

The advantages of the pyruvate to lactate (P-L) assay are: a less expensive assay formulation (because of the much lower concentration of the reactants required); greater absorbency with time (thus allowing more precise measurements); and greater stability of the working reagents once they are prepared as assay solutions (Drent, Cobben et al. 1996).
One unit of LDH activity has been defined as the enzyme activity present in 1.0 ml of body fluid when there is a decrease of 0.001 in optical density per minute in a reaction mixture under specified conditions (Wroblewski 1958).

Enzymes found in cell cytoplasm such as LDH appear to serve no function in body cavity effusions, but can serve as indicators of disturbed cellular integrity induced by pathological conditions (Drent, Cobben et al. 1996). LDH is present in the cytoplasm of almost all cells, and on average tissues have about 500 times the total LDH activity found in the serum, with very high activities in tissues including the liver (9000 U/g), heart (25 000 U/g), skeletal muscle (9000 U/g) and lung (9500 U/g) (Drent, Cobben et al. 1996). Even a small amount of tissue damage can result in significant elevation of LDH in the serum, and its extracellular appearance can therefore be used to detect cell damage or death (Panteghini and Bais 2008). Increased LDH activity of effusions has been found to be a sensitive, though not specific test for pathological conditions in the lungs, especially when applied to pleural or bronchoalveolar lavage fluids (Drent, Cobben et al. 1996). The degree of LDH activity increase in serum following tissue necrosis depends on the amount of LDH present in that tissue and the severity and chronicity of the injury (Wroblewski 1958; Kaneko, Harvey et al. 2008).

LDH does not readily pass through serous membranes, although it appears to increase gradually in body cavity effusions (when serum LDH activity is higher than that in the effusion) (Wroblewski 1958). A study of human pleural fluids in the United Kingdom demonstrated that there was no significant correlation between the serum and pleural fluid concentrations of LDH in transudates or exudates, so it is reasonable to suggest that the serum concentration of LDH does not influence the pleural fluid concentration (Joseph, Badrinath et al. 2001). LDH:albumin ratio was significantly higher in broncho-alveolar lavage fluid than in serum (Smith, Ripps et al. 1988), suggesting that the fluid LDH came from pulmonary tissue rather than reflecting transudation from blood to alveoli.

Wroblewski (1958) first observed that neoplastic cells in culture resulted in increasing amounts of LDH in the medium bathing the cells, and that LDH in pleural fluid containing malignant cells was greater than simultaneous serum LDH activity. Pericardial, pleural, and peritoneal effusions could be considered as in vivo cultures bathing mesothelial or malignant cells. High LDH activity may therefore be expected in effusions bathing neoplastic cells, but not in serous effusions which are not in contact with neoplastic cells (Wroblewski 1958). A trend towards higher LDH activity occurs in malignant tumours compared to the normal tissue of origin (Goldman, Kaplan et al. 1964), and it has been shown that the rapid growth associated with neoplasia resulted in increased LDH activity.
in mice, but increased LDH did not result from non-malignant growth e.g. liver regeneration (Wroblewski 1958).

In a study of 38 human cases with body cavity effusions most inflammatory effusions exhibited a higher LDH activity than seen in the corresponding blood plasma, but LDH was not useful for distinguishing between malignant and benign effusions (Horrocks, King et al. 1962). Light found that LDH activity could not be used to differentiate between different causes of exudate as it is elevated regardless of origin, but it is a reliable indicator of the degree of inflammation in humans (Light 1997). In addition it is possible to find patients with two processes occurring simultaneously, e.g. heart failure with neoplasia, complicating interpretation. Serial increases in LDH indicated increasing inflammation, and decreasing LDH activity suggests that the degree of inflammation was diminishing. If the LDH level increases with repeated thoracocentesis this suggested that the degree of inflammation was becoming progressively worse and an aggressive pursuit should be undertaken (Light 1997; Light 2007).

Light, Girard et al. (1980) found that, although many septic effusions in human patients resolved with antibiotic therapy, those associated with Gram positive bacteria, significantly elevated LDH and a pH <7.2 or glucose <3.33 mmol/L (<60 mg/dL) did not resolve with antibiotic therapy alone, but required surgical intervention.

In human medicine an LDH level of greater than 3 times the upper normal limit for serum is one of the criteria used to decide whether a human patient requires an invasive procedure (Light 2006), however this criterion should not be adhered to rigidly as it has not been validated clinically (Porcel and Light 2009). LDH was found to be a superior test compared to the serum-effusion albumin gradient and serum:effusion albumin ratio for differentiating between transudates and exudates in human pleural effusions (Joseph, Badrinath et al. 2002).

Many sources recommend that total LDH activity be measured on samples that have not been frozen and thawed, however both fresh and frozen and thawed serum have been used for LDH evaluation (Zanatta, Abate et al. 2003). As for blood-related samples, serum is preferred for measurement of LDH, as platelets, which contain high activity of LDH, may be present in plasma samples (Panteghini and Bais 2008). Haemolysed serum samples should not be used as red cells contain 150 times more LDH than serum (Panteghini and Bais 2008), however, one author has found that blood in fluid usually does not adversely affect LDH activity (Light 2007).
1.5.3 LDH ISOENZYMES

LDH has a molecular weight of 134kDa, and is composed of four peptide chains of two types: M and H, each under separate genetic control (Panteghini and Bais 2008). Heart (H) or muscle (M) subunits are so named because of their predominance in the respective tissues. LDH therefore consists of five different isoenzymes, each with different chemical and physical properties. The isoenzymes all catalyse the same biochemical reaction but differ in their molecular structure, and are more or less organ specific (Panteghini and Bais 2008). Different isoenzymes of LDH appear to be elevated in different types of neoplasia, inflammatory conditions and necrosis (Drent, Cobben et al. 1996), for example different LDH isoenzyme patterns may be useful in determining the cause of pulmonary cell damage (Drent, Cobben et al. 1996). The different isoenzymes vary in their sensitivity to cold (-20°C), and it has been found that human serum specimens may be stored at room temperature without loss of activity for up to 3 days (Panteghini and Bais 2008).

Both aerobic and anaerobic glycolytic activities are usually high in malignant neoplasms (Goldman, Kaplan et al. 1964) and there appears to be a change in LDH type (M-LDH which, in contrast to the H-LDH, is better at converting pyruvate to lactate in the presence of high pyruvate concentration) to an enzyme which is geared for pyruvate reduction (Goldman, Kaplan et al. 1964). Light (2007) found that malignant effusions had increased LDH isoenzymes 2, 3 and 4 and benign effusions had increased LDH isoenzymes 4 and 5. LDH isoenzymes 4 and 5 may also originate from white blood cells in the fluid (Light 2007).

1.5.4 REASON FOR MEASURING LDH ACTIVITY IN EFFUSIONS FROM ANIMALS

Although measurement of LDH activity is used extensively in human medicine, few studies have been carried out on the body cavity effusions of veterinary patients. It is especially challenging to determine the pathophysiological cause of effusions that fall into the “modified transudate” category, with minimal diagnostic directions provided by this classification. Tests which may help in this regard would be useful, and may provide a better understanding of the disease process. LDH may help to provide and early diagnosis or prognostic information.

As many veterinary practices currently have dry chemistry analysers that are capable of measuring LDH activity, this test is one that can be used in practice, and could allow early and more targeted treatment than may otherwise have occurred. Although some studies have already been published
in horses (Brownlow, Hutchins et al. 1981; Van Hoogmoed, Rodger et al. 1999; Grosche, Schrodl et al. 2006), cattle (Wittek, Grosche et al. 2010), cats (Zoia, Slater et al. 2009) and dogs (Wroblewski 1958; Zanatta, Abate et al. 2003), a systematic, prospective study evaluating LDH (plus other criteria) in effusions from animals, paying attention to the effects of testing interval, freezing, post mortem interval and sample origin, has not been done.

The purpose of the present study is determine whether measurement of LDH activity in body cavity effusions from different animal species is diagnostically useful, and if so, whether a cut-off point can be selected to differentiate between inflammatory and non-inflammatory processes. In addition, it may be useful to investigate prognosis in effusions from horses. Although Light’s criteria use an upper cut-off of 200 IU/L for transudative effusions in humans, the method of LDH measurement is not mentioned, which may result in substantial differences in activity measured. In addition other authors have suggested modifying Light’s criteria (Joseph, Badrinath et al. 2001), although some have found no advantage to altering the cut-off point (Vives, Porcel et al. 1996).

### 1.6 SUMMARY OF DIAGNOSTIC METHODS

In some cases the cause of an effusion remains undetermined, both in human and veterinary medicine (Ferrer, Munoz et al. 1996; Beatty and Barrs 2010). Traditional fluid analysis is variably useful in determining the aetiology of an effusion, and modified transudates especially need to be interpreted in conjunction with other clinical data. Diagnosis should not be based solely on fluid evaluation, but should be used in conjunction with the clinical presentation and other findings (Cowell, Tyler et al. 1987; Burgess, Maritz et al. 1995). Further studies for veterinary patients that validate potential markers for determining the aetiology of effusions need to be investigated (Beatty and Barrs 2010). Although there is good correlation between biochemical characteristics (especially albumin and LDH activity) and the pathophysiology resulting in exudative or transudative processes of human body cavity effusions, controlled studies correlating fluid characteristics and aetiology in veterinary medicine are limited.
2 MATERIALS AND METHODS

2.1 SAMPLES COLLECTED

Use of samples complied with the Murdoch University animal ethics protocols for use of cadavers and excess samples obtained for diagnostic purposes. Initially samples of body cavity effusions were also collected from animals submitted to the Murdoch University pathology department for post mortem investigation, but this was discontinued following early evaluation of results (14 samples). These samples were not included in any of the other studies. Body cavity effusions included in the present study were from submissions to the Murdoch University Veterinary Hospital clinical pathology laboratory made between July 2011 and September 2012.

Effusions were obtained from dogs (57), horses (31), cats (19) and one sheep (total 107). Canine and feline samples were often submitted in both K$_3$EDTA and in plain (serum) tubes, though in some cases either EDTA or plain tube samples were submitted. Effusion locations included abdominal, thoracic and pericardial. Samples from horses were usually from peritoneal taps and were usually submitted in EDTA only. Concurrent serum samples were analysed if submitted to the laboratory as part of the patient’s clinical workup (Table 1).

**TABLE 1: NUMBERS OF EFFUSION AND SERUM SAMPLES ANALYSED**

<table>
<thead>
<tr>
<th>Species</th>
<th>Abdominal effusion</th>
<th>Pleural effusion</th>
<th>Pericardial effusion</th>
<th>Total effusions</th>
<th>Serum samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horses</td>
<td>28</td>
<td>3</td>
<td>0</td>
<td>31</td>
<td>7</td>
</tr>
<tr>
<td>Dogs</td>
<td>37</td>
<td>17</td>
<td>3</td>
<td>57</td>
<td>23</td>
</tr>
<tr>
<td>Cats</td>
<td>7</td>
<td>9</td>
<td>3</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>29</td>
<td>6</td>
<td>107</td>
<td>36</td>
</tr>
</tbody>
</table>

Following routine analysis all samples from dogs, cats and horses were analysed for LDH activity using RANDOX wet chemistry lactate to pyruvate (L-P) and pyruvate to lactate (P-L) tests using a Randox Daytona biochemistry analyser (Antrim, Northern Ireland, UK), and samples from 26 horses, 7 cats and 36 dogs were tested using IDEXX LDH dry chemistry technology using a VetTest® Chemistry analyser (IDEXX laboratories, Westbrook, ME, USA). Of the 57 samples
collected from dogs, 23 had concurrently collected serum samples which were tested using the Randox wet LDH L-P and P-L tests, and 7 of the serum samples were also tested using IDEXX LDH dry chemistry. Of the 31 horses which had effusions, 7 had concurrently collected serum samples, which were tested using the Randox wet LDH L-P and P-L tests only.

### 2.2 ROUTINE ANALYSIS OF EFFUSIONS

Routine analysis was performed on submitted effusions. This included:

- total nucleated cell count and red cell count by automated methods (flow cytometry, Advia® 120 haematology analyser, Siemens Health Care Diagnostics, Erlangen, Germany) if the sample was not clotted;
- packed red cell volume (PCV) by spinning the fluid in a capillary tube in a Biofuge Haemo centrifuge (Heraeus instruments, Kendro, Germany).
- The supernatant from the PCV capillary tube was used for measurement of total protein using a temperature calibrated refractometer (Reichert Vet 360, NY, USA).
- A direct smear and a cytocentrifuge preparation were made of the effusion for cytological examination.
  - The smears were air dried and stained with Wright-Giemsa (HEMA-TEK modified Wright-Giemsa stain pack, Bayer, North Rhine-Westphalia, Germany) using a Hema-tek slide stainer (Ames, USA) and coverslipped. Smears were evaluated microscopically by a clinical pathologist.
  - For the cytocentrifuge preparation between 50 and 200μL of fluid was placed in a Shandon centrifuge-compatible Single Cytosep™ cytology funnel (Simport, Spectra Services, Ontario, Canada), depending on the cellularity or visual density of the sample. This was placed in a Shandon cytospin 2 centrifuge (Thermo Electron Corporation, Massachusetts, USA) and spun at 500rpm for 8 minutes at low acceleration.
- Culture and sensitivity were performed by an independent laboratory (Vetpath Laboratory Services, Ascot, Western Australia) on samples with a high neutrophil percentage (usually >70%), evidence of neutrophil degeneration, presence of micro-organisms observed visually on microscopy and/or high clinical suspicion of infection.
• Other routine biochemical analyses such as creatinine, cholesterol, triglyceride and lactate concentrations of effusions were performed on the fluids as requested by the submitting clinician, using Randox Daytona biochemistry analyser (Antrim, Northern Ireland, UK) and Randox reagents.

2.3 GROUPING/CLASSIFICATION OF EFFUSIONS

Each effusion was allocated an individual number and the patient’s name, species, laboratory number, date of collection and analysis and data collected from the routine laboratory analysis were recorded. Samples of body cavity effusions were classified as transudates, high protein (modified) transudates, non-septic exudates and septic exudates or haemorrhagic effusions according to routine analysis using total solids measured by refractometry, total nucleated cell counts, PCV, cytological characteristics, culture results (Cowell, Tyler et al. 2008), final clinical diagnosis and/or post mortem findings. Effusions were classified as transudates (usually high protein transudates) if they were associated with cardiac insufficiency diagnosed clinically and/or at post mortem (Zoia, Slater et al. 2009). The animal’s age at diagnosis and whether the animal survived or died (naturally or by euthanasia) as a result of the condition causing the effusion was recorded. Causes for effusion included hypoproteinaemia (protein loss or decreased hepatic production), cardiac insufficiency, neoplasia, non-septic inflammation, intestinal leakage and chylothorax (Table 2). Chylous effusions had increased triglyceride concentration and cytologically contained predominantly small mature lymphocytes. Samples with positive bacterial culture results and/or visible bacteria in the cytological preparations were classified as septic exudates regardless of cell count or protein concentration.

<table>
<thead>
<tr>
<th>Effusion type</th>
<th>Colour</th>
<th>Total nucleated cell count</th>
<th>Total protein</th>
<th>PCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure transude</td>
<td>Clear</td>
<td>Dogs $&lt;2.5 \times 10^9$ cells/L</td>
<td>$&lt;25$g/L</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Horses $&lt;5 \times 10^9$ cells/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High protein transude</td>
<td>Clear/turbid</td>
<td>$2.5-7 \times 10^9$ cells/L</td>
<td>$25-75$g/L</td>
<td>N/A</td>
</tr>
<tr>
<td>Exudate (non-septic)</td>
<td>Turbid</td>
<td>$&gt;5 \times 10^9$ cells/L</td>
<td>$&gt;25$g/L</td>
<td>N/A</td>
</tr>
<tr>
<td>Exudate (septic – bacteria present)</td>
<td>Turbid/clear</td>
<td>$&gt;5 \times 10^9$ cells/L</td>
<td>$&gt;25$g/L</td>
<td>N/A</td>
</tr>
<tr>
<td>Haemorrhagic effusion</td>
<td>Red</td>
<td>Variable</td>
<td>Variable</td>
<td>$&gt;0.05$g/L</td>
</tr>
</tbody>
</table>

N/A = not applicable
LDH activities measured by three methods (see below) were compared in both transudates and exudates (septic, non-septic and neoplastic). In addition the animals’ final clinical diagnoses was recorded, and where possible, whether the animal survived or succumbed from the condition causing the effusion. When available, serum LDH was measured and compared to effusion LDH to obtain a fluid LDH:serum LDH ratio (35 samples).

### 2.4 MEASUREMENT OF LDH ACTIVITY USING THREE METHODS

An aliquot of each effusion sample was spun at 3000rpm (1509 G) in a Jouan CR3i multifunction centrifuge (Thermo Fisher Scientific, USA) at 4°C for 10 minutes, the supernatant placed in a fresh 1ml plastic tube and the sediment discarded. The supernatant was kept at 4°C and analysed for LDH activity either on the day of collection or early the following day, within 24 hours of sample submission.

LDH was measured on each sample (including with and without EDTA as an anticoagulant, if collected into both tube types) using three different methods. Firstly LDH activity was measured via wet chemistry using the pyruvate to lactate reaction (LDH P-L) and LDH lactate to pyruvate reaction (LDH L-P) using a Randox Daytona biochemistry analyser (Antrim, Northern Ireland, UK) and Randox reagents (catalogue numbers 3818 and 3842, respectively). The Randox analyser was calibrated daily using high and low control sera (Randox Bov Asy control 1 and Randox Bov Asy control 3) to ensure accuracy of test procedures. If results obtained from samples were unreadable or out of analytic range, the samples were diluted 1 in 20 in distilled water (50μL of sample in 950μL of distilled water) and the analysis repeated.

LDH P-L was then measured on a VetTest® Chemistry analyser (IDEXX laboratories, USA) using dry-slide technology. This was run on undiluted samples if the wet chemistry results did not require dilution, or the diluted sample if dilution had been required for the Randox Daytona analyser. Fewer samples were tested using IDEXX LDH dry chemistry than the wet chemistry methods due to prolonged delays in receiving test cartridges. Fewer samples were tested using IDEXX LDH dry chemistry than the wet chemistry methods due to prolonged delays in receiving test cartridges.
2.5 STABILITY OF LDH ACTIVITY AT 4°C

A subset of samples was analysed using the Randox Daytona analyser daily using both methods (LDH P-L and LDH L-P) to determine the stability of the enzyme in specimens stored at 4°C. Ten samples were analysed at days 0, 1, 2 and 3. Nineteen additional samples were analysed at days 0 and 7. A further twelve samples were analysed at day 0 and 14.

2.6 EFFECT OF FREEZING AND THAWING ON LDH ACTIVITY

In order to determine whether total LDH activity was adversely affected by freezing and thawing, a subset of 12 samples was separated into two aliquots, one of which was frozen at -20°C and thawed and the other stored at 4°C overnight. Results from the thawed sample were compared to those from the matching refrigerated aliquot. In addition 8 samples were frozen at -20°C for 12 months and LDH re-tested and activity compared to those obtained from the fresh samples. Both wet LDH L-P and LDH P-L were analysed using the Randox Daytona analyser only.

2.7 COMPARISON OF LDH ACTIVITY IN EFFUSIONS COLLECTED INTO EDTA VERSUS PLAIN TUBES

Because many samples were submitted in EDTA anticoagulant only, a comparison of LDH activity was performed. Samples of abdominal, pleural and pericardial effusions (47 total: 14 from horses, 6 from cats and 27 from dogs) were collected into both K₂EDTA and into plain (serum) tubes. LDH activity was assayed using both the Randox wet LDH L-P and P-L tests (but not with dry chemistry due to cost and supply issues).

In order to minimise any pre-analytical factors, the IDEXX VetTest dry slide technology was used to analyse samples collected into plain tubes whenever possible from dogs and cats. Because most of the equine peritoneal effusion samples were submitted to the laboratory in EDTA, the IDEXX dry slide technology was run on samples collected into EDTA tubes for horses.
2.8 DIFFERENTIATING BETWEEN TRANSUDATES AND EXUDATES IN DOGS USING LDH ACTIVITY

Samples of abdominal (37) and pleural (17) effusions were collected from dogs. The classification of effusions was based on routine laboratory methods (Table 2) with minimal modification depending on final clinical diagnosis or post mortem findings. Abdominal effusions included 13 transudates (including high protein modified transudates associated with chronic heart failure), 23 exudates (8 non-septic inflammatory effusions, 11 septic and 4 neoplastic), and 1 haemorrhagic effusion. Pleural effusions included 6 transudates and 2 chylous effusions, 7 non-septic exudates (including 3 neoplastic), 1 septic exudate and 1 haemorrhagic effusion.

LDH results using all three methods were graphed and interpret in light of the diagnosis of the routine laboratory methods. Receiver operating characteristic (ROC) curve analysis was performed on effusions (combined pleural and abdominal effusions) of dogs to establish a cut-off value to differentiate between transudates and exudative effusions. There were insufficient numbers of samples from other species for such analysis.

2.9 LDH ACTIVITY FROM EFFUSION SAMPLES COLLECTED POST MORTEM

LDH activity was measured in 10 body cavity effusions which were collected post mortem from 5 dogs, 3 cats and 2 horses with thoracic or abdominal effusions. These samples were collected within 24-36 hours of death, and all of the cadavers had been refrigerated soon after death. In two of the animals samples were also analysed pre-mortem, and samples from these two animals were collected post mortem within 12-24 hours of death.

2.10 EFFUSION LDH:SERUM LDH RATIO

LDH activity was measured in concurrently collected effusion and serum/plasma samples using both Randox LDH L-P and P-L wet chemistry reactions. The ratio was calculated by dividing the value obtained for the serum or plasma LDH activity into that obtained from the effusion. Results were analysed using ROC analysis.
2.11 LACTATE CONCENTRATION AND LDH ACTIVITY IN ABDOMINAL EFFUSIONS FROM HORSES

Lactate measurements were obtained from the abdominal samples from 11 horses using a radiometer ABL 700 (Radiometer Medical ApS, Denmark) blood gas analyser using samples collected into EDTA anticoagulant and analysed within 15-30 minutes. LDH activity of these samples was measured by wet chemistry LDH L-P and LDH P-L reactions.

2.12 LDH ACTIVITY FOR MONITORING DISEASE

There were few cases (6 animals: 3 dogs, 2 horses and one cat) where samples were collected on two or more consecutive occasions. Samples were analysed routinely and LDH activity measured by wet chemistry LDH L-P and LDH P-L reactions was compared to the clinical and cytological findings and culture results.

2.13 STATISTICAL ANALYSIS

The three different methods for measurement of LDH were compared using Excel 2007 (Microsoft, Redmond, WA, USA) and Medcalc (Version 12.3.0.0, MedCalc Software, Mariakerke, Belgium). Paired and independent sample t-tests were used to determine statistical differences (p <0.05) between results including samples in EDTA and serum tubes, frozen and refrigerated samples, transudates and exudates. The correlation coefficient r was calculated using the Pearson correlation function to compare different methods of LDH measurement. Receiver operating characteristic (ROC) curves were used to calculate cut-off values to help differentiated between different fluid categories. Histograms, scatter plots and Bland Altman plots were produced using Excel 2007.
3 RESULTS

3.1 RESULTS OF ROUTINE ANALYSIS OF EFFUSIONS

3.1.1 DOGS

Of the 57 samples from dogs, 37 were abdominal, 17 pleural and 3 pericardial effusions. Table 3 details total nucleated cell count and total protein concentration of canine effusions.

<table>
<thead>
<tr>
<th>Type of effusion</th>
<th>Number of samples</th>
<th>Nucleated cell count (x10^9/L)</th>
<th>Total protein (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td>Range</td>
</tr>
<tr>
<td>Chylous</td>
<td>2</td>
<td>2.65</td>
<td>0.9 - 4.4</td>
</tr>
<tr>
<td>Transudates</td>
<td>20</td>
<td>1.1</td>
<td>0.1 - 3.6</td>
</tr>
<tr>
<td>Non-septic exudate</td>
<td>20</td>
<td>29.7</td>
<td>0.7 - 105</td>
</tr>
<tr>
<td>Septic exudate</td>
<td>12</td>
<td>35</td>
<td>2.2 - 152</td>
</tr>
<tr>
<td>Haemorrhagic (PCV &gt;5%)</td>
<td>3</td>
<td>6.1</td>
<td>3 - 9.2</td>
</tr>
</tbody>
</table>

Table 3: Cell counts and total protein (refractometry) in canine effusions (N=57)

Six of 20 effusions were classified as transudative based on a diagnosis of congestive cardiac abnormalities (regardless of cell count or protein content). Seventeen of 20 transudative effusions had a nucleated cell count of <1 x 10^9/L. Septic (n=12) and non-septic (n=20) exudates had variable protein concentrations, but usually high cell counts (average of 33 x 10^9 /L +/- standard deviation (SD) of 34 x 10^9 /L) and only 4 septic exudates had cell counts of ≤7 x 10^9 cells/L. Nine of the 20 non-septic exudates and 4 of the 12 septic exudates had a protein concentration of <25g/L.

In dogs in the present study effusions fell predominantly within the exudate range (septic and non-septic), and only 16% of them (9/57) were classified as transudates by traditional analytic methods. Almost 20% (11 of 57) were high protein (or modified) transudates, 6 of which were determined to be associated with congestive heart failure either clinically or at post mortem. The two septic effusions with relatively low total nucleated cell counts also had peripheral leucopenia.
3.1.2 CATS

The 19 samples collected from cats consisted of 9 pleural effusions, 7 abdominal effusions and 3 pericardial effusions, summarised in table 4.

**TABLE 4: CELL COUNTS AND TOTAL PROTEIN (REFRACTOMETRY) IN FELINE PLEURAL AND ABDOMINAL EFFUSIONS (N=16)**

<table>
<thead>
<tr>
<th>Type of effusion</th>
<th>Number of samples</th>
<th>Cell count (x10^9/L)</th>
<th>Total protein (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylous/Lymphocyte rich</td>
<td>6</td>
<td>11.8</td>
<td>7.3 - 17.1</td>
</tr>
<tr>
<td>Transudates</td>
<td>7</td>
<td>1.8</td>
<td>0 - 3.5</td>
</tr>
<tr>
<td>Non-septic exudate/neoplasia</td>
<td>3</td>
<td>4.3</td>
<td>2.9 - 5.8</td>
</tr>
</tbody>
</table>

No septic exudates were collected from cats in the present study. Pericardial effusions were associated with cardiomyopathy and had cell counts between 2 and 3.5 x10^9/L and protein concentration between 59-61g/L. Chylous/lymphocyte rich effusions were associated with congestive heart failure, lymphatic obstruction, splenic haemangiosarcoma or thymoma and transudates were associated with renal or cardiac disease.

3.1.3 HORSES

The 31 samples from horses consisted of 28 abdominal effusions and 3 pleural effusions. The 28 abdominal effusions consisted of 13 transudates and 15 exudates (6 of which were septic). Two of the 3 pleural effusions were septic exudates and one was a non-septic inflammatory exudate. The classification of the equine effusions is summarised in table 5.
TABLE 5: COMPARISON OF CELL COUNT AND TOTAL PROTEIN (MEASURED BY REFRACTOMETER) IN ABDOMINAL AND PLEURAL FLUID FROM HORSES (N=31)

<table>
<thead>
<tr>
<th>Type of effusion</th>
<th>Number of samples</th>
<th>Cell count (x10^9/L)</th>
<th>Total protein (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Range</td>
<td>Range</td>
</tr>
<tr>
<td>Transudates</td>
<td>13</td>
<td>1.7</td>
<td>0.1 – 4.9</td>
</tr>
<tr>
<td>Non-septic exudates</td>
<td>10</td>
<td>26.5</td>
<td>0.7 - 60.2</td>
</tr>
<tr>
<td>Septic exudates</td>
<td>8</td>
<td>96.5</td>
<td>0.3 - 500</td>
</tr>
</tbody>
</table>

All transudates had a cell count of <3 x 10^9/L and 11 transudates had a protein of <25g/L whereas 13 exudates had a cell count of >5 x 10^9/L and a protein content >30g/L.

Of the 28 abdominal effusions obtained from horses, 10 were from horses that recovered without the need for surgery, 7 from horses that recovered following surgery and 11 from horses that died or were euthanased due to sepsis or neoplasia. Of those that recovered following surgery, the total protein of the effusion was ≤26g/L and the total nucleated cell count <2.6 x 10^9 cells/L (only 1 fluid had a cell count <1.2 x 10^9/L).

TABLE 6: TABLE COMPARING CELL COUNT (P=0.06) AND TOTAL PROTEIN (P<0.001) BETWEEN HORSES THAT DIED AND THOSE THAT SURVIVED (WITH OR WITHOUT SURGICAL INTERVENTION)

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of samples</th>
<th>Cell count (x10^9/L)</th>
<th>Total protein (refractometer) in g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Range</td>
<td>Range</td>
</tr>
<tr>
<td>Survived</td>
<td>17</td>
<td>3.9</td>
<td>0.1 – 39.1</td>
</tr>
<tr>
<td>Died</td>
<td>11</td>
<td>76</td>
<td>0.3 - 500</td>
</tr>
</tbody>
</table>

Of the horses that recovered without the need for surgery (usually inflammation present in the abdominal cavity, including intestinal and uterine inflammation) the cell count was <4 x 10^9/L in 15 of 18 samples and the total protein was variable, with 6 effusions having a protein concentration between 28-39g/L. In horses that survived with or without surgery the effusions had <5 x 10^9 cells/L, whereas the cell count was >8 x 10^9 cell/L in 9 of 11 horses that did not survive (however,
this difference was not significantly different, p=0.06) and the protein concentration was >30g/L. Total protein concentration was usually >25g/L in those horses with a poorer prognosis, approximately 30% greater than in those that survived. Neoplasia was rarely detected using cytology of the effusion alone and this was a specific though not a sensitive means of diagnosing neoplasia.

3.2 LDH ACTIVITY USING THREE DIFFERENT METHODS

The results of the three different methods of measuring LDH activity were compared on 83 samples regardless of the classification of the effusion (Figure 1).

![Figure 1: Comparison of average LDH results (N=83) using Randox wet chemistries (LDH L-P and LDH P-L) and IDEXX dry slide technology (P-L). Bars represent standard error of mean.](image)

The different methods of testing LDH activity provided significantly different mean results from each other using a paired t-test. The Randox wet chemistry LDH L-P reaction provided the lowest values, Randox wet chemistry LDH P-L values were approximately double those of the LDH L-P reaction and IDEXX LDH dry slide technology results (LDH P-L) were approximately double those of wet LDH P-L. The ratio of Randox wet LDH L-P : Randox wet LDH P-L : IDEXX dry LDH P-L was almost 1:2:4.
While there were significantly different LDH results between the methods, the correlation between them was moderate (Figure 2).

![Graph 2a](image1.png)

![Graph 2b](image2.png)

![Graph 2c](image3.png)

**FIGURE 2A, B AND C: COMPARISON OF DIFFERENT METHODS OF MEASURING LDH ACTIVITY**

Results from testing LDH activity Randox wet LDH (L-P and P-L) wet chemistries correlated moderately well with each other ($r = 0.75$, with 95% CI ±0.065, Figure 2). Correlation between Randox wet P-L and IDEXX dry P-L was $r = 0.67$ (95% CI ±0.067). Correlation between Randox wet
LDH L-P and IDEXX LDH P-L dry technology was poor with a correlation coefficient of 0.38 (95% CI ±0.064).

3.3 STABILITY OF LDH ACTIVITY AT 4°C

Ten samples from a variety of effusion types were measured using Randox LDH wet chemistry L-P and P-L reagents at 0, 1, 2, and 3 days after storage at 4°C (Figure 3). A further 19 samples were tested at 1 week and 12 samples tested after 2 weeks of storage at 4°C.

FIGURE 3: LDH L-P AND LDH P-L MEASURED OVER 4 DAYS IN SAMPLES (N=10) STORED AT 4°C SHOWS A NON-SIGNIFICANT DECREASE IN LDH ACTIVITY OVER THAT TIME

There was a small but non-significant decrease (p>0.2) in LDH activity comparing day 0 samples to those stored at 4°C and tested after 3 days.

Correlation between day 0 and day 3 was also compared (Figure 4).
FIGURE 4: LDH ACTIVITY MEASURED BY A) WET LDH L-P AND B) WET LDH P-L METHODS SHOWS THAT THERE IS GOOD CORRELATION IN LDH MEASUREMENTS AT THE TIME OF COLLECTION AND AFTER 3 DAYS STORAGE AT 4°C (N=10)

There was good correlation and no significant difference in LDH activity between samples tested at day 0 and the same samples tested 3 days later (Wet LDH L-P: r = 0.99, 95% CI ±0.02; Wet LDH P-L: r = 0.96, 95% CI ±0.06). The comparison between day 0 and day 7 showed similar results (Figure 5).

FIGURE 5: COMPARISON OF LDH ACTIVITY MEASURED BY A) WET LDH L-P AND B) WET LDH P-L AT THE TIME OF COLLECTION AND AFTER 1 WEEK USING SAMPLES STORED AT 4°C (N=19)
There was no significant difference in LDH activity between original values after storage at 4°C for 1 week (p<0.05) and results correlated well (Wet LDH L-P: r = 0.90, 95% CI ±0.14; Wet LDH P-L: r = 0.97, 95% CI ±0.04).

Similar changes were seen in the samples stored for 2 weeks at 4°C, with greater similarity in LDH activity between repeat measurements from samples with lower LDH activity and a decrease in LDH activity in samples with high initial LDH activity (correlation coefficients for all samples evaluated after 2 weeks: LDH L-P = 0.97, 95% CI ±0.09, LDH P-L = 0.98, 95% CI ±0.05).

3.4 EFFECT OF FREEZING AND THAWING ON LDH ACTIVITY

Figures 6 and 7 show the comparison of LDH L-P and LDH P-L results between split aliquots of samples that were either refrigerated at 4°C overnight or were frozen at -20°C overnight, then thawed immediately before analysis.
The percentage difference was calculated between the fresh and frozen-and-thawed samples and plotted (Figure 7).

There was no significant difference between samples stored at 4°C and those frozen overnight and thawed using either the Randox wet LDH L-P or P-L wet chemistry reactions (t-test gave a two tailed probability of \( p = 0.977 \) for both L-P and P-L reactions).
Seven samples were frozen for 12 months and LDH re-tested using LDH wet chemistry reactions for L-P and P-L. Results were compared to initial values (Figure 8).

**FIGURE 8**: WET CHEMISTRY LDH L-P AND P-L RESULTS FROM 7 EFFUSION SAMPLES FROZEN FOR 12 MONTHS COMPARED TO RESULTS FROM FRESH SAMPLES. BARS REPRESENT STANDARD ERROR OF MEAN.

Effusion samples that were frozen for prolonged periods (12 months) showed a significant decrease in LDH activity (p<0.05).

3.5 COMPARISON OF LDH ACTIVITY IN EFFUSIONS COLLECTED INTO EDTA VERSUS PLAIN TUBES

Figure 9 shows the range of LDH values in samples collected into EDTA compared to those collected into plain (serum) tubes.
FIGURE 9 A AND B: LDH ACTIVITY IN SAMPLES COLLECTED INTO PLAIN (SERUM) TUBES COMPARED TO THOSE COLLECTED INTO EDTA ANTICOAGULANT
FIGURE 10: LDH ACTIVITY IN EFFUSIONS COLLECTED INTO PLAIN (SERUM) TUBES COMPARED TO THOSE COLLECTED INTO EDTA ANTICOAGULANT

Results from samples collected into EDTA correlated well with those collected into serum tubes (Figure 9) and showed no significant difference (p>0.8, Figure 10) in total LDH activity using either the wet LDH L-P or LDH P-L reactions compared to samples collected into plain (serum) tubes (correlation r of 0.99, 95% CI of 0.009 for the Randox LDH L-P reaction and 0.99, 95% CI of 0.006 for the Randox LDH P-L reaction).

3.6 DIFFERENTIATION BETWEEN TRANSUDATES AND EXUDATES USING LDH ACTIVITY

3.6.1 DOGS

LDH activity in dogs from both pleural and abdominal effusions using all three methods was compared between transudates, inflammatory non-septic exudates, septic exudates and neoplastic effusions. Mean +/- SEM LDH activity in canine pleural plus abdominal fluids, using all 3 methods for transudates, non-septic exudates, septic exudates and neoplastic effusions are shown in Figure 11 and 12.
FIGURE 11: COMPARISON OF LDH ACTIVITY IN TRANSUDATES, NON-SEPTIC INFLAMMATORY, SEPTIC AND NEOPLASTIC EXUDATES IN ABDOMINAL AND PLEURAL EFFUSIONS FROM DOGS. BARS REPRESENT STANDARD ERROR OF MEAN.

LDH activity was approximately 10 times higher in non-septic inflammatory exudates than in transudates, and on average approximately 20 times higher in septic and neoplastic effusions than in transudates. The two chylous effusions had LDH activity similar to that from the transudates, and the two haemorrhagic effusions had LDH activity similar to that of the exudates (data not shown). There was no significant difference between LDH activity in septic versus non-septic inflammatory and neoplastic effusions.

FIGURE 12: LDH ACTIVITY IN ALL CANINE TRANSUDATES AND EXUDATES (SEPTIC AND NON-SEPTIC). BARS REPRESENT STANDARD ERROR OF MEAN.
LDH activity using all three methods of measurement was significantly lower (p<0.001) in transudates than in exudates of all kinds, when the latter were considered as a single group (i.e. non-spetic, septic and neoplastic combined). The ratio between LDH L-P, LDH P-L, and dry chemistry LDH P-L results in these effusions was 1:2:4).

ROC curves were used to calculated cut-off values for LDH activity (all three methods) from canine abdominal and pleural effusions to differentiate between transudates and exudates (Figure 13 and Table 7).

![ROC curve showing the area under the curve for RANDOX WET LDH L-P, WET LDH P-L and IDEXX DRY CHEMISTRY MEASUREMENTS](image_url)

**FIGURE 13:** ROC curve showing the area under the curve for RANDOX WET LDH L-P, WET LDH P-L and IDEXX DRY CHEMISTRY MEASUREMENTS

**TABLE 7:** PROPOSED CUT-OFF VALUES TO DIFFERENTIATE BETWEEN TRANSUDATES AND EXUDATES IN DOGS, USING THREE DIFFERENT METHODS OF MEASURING LDH ACTIVITY

<table>
<thead>
<tr>
<th>Method of LDH measurement</th>
<th>Dogs</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet LDH L-P</td>
<td>&gt;200</td>
<td>92%</td>
</tr>
<tr>
<td>Wet LDH P-L</td>
<td>&gt;350</td>
<td>91%</td>
</tr>
<tr>
<td>Dry IDEXX LDH P-L</td>
<td>&gt;500</td>
<td>94%</td>
</tr>
</tbody>
</table>
3.6.2 CATS

Samples were tested from 19 cats using Randox wet LDH L-P and LDH P-L wet chemistry methods and 7 samples were also tested using IDEXX P-L dry chemistry. There were 7 abdominal effusions, 9 pleural effusions and 3 pericardial effusions. No septic effusions were tested from cats. LDH activity in pleural and peritoneal samples is summarised in Figure 14.

**Figure 14:** LDH activity in abdominal and pleural transudates, lymphocytic effusions and non-septic exudates from cats of pleural and abdominal origin. Bars represent SEM.

Lymphocytic (chylous and associated with neoplasia) effusions had LDH activity greater than found in transudates, but less than from exudates, despite the fact that the cell count was often higher in lymphoid effusions than in exudates associated with other causes. Group sizes were too small for statistical analysis, but of the samples tested LDH activity was lower in abdominal and thoracic transudates (wet LDH L-P < 20 IU/L, wet LDH P-L < 45 IU/L, IDEXX dry chemistry < 200 IU/L) and higher in exudates (wet LDH L-P > 70 IU/L, wet LDH P-L > 100 IU/L, IDEXX dry chemistry > 400 IU/L).
3.6.3 PERICARDIAL EFFUSIONS IN DOGS AND CATS

There were too few pericardial effusions from both cats and dogs (n=6: 3 dogs and 3 cats) to reach a statistically significant separation between transudates and exudates, however, of the 6 pericardial effusions classified as either transudates and exudates, 5 had very high LDH activity (>1800 IU/L for wet LDH L-P, >2800 IU/L for P-L and >8000 IU/L for IDEXX dry chemistry) and the sixth (with a packed cell volume of 0.24L/L) was on the borderline between transudate and exudate classifications. The three pericardial effusions (from 2 cats) had high LDH activity (both wet LDH L-P and P-L methods >2500 IU/L). Because of the high LDH activity in these effusions, they were not evaluated together with pleural and abdominal effusions.

3.6.4 HORSES

Peritoneal fluid was collected from equine patients presenting to the hospital with colic (n=28) and pleural effusions (n=3). LDH activity was significantly higher in abdominal and thoracic exudates than transudates (p <0.05) using all three methods of LDH activity measurement (Figure 15).

![Figure 15: Comparison of LDH activity in transudates and exudates from equine abdominal and pleural effusions using 3 different methods of LDH activity measurement. Bars represent SEM.](image)

LDH activity was also compared in abdominal fluid from equine patients that died, or survived with or without surgical intervention.
LDH activity in abdominal fluid was compared between the 28 horses with colic that survived without surgery, those that survived following surgery, and those that had septic/neoplastic effusions and did not survive (Figure 16).

**FIGURE 16: COMPARISON OF LDH ACTIVITY IN ABDOMINAL EFFUSIONS FROM HORSES WITH NON-SURGICAL AND SURGICAL COLIC THAT SURVIVED AND THOSE WITH EFFUSIONS AND COLIC SIGNS ASSOCIATED WITH SEPSIS OR NEOPLASIA THAT DIED**

The LDH activity of the abdominal effusions was significantly lower in horses that survived with or without surgery compared to those that died (due to sepsis or neoplasia) using all three methods of measuring LDH activity (p<0.05). There was no significant difference in LDH activity between colic horses that survived without surgery compared to those that survived but where surgery was considered necessary (p > 0.05 for all three methods of measuring LDH activity).

A cut off value was attempted to aid in determining prognosis for horses with colic based on LDH activity. The suggested cut-off values are shown in Table 8 and reasoning explained below.

**TABLE 8: TABLE SHOWING SUGGESTED CUT OFF VALUES OF LDH ACTIVITY FOR HORSES ABOVE WHICH THE PROGNOSIS IS POOR**

<table>
<thead>
<tr>
<th>Equine effusion samples</th>
<th>LDH activity cut off</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet LDH L-P</td>
<td>280 IU/L</td>
<td>90%</td>
</tr>
<tr>
<td>Wet LDH P-L</td>
<td>480 IU/L</td>
<td>90%</td>
</tr>
<tr>
<td>IDEXX LDH P-L dry chemistry</td>
<td>900 IU/L</td>
<td>85%</td>
</tr>
</tbody>
</table>
Of the horses that survived with medical management only, LDH activity was usually <701 IU/L (IDEXX LDH, 8 out of 9 horses), <250 IU/L (Randox wet chemistry LDH L-P, 10 out of 11 horses) or <452 IU/L (Randox wet chemistry LDH P-L, 10 out of 11 horses).

Death was associated with high LDH activity (IDEXX LDH >900 IU/L; 2 out of 15 horses survived, 9/11 died, sensitivity 82%, specificity 86%; wet LDH L-P >280 IU/L, 2/18 horses survived, 12/13 died, sensitivity 91%, specificity 88%; LDH P-L >480 IU/L, 2/18 horses survived, 12/13 horses died, sensitivity 91%, specificity 82%) and was most commonly associated with conditions such as gastro-intestinal necrosis, neoplasia and/or sepsis and almost all horses in the present study with septic (often associated with gastro-intestinal rupture) or neoplastic effusions died, or were euthanased, as a result of the disease. Accuracy was calculated as the number of correctly classified outcomes using that cut-off value.

In contrast, horses with an effusion LDH activity of <750 IU/L (IDEXX dry LDH, only 4/15 >750 IU/L survived, specificity 71%), <250 IU/L (Randox wet LDH L-P, 4/18 horses >250 IU/L survived, specificity 81%) or <480 IU/L (Randox wet LDH P-L, 3/18 >480 IU/L horses survived, specificity 82%) had a good chance of survival if they received appropriate treatment.

### 3.7 LDH ACTIVITY FROM SAMPLES COLLECTED POST MORTEM

Body cavity effusions were collected post mortem from 12 animals including from 2 horses, 6 dogs and 4 cats. Post mortem interval varied from 12 to 36 hours. LDH activity was measured using Randox wet LDH L-P and P-L tests only (Figure 17).
There was no significant difference between LDH activity in transudates or exudates in samples collected post mortem. LDH activity from samples collected post mortem (transudates and exudates) was high (3-10 times greater than in transudative effusions from living animals, i.e. in the range of exudates in living animals), and dilution was usually required to obtain a result. There was no significant difference in the LDH activities between transudates and exudates from dead animals using either test. In two animals (one cat and one dog) effusions were tested both pre-mortem and 24 hours after death. The LDH activity of was increased 10 fold in an exudate (cat) and 220 fold in a transudate (dog).

Because of these findings no further samples were evaluated from dead animals.

3.8 RATIO OF EFFUSION LDH:SERUM LDH

LDH activity was measured in both serum and fluid from only 22 dogs (6 non-septic exudates, one haemorrhagic effusion, 8 septic effusions, 6 transudates and one chylous effusion) and the effusion LDH : serum LDH ratio was calculated (Figure 18). The chylous effusion was included with the transudates and the haemorrhagic effusion was excluded from calculations.
FIGURE 18: RATIO OF EFFUSION LDH: SERUM LDH RATIO, COMPARING TRANSUDATES, NON-SEPTIC AND SEPTIC EXUDATES IN 22 DOGS. BARS REPRESENT STANDARD ERROR OF MEAN.

LDH L-P and LDH P-L wet chemistry measurement of LDH activity in all the animals with transudative effusions gave a ratio of less than 0.7, i.e. the LDH activity was substantially higher in the serum than in the transudate. A ratio >1.9 was obtained for 5 of 6 non-septic exudates (0.7-5.9) and >4 for all 8 septic exudates (4.1-68.2). There was a significant difference in the ratio between transudates and exudates (p<0.01).

In 5 effusions (2 pleural and 3 abdominal) from cats, ratios of <0.7 in transudates and >0.8 in exudates were calculated. A pericardial effusion (which was the result of congestive heart failure) had an effusion LDH:serum LDH ratio of >30.

Serum and effusion LDH activities were also obtained from 6 horses. The effusion LDH: serum LDH ratio in horses was similar to that from dogs and cats, with transudates and effusions in horses that recovered without surgery (n=4) resulting in an effusion LDH: serum LDH ratio of <0.7 and one septic exudate and one neoplastic effusion (exfoliative squamous cell carcinoma) producing a ratio of >0.7.
3.9 EFFUSION LACTATE AND LDH ACTIVITY IN HORSES

Effusion lactate level was measured in abdominal effusions from 11 horses, 9 with effusions classified routinely as transudates and 2 as septic exudates (Figure 19). Lactate content ranged from 1.6-13.6mmol/L (average 7.3mmol/L, SE 1.1 mmol/L).

Interestingly, while there was poor positive correlation between lactate concentration and the IDEXX LDH dry chemistry P-L activity in 8 abdominal effusion (r = 0.24), there was moderate correlation between lactate and wet chemistry LDH L-P and P-L activity measurements in 11 abdominal effusions (r = 0.73)

3.10 LDH ACTIVITY FOR MONITORING DISEASE

Six animals with different diseases had more than one effusion sample collected at different times. Due to the low number of results these were not analysed statistically.
In a horse with an abdominal *Actinobacillus spp.* infection LDH activity of the abdominal fluid was measured by all methods approximately every 3 weeks over a period of 3 months to monitor for infection (Figure 20).

**FIGURE 20: FLUCTUATING LDH ACTIVITY IN THE ABDOMINAL FLUID FROM A HORSE, ASSOCIATED WITH FLUCTUATING PERITONEAL INFLAMMATION CULMINATING IN SEPSIS (EFFUSIONS WERE CLASSIFIED AS NON-SEPTIC IF NO BACTERIA WERE CULTURED, AND SEPTIC IF BACTERIA WERE SEEN OR CULTURED IN THE FLUID)**

LDH activity measured by all three methods was initially mildly increased compared to normal values (*Actinobacillus spp.* was cultured). Following successful antibiotic treatment (3 negative cultures, no bacteria seen cytologically) LDH activity decreased marginally, only to increase dramatically when septic peritonitis recurred.

LDH activity remained constant in one cat with chylothorax over a period of 2 weeks (n=3). In a dog with gall bladder inflammation the LDH activity increased markedly following surgery. In a dog and foal with ongoing septic peritonitis LDH activity remained consistently high (n=2). In one dog
with septic peritonitis associated with a multi-resistant bacterial strain the LDH activity increased over a period of hours when wound breakdown increased (n=2), and increased bacterial numbers and leucocyte degeneration was detected cytologically.

4 DISCUSSION

Fluids obtained from body cavities (abdominal, pleural and pericardial) are often evaluated in order to determine a possible cause for the effusion, prognosis or provide information to direct further diagnostic testing. In dogs, cats and horses the evaluation of effusions may help to elucidate the pathophysiology of the effusion, or in some cases can be diagnostic (e.g. if neoplastic cells, bacteria or contents from a ruptured organ are detected). Measuring LDH activity may be a useful diagnostic test to help in the identification of transudative or exudative effusions, especially if in-house biochemistry is available and before cytological evaluation is obtained. In addition, LDH measurement may also provide prognostic information.

Traditional methods for classification of effusions (total protein measured by refractometry, total nucleated cell count, red cell count and cytology) are useful and practical, but may fail to classify effusions according to their inciting cause (Stockham and Scott 2008; Dempsey and Ewing 2011). In addition, the cytological interpretation depends on the experience of the evaluator.

Although traditional methods help to investigate the pathophysiology of the fluid formation, e.g. transudative, exudative, haemorrhagic, leakage from a hollow organ, or neoplastic, there is a degree of overlap, especially when a fluid falls into the category of modified transudate (high protein transudate), and interpretation must always be made in conjunction with clinical signs in the patient. Because of this overlap, other tests are warranted and can be performed on the fluid for further diagnostic and prognostic information.

Although LDH is commonly used in classifying human pleural, abdominal and pericardial effusions as exudates or transudates, this enzyme has been used in only a limited number of studies in dogs (Wroblewski 1958), cats (Zoia, Slater et al. 2009) and horses (Brownlow, Hutchins et al. 1981; Van Hoogmoed, Rodger et al. 1999; Grosche, Schrodl et al. 2006). The original aim of the present study was to evaluate applicability of LDH activity in characterisation of effusions. However, it was soon realised that before doing this many further, mostly methodological tests needed to be done.
In the present study LDH was found to be stable in vitro for up to three days at 4°C, and could be measured with comparable results using samples collected into plain tubes or into EDTA anticoagulant. This is an advantage over other analytes, including glucose (which decreases in the presence of cells or bacteria in the absence of added fluoride if not separated from cells) and lactate (which requires anaerobic sample conditions and should be analysed as soon as possible (Latson, Nieto et al. 2005), and is therefore best tested if in-house measurement (including hand-held instruments) is available). The advantage of being able to measure LDH activity in older samples (stored in the refrigerator or freezer) is that a progression in disease, improving or deteriorating, might be possible. In the present study very few cases were tested more than once, and further studies are necessary for more definitive information on progression of disease and to provide prognostic information.

No evaluation of the effect of haemolysis was performed on our samples. This is a weakness of the present study and further investigation is needed for different animal species. Haemolysed serum samples may have increased LDH activity as red cells contain 150 times more LDH than serum in some species (Panteghini and Bais 2008), although one author found that bloody pleural fluid in humans usually does not adversely affect LDH activity (Light 2007).

In the present study LDH activity was usually low in transudates, including modified transudates, and high in exudates, even those with low cell counts, and consequently LDH may be useful in helping to distinguish between these fluid types, especially before cytology and culture results are available. Although LDH appears to be useful in differentiating between transudates and exudates in dogs, cats and horses (and most likely in other species too), the present study has shown that different cut-off values are required from those used in human medicine, and different cut-off values are required for differentiation between transudates and exudates in each species.

Chylous effusions had cell counts 2.5 times greater than non-septic exudates/neoplasia and 7 times greater than in transudates. The average total protein concentration (by refractometry) was also the highest in chylous effusions (which is not surprising, and likely an unreliable result). The biuret method of measuring protein concentration may be better for these); transudates and non septic exudates had similar total protein results.

Special attention was paid to the abdominal effusions of horses. LDH may provide information regarding the prognosis in horses with colic. Measuring LDH activity may be a useful adjunct to routine analysis of effusion fluid. This includes total protein, cell count and cytology, clinical signs
and effusion lactate concentration (Furr, Lessard et al. 1995; Latson, Nieto et al. 2005). In the present study the effusions collected from horses were evaluated to investigate prognosis. The fluid was often collected from colic horses without body cavity effusions per se, but rather sampled from a normal amount of abdominal fluid in order to gain diagnostic and prognostic information regarding the cause and severity of colic. Abdominal fluid is most frequently collected from horses in order to investigate the cause and severity of colic, and to help in determining a prognosis (Cowell, Tyler et al. 1987; Furr, Lessard et al. 1995). Questions that may be asked include whether there is abdominal inflammation and how severe it is, whether the intestinal wall is viable or necrotic, the presence or absence of microbial leakage, whether surgery is required and the likelihood of survival. Further studies should be undertaken to compare the prognostic value of LDH activity in abdominal fluid in horses with colic to that of other variables with known prognostic merit, such as degree of haemoconcentration, heart rate, blood pressure and blood lactate.

To our knowledge this is the first time that LDH activity in abdominal fluid from colic horses has been evaluated to differentiate between horses that survived the disease process for which they were admitted to the hospital and those that did not survive. LDH activity was increased in the abdominal fluid from horses with a poor to grave prognosis, but could not be used to differentiate between cases that required surgery or only medical management. Very high LDH activity in our study was usually associated with sepsis (due to intestinal leakage or rupture) or advanced intra-abdominal neoplasia, and clinically the prognosis was considered to be hopeless. LDH activity in abdominal fluid from horses with colic may provide prognostic information, especially before other laboratory data is available.

In the present study the method used for measurement of LDH activity seriously influenced results. There are a number of different ways of measuring LDH activity; however, in most papers that discussed measurement of LDH activity in effusions from either humans or animals, the method of LDH measurement is not mentioned. Kits for analysis of the lactate to pyruvate reaction are also available for many wet biochemistry analysers.

Three methods were compared in the present study, and they resulted in significantly different values. The forward and reverse reactions using wet chemistry resulted in significantly different values in our study, although they did follow a similar pattern. The IDEXX Vettest analyser measures LDH using the pyruvate to lactate reaction, however, it uses dry chemistry, resulting in
different values to those obtained from either of the wet chemistry reactions used here. The cut-off values determined in the present study showed that different cut-off points can be used for differentiation between transudates and exudates depending on the test method used. This therefore means that it is important to know how LDH activity was measured and to use one method consistently. Only two analysers were used in the present study, and further investigation should be done into other available methods. However, results should be repeatable or comparable if the same method of measurement is used.

Different cut-off values for LDH activity in effusions have been calculated for differentiation between transudates and exudates in people (Light, Macgregor et al. 1972; Burgess, Maritz et al. 1995). The variation in cut-off values obtained in studies conducted in different countries and using different laboratories may be partially explained by differences between analysers and test methods. LDH activity measured by all three methods trialled in the present study was lower in transudates than exudates when pleural and abdominal effusions were considered collectively in dogs, though different cut-off values had to be calculated for each method of LDH measurement and in each species.

LDH has previously been found to be stable at 4°C for 3 days and in human serum specimens stored at room temperature for up to 3 days (Kaneko, Harvey et al. 2008; Panteghini and Bais 2008). This is similar to the findings in body cavity effusions evaluated in the present study. We found no significant decrease in LDH activity in samples kept at 4°C for 3 days, and only a 10-20% decrease in LDH activity in samples stored for up to 2 weeks at 4°C, provided the supernatant had been separated from the cells.

LDH test kit manufacturers suggest that LDH be measured on unfrozen samples (Randox information leaflet accessed 2011, Panteghini and Bais 2008). In the present study the LDH results of samples stored at 4°C overnight and those frozen overnight at -20°C and thawed were not significantly different, and a single freeze-thaw cycle therefore does not appear to decrease the LDH activity significantly. However, we found that there was a significant decrease in LDH activity after storage for 1 year at -20°C. Freezing samples for short lengths of time may therefore result in reliable results if testing is required at a later date (e.g. to determine progression of disease), though long-term freezing does not prevent a decrease in LDH activity.

There are no available comparisons of samples collected into EDTA anticoagulant and those collected into plain (serum) tubes, although this can be important for practitioners. In the present
study there was no significant difference between 47 fluid samples collected into plain (serum) tubes and those collected into EDTA. Serum is the preferred specimen for measurement of LDH, as platelets, which contain high activity of LDH, may be present in plasma samples (Panteghini and Bais 2008). In our study all the samples were spun down within 1 hour of sample submission and the supernatant used for analysis, removing platelets, red cells and other cellular fragments. Our results showed no significant difference between LDH activities in plain and EDTA anticoagulated samples.

LDH activity was 2 to 20 times higher in exudates than in transudates from pleural and abdominal effusions in dogs and cats, although there was some overlap between the groups using the 3 different methods of testing LDH activity. Lymphocytic effusions in cats, although they often had a high cell count, often had relatively low LDH activity compared to inflammatory exudates. This may suggest that lymphocytes have low LDH activity, however, this hypothesis needs further investigation. Another hypothesis for the high LDH activity in exudates might be damage to organs within the body cavity concerned.

Only a small number of pericardial effusions were collected in our study, however, five out of six of these effusions had high LDH activity regardless of whether they were related to congestive heart failure (transudates) or exfoliative cardiac neoplasia or whether or not they contained a substantial amount of blood. One hypothesis for the high LDH activity in the present study may be the due to the presence of high LDH activity in cardiac muscle, which may leak from the damaged myocardium into the relatively small volume of pericardial fluid. Low LDH activity was detected in one pericardial effusion associated with peracute intrapericardial haemorrhage (PCV 0.24L/L), possibly resulting in the LDH activity measured being more representative of that in the blood than associated with an effusion forming in the pericardium. Further investigation of a larger number of pericardial effusions is necessary, however the present study suggests that caution should be exercised when interpreting LDH activity in pericardial effusions until further data is available. Our findings may be different, mainly due to insufficient samples, to those of Burgess, Reuter et al. (2002), who found that human pericardial effusions can be reliably differentiated into transudates and exudates using Light’s criteria.

LDH activity was not significantly different between effusions resulting from neoplasia, sepsis or inflammation in dogs and cats, and could not be used to differentiate between these conditions.
This is similar to conclusions drawn from effusion samples in humans (Horrocks, King et al. 1962; Light 1997).

Although sequential testing was only performed on a few animals, LDH activity appeared to vary directly with clinical and cytological findings in these effusions. Measuring LDH activity may therefore be a useful additional test especially in monitoring the progression of disease, however, further investigation is required.

In the present study the LDH activity were significantly increased in horses with abdominal and pleural sepsis and neoplasia. The abdominal effusions were often associated with necrosis and rupture of the intestinal tract and commonly resulted in euthanasia or death despite therapeutic intervention. LDH activity could not be used to reliably differentiate between those horses which were deemed clinically to require surgical intervention and those that could be managed medically in the present study as there was considerable overlap in LDH activity between these patients, however, subjectively the higher the LDH activity in the effusion the greater the likelihood of surgical intervention and the poorer the prognosis appeared to be.

One of the limitations of the present study is the absence of a normal control population of equine abdominal and pleural fluid and the relatively small number of samples tested, especially using the IDEXX Vettest analyser. A previous study found that LDH activity in the peritoneal fluid of 20 normal horses was 143.0 ± 106.1 IU/L (Brownlow, Hutchins et al. 1981), though the method of LDH measurement was not mentioned. Further investigation of equine effusions is necessary, including evaluation of differences between laboratories.

In the samples collected from animals at post mortem, the effusions were classified as transudates and exudates based on clinical and post mortem findings. LDH activity from the effusions of dead animals, even with a relatively short post mortem interval, was high in all 12 effusions examined, and considerably increased from that measured in the pre-mortem effusion samples in the two animals evaluated in the present study. This may be due to breakdown of mesothelial and endothelial cell barriers, resulting in leakage of LDH into the effusion fluid from lysed red blood cells, autolysis of muscle fibres, hepatocytes, gastro-intestinal tract and other cells and bacteria. Measurement of LDH activity in effusions from dead animals was not found to be useful in differentiating between transudates and exudates in dead animals.
Lactate concentration has been used to investigate for intestinal ischaemia and prognosis in horses with colic (Latson, Nieto et al. 2005; van den Boom, Butler et al. 2010). Lactate should be measured soon after sample collection for accurate results and can therefore only be done at referral hospitals with on-site laboratories or places with point-of-care monitors (Tennent-Brown, Wilkins et al. 2007). In our study on horses there was moderate correlation between LDH activity and lactate concentration in abdominal fluid from horses with colic using wet chemistry tests to measure LDH activity, but poor correlation with IDEXX LDH. The latter may be due to the small number of samples tested. Measuring lactate concentration and LDH activity may complement each other in their diagnostic value to the practitioner.

In dogs (n=22) the ratio between LDH activity in the effusion fluid compared to that in the blood (serum or plasma) in the present study resulted in more accurate separation of transudates from exudates using a cut off value of 0.7 than the measurement of LDH activity alone in the effusion fluid. This finding is similar to findings in some human studies (Burgess, Reuter et al. 2002) who found that Light’s criteria were the most accurate at identifying exudates in pericardial effusions. However, others found that LDH activity in the fluid combined with the ratio of total protein in fluid to total protein in serum (TPR) is the most accurate marker for the diagnostic separation of transudates and exudates and that the fluid LDH: serum LDH ratio has no role in this process (Joseph, Badrinath et al. 2001).

In the 6 animals for which serial samples were submitted in the present study, LDH activity increased with increased inflammation or development of sepsis, and decreased as disease resolved. This is similar to findings in other studies in humans which found that increased LDH activity in repeat/successive thoracocentesis samples suggested that the degree of inflammation was becoming progressively worse and an aggressive pursuit should be undertaken (Light 1997; Light 2007).

In the present study, fewer samples were tested using IDEXX LDH dry chemistry than the wet chemistry methods due to prolonged delays in receiving test cartridges. Samples were frozen for months for batch processing once the cartridges arrived; however, LDH activity was found to have decreased significantly from initial results using wet chemistry testing of those samples stored frozen (-20°C) for 9-12 months. These samples were therefore no longer suitable for testing using the IDEXX LDH dry chemistry tests for inclusion in the present study. Further work is therefore required in evaluating LDH activity if effusion fluids using the IDEXX LDH dry chemistry method.
5 CONCLUSIONS

The findings in the present study are all new scientific results and as follows:

1. LDH in effusion fluid provides significantly different results using different test methods. Therefore the methods should be always mentioned in further studies otherwise these cannot be compared with each other.

2. LDH is stable when stored in effusion supernatant from dogs, cats and horses at 4°C for at least 3 days and 2 weeks at -20°C, however, activity decreased in samples stored for 12 months at -20°C.

3. LDH activity can be measured using samples collected into plain (serum) tubes or into EDTA without significantly different results using either the lactate-to-pyruvate or pyruvate-to-lactate reactions.

4. Different cut-off values of LDH activity are needed for different animal species as well as for different test methods when differentiating between transudates and exudates.

5. LDH activity in effusion fluid can aid in differentiating between transudates and exudates and may be especially useful for interpretation before routine laboratory results including cytology are available.

6. LDH activity in abdominal effusion fluid may provide additional useful prognostic information in horses with colic.

7. Effusion LDH : serum LDH ratio of 0.7 may be a sensitive and specific test for separating exudates from transudates in dogs and a ratio of >4 may help to differentiate septic from non-septic effusions in pleural and peritoneal cavities. Too few samples were available to draw conclusions in cats and horses.

8. The ratio may be preferable to cut-off values as it overcomes the variation found with the cut-off value of single LDH measurements, and further exploration is recommended.

9. LDH activity in effusion fluid collected from animals post mortem did not provide useful information for separating transudates from exudates as LDH activity increased markedly in samples after death.
6 REFERENCES


