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The utility of uric acid assay in dogs as an indicator of functional hepatic mass

by

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CONTENTS

SUMMARY

Laboratory serum biochemical tests are regarded by the Liver Study Group (LSG) of the WSAVA as an essential component of any liver investigation. The LSG categorised liver disease into four groups: vascular disorders, biliary disorders; parenchymal disorders and neoplasia. The laboratory tests that evaluate the liver have three categories. The cytosolic enzymes assess hepatocellular integrity; the cholestatic or inducible enzymes assess the biliary tree, liver excretory pathways and possible enzyme induction. The third category is the liver function tests which assess overall hepatic functional mass and portovascular integrity. The liver function tests commonly used include plasma ammonia concentration, serum bile acid concentrations and various tests that evaluate the uptake and conjugation of metabolites by the liver.

Uric acid was once used as a liver test in the late 1950's early 1960's. Physiologically, uric acid is an attractive candidate for a liver function test. In most mammalian species serum uric acid levels only increase to the levels encountered in humans when there is hepatic dysfunction. Uric acid fell out of favour as a liver function test following the publication of two studies and one case report in the late 1950's. The differences between hepatocellular integrity tests, cholestatic tests and tests of liver function were not fully understood at that time. The authors unfairly compared uric acid, essentially a liver function test, to a test of cholestasis. In addition the authors had very vague inclusion criteria for their liver disease cases. Despite the short-comings in these studies several prominent reference texts have since perpetuated their findings and uric acid fell out of the reckoning as a test of liver function.

Many tests of liver function have been used over the years. Dynamic function tests have gained popularity again. Plasma ammonia concentration is a very reliable test of liver function but has very stringent sample-handling requirements which often make its application in the average clinic setting impractical. Serum bile acid concentrations, while not as sensitive or specific for portovascular shunting as ammonia, are widely regarded as the best test of overall liver function, especially with respect to non vascular-associated liver disease. However bile acid assays are not widely available in South Africa resulting in delays in turn-around times. In today's climate of ever increasing costs, and demand for rapid turn around times, it would be very useful to veterinarians if a simple, rapid, cheap and robust assay could be found for evaluating functional hepatic mass. Uric acid would seem to have this potential and it is performed by most medical laboratories.

In this study the serum uric acid concentrations and concentration of bile acids of a control group of normal dogs was used to compare to those in three other groups of dogs. Two of these groups had liver disease, and the third was a renal disease group. The one group of liver disease was comprised of dogs with congenital vascular anomalies while the second liver disease group was made up of dogs with various parenchymal liver diseases. Serum bile acid concentrations in the four groups were compared to the serum uric acid levels to assess the utility of uric acid as a test of liver function; and to measure the affects of diminished renal function on serum uric acid concentrations. There were significant differences in the serum bile acid concentrations between the two liver disease groups and the non-liver disease groups. Uric acid concentrations between all four groups did not differ significantly however. Serum uric acid was elevated in dogs with renal impairment. Therefore the findings in this study indicate that uric acid cannot be used as a test of liver function and is not comparable to serum bile acids in this regard.

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LIST OF ABBREVIATIONS

ABT	Aminopyrine Breath Test
ACTH	Adrenocorticotropic Hormone
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
APTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
ATP	Adenosine Tri-phosphate
AUS CAT D	Australian cattle dog
BORD COLLIE	border collie
BSAVA	British Small Animal Veterinary Association
BSP	Bromo-sulphophthalein
BULL TERR	bull terrier
C-AMP	Cyclic Adenosine Monophosphate
C-GMP	Cyclic Guanosine Monophosphate
CO2	Carbon Dioxide
CREAT	Creatinine
DIC	Disseminated Intra-vascular Coagulation
DiffBA	difference between pre- and post-prandial bile acids
DiffUA	difference between pre- and post-prandial uric acid
FOX TERR	fox terrier
GGT	Gamma Glutamyltransferase
GLDH	Glutamate Dehydrogenase
GSD	German shepherd dog
ICG	Indocyanin Green
IFCC	International Federation of Clinical Chemistry
KG	Kilograms
LAB	labrador
LSG	Liver Study Group
MIN DACHS	miniature dachshund
MIN DOB	miniature doberman
MIN PINSCH	miniature pinscher
MRP4	Multi-drug Resistant Protein 4
MVD	Microvascular dysplasia
NPV	Negative Predictive Value
OAT1	Organic Anion Transporter 1
OAT2	Organic Anion Transporter 2
OVAH	Onderstepoort Academic Veterinary Hospital
PPV	Positive Predictive Value
Pre-BA	Pre-prandial bile acids
Pre-UA	Pre-prandial uric acid
Pst-BA	Post-prandial bile acids
Pst-UA	Post-prandial uric acid
PSVA	Porto-systemic Vascular Anomalies
PT	Prothrombin Time
Ref.Ran	Reference range
RTE	Renal Tubular Epithelial
SDH	Sorbitol Dehydrogenase

SI Units	International System of Units
SPAN	spaniel
STAFF BT	Staffordshire bull terrier
StdDev	Standard deviation
UA	Uric acid
WLDCG	WSAVA Liver Disease Classification Group
WSAVA	World Small Animal Veterinary Association
WWA	William Woodard Associates
YORK TERR	Yorkshire terrier

CHAPTER 1

1. LITERATURE REVIEW AND HISTORY

The evaluation of the hepatobiliary system is required in many canine patients presented to a veterinarian. Hepatobiliary disease has many varied clinical presentations which include weight loss, anorexia, depression, vomiting, diarrhoea, polyuria/polydipsia, icterus, ascites, abdominal pain, abdominal distension, dermatitis and nervous signs. Inadequacies in liver function can be classified the following ways (1;2):

1. Disturbances in bilirubin excretion;
2. Disturbances in bile secretion;
3. Inadequate conversion of ammonia to urea;
4. Abnormal carbohydrate metabolism;
5. Protein synthetic deficits;
6. Diminished Kupffer cell activity;
7. Portosystemic shunting;
8. Portal hypertension; and,
9. Impaired drug and toxin metabolism and excretion.

History and physical examination will give the clinician some idea of the possibility of hepatic disease. Biochemical evaluation of the liver will be discussed in greater detail below. Once these tests have confirmed the presence of liver disease, further diagnostic procedures are required to make definitive diagnoses. Radiography is of limited use in that it can only identify hepatomegaly and sometimes microhepatia but may appear normal in the face of

severe hepatic disease (1). Emaciation or abdominal effusion reduce the usefulness of radiography (1). Ultrasonography is a useful diagnostic adjunct in assessing liver disease (1). A skilled ultrasonographer can visualize the hepatic parenchyma, portal and hepatic veins, gallbladder and biliary system (1). The detection of focal disease is easier than diffuse change (1). It is also able to identify areas of necrosis, neoplastic masses and ascitic fluid (1). In academic institutions and some referral practices hepatic scintigraphy may be available for measurement of relative arterial and portal blood flow (1). Ultimately, in many cases, a definitive diagnosis is often only made after liver biopsy with histopathologic evaluation of tissue has been performed (1;3-6). It is of paramount importance to note that hepatic damage does not necessarily mean hepatic failure (1;4-8). Tests indicating hepatic damage do not reflect the functional status of the liver as a whole.

Biochemical evaluation of the liver is made complex by the wide array of functions that the liver has in carbohydrate, lipid and protein metabolism; in biosynthesis and homeostasis; in endogenous hormone degradation and toxin detoxification and elimination (1;2;5;6;9;10). The process of diagnosis is complicated by the fact that many non-hepatic diseases may result in clinicopathological abnormalities in routine biochemistry panels that evaluate the liver (1;2;4-12). The veterinarian must decide if there is a primary hepatic disease or if there is another disease process causing secondary hepatobiliary abnormalities. These so-called reactive hepatopathies may or may not cause hepatobiliary dysfunction (1;2;4-12). There are a variety of reasons why an extrahepatic disease may secondarily involve the liver. These can be broadly divided into anatomical or functional/physiological mechanisms (1;2;5;6;9;10). The liver has two blood supplies; the hepatic artery supplying oxygenated blood and the portal vein draining the visceral organs (1;9;13). The portal vein supplies 80% of the hepatic

blood flow (1;9;13). Consequently, the liver can be secondarily affected by cardiovascular dysfunction, anaemia, portosystemic shunting, pancreatitis, enteritis, other gastrointestinal disease and exposure to substances in the systemic circulation from endocrinopathies, extrahepatic inflammation, neoplasia or infections (1;4;9-11;13;14). Compounding the clinician's evaluation is the huge functional reserve capacity of the liver. Sixty- to 80% of the liver mass must be non-functional before there is evidence of reduced function (1;4-6;8;9;14).

A major problem in evaluating studies of the hepatobiliary system is the variation in nomenclature that has been used over the years. To this end the World Small Animal Veterinary Association (WSAVA) has appointed a group of experts, The Liver Diseases and Pathology Standardisation Research Group, to standardise and clarify the nomenclature (3;4;15;16). This group published a book in 2006 called the "Standards for the Clinical and Histological Diagnosis of Canine and Feline Liver Diseases" with an aim to describe the world-wide-accepted criteria for the diagnosis of all known liver diseases of dogs and cats (17). Although the Liver Study Group (LSG) primarily concentrated on the pathological interpretation of liver diseases, they concluded that ultrasonographic examination is an essential cornerstone of the diagnosis of liver disease, particularly the vascular liver diseases. Although haematological and serum biochemical results were accepted as an integral part of the diagnostic process, they were considered to be more useful for differentiating primary liver disease from other diseases with similar clinical signs (17). As soon as liver involvement in a disease process has been demonstrated by the haematological and biochemical results, the usefulness of this data in making a specific diagnosis is very limited, except in the case of vascular liver diseases (17).

The LSG divided liver diseases into 4 groups (17):

1. Vascular Liver Disorders.
2. Biliary Tract Disorders.
3. Parenchymal Disorders (including Stellate cells and Kupffer cells).
4. Neoplasia.

From a clinicopathological/biochemical perspective, the clinician is armed with three'limbs' of diagnostic testing:

1. The cytosolic or 'leakage' enzymes.
2. The cholestatic enzymes.
3. The tests of hepatic function and circulation.

It is important to note that there are distinct differences between the first two limbs which are the tests used to assess hepatobilary *integrity* ('cytosolic' and cholestatic enzymes) as apposed to those used in the assessment of hepatobiliary *function*.

1.1 The three branches of biochemical liver evaluation

1.1.1 The cytosolic enzymes

These include alanine aminotransferase (ALT), aspartate aminotransferase (AST), sorbitol dehydrogenase (SDH) and glutamate dehydrogenase (GLDH) – ALT and AST are widely available and commonly used whereas the latter two are not all that widely used (1;5;6;8;11;13;18-20).

1.1.2 Enzymes that indicate cholestasis or that are inducible

These enzymes may be induced by drugs or endogenous hormones and include alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT). They are widely utilised in routine medical profile panels as part of a minimum database or for specific investigation for liver disease (1;5;6;8;11;13;18-20) .

High serum enzyme activity (cytosolic or cholestatic/inducible) is non-specific, as it may reflect reversible or irreversible change in the hepatocellular membranes or hepatic enzyme induction.

1.1.3 Liver function tests

These tests measure substances taken up, conjugated or excreted by the liver (1;5;6;8;11;13;18-20). Liver function can be compromised by decreased functional hepatic mass either because too few hepatocytes are present or because there is dysfunction of existing hepatocytes, or due to altered hepatic circulation (1;2;5-7;9;11;19-21). Bilirubin, bile acids, ammonia levels and, less commonly, excretion of exogenous dyes (bromosulphophthalein and indocyanine green) are well-described and accepted tests used to assess hepatobiliary function (5;7;8;19;22-25) .

1.2 The investigation of liver disease

In the course of investigating liver disease, the clinician must ask (1;5;6;23;26):

1) is this primary or secondary hepatic disease;
2) is there hepatocellular injury or necrosis;

3) is there cholestasis or enzyme induction;

4) is there a portosystemic shunt;

5) is there loss of functional hepatic mass?

From scrutiny of numerous widely used veterinary references (1;2;6;9;11;13;18-20;26-29) it is apparent that the biochemical tests mentioned as useful in evaluation of liver disease are extensively researched and widely applied. Making use of the three limbs of diagnosis described above together with history, physical examination and pattern recognition techniques, differential diagnosis lists can be created and further diagnostic procedures can be planned accordingly (1;6;8;11;13;19). In two of the above-mentioned references (1;28), uric acid is mentioned as a test of liver function. In both cases however, the authors are not in favour of its use as a test of functional hepatic mass (1;28).

1.3 Why uric acid should be revisited as a measure of hepatic function.

Uric acid is a nitrogenous end product of the catabolism of purine metabolites from endogenous and dietary sources in vertebrates (1;28;30-34). In most mammalian species, excluding humans and certain primates (chimpanzees, gorillas, orangutans and gibbon monkeys (35), uric acid (which has poor water solubility), is taken up by hepatocytes and converted via decarboxylation by the enzyme uricase to allantoin, a highly water soluble compound, which is easily excreted by renal filtration (1;28;30-34;36). It appears that in the evolution of man and other primates the uricase enzyme system was lost due to mutational silencing of the uricase gene (35;37;38). Therefore human serum urate levels are

approximately 100-fold higher as compared to other mammals (37). It is postulated that this is an evolutionary advantage because uric acid has well documented antioxidant action and inhibits damage to DNA (37;39). Consequently, in most mammalian species, serum uric acid concentrations only rise to the level encountered in humans (or higher) when there is a reduction in functional hepatic mass (1;32). There are exceptions to this rule among domestic dog breeds. Dalmatians and certain inbred lines of English bulldogs appear to have sufficient hepatic uricase to cope with the normal load of uric acid, but due to an ineffective hepatic cell membrane transport mechanism they have higher serum uric acid concentrations which predisposes these breeds to urate urolithiasis (1;30;32;35;40-42). Yorkshire terriers have also been reported to have a higher incidence of urate urolithiasis (32). The pathophysiology behind this finding in the Yorkshire terrier has however not yet been explained. It is important to note that in man and in Dalmatian dogs and probably other breeds, uric acid is excreted through the kidney via renal tubular excretion where it undergoes bi-directional active transport (32;43;44). It has been shown that the reabsorption in the proximal tubule is mediated by an organic anion transporter known as URAT1 (37) by exchanging urate for any of several anions that have a cell-to-lumen electrochemical gradient (45). But there are species differences with humans, rats, monkeys and most dog breeds favouring urate reabsorption, which is in contrast to the situation in rabbits, pigs, snakes and the Dalmatian dog where net excretion of urate is favoured, probably due to a lack of URAT1 (37). Studies of the apical and basolateral membrane vesicles of the renal tubular epithelial (RTE) cells have shown that at the basolateral surface, organic anion transporters OAT1 and OAT2 mediate uptake of urate from the blood, via the interstitial space, into the RTE cells (37;46). Van Aubel et al (37) have shown that a multi-drug resistant transporter protein MRP4, acts as an organic anion transporter mediating low-affinity ATP-dependant urate efflux into the renal

tubule lumen (37). MRP4 has been shown to be highly expressed basolaterally in hepatocytes, and thus is probably responsible for efflux of uric acid from hepatocytes into blood circulation (37). This protein transports various anions including c-GMP and c-AMP via complex interactions of multiple allosteric binding sites (37). Other renal factors involved in handling of organic acids may affect net uric acid excretion in dogs (32;37). Uric acid has been found to increase along with urea in cases of experimental and spontaneous uraemia and in cases of uraemia following strenuous exercise (47-49). Unlike in man, the gastrointestinal tract does not appear to be a significant route of uric acid excretion in dogs (32).

Many drugs in man affect serum uric acid levels. Many of the older generation diuretics including loop diuretics (furosemide and thiazide diuretics) lead to hyperuricaemia after prolonged use (50-54). Alcoholism causes oxidative liver damage which interferes with uric acid metabolism and leads to hyperuricaemia, although probably not important in veterinary medicine (55). The xanthine derivatives, theophylline and aminophylline, used in treatment of asthma and hypertension have been associated with the development of hyperuricaemia (53;56;57). Cholinergic drugs have been reported to result in increased serum uric acid levels in rats (51). Many anti-cancer drugs, especially those used to treat lymphoma and leukaemia in children, induce tumour lysis with subsequent hyperuricaemia (58). Potassium oxonate is a well-documented inhibitor of uricase, and in fact, is used in animal models to create hyperuricaemic conditions for the study of human hyperuricaemia (38). Allopurinol is the major commercially available xanthine oxidase inhibitor that is used to treat hyperuricaemia in man by inhibiting the production of uric acid by hepatocytes (38). However due to its side effects of hepatitis, nephropathy and allergic reactions, various natural compounds have been investigated for their ability to lower serum uric acid concentrations (38;39). These include

procyanidins (39), flavonoids and caffeoylquinic acid derivatives (38) which all have xanthine oxidase inhibitory action. Alternatively, increasing renal uric acid excretion is used to treat hyperuricaemia using drugs such as salicylates, benzbromarone, sulfinpyrazone, probenecid, losartan (46) and the tuberculostatics ethambutol and pyrazinamide (45;46). Interstingly these drugs can have a paradoxical hypouricosuric effect at low doses. At high doses the concentrations achieved in the tubular lumen are sufficient to either directly compete with urate binding to, and transport by URAT1, or indirectly by binding to the receptor without being transported and thus block urate transport (45;46). Whereas at low doses these compounds can accumulate in the RTE cells via Na^+-cotransport across the luminal membrane and once their concentration rises intracellullarly, they can serve as anion partners for urate reabsorption mediated by URAT1 (45;46).

1.3.1 Why did uric acid fall out of favour?

As a result of this hepatocyte-dependant degradation, uric acid initially became known as a reliable indicator of liver function (59). However, in the late 1950's two studies and one case report criticised it's use in dogs. Morgan (60) in his study of the bromosulphophthalein (BSP) test concluded that the BSP test was a more effective test than uric acid as a test of liver function. Malherbe (61) reported on a dog with intrahepatic bile duct obstruction and compared ALP and ALT levels to uric acid and stated that, "blood uric acid appeared to be a less useful test" and he went on to quote Morgan's BSP study. He did, however, state that, "values were elevated during the 'toxic' period", but did not elaborate on this sentence (61). Hoe and Harvey (62), published an extensive comparative evaluation of a number of liver 'function' tests and arrived at the following conclusion for serum uric acid -"If one compares the summary of the uric acid results with those of ALP it can be seen that the uric acid test

does not appear to be such a valuable index of liver function." (62). At this time in the development of hepatic enzymology testing, the necessity for distinction between hepatocellular integrity tests and those of liver function was not fully understood. This study based its conclusions on the ALP serum activity of 436 serum samples and the concentration of uric acid in 245 samples (62), which on the face of it appears reasonably comprehensive. In the next section it will be shown why the comparison of ALP and uric acid is inappropriate.

The two dated studies by Malherbe and Hoe et al. and one case report have been widely accepted. Uric acid, as a test for liver function, fell into even more disfavour in the 2nd, 3rd, and 4th editions of Kaneko's Clinical Biochemistry of Domestic Animals (1970, 1980 and 1989, respectively) (28;59;63). This well respected reference book has subsequently been quoted by other respected authors like Hardy (40) and Center (1) and thus there has been no respite in the criticism of the uric acid test. Although Hardy (40), when referring to portosystemic shunts, stated that uric acid concentrations were often increased in serum in these patients, since a major hepatic function was the conversion of uric acid to allantoin. He did not, however, elaborate on any diagnostic utility of uric acid. Center (1) did mention, that in animals with normal uric acid metabolism but insufficient hepatic function, serum uric acid may be increased because of impaired availability or function of hepatic urate oxidase – citing Kruger & Osborne (32). She concluded by saying that its use had largely been abandoned because of limited clinical utility (1).

In today's climate of ever increasing costs, and demand for rapid turn-around times, it would be very useful to veterinarians if a simple, rapid, cheap and robust assay could be found for

evaluating functional hepatic mass. Uric acid has this potential, if it were not for the poor evidence from the three very dated studies listed above. Uric acid, particularly because of its availability, cheap assay methodology, stability in serum and simplicity, deserves re-evaluation.

1.3.2 A review of the literature critical of the use of serum uric acid concentration as a measure of hepatic function

Firstly, it is important to differentiate between hepatic function tests and tests of hepatocellular integrity. At the time when the studies by Morgan (60) and Hoe et al. (62) were conducted, there was no such clear differentiation. This lack of discrimination between tests evaluating residual functional hepatic mass and hepatocyte damage can mislead the clinician into believing that alterations in either group of tests have the same clinical meaning. It appears that Hoe et al. (62) and Malherbe (61) made no distinction. Unfortunately this trend has continued into the fairly recent past with Tenant "lumping" all the tests under the heading of "Liver Function" (63). A notable exception is Center, who specifically differentiates between those tests advocated for the diagnosis of acute hepatic disease and those for chronic hepatopathies (4;27). Other authors such as Stockham (13), Willard & Tvedten (20), Bain (11), Meyer (19;26), Hess (6), Sevelius (7), Huxtable (2), Hayes (9), Sutherland (8) and Thrall (18), amongst others, clearly define the difference between tests for hepatocellular disease and those for hepatic insufficiency. The cytosolic and cholestatic enzymes have no diagnostic use with respect to the residual functional hepatic mass of the liver (1;2;5;6;11;26).

Making closer evaluation of the two 1959 publications, raises questions about their criticism of serum uric acid. Morgan administered carbon tetrachloride to six dogs (60). This compound causes severe hepatic degeneration and cellular swelling, particularly in the centrilobular hepatocytes (9;22;64). This would have caused a severe and rapid compromise of the bile canaliculi (1;9;13;22;27), which explains the apparent greater sensitivity of BSP in detecting liver dysfunction (compared to uric acid) that Morgan reported (24;60). In the case of the dog with bile duct obstruction that Malherbe reported (61), the induction of ALP isoenzymes (only discovered subsequent to Malherbe's time) is now recognised as an extremely sensitive indicator of increased bile duct pressure (5;9;11;28;65) and is not in fact related to hepatic function at all (2;5;65-67). Therefore, in neither situation, was it reasonable to expect serum uric acid concentrations to be a better modality than the tests advocated by the authors which were actually tests of hepatocellular damage. In fact, neither author invalidated serum uric acid as a reasonable test of liver function (particularly of reduced functional hepatic mass). Morgan does provide two graphs illustrating a sequential increase in serum uric acid as the study progressed but reported that it only exceeded the normal reference range in one dog (60). It is not unreasonable to assume that, with modern test methods and improved sensitivity in analytical equipment, that a narrower normal range would improve the sensitivity of uric acid in detecting impaired hepatic function.

Closer inspection of the Hoe et al. (62) article reveals two critical flaws. First, it becomes evident that the cases (and the sera), which were used for the evaluation of ALP, were different from those used to evaluate uric acid. Second, the definition of "definite liver involvement", was based on the presence of jaundice in 15 cases (which was not further investigated) or the presence of diabetes mellitus (23 cases) or confirmed histopathological

evidence of hepatopathy at autopsy (number not given but they would appear to represent only about seven cases). In their conclusion the authors admitted that the classification of liver damage that was used was somewhat arbitrary. This impacted negatively on their ability to evaluate tests of hepatic disease and/or function. The definition they used for the cases of "suspected liver involvement" was even less convincing and they included dogs suffering from virus diseases, pyometritis, pseudo-Hodgkin's disease, toxaemias, and cases of abdominal neoplasia that were suspected of having liver damage clinically only, without any histological or other proof of liver disease. Furthermore, the comparison between ALP and uric acid, on the basis of which the above statement was made, is made on only two of these disease categories, namely jaundice (ALP 100% sensitive, uric acid 86% sensitive) and diabetes mellitus (ALP 100% sensitive, uric acid 9% sensitive). In 1956, Ott, cited by Cornelius (28), reported that blood uric acid levels were significantly elevated in hepatocellular jaundice, but normal in haemolytic and obstructive jaundice. Consequently, comparing ALP and uric acid on the basis of "undefined" jaundice cases is not reliable at all. Adding to the weakness of this comparison is the "uncritical" association of diabetes mellitus with "definite evidence" of liver disease (62). Very few diabetic patients have liver complications (principally fatty infiltration) that would bring about a significant reduction in functional hepatic mass, although there may well be some disturbance in membrane stability, releasing enzymes such as the transaminases (ALT, AST) (10;12). There is also ample evidence to suggest that a proportion of canine diabetics are associated with (or possibly even the result of) hyperadrenocorticism (12). The most common cause of insulin-resistance in the dog is hyperadrenocorticism, whether it be endogenous or iatrogenic (10;12). In these dogs, diabetes mellitus is usually diagnosed first because hyperglycaemia and glycosuria are easily diagnosed, whereas the physical findings of alopecia, dermal atrophy, abdominal enlargement

associated with hyperadrenocorticism may only become apparent to the clinician later in the course of disease (12). Of the non-hormonal abnormalities in hyperadrenocorticism (in the dog), elevation of ALP is the most common laboratory abnormality (5;10-12;65;68;69). This elevation is due to the production of a steroid-induced or ACTH-responsive isoenzyme of ALP and not hepatic damage (10;11;65-69). Consequently, comparing the elevation of ALP and uric acid in diabetic dogs is probably misleading if that comparison is designed to compare the two tests for their ability to identify hepatic disease, particularly as the two tests are not measuring the same parameters. Also in the article by Hoe et al., the authors acknowledged that during the course of their study, high uric acid values frequently appeared to be associated with a poor prognosis, and were found in moribund animals (62). The question must be asked whether these were the cases that had true hepatic insufficiency, while others in the study had secondary hepatopathies or reversible primary hepatic disease?

Center (1) did mention uric acid but stated that uric acid as a clinical test for liver disease in the dog is a relatively insensitive measure of liver disease or function. She further stated that its use has largely been abandoned because of limited clinical utility (1). This review of the very small body of old research literature available on uric acid as a measure of liver function in dogs makes it clear that there is so little evidence either for or against uric acid as a good measure of hepatic function and that further work is necessary before finally condemning this assay.

1.3.3 The issue of test complexity

The situation in South Africa, and in many other countries, is that a reliable test of functional hepatic mass is not always readily available. The older uric acid assays were rather complex.

Morgan (60) and Hoe et al. (62) articles both alluded to the technical difficulty of the "Brown" method of uric acid assay, thus creating the impression that if it were technically easier, they would have been less inclined to criticise it. Hoe et al. (62) listed as one of their objectives to use simple tests to assess liver function, and the complexity of the uric acid assay (used at that time), appeared to have added to their negative perception of the test. Morgan (60) stated that if it had been found that uric acid determination was of diagnostic value, it would still have been of little value to the veterinary practitioner due to the cost of reagents, the complexity of reagent mixing, the short shelf life of some reagents and the amount of time consumed on the test which would have made it of academic value only. Similarly, Cornelius (28) left no doubt that the technical issue played an important role in his negative stance towards it.

Currently however serum uric acid assay is available from almost any clinical pathology laboratory, owing to its regular use for the diagnosis and monitoring of gout in humans (31;33;34). The old assay method made use of phosphotungstic acid reduction by uric acid in an alkaline medium, producing tungsten blue with the colour change measured by absorbance at certain wavelengths (33;34). With this method, protein removal was obligatory to prevent interference in the test (33;34;61). It was probably this step which Cornelius regarded as "tedious" (28;59). Much simpler uric acid methods have since been developed and these have become popular and feasible as a result of the availability of high-quality, low-cost uricase from bacterial sources (31;33;34). These are easily adapted for automated use and have been incorporated into dry chemistry systems (31;33;34). Most current uric acid methods use a peroxidase reaction coupled with one of a number of oxygen acceptors to produce a chromagen (33;34). The majority suffer interference from only guanine, xanthine and a few

structural analogues of urate, but usually only at concentrations not expected in biological fluids (33). Some methods have been reported to suffer interference from various metabolites in serum including bilirubin, haemoglobin, ascorbic acid, glutathione, lipids and peroxide (91). The most important of these is bilirubin which can cause both positive and negative interference with the peroxidase-coupled assays, depending on its serum concentration (91). This interference occurs in 2 ways; chemical interference when bilirubin competes with the peroxidise reaction as a hydrogen donor, and thus less chromophore is produced, underestimating uric acid concentration (91). And secondly a spectrophotometric interference when bilirubin in high concentrations is measured at wavelengths less than 540nm which overlaps with the measured chromophore's wavelentgh and causes a positive interference, overestimating uric acid concentrations (91). Steps that can be taken to combat this interference include measuring at wavelengths greater then 540nm (often not practical), addition of ferrocyanide or bilirubin oxidase to block bilirubin's reduction of peroxide (91). A high-performance liquid chromatography method has been utilized in serum from dogs but is not routinely used due to the cost of chromatography (70).

1.4 Other liver function tests

Bilirubinaemia has been used for a long time as an indicator of a hepatopathy. Bilirubin is the by-product of the break-down of porphyrin-ring containing compounds, mainly haemoglobin but also myoglobin, cytochrome P_{450}, peroxidase and catalase (8;18). Briefly, these molecules are degraded into a protein portion, iron and protoporphyrin in macrophages (18). This protoporphyrin is converted firstly into biliverdin and then bilirubin (18). This unconjugated

(not water soluble) bilirubin is secreted into blood by the macrophages and carried to the liver bound to protein molecules. In the liver it is detached from the protein carriers and enters the hepatocytes. In the hepatocytes it is conjugated to a sugar group to make it water soluble (13;18). The most common sugar is glucoronic acid and bilirubin glucoronide is the predominant form in dogs (13;18). The conjugated bilirubin is water soluble and the majority is excreted in the bile (11;13;18). For this reason, bilirubinaemia may indicate inadequate liver function. Theoretically, because unconjugated and conjugated bilirubin can be identified separately (by calculation), one should be able to differentiate pre-hepatic bilirubinaemia with a unconjugated bilirubin excess, from hepatic or post-hepatic causes of bilirubinaemia in which the conjugated form should predominate (11;13;18). Apart from haemolytic anaemia, where a strongly regenerative anaemia adds weight to a suspected pre-hepatic cause of bilirubinaemia, the separation of the bilirubin fractions lack sensitivity with respect to the site of bilirubin accumulation (11;13;18). Due to the structure of the liver it has been recognised that hepatic and post-hepatic causes eventually lead to increases in both conjugated and unconjugated bilirubin (13;18). In addition, bilirubinaemia ocurrs much later in liver disease than do bile acid or ammonia elevations, and thus the test lacks sensitivity as a liver function test (13;18). The situation is further complicated by the fact that some conjugated bilirubin filters into the blood and remains bound to albumin and is called biliprotein (18). This fraction is measured in the total bilirubin test and has a half-life of about two weeks (similar to albumin) and this complicates the interpretation of a hyperbilirubinaemia (18). When pre-hepatic causes (usually haemolysis or excess erythrocyte break-down) can be excluded, hyperbilirubinaemia has a reasonable specificity as a test of liver function, but is still not as good as bile acids or ammonia levels (18). Bilirubin is however useful when included in a test panel to evaluate for hepatobiliary disease. Particularly in cases of portosystemic

shunting where it may help to differentiate congenital portovascular anomalies (not associated with cholestasis) from acquired portosystemic shunting in which some degree of cholestasis and thus bilirubin elevation is present (71). In cases of possible steroid-induced ALP elevation, bilirubin should not be induced (until later on in the disease course when it may increase due to hepatocyte swelling), whereas in ALP increase due to cholestasis, bilirubin concentration should also be raised (72).

Many other tests of liver function lack sensitivity. These include serum concentrations of cholesterol, urea, glucose and albumin (1;2;5;8;11;13;19;26;28). BSP retention is very sensitive but its limited availability, high cost and the influence of non-hepatic factors on its interpretation have all but rendered it defunct in the practice setting (1;8;11;13;19;20;22;24). Severe perivascular inflammation associated with extravasation of BSP has been reported in humans (73). Indocyanin green (ICG), another liver clearance test, is prohibitively expensive and thus impractical (1;11;13;19;20). Preparation and measurement of ICG is complicated and not practical (73). ICG and BSP are sensitive for abnormalities in hepatic perfusion and function; however they lack specificity for loss of hepatic function, because they are influenced by multiple non-hepatic variables, including fever, congestive heart failure and hyperbilirubinaemia (73).

The search for hepatic function tests has regained momentum in recent years in both human and veterinary medicine as the limitations of older tests are recognised and the establishment of specific histopathological classification of liver diseases is leading to extensive research on treatments and monitoring for prognostic purposes (73). Dynamic tests that entail administration of substances that are taken up and metabolised by the liver and the

subsequent monitoring of their kinetics have regained attention (74). One such test in man is the galactose load test which showed some promise in dogs when investigated by Bernardini et al (74). However, the study groups were small and the authors stated that further research was required. Similar dynamic breath-tests are popular in man for evaluating liver function. The aminopyrine breath test (ABT) is one of these that has been shown to be useful in quantifying hepatic microsomal enzyme function and is of use diagnostically in identifying chronic hepatitis and cirrhosis (73). The test involves oral administration of aminopyrine, labeled with a radioactive carbon isotope (C^{12}, C^{13} or C^{14}), which is taken up and metabolised by the hepatocytes (73;75). The aminopyrine is demethylated by microsomal enzymes and the resultant methyl groups are oxidised to carbon dioxide (CO_2) which diffuses into the blood and is expelled in expired breath (73;75). The measurement of the irradiated carbon in the expiratory air allows for quantification of that fraction of CO_2 derived from the microsomal demethylation by the liver (73). The amount of irradiated carbon is recorded as a percentage dose of the aminopyrine administered. The test has been adapted for use in dogs by giving the C-aminopyrine intravenously and collecting a blood sample after 45 minutes for analysis. The CO_2 is extracted from the sample by addition of strong hydrochloric acid. After addition of the acid, samples are immediately vortexed to prevent acid coagulation and to promote maximum CO_2 release. Gas samples are then analysed by fractional mass spectrometry using an automated breath-carbon analyser to determine the fraction of irradiated C in the extracted CO_2 (73;75). This method has been tested for safety of the C-aminopyrine and doses in dogs recommended, but further studies are needed to evaluate it in dogs with various types of liver disease (76;77). Another radioactive carbon-labelled breath test used in humans is C-phenylalanine (78). L-phenylalanine is an essential amino acid that is metabolized by the liver and plasma levels are elevated in humans with liver diseases (78).

Neumann et al compared plasma L-phenylalanine concentrations to pre-prandial bile acids in dogs with hepatitis, liver tumours, non-hepatic disease and a normal control group. They found that L-phenylalanine was significantly elevated in the dogs with liver disease compared to the normal and non-hepatic disease groups and that the results were comparable to bile acids (78). However, there was overlap between the L-phenylalanine results for the non-liver disease groups and the hepatic disease groups which warranted further research (78). In a similar study on a liver metabolite, L-carnitine was shown to be elevated in dogs with liver disease compared to normal healthy dogs (79). However, considerable over-lap occurred between the groups and the authors recommended further studies to refine the test (79). In a recent study by researchers at Cornell University (80) the usefulness of the vitamin K-dependent anticoagulant protein C as a measure of liver function was investigated. The liver is the primary site for synthesis of many of the coagulation proteases (factors I, II, V, VII, IX, X, XI, XIII) and many of the components of the fibrinolytic system (81). Although coagulation assays are routinely assessed in dogs with hepatobiliary disease to check for risk of haemorrhage if surgery is required, the traditional prothrombin time (PT) and activated partial thromboplastin time (aPTT) are relatively insensitive in that they are only prolonged when clotting factors have activity below 35% of normal (80;81). The authors of this study found that only the subset of dogs with severe, diffuse parenchymal loss, complete extrahepatic bile duct obstruction, hepatic failure or fulminant DIC had prolonged PT and aPTT (80). Many dogs with histologically confirmed hepatobiliary disease lacked abnormal coagulation variables, but almost 60% of them had below-normal levels of protein C (80). The authors concluded that protein C activity functioned reliably as a biomarker of hepatic function and hepatoportal perfusion. Incorporation of protein C determination into conventional routine clinicopathologic testing panels will help clinicians in the recognition of

acquired and congenital portosystemic shunts, hepatic failure, and other forms of severe hepatobiliary disease (80). Their results suggested that protein C activity has a specific clinical application as a non-invasive indicator of hepatoportal blood flow. Measurement of protein C activity may provide an economical and non-invasive means of differentiating porto-systemic vascular anomalies (PSVA) from microvascular dysplasia (MVD) in those breeds in which these disorders appear to be inherited and, possibly, for determining the severity of any shunt present. Additionally, evaluation of protein C activity before and after shunt ligation may be helpful for monitoring the success of the restoration of hepatoportal blood flow (80).

Plasma ammonia level is also a sensitive assay but more costly than serum bile acids and the handling requirements make it largely impractical for many veterinarians in private practice (1;11;13;19;20;23). The samples have to be transported on ice and processed within 30 minutes (5;6;11;13;19;20;23). Serum bile acids are widely regarded as the most useful test of hepatic functional mass and circulation (5;6;11;13;19-21;23;25;64;82-85). Bile acids are available but not necessarily in the practitioner's region (often the case in South Africa); and, because of the cost of the assay, samples are stored and run in batches thus prolonging turn-around times (author's observation). The accepted recommendation to use pre- and post-prandial samples to improve sensitivity also adds to the labour and cost involved (some authors dispute the additional sensitivity gained by running the extra sample, whether it be the pre- or post-prandial assay (1;22;84).

CHAPTER 2

2.1 PROBLEM STATEMENT

1. Serum uric acid, as a test of hepatic function, appears to have been rejected on potentially spurious and very dated evidence.

2. The rejection of uric acid as a hepatic function test was principally based on comparisons with serum liver enzyme activities rather than other liver function tests.

3. One study comparing it to a liver function test, BSP, did not evaluate uric acid fairly

4. There is a need for a sensitive, inexpensive, easily applied and readily available test of hepatic function for dogs in South Africa and other developing world counties where there is heavy dependence on human clinical pathology laboratories for veterinary diagnostic work.

2.2 HYPOTHESIS

1. Serum uric acid performs at least as well as serum bile acid or ammonia measurement for the diagnosis of reduced hepatic functional mass or vascular shunting (except in the Dalmation breed).

2. Serum uric acid concentrations will be elevated in azotaemic dogs without hepatic hypofunction

2.3 RESEARCH OBJECTIVES

To retrospectively evaluate the diagnostic efficiency of canine serum uric acid for a decrease in functional hepatic mass in relation to serum bile acids and/or plasma ammonia levels.

2.4 RESEARCH QUESTIONS

I. What is the normal range of uric acid in healthy dogs?

II. How does uric acid compare with serum bile acids and plasma ammonia as tests of reduced functional hepatic mass with respect to sensitivity, specificity, positive predictive value and negative predictive value?

III. How does uric acid compare to serum bile acids and plasma ammonia as tests for congenital porto-systemic shunting and other vascular anomalies with respect to sensitivity, specificity, positive predictive value and negative predictive value?

IV. How does uric acid compare to serum bile acids and plasma ammonia in cases of secondary hepatobiliary disease with respect to sensitivity, specificity, positive predictive value and negative predictive value?

V. Do dogs with uraemia or azotaemia without liver disease have elevated uric acid levels?

CHAPTER 3

3.1 MATERIALS AND METHODS

3.1.1 Experimental Design

The investigation consisted of a retrospective component and smaller prospective component. The retrospective study evaluated clinical records from the William Woodard Associates (WWA), laboratory program data base of the Onderstepoort Veterinary Academic Clinical Pathology Laboratory and the clinical records of the Onderstepoort Veterinary Academic Hospital (OVAH). Cases with complete diagnostic evaluations and histopathological confirmation of hepatobiliary disease and either bile acid and/or ammonia results from 1998 to 2004 were included. The prospective component consisted of uric acid and bile acid comparisons in healthy dogs that were presented to the Pietermaritzburg SPCA for vaccination and/or sterilisation.

Serum uric acid concentrations were determined on selected frozen serum samples from the databank of the Clinical Pathology Laboratory, on which either plasma ammonia assays and/or serum bile acid determinations were performed. Uric acid is stable in urine stored at -20 degrees centigrade according to Bartges et al (86). Uric acid was reliably measured in frozen serum samples in rats by Nguyen et al (38) and in frozen dog plasma samples by Hinchcliff (48) and therefore should be reliable for the samples selected in this study.

3.1.2 Case Selection

Cases selected for the study were divided into four groups of at least 25 cases each:

GROUP 1:

Included healthy dogs presented to the Pietermaritzburg SPCA for routine ovariohysterectomy or vaccination. This group served as the control group.

Inclusion criteria:

- Normal clinical examination (temperature, pulse, respiration, capillary refill time, cardiac auscultation, abdominal palpation and peripheral lymph node palpation)
- Haematology and biochemistry results within the reference range according to Canine Medical Profile of Vetdiagnostix Laboratory (see Appendix A).

Exclusion criteria:

- Any dog that did not appear clinically normal or had elevated liver enzymes or pre-prandial bile acids (>15 µmol/l).
- Dalmatians and Bull Dogs were excluded.

GROUP 2:

Dogs were included in this group if they had congenital vascular anomalies including definite single intra- or extra-hepatic porto-systemic shunts, microvascular dysplasia, portal vein hypoplasia or arterio-venous fistulae. Initial suspicion of vascular shunting was based upon clinical history and screening clinical pathology laboratory tests. Suggestive history included stunted growth, poor weight gain, microhepatia, intermittent anorexia, pica, polyphagia, polydipsia-polyuria, pollakiuria, urate urolithiasis, vague neurological signs post-eating or intolerance to anaesthetic drugs. Suggestive clinical pathology findings included mild microcytic normochromic anaemia, mild hypoalbuminaemia, hyperglobulinaemia,

hypocholesterolaemia, hypoglycaemia, elevated bile acids, raised plasma ammonia concentration and low or low-normal urea and creatinine. Liver enzymes were commonly within reference limits in these conditions.

Inclusion criteria:

- Dogs less than one year of age with a porto-systemic shunt confirmed by any one of the following modalities: ultrasonography, contrast radiography, scintigraphy or visually during surgery.
- Dogs with clinical work-ups that warranted liver biopsies which had histopathological evidence of congenital vascular anomalies
- Dogs that were euthenased at the owners' request or due to poor prognosis, following a clinical work up which had histopathological evidence of congenital vascular anomalies at necropsy, were also included.

Exclusion criteria:

- Dogs were excluded if there was a history that made a congenital shunt less likely than an acquired shunt and if there was any medical evidence for other non-related disease.
- All dogs in this group were confirmed to be non-azotaemic (azotaemia is defined as urea and creatinine raised more than twice top-normal i.e. urea >20mmol/l; creatinine >260 µmol/l)
- Dalmatians and Bull Dogs were excluded.

GROUP 3:

Dogs diagnosed with any hepatopathy causing hepatic hypofunction except those dogs that had congenital porto-systemic shunts.

Inclusion criteria:

- Cases with pre-prandial serum bile acids above 15 µmol/l or post-prandial serum bile acids above 20 µmol/l (the cut-off value above which histopathological evidence of an hepatopathy may occur according to the BSAVA Clinical Pathology manual) (87).
- Cases with random plasma ammonia levels or the ammonia 30 minutes later, after a tolerance test, greater than 30 µmol/l.
- Ultrasound or histological confirmation of the hepatopathy.

Exclusion criteria:

- Cases that had biochemical evidence of significant azotaemia defined as urea and creatinine raised more than 2× top-normal (urea >20 mmol/l; creatinine >260 µmol/l).
- Cases that had histories of being on prolonged diuretic treatment, treatment with xanthine derivatives or large doses of anti-cancer drugs.
- Dalmatians and Bull Dogs were excluded. .

GROUP 4:

Dogs with azotaemia and/or uraemia without elevated liver enzymes (greater than 2× top-normal) and serum pre-prandial bile acids less than 20 µmol/l or normal plasma ammonia levels (<30 µmol/l).

Inclusion criteria:

Cases with significant azotaemia defined as urea and creatinine raised more than 2× top-normal (urea >20 mmol/l; creatinine >260 µmol/l) with supportive findings such as inappropriately dilute urine specific gravity and/or hyperphosphataemia and liver enzymes

NOT greater than 2× top-normal (ALT >150 IU/l; ALP >600 IU/l; GGT >20 IU/l) and normal bile acids (<15 µmol/l).

Exclusion criteria:

- Cases with elevated serum bile acids (>20 µmol/l) or plasma ammonia levels (>30 µmol/l).
- Cases with liver enzymes raised more than 2× top-normal.
- Any history of dehydration in the clinical assessment, or cases with a urine specific gravity greater than 1.030.
- Dalmatians and Bull Dogs were excluded.

3.2 LABORATORY METHODS FOR DETERMINATION OF SERUM URIC ACID AND BILE ACIDS

3.2.1 Bile Acids

The bile acid assays were conducted using an enzymatic end-point formazan method. Basically the bile acids in the sample are converted into their corresponding ketones by 3-apha-hydroxysteroid dehydrogenase. The ketones, catalysed by diaphorase, lead to reduction of NAD to NADH which reacts with Nitroblue tetrazolium producing a formazan that is blue. The absorbance by the blue colour is measured at 540nm and is directly proportional to the concentration of bile acids in the serum (Materlab bile acid assay Cod BIL8900).

3.2.2 Uric Acid

The uric acid assays were conducted using a colorimetric uricase enzymatic two-stage end-point method which catalyses the reaction of water, oxygen and uric acid in the sample to

allantoin, peroxide and carbon dioxide. The peroxide, catalysed by peroxidase, then effects the oxidative coupling of 2,4 dicholorophenol and 4-aminophenazone. A red dye is formed that increases absorbance at 505nm, which is directly proportional to the uric acid in the serum sample. (NEXCT uric acid assay, Alfa Wassermann)

3.3 STATISTICAL ANALYSIS

Files from the OVAH database were searched and data from suitable cases was extracted and entered into Microsoft Excel® spreadsheets (Microsoft Corporation). The statistical analysis was performed by the Department of Statistics, University of Pretoria. The software packages used included STATISTICA® (Release 7), BMDP Statistical Software® (Release 7.1) and SAS® (version 9.1).

Descriptive statistics included the mean, median and standard deviation for the variables common to the majority of cases in all 4 groups and are listed in the tables 1, 2, 4 and table 6. These variables included: age, weight, ALT, ALP, urea, creatinine, pre-prandial bile (pre-BA), post-prandial bile acids (post-BA) and uric acid (UA). In the control group (Group 1) there were 2 uric acid values available for each dog - one uric acid result value from the pre-prandial sample and one from the post-prandial sample. A variable called 'Diff-uric acid' (Diff-UA) was created, which was the difference between these two values for each dog. Similarly, a variable called 'Diff-bile' (Diff-BA) was created, which was the difference between the pre-prandial and post-prandial bile acid results in groups 1, 2 and 3. Post-prandial bile acid tests were not available for dogs from group 4.

The medians for each variable except Diff-UA were compared between all 4 groups using the non-parametric Kruskal-Wallis test. Within-group comparisons were done for those variables that had 2 comparable results including pre- and post-prandial uric acid in group 1; pre- and post-prandial bile acids in groups 1,2 and 3; and, Diff-BA for groups 1, 2 and 3. The method used for these within-group comparisons was the Wilcoxon non-parametric method for paired differences. Also a Chi-Squared test was performed to see if the uric acid values were within or above the reference range of the Clinical Pathology laboratory of the OVAH (0 – 0.06 mmol/l) in each group and for comparison of the relationship across the groups. Another Chi-Squared test was used to test the relationship between pre-prandial bile acids and uric acid when results were categorised as within normal and above normal across all four groups.

Sensitivities and specificities were calculated for uric acid and pre-prandial bile acids in identifying liver disease (groups 2 and 3 combined) versus non-liver disease (groups 1 and 4). In addition, the sensitivities and specificities for uric acid and pre-prandial bile acids in identifying congenital vascular anomalies (group 2 cases only) versus non-liver disease were also calculated. Similarly, the sensitivities and specificities for uric acid and pre-prandial bile acids in identifying primary parenchymal or secondary liver disease (group 3 cases only) compared to non-liver disease were calculated.

CHAPTER 4

4. RESULTS

4.1 RESULTS PER GROUP

Insufficient data for serum ammonia was available for meaningful comparison to uric acid and therefore serum uric acid results were only compared to those of serum bile acid results. The results that were available for the majority of the cases after data collection, allowed the variables of ALT, ALP, urea and creatinine to be recorded for completeness in the four groups. The parameters pre-prandial bile acids (pre-BA), post-prandial bile acids (post-BA), uric acid and the value which was denoted Diff-bile (DIFF-BA), the difference between pre-BA and post-BA values, were used in statistical analysis for comparison between all 4 groups. Groups 2 and 3 were grouped together and compared to groups 1 and 4 for a comparison of a general liver disease group versus a non-liver disease group, respectively. Group 1, the control group, also had an additional variable Diff-uric acid (DIFF-UA) available. The 4 groups included the control group (Group 1), the congenital hepatic vascular anomaly group (Group 2), the liver disease group (Group 3) and the renal disease group (Group 4).

4.1.1 Group 1

The control group was selected from dogs that were presented to the local welfare society for routine neutering or vaccination. The dogs selected for this group underwent a thorough physical examination by the veterinarian on duty. Blood samples were submitted from the dogs that had no abnormalities in the physical examination for haematology and biochemical screening, which included alanine aminotransferase (ALT), alkaline phosphatase (ALP),

urea, creatinine, pre-prandial bile acid (pre-BA) and post-prandial bile acid (post-BA). Uric acid was determined on both the pre- and post-prandial samples to investigate if uric acid had an increase post-prandially. Dogs were included if their haematology or biochemistry parameters were within normal limits, and pre-prandial bile acids <15 µmol/l. The pre-BA results alone were used in the context that in practice, routine biochemical screening in a healthy animal would not include post-BA.

The control group consisted of 25 dogs, of which 11 were females and 14 were males. The age range was from 5 months to 7 years. The average age was 29.2 months and the median age was 18 months. Their masses ranged from 9 – 26 kilograms. The average mass was 15.8 kilograms, the median mass 15.2 kilograms (kg). The breeds consisted of 11 cross-breed dogs, 5 Staffordshire bull terrier crosses, 2 Labrador crosses and one case each of Australian cattle dog, beagle, Boxer cross, bull terrier, pointer cross, German shepherd cross and a spaniel cross.

The data from group 1 for the significant variables are given in Table 1. The descriptive statistics for those variables are given in the bottom of the table. Briefly, for the dogs in group 1 the medians for the liver enzymes were within reference intervals (ALT median – 27 IU/l; ALP – 81 IU/l) according to the reference ranges for Vetdiagnostix (see appendix A). The medians for serum urea and creatinine were within reference intervals (urea – 6 mmol/l; creatinine 86 µmol/l) and only one dog had a mildly elevated creatinine concentration of 146 µmol/l (ref int. 70 - 130 µmol/l) which was not considered significant with respect to this dog's clinical examination. Two dogs had mildly elevated urea concentrations but normal creatinine concentrations. The pre-BA values were all within the reference interval (pre-BA

ref int. <15µmol/l). Although the median for post-BA was within the reference interval two dogs had significantly raised post-BA. The median for the value designated post-UA, (a uric acid result run on the samples used for the post-prandial bile acid test) was greater than the median for pre-UA (0.08 mmol/l compared to 0.06 mmol/l for pre-UA). The complete haematology and biochemistry data set for these dogs is available in Appendix A.

4.1.2 Group 2

The dogs included in this group were selected on the basis of suffering from a congenital hepatic vascular anomaly. According to the WSAVA Liver Disease Classification Group (WLDCG) such conditions include single intra- or extra-hepatic porto-systemic shunts, portal vein hypoplasia, microvascular dysplasia (considered by the WLDCG to be a form of portal vein hypoplasia) and arteriovenous fistulas. Cases were included if they had appropriate signalment, suggestive clinical history, clinical presentation, laboratory findings, ultrasound or other imaging studies, or histopathological evidence that supported a diagnosis of a congenital vascular anomaly. Suggestive historical findings included stunted growth, poor weight gain, microhepatica, intermittent anorexia, pica, polyphagia, polydipsia and polyuria, pollakiuria, urate urolithiasis, ataxia, circling, vague post-prandial neurological signs or intolerance to anaesthetic drugs. Suggestive laboratory findings in all cases included one or more of the following: microcytic normochromic anaemia, hypoalbuminaemia, hyperglobulinaemia, hypocholesterolaemia, hypoglycaemia, raised serum bile acids, raised plasma ammonia concentration and normal serum urea and creatinine levels. It must be noted that at the time that most of these cases presented, microvascular dysplasia was the preferred morphological diagnostic term for the typical histopathology seen in all these disorders.

The congenital vascular anomaly group consisted of 19 dogs, of which 13 were females and 6 were males. The age range was from 2 to 11 months (average age was 7.2 months; median age was 7 months). Their masses ranged from 1kg to 27.7kg. The average mass was 10.2kg, the median 7.8kg. The breeds consisted of 3 German shepherd dogs; 2 bull dog crosses and 2 dachshunds; and one case each of miniature doberman, Boxer, bull terrier, greyhound, rottweiler, Labrador, spaniel, border collie, schnauzer, pug, Yorkshire terrier and a cross-breed.

The biochemistry data for the dogs in this group are provided in table 2. The descriptive statistics for these variables are given in the bottom of table 2. Briefly, for the dogs in group 2 the median for ALT (76.5 IU/l) was only mildly raised above the upper reference limit of 73 IU/l for the clinical pathology laboratory at the OVAH but 8 dogs had values greater than the reference interval. For ALP the median was within the reference interval of 65 – 311 IU/l but 3 dogs had values exceeding the upper limit. The median for urea (2.8 mmol/l) was below the reference interval of 3.6 – 8.9 mmol/l. The creatinine median (50.5 µmol/l) was near the bottom of the reference interval of 40 - 133 µmol/l. The medians for pre-BA and post-BA were significantly elevated above the reference interval. Uric acid was mildly elevated (0.06 mmol/l) above the reference interval (>0.06 mmol/l). Table 3 provides a summary of the histopathology and ultrasound findings on those cases in which these modalities were used to select cases to this group. The biochemistry results related to the liver are also included in table 3.

4.1.3 Group 3

Dogs in group 3 were cases that either had primary hepatic disease other than congenital vascular anomalies or secondary hepatic dysfunction due to other systemic conditions excluding renal disease. Initial case selection was based on raised bile acids with ultrasound or histopathological evidence of a hepatopathy. Supportive findings included history, clinical presentation, liver enzyme elevations, ultrasound findings or histopathological evidence of a hepatopathy that did not include any evidence or history of congenital vascular shunting. Some cases may have had acquired vascular shunts but cases were only included when ultrasound or histopathology did not identify evidence of shunts were included.

The acquired liver disease group consisted of 27 dogs, of which 12 were females and 15 males. The age range was from 3 months to 18 years (average age was 7years 3 months; median age was 7 years 10 months). Their masses ranged from 1.72kg to 36kg. The average mass was 12.2kg, the median 8.6kg. The breeds consisted of 5 Maltese and 5 cross-breed dogs, 4 dachshunds, 3 bull terriers, 2 spaniels and one each of German shepherd, Labrador, pug, Staffordshire bull terrier, miniature doberman, chow chow, Yorkshire terrier and fox terrier. Two cases (dogs 4 and 11) had elevated bile acid levels due to passive congestion as a result of right-sided heart failure which was confirmed by echocardiography. Two cases of hyperadrenocorticism, that had typical diffuse hyperechogenicity of a steroid hepatopathy on ultrasound, were confirmed by stimulation testing (dog 2) and presence of an adrenal tumour (dog 1). Thirteen cases had histopathological evidence of a hepatopathy. Twenty-four cases had ultrasound findings indicating a hepatopathy.

The data from group 3 for the signalment and pertinent biochemistry variables are given in table 4. The descriptive statistics for those variables are given in the bottom of the table. Briefly, for dogs in group 3 the median for ALT was not elevated but that for ALP was elevated (341 IU/l) compared to the reference interval of 65- 311 IU/l. Urea and creatinine for this group of dogs had medians within the reference interval. The medians for pre-BA and post-BA were elevated above the reference interval. The uric acid median was only mildly elevated at 0.07 mmol/l. Table 5 provides a summary of the histopathology and ultrasound findings on those cases in which these modalities were used. The biochemistry results related to the liver are also included in table 5.

4.1.4 Group 4

Cases for the renal disease group were selected based on laboratory results which included azotaemia (twice top-normal urea and creatinine; ref range 3.6-8.9 mmol/l and 40-133 µmol/l respectively) with inappropriately dilute urine specific gravity, elevated serum inorganic phosphate (>1.6 mmol/l; ref range 0.9-1.6 mmol/l), ultrasound findings consistent with a nephropathy or histopathological confirmation of renal pathology (when available). Liver disease was excluded based on history, clinical examination (or lack of clinical evidence that suggested liver disease), and liver specific biochemistry (when available) or bile acid results ≤20 µmol/l. The latter cut-off point was chosen in accordance with the BSAVA recommendation that values greater than 20 µmol/l may be associated with histopathological evidence of an hepatopathy (87).

Only 12 dogs met the criteria of having azotaemia, with either ultrasound or histopathology indicating a nephropathy and pre-prandial bile acid values ≤20 µmol/l. Four dogs were

females and 8 were males. The age range was from 12 months to 13 years (average age was 5.8 years; median age was 5 years). Their masses ranged from 2.92kg to 45kg. The average mass was 26.45kg, with a median of 28.1kg. The breeds consisted of 2 cases each of cross-breed dogs, rottweilers, German shepherds and Boerboels; and, one case each of Rhodesian ridgeback, miniature pinscher, fox terrier and Yorkshire terrier.

The data from group 4 for the signalment and pertinent biochemistry variables are given in table 6. The descriptive statistics for those variables are given in the bottom of the table. Only four dogs in group 4 had liver enzyme results available. ALT median was mildly elevated (78 IU/l) above the upper reference limit of 73 IU/l but only 2 of the dogs had values greater than the reference interval. ALP median was within the reference interval. Azotaemia was present in all the dogs in this group due to the selection criteria for this group. The pre-BA median was 17 µmol/l (post-BA values were not available for this group), only slightly elevated above the reference interval (<15 µmol/l). The uric acid median was top-normal at 0.06 mmol/l. The ultrasound and histopathological findings for these cases together with the urine specific gravity, urea, creatinine and phosphate concentrations are given in Table 7.

4.2 GROUP COMPARISONS AND STATISTICAL ANALYSIS

4.2.1 Between-Group Comparisons For Selected Variables

The distribution of all the variables was not normal and therefore the non-parametric Kruskal-Wallis test was used for statistical analysis between the groups for each variable.

URIC ACID

Uric acid (UA) had a mean of 0.06 mmol/l and a median of 0.06 mmol/l for group 1 dogs. The mean for group 2 dogs was 0.07 mmol/l and the median 0.07 mmol/l. For group 3 the mean for UA was 0.09 mmol/l and the median 0.07 mmol/l. In group 4 the mean for UA was 0.08 mmol/l and the median 0.06 mmol/l. The frequency for UA in all four groups was 100% (Table 8). The relative distribution for UA in all four groups is given in box plot 1.

The medians were used to compare UA results between groups using the Kruskal-Wallis method. The median of UA was not significantly different for any of the groups ($p = 0.1605$) as indicated in table 9.

PRE-PRANDIAL BILE ACIDS

Pre-prandial bile acids (pre-BA) had a mean of 9.57 µmol/l and a median of 9.24 µmol/l for group 1 dogs. The mean for group 2 dogs was 93.46 µmol/l and the median 48.2 µmol/l. For group 3 the mean for pre-BA was 67.72 µmol/l and the median 45.01 µmol/l. In group 4 the

mean for pre-BA was 16.96 µmol/l and the median 17.02 µmol/l. The frequency for pre-BA in all four groups was 100% (Table 8). The relative distribution for pre-BA in all four groups is given in box plot 2.

The medians were used to compare pre-BA results between the groups using the Kruskal-Wallis method. The median of pre-BA was not statistically significantly different between groups 2 and 3 or between groups 1 and 4. Groups 1 and 4 had a significantly lower median than groups 2 and 3 ($p < 0.0001$) as indicated in table 9.

POST-PRANDIAL BILE ACIDS

Post-prandial bile acids (post-BA) had a mean of 26.76 µmol/l and a median of 23.82 µmol/l for group 1 dogs. All 25 dogs had a post-BA result (100% frequency) as indicated in table 8. The mean for group 2 dogs was 207.03 µmol/l and the median 202.5 µmol/l. The frequency for post-BA in group 2 was 68.4% (Table 8). For group 3 the mean for post-BA was 88.16 µmol/l and the median 66.04 µmol/l. The frequency for post-BA in group 3 was 51.9% (Table 8). No dogs in group 4 had a post-BA result and the frequency was thus 0% (Table 8). The relative distribution for post-BA in groups 1, 2 and 3 is given in box plot 3.

The medians were used to compare post-BA results between groups 1, 2 and 3 using the Kruskal-Wallis method. The median of post-BA was not statistically significantly different between groups 2 and group 3. The median post-BA in group 1 was significantly lower than groups 2 and 3 ($p < 0.0001$) as indicated in table 9.

DIFF-BA

The difference between post-BA and pre-BA was assessed between the groups. DIFF-BA had a mean of 17.2 µmol/l and a median of 12.4 µmol/l for group 1 dogs. All 25 dogs had a DIFF-BA result (100% frequency) as indicated in table 8. The mean for group 2 dogs was 175.4 µmol/l and the median 166.2 µmol/l. The frequency for DIFF-BA in group 2 was 68.4% (Table 8). For group 3 the mean for DIFF-BA was 25.23 µmol/l and the median 11.91 µmol/l. The frequency of DIFF-BA in group 3 was 51.9% (Table 8). No dogs in group 4 had a post-BA result and therefore no DIFF-BA result and the frequency was thus 0% (Table 8). The relative distribution for DIFF-BA in groups 1, 2 and 3 is given in box plot 4.

The medians were used to compare DIFF-BA results between groups 1, 2 and 3 using the Kruskal-Wallis method. The median of DIFF-BA was not statistically significantly different between groups 1 and group 3. Group 2's DIFF-BA median was significantly higher than groups 1 and 3 ($p = 0.0003$) as indicated in table 9.

4.2.2 Comparisons Within Group 1 For Uric Acid

At the time of sampling of the group 1 dogs, samples were collected for both the pre-, and post-prandial bile acid assays. Both samples were analysed for uric acid levels. The median for the pre-prandial UA was 0.06 mmol/l and that for the post-prandial UA was 0.08 mmol/l. The comparisons between the 2 sets of results were done by using the Wilcoxon non-parametric test for paired differences. A significant difference ($p = 0.0007$) between the two sets of results was identified (Table 10).

4.3 SENSITIVITY AND SPECIFICITY FOR URIC ACID

The sensitivity and specificity for uric acid as a test to identify liver disease (both congenital vascular anomalies and other primary and secondary hepatic disease) in this study was calculated. The reference range for uric acid that the Clinical Pathology laboratory of the OVAH uses is 0 – 0.06 mmol/l. In this study the sensitivity of uric acid for diagnosing liver disease overall (UA = 0.07 mmol/l or greater) was 65%; and the specificity 59% (Table 11).

Chi-Square analysis of uric acid being within the normal range or above normal range showed a similar pattern across all 4 groups ($p = 0.1595$), confirming the lack of sensitivity and specificity of the test (Table 12).

The sensitivity and specificity for uric acid to detect vascular anomalies (Group 2 cases only) in this study was calculated. Sensitivity was 68% and specificity was 59% (Table 13). The sensitivity and specificity for detecting parenchymal primary or secondary hepatopathies (Group 3 cases only) was 63% and 60% respectively (Table 14). At the 95% confidence interval, the lower and upper limits for these two calculations overlapped indicating no statistical difference.

4.4 SENSITIVITY AND SPECIFICITY FOR BILE ACIDS

The sensitivity and specificity for bile acids using the pre-BA results, as a test to identify liver disease (both congenital shunts and other primary and secondary hepatic disease), in this study was calculated. The Clinical Pathology laboratory of the OVAH uses <15 µmol/l as its

reference range for pre-prandial bile acids. In this study the sensitivity for diagnosing liver disease overall (pre-BA <15 µmol/l) was 95.8%; and the specificity 71% (Table 15).

The sensitivity and specificity for pre-BA to detect vascular anomalies in this study was then calculated. Sensitivity was 100% (skewed by the selection criteria) and specificity was 73% (Table 16). The sensitivity and specificity for detecting parenchymal primary or secondary hepatopathies was 96% and 77% respectively (Table 17). At the 95% confidence interval, the lower and upper limits for these two calculations overlapped indicating no statistical difference.

4.5 POSITIVE AND NEGATIVE PREDICTIVE VALUES FOR URIC ACID AND PRE-PRANDIAL BILE ACIDS

The positive predictive value (PPV) for uric acid as a test of liver function for groups 2 and 3 combined was 66% (Table 11) and the negative predictive value (NPV) was only 58%. When only group 2 was evaluated to test the predictive values for identifying congenital vascular anomalies the PPV was only 46% and the NPV was 79% (Table 13). Similarly with group 3 dogs alone, the predictive values for identifying primary and secondary parenchymal disease were calculated and the PPV was 53% and the NPV was 69% (Table 14).

The PPV for pre-BA as a test of liver function for groups 2 and 3 combined was 82% (Table 15) and the NPV was 92%. When only group 2 was evaluated to test the predictive values for identifying congenital vascular anomalies the PPV was only 66% and the NPV was 100% (Table 16). Similarly with group 3 dogs alone, the predictive values for identifying primary

and secondary parenchymal disease were calculated and the PPV was 72% and the NPV was 96% (Table 17).

4.6 RELATIONSHIP BETWEEN URIC ACID AND PRE-PRANDIAL BILE ACIDS

The levels of uric acid and pre-BA can be categorized as falling above normal or within reference range. For uric acids the within reference range is ≤0.06 mmol/l and for pre-BA it is ≤15 μmol/l.

A chi-square analysis was done to determine whether there was any significant relationship between the levels of uric acid and pre-BA when cases were categorized as above normal or within reference range. The frequencies for the 4 groups combined are shown in table 18. The p-value was 0.3096, indicating that there was no significant relationship between the levels of uric acid and pre-BA.

CHAPTER 5

5. DISCUSSION

The results of this study showed that there were significant differences between the groups for the medians of pre- and post-prandial bile acids but not for uric acid (Table 9). With respect to uric acid, there was no statistical difference between the medians for the liver disease groups (groups 2 and 3) compared to the non-liver disease groups (groups 1 and 4). Plasma ammonia level is widely regarded as the most sensitive assay for detecting congenital porto-systemic shunts (71;88). Unfortunately not enough cases with congenital portovascular anomalies or liver disease in this retrospective study had plasma ammonia data. Therefore uric acid was only compared to bile acids between the four groups in the end. And for the purposes of using a single test as a test of liver function the main focus was comparing pre-BA results to those of uric acid.

5.1 THE NORMAL RANGE OF URIC ACID

In this study the control group served as the normal dog population. There were 25 dogs in this group which had no evidence of disease after a full clinical examination and routine haematology and biochemistry investigation. Uric acid was measured on the same serum samples collected for pre- and post-prandial bile acids determinations; starved and two hours after a small meal. Interestingly, the two different sets of samples produced statistically significantly different medians for uric acid as illustrated in table 10. The pre-prandial uric

acid had a median of 0.06 mmol/l while the median for the post-prandial uric acid was 0.08 mmol/l. The means were 0.06 mmol/l and 0.093 mmol/l respectively. The published reference range for uric acid varies between laboratories. The Animal Health Diagnostic Centre at Cornell University gives 0.012 – 0.053 mmol/l (0.2 – 0.9 mg/dl); University of Florida Veterinary Medical Centre gives <0.029 mmol/l (<0.5 mg/dl) and the veterinary Clinical Pathology Laboratory at the Onderstepoort Veterinary Academic Hospital, that did the assays for this study, uses a reference range of < 0.06 mmol/l. The data in this study suggest that the normal (control) dogs had uric acid means and medians above the reference ranges given for all 3 laboratories. Alternatively, using the International Federation of Clinical Chemistry (IFCC) convention of a mean ± 2 standard deviations for the 95% confidence interval, the control group had a normal range of 0 – 0.15 mmol/l. The size of this population (n = 25) was too small to be a reliable measure of a reference range for normal dogs. The IFCC recommends that at least 50 subjects in a population are required in order to establish a reference range. Also, when compared to the reference ranges of the laboratories listed previously, these results raise concerns that some dogs in this group may have been harbouring occult liver disease. A limitation of using this population of SPCA dogs waiting for adoption meant that biopsies of the liver could not be done to confirm absence of liver disease. The pre-BA results from the control group were all within the normal range (<15 µmol/l) for this set of dogs. As indicated in table 1, 14 of the 25 dogs (56%) had a pre-BA result less than 10 µmol/l, but 10 of the 25 dogs (40%) had a post-BA result greater than 25 µmol/l. Of the pre-BA results, one case had a higher pre-BA result than post-BA result. This outcome could be due to early gall bladder contraction or delayed intestinal transit, which are known factors that can affect the endogenous bile acid challenge test (84). Bile acids can be elevated in dogs without hepatic disease as was reported in a study

investigating sub-clinical porto-systemic shunts in Maltese poodles (88). No poodles were present in this study's control group however. Two cases, both Staffordshire Bull terrier-crosses (dogs 4 and 14), had marked increases from pre-prandial to post-prandial bile acid levels (post-BA of 51.2 and 102.3 µmol/l, respectively) without any other signs of a hepatopathy. It is possible that these dogs may have had a sub-clinical porto-systemic shunt or other portovascular anomalies, or sub-clinical parenchymal disease. The respective pre-prandial uric acid values for these 2 dogs were 0.11 mmol/l (elevated) and 0.04 mmol/l (within reference range). The post-prandial uric acid concentrations were 0.15 mmol/l and 0.14 mmol/l respectively, both above the reference range. A third dog, also a Staffordshire Bull terrier-cross (dog 2), had an increase in bile acids from 5.9 µmol/l to 38.7 µmol/l post-prandially. The post-BA sample from this dog was reported to be haemolysed which may have contributed to this elevation. This dog's pre- and post-prandial uric acid results were 0.12 mmol/l (elevated) and 0.06 mmol/l (within the reference range), respectively. All three of these dogs fall into a category of having a DIFF-BA which is greater than 25 which was used by Kerr and Doorn in their study to identify Irish wolfhounds with congenital porto-systemic shunts (DIFF-BA <20 unlikely to have a shunt) (89). The DIFF-BA for 19 of the remaining 22 cases was less than 20. Three cases (dogs 12, 16, 24) had DIFF-BA above 20 but less than 25, considered an inconclusive result by Kerr and Doorn (89). Dogs 12 and 16 however, had both pre-UA and post-UA results within the reference range. Dog 24 had an elevated pre-UA but lower post-UA, which was still above the reference range. However, the difference between the pre- and post-prandial uric acid results for dogs 2, 4 and 14 did not follow the same pattern as the bile acid result elevation from pre- to post-prandial. This together with the difference between the medians of the pre-prandial and post-prandial uric acid results for the group was unexpected and difficult to explain physiologically. Increased

splanchnic blood-flow post-prandially and increased metabolic activity in the hepatocytes might explain this increase in serum uric acid, but concerns of natural variation in serum uric acid concentration similar to those seen with cortisol are raised by this finding. It is possible that the group used in this study may not have been a true reflection of a normal population. Nevertheless, the median for uric acid for the renal disease group (group 4) was not statistically different from this 'normal' group or those of the two liver disease groups.

5.2 SENSITIVITY AND SPECIFICITY OF URIC ACID COMPARED TO PRE-PRANDIAL BILE ACIDS FOR IDENTIFYING LIVER DISEASE

In this study the overall sensitivity of uric acid for diagnosing abnormal liver function (congenital portosystemic vascular diseases, primary parenchymal diseases and secondary hepatopathies) was only 65% while the specificity was only 59%. The sensitivity and specificity for pre-BA was 96% and 71% respectively. It must be remembered however, that elevated bile acid results were used as selection criteria for both group 2 and group 3 cases (all liver disease cases) and therefore a bias was created by this selection process. When assessing the cases in group 2 (congenital vascular anomalies) alone, the sensitivity of uric acid was 68% and specificity 59%, while for pre-BA it was 100% and 73%, respectively. Although the sensitivity percentage will have been skewed by the selection criteria it is consistent with findings in another study (90). Similarly when only cases in group 3 (primary and secondary parenchymal liver diseases) were evaluated the sensitivity and specificity of pre-BA was far superior (96% and 77% respectively) compared to uric acid sensitivity of 63% and specificity of 60%. As illustrated in table 9, the medians of pre-BA and post-BA for groups 2 and 3 differed significantly from that of group 1. Whereas for DIFF-BA only the

median of group 2 differed significantly from group 1 while the medians for group 3 and 1 were not statistically different. This suggests that DIFF-BA may only be of clinical use in porto-systemic shunting cases, as used by Kerr and Doorn. However, the medians of uric acid for all groups were not statistically different suggesting that its ability to diagnose liver disease is questionable. The particularly poor specificity of uric acid is mainly due to the effects of renal disease in this study despite the fact that the renal disease group was only comprised of 12 cases. Uric acid is excreted almost exclusively by the kidney in dogs, unlike man where some excretion is via the gastrointestinal tract. The RTE cells in the canine kidney appear to have the same carrier molecules for uric acid transport as those reported in man which may not function in renal disease resulting in hyperuricaemia. The results of this study showed that there was no statistical relationship between uric acid and pre-BA for values falling within normal range or those above normal range across all 4 groups (table 18). This is significant in that it indicates that the tests were not congruent with each other in this population of dogs.

5.3 PREDICTIVE VALUES OF URIC ACID COMPARED TO PRE-PRANDIAL BILE ACIDS

The predictive values for uric acid and pre-BA were calculated for both the liver disease groups (groups 2 and 3 combined) verses the non-liver disease groups (groups 1 and 4). The predictive values were also calculated for the congenital vascular anomalies group (group 2) verses the non-liver disease groups, and for the parenchymal liver disease group (group 3) compared to the non-liver disease groups. The PPV of uric acid in all three scenarios were

not high enough for a diagnostic test. The pre-BA however performed much better although for the congenital vascular anomalies the PPV for pre-BA was just 66%.

The NPV for uric acid were better than the PPV's but only the NPV for congenital vascular anomalies was approaching a useful level of almost 80%. The NPV of bile acids for all three scenarios was much better although the selection criteria would have biased this statistic.

CHAPTER 6

CONCLUSIONS

Assessment of liver disease for the veterinary practitioner involves clinical pathology, ultrasound, and ultimately histopathology. In clinical pathology there are three branches of tests; tests of cellular integrity such as liver enzymes ALT and AST, liver enzymes that assess the biliary tree and cholestasis such as ALP and GGT, and, liver function tests that assess the overall liver functional mass and blood circulation such as bile acids and plasma ammonia concentrations. A single pre-prandial bile acid test is the cheapest and easiest option for most practitioners in private practice as a test of liver function. At the advent of enzymology in the 1960's uric acid was evaluated as a 'liver test' but was condemned by the authors mentioned previously. Plasma ammonia concentration is widely regarded as the most reliable liver function test especially with respect to portovascular anomalies. However the sample handling requirements often make this test impractical for many practitioners. In South Africa and probably many other developing countries bile acid assays are not widely available and it would be convenient if a simple once-off blood test such as uric acid could be used as a screening test for liver function.

This study was implemented to test the utility of uric acid in comparison with bile acids to identify liver disease in dogs. Four groups were created with two separate liver disease groups which were congenital vascular anomalies in one group and primary and secondary parenchymal liver diseases in the other liver disease group. Plasma ammonia concentration is the test of choice for diagnosing porto-vascular shunting and other vascular anomalies but unfortunately not enough cases with plasma ammonia results could be found on the database

of the OVAH. Therefore bile acids were compared to uric acid in this study. Bile acids are used widely and numerous studies have shown a diagnostic difference when congenital vascular anomalies are diagnosed with bile acids compared to parenchymal liver disease. Therefore the two groups were separated to assess if uric acid behaved in a similar way. However the statistical analysis for sensitivity and specificity of both tests were calculated on both groups combined compared to the non-liver disease groups. Group one was a control group of clinically normal dogs and group four was a renal disease group. This group was included in the study because concerns of impairment of uric acid excretion via the kidneys impacting on the serum uric acid levels were raised by various observers.

The results of the study showed no statistical difference in the medians for uric acid between groups of dogs that were normal (controls), diagnosed with congenital porto-systemic shunts, diagnosed with primary or secondary hepatopathy, and dogs with renal disease (azotaemic). In fact, the control group had a uric acid median above the normal reference range. As previously discussed the control group may have contained dogs with occult liver disease. There was also a variation in uric acid samples on the same dog pre- and post-prandially. This was an unexpected finding and raises concerns of a natural daily fluctuation in the serum uric acid levels that may impact negatively on its use as a random liver function test. Nevertheless the uric acid elevations in these cases were not consistent with any changes in pre- and post bile acids results. One limitation of this study was that uric acid was only compared to pre-prandial bile acids in all the groups. It is not unusual in modern times to use post-prandial bile acids as a stand-alone screening test, and comparison of uric acid versus post-prandial bile acids for groups 1, 2 and 3 could have been carried out. However considering the lack of statistically significant difference in the medians of uric acid for these

three groups and the distinctly significant difference in post-prandial bile acids medians for these groups, the final outcome is unlikely to be any different.

The major finding in the study however was that of the raised uric acid levels in the renal disease group. The median of this group was not statistically significantly different from that of the liver disease groups that led to a poor specificity for uric acid in identifying liver disease. When sensitivity and specificity for uric acid was assessed for congenital vascular diseases versus non-liver disease and then parenchymal liver disease versus non-liver disease, there was no significant improvement in the sensitivity and specificity. Bile acids performed much better in all three scenarios with sensitivities and specificities, which were diagnostically useful and similar to other reported studies. The positive predictive values for uric acid as a test of liver function overall and then specifically for congenital vascular anomalies or primary and secondary parenchymal diseases were disappointingly low and therefore suggest that it is not a reliable diagnostic test. However the negative predictive value for uric acid identifying congenital vascular anomalies was 79%, which would be feasible as a screening test. There may be a case for using uric acid in very specific conditions of screening a young dog, with no signs of renal disease, for portovascular anomalies. However, the lengths that one would need to go to, to exclude renal disease would probably be as expensive and time-consuming as conducting pre- and post-prandial bile acids tests.

REFERENCE LIST

(1) Center SA. Pathophysiology and Laboratory Diagnosis of Hepatobiliary Disorders. In: Ettinger SJ, Feldman EC, editors. Textbook of Veterinary Internal Medicine. Philadelphia: W.B. Saunders Co., 1995: 1261-312.

(2) Huxtable CRR. The Liver & Exocrine Pancrease. In: Robinson WF, Huxtable CRR, editors. Clinicopathologic Principles for Veterinary Medicine. Cambridge: Cambridge University Press., 2003: 194-215.

(3) Boomkens SY, Penning LC, Egberink HF et al. Hepatitis with special reference to dogs. A review on the pathogenesis and infectious etiologies, including unpublished results of recent own studies. Vet Q 2004 Sept.; 26(3):107-14.

(4) Center SA. Chronic liver disease: current concepts of disease mechanisms. J Small Anim Pract 1999 Mar.; 40(3):106-14.

(5) Dial SM. Clinicopathologic evaluation of the liver. Vet Clin North Am Small Anim Pract 1995 Mar.; 25(2):257-73.

(6) Hess PR, Bunch SE. Diagnostic Approach to Hepatobiliary Disease. In: Bonagura DG, editor. Kirk's Current Veterinary Therapy XIII. Philadelphia: W.B. Saunders Co., 2000: 659-64.

(7) Sevelius E. Diagnosis and prognosis of chronic hepatitis and cirrhosis in dogs. J Small Anim Pract 1995 Dec.; 36(12):521-8.

(8) Sutherland RJ. Biochemical evaluation of the hepatobiliary system in dogs and cats. Vet Clin North Am Small Anim Pract 1989 Sept.; 19(5):899-927.

(9) Hayes MA. Pathophysiology of the Liver. In: Dunlop RH, Malbert CH, editors. Veterinary Pathophysiology. Iowa: Blackwell Science Co., 2004: 371-99.

(10) Meyer DJ, Twedt DC. Effect of Extrahepatic Disease on the Liver. In: Bonagura DG, editor. Kirk's Current Veterinary Therapy XIII. Philadelphia: W.B. Saunders Co., 2000: 668-71.

(11) Bain PJ. Liver. In: Latimer KS, Mahaffey EA, Prasse KW, editors. Duncan & Prasse's Veterinary Laboratory Medicine : Clinical Pathology. Ames: Blackwell Publishing, 2003: 193-214.

(12) Feldman EC, Nelson RW. Canine Diabetes Mellitus. In: Feldman EC, Nelson RW, editors. Canine and Feline Endocrinology and Reproduction. St. Louis: Elsevier Science., 2004: 486-538.

(13) Stockham SL, Scott MA. Liver Function. In: Stockham SL, Scott MA, editors. Fundamentals of Veterinary Clinical Pathology. Ames: Blackwell Publishing, 2002: 461-86.

(14) Center SA, Magne ML. Historical, physical examination, and clinicopathologic features of portosystemic vascular anomalies in the dog and cat. Semin Vet Med Surg (Small Anim) 1990 May; 5(2):83-93.

(15) Rothuizen J. Canine chronic liver disease: where are we now? Four pointers for future research. Vet J 2004 May; 167(3):219.

(16) Watson PJ. Chronic hepatitis in dogs: a review of current understanding of the aetiology, progression, and treatment. Vet J 2004 May; 167(3):228-41.

(17) Rothuizen J, Bunch SE, Charles JA et al. WSAVA Standards for Clinical and Histological Diagnosis of Canine and Feline Liver Diseases. Saunders Limited, 2006.

(18) Lassen ED. Laboratory Evaluation of the Liver. In: Thrall MA, Baker DC, Campbell TW et al, editors. Veterinary Haematology in Clinical Chemistry. Philadelphia: Lippincott Williams & Wilkins., 2004: 355-76.

(19) Meyer DJ, Williams DA. Diagnosis of hepatic and exocrine pancreatic disorders. Semin Vet Med Surg (Small Anim) 1992 Nov.; 7(4):275-84.

(20) Willard MD, Twedt DC. Gastrointestinal, Pancreatic & Hepatic Disorders. In: Willard MD, Tvedten H, editors. Small Animal Clinical Diagnosis by Laboratory Methods. St. Louis: Saunders Elsevier, 2004: 208-46.

(21) Center SA, Magne ML. Historical, physical examination, and clinicopathologic features of portosystemic vascular anomalies in the dog and cat. Semin Vet Med Surg (Small Anim) 1990 May; 5(2):83-93.

(22) Aquilera-Tejero E, Meyer-Volar R, Gomes-Cardenas G. Plasma bile acids, lactate dehydrogenase and sulphabromophthalein retention test in carbon tetrachloride intoxication. Journal of Small Animal Practice 1983; 29:711-7.

(23) Center SA. Liver function tests in the diagnosis of portosystemic vascular anomalies. Semin Vet Med Surg (Small Anim) 1990 May; 5(2):94-9.

(24) Center SA. BSP--a retired test revisited. J Vet Intern Med 2000 Nov.; 14(6):557-9.

(25) Winkler JT, Bohling MW, Tillson DM et al. Portosystemic shunts: diagnosis, prognosis, and treatment of 64 cases (1993-2001). J Am Anim Hosp Assoc 2003 Mar.; 39(2):169-85.

(26) Meyer DJ, Harvey JW. Evaluation of hepatobiliary System and Skeletal Muscle and Lipid Disorders. In: Meyer DJ, Harvey JW, editors. Veterinary Laboratory Medicine: Interpretation & Diagnosis. Philadelphia: W.B. Saunders.Co., 1998: 157-86.

(27) Center SA. Acute hepatic injury: Hepatic necrosis and fulminant hepatic failure. In: Guilford WG, Center SA, Strombeck DR et al, editors. Strombeck's Small Animal Gastroenterology. Philadelphia: W.B. Saunders Co., 1996: 654-704.

(28) Cornelius CE. Liver Function. In: Kaneko JJ, editor. Clinical Biochemistry in Domestic Animals. San Diego: Academic Press, 1989: 364-97.

(29) Kerr MG. Investigation on an Individual Organ Basis. In: Kerr MG, editor. Veterinary Laboratory Medicine. Oxford: Blackwell Scientific, 2002: 199-208.

(30) Giesecke D, Tiemeyer W. Defect of uric acid uptake in Dalmatian dog liver. Experientia 1984 Dec.; 40(12):1415-6.

(31) Gregory LC. Metabolic Analytes. In: Christenson RH, Gregory LC, Johnson LJ, editors. Appleton & Lange's Outline Review: Clinical Chemistry. New York: McGraw-Hill Co., 2001: 121-4.

(32) Kruger JM, Osborne CA. Etiopathogenesis of uric acid and ammonium urate uroliths in non-Dalmatian dogs. Vet Clin North Am Small Anim Pract 1986 Jan.; 16(1):87-126.

(33) Newman DJ, Price CP. Non-protein Nitrogen Metabolites. In: Burtis CA, Ashwood ER, editors. Tietz's Fundementals of Clinical Chemistry. Philadelphia: W.B. Saunders Co., 2001: 422-6.

(34) Smith ST. Non-protein Nitrogen. In: Bishop ML, Duben-Engelkirk JL, Foden FP, editors. Clinical Chemistry: Principles, Procedures and Correlations. Philadelphia: Lippencot-Raven Publishers, 1996: 348-50.

(35) Safra N, Schaible RH, Bannasch DL. Linkage analysis with an interbreed backcross maps Dalmatian hyperuricosuria to CFA03. Mamm Genome 2006 Apr.; 17(4):340-5.

(36) Candlin FT. Diseases of the Liver, Pancreas and Peritoneum. In: Catcott EJ, editor. Canine Medicine. Illinois: American Veterinary Publications, 1968.

(37) Van Aubel RA, Smeets PH, van den Heuvel JJ, Russel FG. Human organic anion transporter MRP4 (ABCC4) is an efflux pump for the purine end metabolite urate with multiple allosteric substrate binding sites. Am J Physiol Renal Physiol 2005 Feb.; 288(2):F327-F333.

(38) Nguyen MT, Awale S, Tezuka Y et al. Hypouricemic effects of acacetin and 4,5-o-dicaffeoylquinic acid methyl ester on serum uric acid levels in potassium oxonate-pretreated rats. Biol Pharm Bull 2005 Dec.; 28(12):2231-4.

(39) Wang Y, Zhu JX, Kong LD et al. Administration of procyanidins from grape seeds reduces serum uric acid levels and decreases hepatic xanthine dehydrogenase/oxidase activities in oxonate-treated mice. Basic Clin Pharmacol Toxicol 2004 May; 94(5):232-7.

(40) Hardy RM. Diseases of the Liver. In: Ettinger SJ, editor. Textbook of Veterinary Internal Medicine. Philadelphia: W.B. Saunders Co., 1983: 1373-474.

(41) Houston DM, Moore AE, Favrin MG, Hoff B. Canine urolithiasis: a look at over 16 000 urolith submissions to the Canadian Veterinary Urolith Centre from February 1998 to April 2003. Can Vet J 2004 Mar.; 45(3):225-30.

(42) Safra N, Ling GV, Schaible RH, Bannasch DL. Exclusion of urate oxidase as a candidate gene for hyperuricosuria in the Dalmatian dog using an interbreed backcross. J Hered 2005; 96(7):750-4.

(43) Lathem W, Davis BB, Rodnan GP. Renal tubular secretion of uric acid in the mongrel dog. Am J Physiol 1960 July; 199:9-12.

(44) Miller GE, Danzig LS, Talbott JH. Urinary excretion of uric acid in the Dalmatian and non-Dalmatian dog following administration of diodrast, sodium salicylate and a mercurial diuretic. Am J Physiol 1951 Jan.; 164(1):155-8.

(45) Guggino SE, Aronson PS. Paradoxical effects of pyrazinoate and nicotinate on urate transport in dog renal microvillus membranes. J Clin Invest 1985 Aug.; 76(2):543-7.

(46) Roch-Ramel F, Guisan B, Diezi J. Effects of uricosuric and antiuricosuric agents on urate transport in human brush-border membrane vesicles. J Pharmacol Exp Ther 1997 Feb.; 280(2):839-45.

(47) Burr JR, Reinhart GA, Swenson RA et al. Serum biochemical values in sled dogs before and after competing in long-distance races. J Am Vet Med Assoc 1997 July; 211(2):175-9.

(48) Hinchcliff KW. Performance failure in Alaskan sled dogs: biochemical correlates. Res Vet Sci 1996 Nov.; 61(3):271-2.

(49) Kawamura M, Ohashi F, Nishimura R et al. Plasma concentrations of substances suspected as uremic toxins in experimentally induced and spontaneous uremic dogs. J Vet Med Sci 1994 Apr.; 56(2):381-3.

(50) Greenberg A. Diuretic complications. Am J Med Sci 2000 Jan.; 319(1):10-24.

(51) Iwaki K, Ishii M, Yonetani Y. Hypotensive and uric acid-retaining effects of trichlormethiazide under dietary sodium restriction in spontaneously hypertensive rats. Jpn J Pharmacol 1984 Nov.; 36(3):301-9.

(52) Leary WP, Reyes AJ. Angiotensin I converting enzyme inhibitors and the renal excretion of urate. Cardiovasc Drugs Ther 1987; 1(1):29-38.

(53) Pea F. Pharmacology of drugs for hyperuricemia. Mechanisms, kinetics and interactions. Contrib Nephrol 2005; 147:35-46.

(54) Yonetani Y, Ishii M, Iwaki K. Hyperuricemia induced by some antihypertensives and uricosuric drugs in oxonate-treated rats. Jpn J Pharmacol 1980 Dec.; 30(6):829-40.

(55) Lieber CS. Alcohol and the liver: metabolism of alcohol and its role in hepatic and extrahepatic diseases. Mt Sinai J Med 2000 Jan.; 67(1):84-94.

(56) Morita Y, Nishida Y, Kamatani N, Miyamoto T. Theophylline increases serum uric acid levels. J Allergy Clin Immunol 1984 Nov.; 74(5):707-12.

(57) Toda K, Goriki K, Ochiai M et al. Gout due to xanthine derivatives. Br J Rheumatol 1997 Oct.; 36(10):1131-2.

(58) Laine J, Holmberg C. Mechanisms of hyperuricemia in cyclosporine-treated renal transplanted children. Nephron 1996; 74(2):318-23.

(59) Cornelius CE. Liver Function. In: Kaneko JJ, editor. Clinical Biochemistry of Domestic Animals. San Diego: Academic Press, 1970: 161-230.

(60) Morgan HC. A comparison of Uric acid determinations and Sulphobromophthalein Sodium retention tests as an index to canine liver dysfunction. American Journal of Veterinary Research 1959; 20:372-7.

(61) Malherbe WD. Intrahepatic cholestasis in a Rhodesian Ridgeback dog: a clinicopathological study. Journal of the South African Veterinary Medical Association 1959; 30:113-24.

(62) Hoe CM, Harvey DG. An Investigation into Liver Function Tests in Dogs; Part2; Tests other than Transaminase Estimations. Journal of Small Animal Practice 1961; 2:109-27.

(63) Tenant BC. Hepatic Function. In: Kaneko JJ, Harvey JW, Bruss ML, editors. Clinical Biochemistry in Domestic Animals. San Diego: Academic Press, 1997: 327-52.

(64) Turgut K, Demir C, Ok M, Ciftci K. Pre- and post-prandial total serum bile acid concentration following acute liver damage in dogs. Zentralbl Veterinarmed A 1997 Mar.; 44(1):25-9.

(65) Center SA, Slater MR, ManWarren T, Prymak K. Diagnostic efficacy of serum alkaline phosphatase and gamma-glutamyltransferase in dogs with histologically confirmed hepatobiliary disease: 270 cases (1980-1990). J Am Vet Med Assoc 1992 Oct.; 201(8):1258-64.

(66) Wiedmeyer CE, Solter PE, Hoffmann WE. Alkaline phosphatase expression in tissues from glucocorticoid-treated dogs. Am J Vet Res 2002 Aug.; 63(8):1083-8.

(67) Wiedmeyer CE, Solter PE, Hoffmann WE. Kinetics of mRNA expression of alkaline phosphatase isoenzymes in hepatic tissues from glucocorticoid-treated dogs. Am J Vet Res 2002 Aug.; 63(8):1089-95.

(68) Syakalima M, Takiguchi M, Yasuda J, Hashimoto A. The age dependent levels of serum ALP isoenzymes and the diagnostic significance of corticosteroid-induced ALP during long-term glucocorticoid treatment. J Vet Med Sci 1997 Oct.; 59(10):905-9.

(69) Teske E, Rothuizen J, de Bruijne JJ, Rijnberk A. Corticosteroid-induced alkaline phosphatase isoenzyme in the diagnosis of canine hypercorticism. Vet Rec 1989 July; 125(1):12-4.

(70) Felice LJ, Dombrovskis D, Lafond E et al. Determination of uric acid in canine serum and urine by high performance liquid chromatography. Vet Clin Pathol 1990; 19(3):86-9.

(71) Gerritzen-Bruning MJ, van den Ingh TS, Rothuizen J. Diagnostic value of fasting plasma ammonia and bile acid concentrations in the identification of portosystemic shunting in dogs. J Vet Intern Med 2006 Jan.; 20(1):13-9.

(72) Pinches M. Getting results in clinical pathology 4. Influence of medications on biochemical parameters. In Pract 2006 May; 28(5):274-8.

(73) Moeller EM, Steiner JM, Williams DA, Klein PD. Preliminary studies of a canine 13C-aminopyrine demethylation blood test. Can J Vet Res 2001 Jan.; 65(1):45-9.

(74) Bernardini D, Poser H, Giuliani A et al. Liver function evaluation in dogs by the galactose load test. Vet Res Commun 2005 Aug.; 29 Suppl 2:277-80.

(75) Chiaramonte D, Steiner JM, Broussard JD et al. Use of a 13C-aminopyrine blood test: first clinical impressions. Can J Vet Res 2003 July; 67(3):183-8.

(76) Moeller EM, Steiner JM, Williams DA et al. Kinetic analysis of demethylation of 13C-aminopyrine in healthy dogs. Am J Vet Res 2004 Feb.; 65(2):159-62.

(77) Moeller EM, Steiner JM, Williams DA et al. Comparison of various doses of carbon 13-labeled aminopyrine for a carbon 13-labeled aminopyrine demethylation blood test in healthy dogs. Am J Vet Res 2006 July; 67(7):1110-4.

(78) Neumann S, Welling H, Thuere S. Evaluation of Serum L-phenylalanine Concentration as Indicator of Liver Disease in Dogs: A Pilot Study. J Am Anim Hosp Assoc 2007 July; 43(4):193-200.

(79) Neumann S, Welling H, Thuere S, Kaup FJ. Plasma L-carnitine concentration in healthy dogs and dogs with hepatopathy. Vet Clin Pathol 2007 June; 36(2):137-40.

(80) Toulza O, Center SA, Brooks MB et al. Evaluation of plasma protein C activity for detection of hepatobiliary disease and portosystemic shunting in dogs. J Am Vet Med Assoc 2006 Dec.; 229(11):1761-71.

(81) Kummeling A, Teske E, Rothuizen J, van Sluijs FJ. Coagulation profiles in dogs with congenital portosystemic shunts before and after surgical attenuation. J Vet Intern Med 2006 Nov.; 20(6):1319-26.

(82) Beher WT, Dienst SG, Lin GJ et al. Canine bile acid enterohepatic circulation. Steroids 1982 Oct.; 40(4):413-24.

(83) Center SA, ManWarren T, Slater MR, Wilentz E. Evaluation of twelve-hour preprandial and two-hour postprandial serum bile acids concentrations for diagnosis of hepatobiliary disease in dogs. J Am Vet Med Assoc 1991 July; 199(2):217-26.

(84) Center SA. Serum bile acids in companion animal medicine. Vet Clin North Am Small Anim Pract 1993 May; 23(3):625-57.

(85) Hauge JG, Abdelkader SV. Serum bile acids as an indicator of liver disease in dogs. Acta Vet Scand 1984; 25(4):495-503.

(86) Bartges JW, Osborne CA, Felice LJ et al. Effects of various methods of preservation on the stability of uric acid in frozen canine urine. Am J Vet Res 1996 June; 57(6):787-90.

(87) Hail EJ, German AJ. Laboratory evaluation of hepatic disease. In: Villiers E, Blackwood L, editors. BSAVA Manual of Clinical Pathology. Blackwell Publishing, 2007.

(88) Tisdall PL, Hunt GB, Tsoukalas G, Malik R. Post-prandial serum bile acid concentrations and ammonia tolerance in Maltese dogs with and without hepatic vascular anomalies. Aust Vet J 1995 Apr.; 72(4):121-6.

(89) Kerr MG, van Doorn T. Mass screening of Irish wolfhound puppies for portosystemic shunts by the dynamic bile acid test. Vet Rec 1999 June; 144(25):693-6.

(90) Poldervaart JH, Favier RP, Penning LC et al. Primary hepatitis in dogs: a retrospective review (2002-2006). J Vet Intern Med 2009 Jan.; 23(1):72-80.

APPENDICES

APPENDIX A

VETDIAGNOSTIX CANINE FULL MEDICAL PROFILE REFERENCE RANGES

HAEMATOLOGY

HAEMOGLOBIN	12.0-18.0 g/dl
RBC	5.5-8.5 $x10^{12}$/l
HCT	0.37-0.55 l/l
MCV	60.0-77.0 fl
MCH	19.5-24.5 pg
MCHC	32.0-36.0 g/dl
RDW	11.5-14.0
PLATELETS	175-500 $x10^9$/l
LEUCOCYTE COUNT	6.0-17.0 $x10^9$/l
Neutrophils %	%
Neutrophils	3.0-11.5 $x10^9$/l
Lymphocytes %	%
Lymphocytes	1.0-4.8 $x10^9$/l
Monocytes %	%
Monocytes	0.1-1.4 $x10^9$/l
Eosinophils %	%
Eosinophils	0.1-1.75 $x10^9$/l
Basophils %	%
Basophils	0.0-0.0 $x10^9$/l
LUC %	0.0-4.0 %
LUC	0.0-0.4 $x10^9$/l
Reticulocyte %	0.0-1.5 %
Retics (Absolute)	10-100 $x10^9$/l

BIOCHEMISTRY

Urea	3.6-8.9 mmol/l
Creatinine	70-130 umol/l
Alkaline phosphatase	56-113IU/l
ALT	14-66 IU/l
Total Protein	53-75 g/l
Albumin	25-35 g/l
Sodium	126-157 mmol/l
Potassium	3.6-5.1 mmol/l
Chloride	100-125 mmol /l
Cholesterol	3.0-6.0 mmol/l
Total Bilirubin	0-7 umol/l
Creatine Kinase	46-94 IU/l
AST	15-62 IU/L
Inorganic Phosphate	0.9-1.6 mmol/l
Calcium	2.0-3.0 mmol/l
Lipase	16-188 IU/l
Bicarbonate	27-29 mmol/l
Random Glucose	3.3-5.5 mmol/l
Bile Acids	< 15 umol/l

REFERENCE RANGES FOR OVAH LABORATORY

ALT	9 – 73 IU/l
ALP	65 – 311 IU/l
UREA	3.6 – 8.9 mmol/l
CREATININE	40 – 133 umol/l
BILE ACIDS	<15 umol/l
URIC ACID	<0.06 mmol/l

APPENDIX C

**** COPY REPORT ****
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PATIENT : SPCA DOG 25,SPANIEL-CROSS SEX: F AGE: 48mnths 12kg

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
FULL BLOOD COUNT					
> HAEMOGLOBIN		12.5			12.0-18.0 g/dl
> RBC	5.36			L	5.5-8.5 x10^12/l
> HCT		0.390			0.37-0.55 l/l
> MCV		72.8			60.0-77.0 fl
> MCH		23.3			19.5-24.5 pg
> MCHC	31.9			L	32.0-36.0 g/dl
> RDW		12.2			11.5-14.0
> PLATELETS		385			175-500 x10^9/l
> LEUCOCYTE COUNT		11.13			6.0-17.0 x10^9/l
> Neutrophils %		73.9			%
> Neutrophils		8.23			3.0-11.5 x10^9/l
> Lymphocytes %		17.9			%
> Lymphocytes		1.99			1.0-4.8 x10^9/l
> Monocytes %		3.1			%
> Monocytes		0.35			0.1-1.4 x10^9/l
> Eosinophils %		4.3			%
> Eosinophils		0.47			0.1-1.75 x10^9/l
> Basophils %		0.3			%
> Basophils		0.04			0.0-0.1 x10^9/l
> LUC %		0.4			0.0-4.0 %
> LUC		0.04			0.0-0.4 x10^9/l
RETICULOCYTE PROFILE					
> Reticulocyte %		1.2			0.0-1.5 %
> Retics (Absolute)		64			10-100 x10^9/l
> Urea	3.2			L	3.6-8.9 mmol/l
> Creatinine		71			70-130 umol/l
> Alkaline phosphatase			170	H	56-113 U/l
> ALT		17			14-66 U/l

PATIENT : SPCA,DOG 25 SEX: F AGE: 48mnths 12kg PRE BA

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		10.13			< 15 umol/l

Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.

PATIENT : SPCA,DOG 25 POST

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		22.53			< 25 umol/l

Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.

*** COPY REPORT **** PAGE: 1

PATIENT : SPCA,DOG 23 CROSS-BREED SEX: F AGE: 60 13.7kg

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
FULL BLOOD COUNT					
> HAEMOGLOBIN		13.2			12.0-18.0 g/dl
> RBC		5.64			5.5-8.5 x10^12/l
> HCT		0.400			0.37-0.55 l/l
> MCV		71.5			60.0-77.0 fl
> MCH		23.4			19.5-24.5 pg
> MCHC		32.8			32.0-36.0 g/dl
> RDW		12.0			11.5-14.0
> PLATELETS		345			175-500 x10^9/l
> LEUCOCYTE COUNT		11.93			6.0-17.0 x10^9/l
> Neutrophils %		73.1			%
> Neutrophils		8.73			3.0-11.5 x10^9/l
> Lymphocytes %		13.9			%
> Lymphocytes		1.65			1.0-4.8 x10^9/l
> Monocytes %		10.5			%
> Monocytes		1.25			0.1-1.4 x10^9/l
> Eosinophils %		1.0			%
> Eosinophils		0.12			0.1-1.75 x10^9/l

> Basophils %					%
> Basophils					0.0-0.1 x10^9/l
> LUC %		0.7			0.0-4.0 %
> LUC		0.09			0.0-0.4 x10^9/l
RETICULOCYTE PROFILE					
> Reticulocyte %		0.4			0.0-1.5 %
> Retics (Absolute)		23			10-100 x10^9/l
> Urea	2.7			L	3.6-8.9 mmol/l
> Creatinine		72			70-130 umol/l
> Alkaline phosphatase			174	H	56-113 U/l
> ALT		18			14-66 U/l

PATIENT : SPCA,DOG 23 PRE

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		14.49			< 15 umol/l

Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

PATIENT : SPCA,DOG 23 : POST

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids			38.99	H	< 25 umol/l

Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

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PATIENT : SPCA,DOG 24, POINTER-CROSS SEX: M AGE: 40mnths 15.2kg:

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
FULL BLOOD COUNT					
> HAEMOGLOBIN		14.2			12.0-18.0 g/dl
> RBC		6.56			5.5-8.5 x10^12/l
> HCT		0.405			0.37-0.55 l/l
> MCV		67.3			60.0-77.0 fl
> MCH		22.3			19.5-24.5 pg
> MCHC		34.7			32.0-36.0 g/dl
> RDW			15.4	H	11.5-14.0
> PLATELETS		442			175-500 x10^9/l
> LEUCOCYTE COUNT		13.47			6.0-17.0 x10^9/l
> Neutrophils %		83.1			%
> Neutrophils		11.19			3.0-11.5 x10^9/l
> Lymphocytes %		9.8			%
> Lymphocytes		1.31			1.0-4.8 x10^9/l
> Monocytes %		4.5			%
> Monocytes		0.61			0.1-1.4 x10^9/l
> Eosinophils %		1.7			%
> Eosinophils		0.23			0.1-1.75 x10^9/l
> Basophils %		0.4			%
> Basophils		0.06			0.0-0.1 x10^9/l
> LUC %		0.5			0.0-4.0 %
> LUC		0.07			0.0-0.4 x10^9/l
RETICULOCYTE PROFILE					
> Reticulocyte %		0.5			0.0-1.5 %
> Retics (Absolute)		32			10-100 x10^9/l
> Urea		5.0			3.6-8.9 mmol/l
> Creatinine			146	H	100-130 umol/l
> Alkaline phosphatase	32			L	56-113 U/l
> ALT		27			14-66 U/l

PATIENT : SPCA,DOG 24: PRE BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		11.93			< 15 umol/l

Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

PATIENT : SPCA,DOG 24 POST BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids			32.08	H	< 25 umol/l

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PATIENT : SPCA,DOG 17, CROSS-BREED SEX: M AGE: 12mnths 15.5kg

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
FULL BLOOD COUNT					
> HAEMOGLOBIN		16.5			12.0-18.0 g/dl
> RBC		8.32			5.5-8.5 x10^12/l
> HCT		0.450			0.37-0.55 l/l
> MCV	54.0			L	60.0-77.0 fl
> MCH		19.9			19.5-24.5 pg
> MCHC			36.8	H	32.0-36.0 g/dl
> RDW			19.7	H	11.5-14.0
> PLATELETS	155			L	175-500 x10^9/l
> LEUCOCYTE COUNT		12.23			6.0-17.0 x10^9/l
> Neutrophils %		43.8			%
> Neutrophils		5.36			3.0-11.5 x10^9/l
> Lymphocytes %		35.5			%
> Lymphocytes		4.34			1.0-4.8 x10^9/l
> Monocytes %		3.7			%
> Monocytes		0.46			0.1-1.4 x10^9/l
> Eosinophils %		15.2			%
> Eosinophils			1.86	H	0.1-1.75 x10^9/l
> Basophils %		1.4			%
> Basophils			0.17	H	0.0-0.1 x10^9/l
> LUC %		0.3			0.0-4.0 %
> LUC		0.04			0.0-0.4 x10^9/l
> Comment					
	EOSINOPHILIA				
> Reticulocyte %			2.0	H	0.0-1.5 %
> Retics (Absolute)			166	H	10-100 x10^9/l
> Sodium		150			126-157 mmol/l
> Potassium		4.9			3.6-5.1 mmol/l
> Urea		7.9			3.6-8.9 mmol/l
> Creatinine		99			70-130 umol/l
> Alkaline phosphatase	29			L	56-113 U/l
> ALT		60			14-66 U/l
> Total Protein		70			53-75 g/l
> Albumin			40	H	25-35 g/l
> Calcium		2.80			2.00-3.00 mmol/l

PATIENT : SPCA,DOG 17 PRE

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		8.76			< 15 umol/l
	Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.				

PATIENT : SPCA,DOG 17 SEX: M AGE: 120 POST

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids			26.20	H	< 25 umol/l
	Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.				

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PATIENT : SPCA,DOG 16, GSD-CROSS SEX: M AGE: 13mnths 17.5kg

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
FULL BLOOD COUNT					
> HAEMOGLOBIN		13.3			12.0-18.0 g/dl
> RBC		7.70			5.5-8.5 x10^12/l
> HCT		0.410			0.37-0.55 l/l
> MCV	52.6			L	60.0-77.0 fl
> MCH	17.2			L	19.5-24.5 pg
> MCHC		32.7			32.0-36.0 g/dl
> RDW			16.4	H	11.5-14.0
> PLATELETS	154			L	175-500 x10^9/l
> LEUCOCYTE COUNT		8.81			6.0-17.0 x10^9/l
> Neutrophils %		49.4			%
> Neutrophils		4.35			3.0-11.5 x10^9/l
> Lymphocytes %		32.6			%
> Lymphocytes		2.87			1.0-4.8 x10^9/l
> Monocytes %		6.9			%
> Monocytes		0.60			0.1-1.4 x10^9/l
> Eosinophils %		9.7			%
> Eosinophils		0.85			0.1-1.75 x10^9/l

> Basophils %				%
> Basophils				0.0-0.1 x10^9/l
> LUC %	0.8			0.0-4.0 %
> LUC	0.07			0.0-0.4 x10^9/l
> Comment				
Thrombocytopenia confirmed on blood film.				
> Reticulocyte %	1.0			0.0-1.5 %
> Retics (Absolute)	77			10-100 x10^9/l
> Sodium	147			126-157 mmol/l
> Potassium	4.0			3.6-5.1 mmol/l
> Chloride	109			100-125 mmol/l
> Urea		14.8	H	3.6-8.9 mmol/l
> Creatinine	89			70-130 umol/l
> Alkaline phosphatase	87			56-113 U/l
> AST	24			15-62 U/l
> ALT	25			14-66 U/l
> Total Protein	73			53-75 g/l
> Albumin	32			25-35 g/l
> Creatine Kinase		146	H	46-94 U/l
> Calcium	2.73			2.00-3.00 mmol/l
> Inorganic Phosphate	1.61			0.9-1.9 mmol/l
> Total Cholesterol	6.2			3.0-8.0 mmol/l
> Lipase	93			16-188 U/l

PATIENT : SPCA,DOG 16 PRE

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids	10.38				< 15 umol/l
Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.					

PATIENT : SPCA,DOG 16 : POST

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids			32.32	H	< 25 umol/l
Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.					

**** COPY REPORT **** PAGE: 1

PATIENT : SPCA,DOG 18, BEAGLE SEX: F AGE: 12mnths 16.5kg

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> HAEMOGLOBIN		13.3			12.0-18.0 g/dl
> RBC		6.47			5.5-8.5 x10^12/l
> HCT		0.390			0.37-0.55 l/l
> MCV		61.1			60.0-77.0 fl
> MCH		20.6			19.5-24.5 pg
> MCHC		33.7			32.0-36.0 g/dl
> RDW		13.7			11.5-14.0
> PLATELETS	90			L	175-500 x10^9/l
> LEUCOCYTE COUNT		13.22			6.0-17.0 x10^9/l
> Neutrophils %		51.5			%
> Neutrophils		6.80			3.0-11.5 x10^9/l
> Lymphocytes %		31.0			%
> Lymphocytes		4.11			1.0-4.8 x10^9/l
> Monocytes %		4.7			%
> Monocytes		0.63			0.1-1.4 x10^9/l
> Eosinophils %		11.5			%
> Eosinophils		1.52			0.1-1.75 x10^9/l
> Basophils %		0.8			%
> Basophils			0.11	H	0.0-0.1 x10^9/l
> LUC %		0.4			0.0-4.0 %
> LUC		0.06			0.0-0.4 x10^9/l
> Comment					
Thrombocytopenia confirmed on blood film.					
> Reticulocyte %			1.6	H	0.0-1.5 %
> Retics (Absolute)			104	H	10-100 x10^9/l
> Lipase		143			16-188 U/l
> Total Cholesterol		4.7			3.0-8.0 mmol/l
> Sodium		147			126-157 mmol/l
> Potassium		4.6			3.6-5.1 mmol/l
> Chloride		108			100-125 mmol/l
> Urea		5.1			3.6-8.9 mmol/l
> Creatinine		83			70-130 umol/l
> Alkaline phosphatase		57			56-113 U/l
> AST		27			15-62 U/l
> ALT		27			14-66 U/l

> Total Protein				53-75 g/l
> Albumin			H	25-35 g/l
> Calcium		2.76		2.00-3.00 mmol/l
> Inorganic Phosphate		1.79		0.9-1.9 mmol/l

PATIENT : SPCA,DOG 18 SEX: F AGE: 12mnths : PRE

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		8.30			< 15 umol/l

Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

PATIENT : SPCA,DOG 18 : POST

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		24.74			< 25 umol/l

Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

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PATIENT: SPCA,DOG, 22, CROSS-BREED AGE/SX: 66mnths /M MASS: 11KG

Test	Result	Flag	Reference
HAEMOGLOBIN	15.7		12.0-18.0 g/dl
RBC	6.97		5.5-8.5 x10^12/l
HCT	0.480		0.37-0.55 l/l
MCV	68.7		60.0-77.0 fl
MCH	22.6		19.5-24.5 pg
MCHC	32.9		32.0-36.0 g/dl
RDW	13.4		11.5-14.0
PLATELETS	310		175-500 x10^9/l
LEUCOCYTE COUN	9.03		6.0-17.0 x10^9/l
Neut %	59.6		%
Neutrophils	5.38		3.0-11.5 x10^9/l
Lymphs %	24.1		%
Lymphs	2.18		1.0-4.8 x10^9/l
Monos %	6.2		%
Monocytes	0.56		0.1-1.4 x10^9/l
Eosinophils %	9.0		%
Eosinophils	0.82		0.1-1.75 x10^9/l
Basophils %	0.4		%
Basophils	0.04	H	0.0-0.1 x10^9/l
LUC %	0.7		0.0-4.0 %
LUC	0.06		0.0-0.4 x10^9/l
Retic %	0.9		0.0-1.5 %
Retics (Abs)	63		10-100 x10^9/l
Sodium	147		126-157 mmol/l
Potassium	5.5	H	3.6-5.1 mmol/l
Chloride	108		100-125 mmol/l
Bicarbonate	26	L	27-29 mmol/l
Urea	4.8		3.6-8.9 mmol/l
Creatinine	83		70-130 umol/l
Total Chol	5.9		3.0-8.0 mmol/l
Alk phosph	133	H	56-113 U/l
AST	42		15-62 U/l
ALT	21		14-66 U/l
Total Protein	60		53-75 g/l
Albumin	36	H	25-35 g/l
CK	160	H	46-94 U/l
Calcium	2.69		2.00-3.00 mmol/l
INORGANIC PHOSP	1.85		0.9-1.9 mmol/l
Lipase	118		16-188 U/l

Test	Result	Flag	Reference
Prepandial DOG 22			
Total Bile Acids	13.85		< 15 umol/l

Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

Test	Result	Flag	Reference
Post-prandial DOG 22			
Total Bile Acids	15.13		< 25 umol/l

Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

** END OF REPORT **

PATIENT: SPCA,DOG 20, CROSS-BREED AGE/SX: 72mnths/M MASS: 17.5KG

Test	Result			
HAEMOGLOBIN	14.3			12.0-18.0 g/dl
RBC	6.38			5.5-8.5 x10^12/l
HCT	0.420			0.37-0.55 l/l
MCV	66.2			60.0-77.0 fl
MCH	22.4			19.5-24.5 pg
MCHC	33.9			32.0-36.0 g/dl
RDW	13.7			11.5-14.0
PLATELETS	141		L	175-500 x10^9/l
LEUCOCYTE COUNT	18.30		H	6.0-17.0 x10^9/l
Neut %	50.8			%
Neutrophils	9.30			3.0-11.5 x10^9/l
Lymphs %	37.8			%
Lymphs	4.83		H	1.0-4.8 x10^9/l
Monos %	8.03			%
Monocytes	1.47		H	0.1-1.4 x10^9/l
Eosinophils %	14.1			%
Eosinophils	2.58		H	0.1-1.75 x10^9/l
Basophils %	0.8			%
Basophils	0.15		H	0.0-0.1 x10^9/l
LUC %	0.4			0.0-4.0 %
LUC	0.07			0.0-0.4 x10^9/l
Retic %	2.0		H	0.0-1.5 %
Retics (Abs)	128		H	10-100 x10^9/l
Sodium	148			126-157 mmol/l
Potassium	5.6		H	3.6-5.1 mmol/l
Chloride	110			100-125 mmol/l
Bicarbonate	21		L	27-29 mmol/l
Urea	4.5			3.6-8.9 mmol/l
Creatinine	84			70-130 umol/l
Total Chol	4.1			3.0-8.0 mmol/l
Alk phosph	109			56-113 U/l
AST	45			15-62 U/l
ALT	22			14-66 U/l
Total Protein	65			53-75 g/l
Albumin	35			25-35 g/l
Calcium	2.44			2.00-3.00 mmol/l
INORGANIC PHOSP	2.15		H	0.9-1.9 mmol/l
Lipase	50			16-188 U/l

TOTAL BILE ACID DOG 20
COMMENTS: PRE

Test	Result	Flag	Reference
Total Bile Acids	10.43		< 15 umol/l

Preprandial | Sample submitted to The Faculty of Veterinary Science, | Onderstepoort for analysis

COMMENTS: POST

Test	Result	Flag	Reference
Total Bile Acids	13.38		< 25 umol/l

Post-prandial | Sample submitted to The Faculty of Veterinary Science, | Onderstepoort for analysis.

**** COPY REPORT **** PAGE: 1

PATIENT : SPCA,DOG 21, AUSTALIAN CATTLE DOG SEX: M AGE: 72mnths 14.5kg

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> HAEMOGLOBIN		17.5			12.0-18.0 g/dl
> RBC		7.33			5.5-8.5 x10^12/l
> HCT		0.510			0.37-0.55 l/l
> MCV		69.7			60.0-77.0 fl
> MCH		23.9			19.5-24.5 pg
> MCHC		34.3			32.0-36.0 g/dl
> RDW		12.5			11.5-14.0
> PLATELETS		244			175-500 x10^9/l
> LEUCOCYTE COUNT		12.64			6.0-17.0 x10^9/l
> Neutrophils %		56.1			%
> Neutrophils		7.09			3.0-11.5 x10^9/l
> Lymphocytes %		28.6			%
> Lymphocytes		3.61			1.0-4.8 x10^9/l
> Monocytes %		6.7			%
> Monocytes		0.85			0.1-1.4 x10^9/l
> Eosinophils %		7.4			%
> Eosinophils		0.94			0.1-1.75 x10^9/l
> Basophils %		1.0			%
> Basophils			0.12	H	0.0-0.1 x10^9/l
> LUC %		0.2			0.0-4.0 %
> LUC		0.02			0.0-0.4 x10^9/l
> Reticulocyte %		0.6			0.0-1.5 %
> Retics (Absolute)		44			10-100 x10^9/l
> Sodium		148			126-157 mmol/l

> Potassium					3.6-5.1 mmol/l
> Chloride					100-125 mmol/l
> Bicarbonate	21			L	27-29 mmol/l
> Urea		6.0			3.6-8.9 mmol/l
> Creatinine	65			L	70-130 umol/l
> Alkaline phosphatase		94			56-113 U/l
> AST		48			15-62 U/l
> ALT		31			14-66 U/l
> Total Protein		65			53-75 g/l
> Albumin			40	H	25-35 g/l
> Calcium		2.44			2.00-3.00 mmol/l
> Inorganic Phosphate		1.85			0.9-1.9 mmol/l
> Total Cholesterol		4.5			3.0-8.0 mmol/l
> Lipase		45			16-188 U/l

PATIENT : SPCA,DOG 21 SEX: M AGE: 72mnths `14.5kg PRE BA

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		9.24			< 15 umol/l

Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.

PATIENT : SPCA,DOG 21 : POST

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		24.81			< 25 umol/l

Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.

PATIENT : SPCA,DOG 19 BOXER-CROSS SEX: M AGE: 84MNTHS 18.6kg

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> HAEMOGLOBIN		13.2			12.0-18.0 g/dl
> RBC		5.74			5.5-8.5 x10^12/l
> HCT		0.380			0.37-0.55 l/l
> MCV		66.8			60.0-77.0 fl
> MCH		23.0			19.5-24.5 pg
> MCHC		34.4			32.0-36.0 g/dl
> RDW			15.1	H	11.5-14.0
> PLATELETS	162			L	175-500 x10^9/l
> LEUCOCYTE COUNT		16.54			6.0-17.0 x10^9/l
> Neutrophils %		71.3			%
> Neutrophils			11.78	H	3.0-11.5 x10^9/l
> Lymphocytes %		11.1			%
> Lymphocytes		1.83			1.0-4.8 x10^9/l
> Monocytes %		9.6			%
> Monocytes			1.59	H	0.1-1.4 x10^9/l
> Eosinophils %		7.1			%
> Eosinophils		1.17			0.1-1.75 x10^9/l
> Basophils %		0.4			%
> Basophils		0.07			0.0-0.1 x10^9/l
> LUC %		0.6			0.0-4.0 %
> LUC		0.09			0.0-0.4 x10^9/l
> Reticulocyte %			1.8	H	0.0-1.5 %
> Retics (Absolute)			103	H	10-100 x10^9/l-
> Total Cholesterol		6.9			3.0-8.0 mmol/l
> Lipase		85			16-188 U/l
> Sodium		152			126-157 mmol/l
> Potassium		4.7			3.6-5.1 mmol/l
> Chloride		113			100-125 mmol/l
> Bicarbonate	24			L	27-29 mmol/l
> Urea		7.9			3.6-8.9 mmol/l
> Creatinine		83			70-130 umol/l
> Alkaline phosphatase			155	H	56-113 U/l
> AST		47			15-62 U/l
> ALT			77	H	14-66 U/l
> Total Protein		58			53-75 g/l
> Albumin		31			25-35 g/l
> Calcium		2.44			2.00-3.00 mmol/l
> Inorganic Phosphate			2.01	H	0.9-1.9 mmol/l

CLINICAL INFO : PRE 19

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		13.59			< 15 umol/l

Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.

PATIENT : SPCA,DOG 19

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids			32.63	H	< 25 umol/l
Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.					

SPCA,DOG 9 CROSS-BREED SEX: M AGE: 18mnths 12.8kg
REF DR : VETDIAGNOSTIX PATH REPORT

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
FULL BLOOD COUNT					
HAEMOGLOBIN		17.2			12.0-18.0 g/dl
RBC		8.44			5.5-8.5 x10^12/l
HCT			0.560	H	0.37-0.55 l/l
MCV		66.6			60.0-77.0 fl
MCH		20.4			19.5-24.5 pg
MCHC	30.7			L	32.0-36.0 g/dl
RDW			16.7	H	11.5-14.0
PLATELETS		199			175-500 x10^9/l
LEUCOCYTE COUNT		15.37			6.0-17.0 x10^9/l
Neutrophils %		55.3			%
Neutrophils		8.49			3.0-11.5 x10^9/l
Lymphocytes %		20.5			%
Lymphocytes		3.15			1.0-4.8 x10^9/l
Monocytes %		4.4			%
Monocytes		0.67			0.1-1.4 x10^9/l
Eosinophils %		18.6			%
Eosinophils			2.86	H	0.1-1.75 x10^9/l
Basophils %		0.9			%
Basophils			0.13	H	0.0-0.1 x10^9/l
LUC %		0.3			0.0-4.0 %
LUC		0.05			0.0-0.4 x10^9/l
Comment					
Slide assessment of platelets in keeping with count. Eosinophilia.					
RETICULOCYTE PROFILE					
Reticulocyte %		1.2			0.0-1.5 %
Retics (Absolute)			101	H	10-100 x10^9/l
Sodium		145			126-157 mmol/l
Potassium		3.8			3.6-5.1 mmol/l
Urea		3.7			3.6-8.9 mmol/l
Creatinine		117			70-130 umol/l
Alkaline phosphatase	41			L	56-113 U/l
ALT		28			14-66 U/l
Total Bilirubin		3			0-7 umol/l
Total Protein			77	H	53-75 g/l
Albumin			36	H	25-35 g/l
Creatine Kinase			295	H	46-94 U/l

PATIENT : SPCA,DOG 9 PRE BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		8.30			< 15 umol/l
Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.					

PATIENT : SPCA,DOG 9 POST BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		19.07			< 25 umol/l
Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.					

**** COPY REPORT ****

SPCA,DOG 10 LABRADOR-CROSS SEX: M AGE: 12mnths 24kg

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
FULL BLOOD COUNT					
> HAEMOGLOBIN		15.9			12.0-18.0 g/dl
> RBC		7.19			5.5-8.5 x10^12/l
> HCT		0.470			0.37-0.55 l/l
> MCV		65.4			60.0-77.0 fl
> MCH		22.1			19.5-24.5 pg
> MCHC		33.8			32.0-36.0 g/dl
> RDW		13.4			11.5-14.0

> PLATELETS					175-500 x10^9/l
> LEUCOCYTE COUNT					6.0-17.0 x10^9/l
> Neutrophils %		38.2			%
> Neutrophils		5.54			3.0-11.5 x10^9/l
> Lymphocytes %		44.9			%
> Lymphocytes			6.52	H	1.0-4.8 x10^9/l
> Monocytes %		14.7			%
> Monocytes			2.14	H	0.1-1.4 x10^9/l
> Eosinophils %		0.3			%
> Eosinophils	0.05			L	0.1-1.75 x10^9/l
> Basophils %		1.2			%
> Basophils			0.18	H	0.0-0.1 x10^9/l
> LUC %		0.5			0.0-4.0 %
> LUC		0.08			0.0-0.4 x10^9/l
> Comment					
	Lymphocytosis.				
RETICULOCYTE PROFILE					
> Reticulocyte %		1.1			0.0-1.5 %
> Retics (Absolute)		79			10-100 x10^9/l

Urea		6.5			3.6-8.9 mmol/l
Creatinine		94			70-130 umol/l
Alkaline phosphatase		81			56-113 U/l
ALT		31			14-66 U/l
Total Protein		59			53-75 g/l
Albumin			37	H	25-35 g/l
Calcium		2.77			2.00-3.00 mmol/l

PATIENT : SPCA,DOG 10 PRE BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		8.13			< 15 umol/l
	Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.				

PATIENT : SPCA,DOG 10 POST BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		23.82			< 25 umol/l
	Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.				

PATIENT : SPCA,DOG 11 LABRADOR-CROSS SEX: F AGE: 24 26kg

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
FULL BLOOD COUNT					
> HAEMOGLOBIN			18.8	H	12.0-18.0 g/dl
> RBC		8.50			5.5-8.5 x10^12/l
> HCT			0.560	H	0.37-0.55 l/l
> MCV		65.4			60.0-77.0 fl
> MCH		22.1			19.5-24.5 pg
> MCHC		33.9			32.0-36.0 g/dl
> RDW			14.4	H	11.5-14.0
> PLATELETS		212			175-500 x10^9/l
> LEUCOCYTE COUNT		11.36			6.0-17.0 x10^9/l
> Neutrophils %		53.8			%
> Neutrophils		6.11			3.0-11.5 x10^9/l
> Lymphocytes %		20.5			%
> Lymphocytes		2.33			1.0-4.8 x10^9/l
> Monocytes %		4.8			%
> Monocytes		0.54			0.1-1.4 x10^9/l
> Eosinophils %		19.0			%
> Eosinophils			2.16	*H	0.1-1.75 x10^9/l
> Basophils %		1.5			%
> Basophils			0.17	H	0.0-0.1 x10^9/l
> LUC %		0.3			0.0-4.0 %
> LUC		0.04			0.0-0.4 x10^9/l
> Comment					
	Eosinophilia.				
RETICULOCYTE PROFILE					
> Reticulocyte %		0.6			0.0-1.5 %
> Retics (Absolute)		51			10-100 x10^9/l

Potassium		5.3			3.6-5.1 mmol/l
Urea		3.7			3.6-8.9 mmol/l
Creatinine	69			L	70-130 umol/l
Alkaline phosphatase		78			56-113 U/l

AST					15-62 U/l
ALT					14-66 U/l
Albumin		25			25-35 g/l
Total Protein		75			53-75 g/l
Creatine Kinase			222	H	46-94 U/l

PATIENT : SPCA,DOG 11: PRE BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		14.55			< 15 umol/l

Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

PATIENT SPCA 11 POST BILE ACIDS

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		8.80			< 25 umol/l

Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

PATIENT : SPCA,DOG 12 CROSS-BREED SEX: F AGE: 12mnths 9kg

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
FULL BLOOD COUNT					
> HAEMOGLOBIN		16.2			12.0-18.0 g/dl
> RBC		6.87			5.5-8.5 x10^12/l
> HCT		0.450			0.37-0.55 l/l
> MCV		65.9			60.0-77.0 fl
> MCH		23.7			19.5-24.5 pg
> MCHC		35.9			32.0-36.0 g/dl
> RDW		13.7			11.5-14.0
> PLATELETS		366			175-500 x10^9/l
> LEUCOCYTE COUNT		6.64			6.0-17.0 x10^9/l
> Neutrophils %		72.0			%
> Neutrophils		4.78			3.0-11.5 x10^9/l
> Lymphocytes %		19.0			%
> Lymphocytes		1.26			1.0-4.8 x10^9/l
> Monocytes %		9.0			%
> Monocytes		0.60			0.1-1.4 x10^9/l
> Eosinophils %		0.0			%
> Eosinophils	0.00			L	0.1-1.75 x10^9/l
> Basophils %		0.0			%
> Basophils		0.00			0.0-0.1 x10^9/l
> LUC %		0.0			0.0-4.0 %
> LUC		0.00			0.0-0.4 x10^9/l
> Reticulocyte %		0.9			0.0-1.5 %
> Retics (Absolute)		62			10-100 x10^9/l
> Urea		7.5			3.6-8.9 mmol/l
> Creatinine		87			70-130 umol/l
> Alkaline phosphatase			182	H	56-113 U/l
> ALT		34			14-66 U/l
> Albumin	24			L	25-35 g/l
> Calcium		2.77			2.00-3.00 mmol/l

PATIENT : SPCA,DOG 12 PRE BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		9.70			< 15 umol/l

Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

PATIENT : SPCA,DOG 12 POST BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids			29.74	H	< 25 umol/L

Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

** END OF REPORT **

PATIENT : SPCA,DOG 13 CROSS-BREED SEX: F AGE: 24mnths 13kg

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
FULL BLOOD COUNT					
> HAEMOGLOBIN		14.2			12.0-18.0 g/dl
> RBC		6.36			5.5-8.5 x10^12/l

> HCT				0.37-0.55 l/l
> MCV				60.0-77.0 fl
> MCH	22.3			19.5-24.5 pg
> MCHC	34.7			32.0-36.0 g/dl
> RDW		14.4	H	11.5-14.0
> PLATELETS	402			175-500 x10^9/l
> LEUCOCYTE COUNT	13.47			6.0-17.0 x10^9/l
> Neutrophils %	83.1			%
> Neutrophils	11.19			3.0-11.5 x10^9/l
> Lymphocytes %	9.8			%
> Lymphocytes	1.31			1.0-4.8 x10^9/l
> Monocytes %	4.5			%
> Monocytes	0.61			0.1-1.4 x10^9/l
> Eosinophils %	1.7			%
> Eosinophils	0.23			0.1-1.75 x10^9/l
> Basophils %	0.4			%
> Basophils	0.06			0.0-0.1 x10^9/l
> LUC %	0.5			0.0-4.0 %
> LUC	0.07			0.0-0.4 x10^9/l
RETICULOCYTE PROFILE				
> Reticulocyte %	1.2			0.0-1.5 %
> Retics (Absolute)	61			10-100 x10^9/l
> Urea		9.2	H	3.6-8.9 mmol/l
> Creatinine	82			70-130 umol/l
> Alkaline phosphatase		158	H	56-113 U/l
> ALT	39			14-66 U/l

: SPCA,DOG 13 PRE BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		12.87			< 15 umol/l

Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.

PATIENT : SPCA,DOG 13 POST BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		18.56			< 25 umol/l

Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.

PATIENT : SPCA,DOG 14 STAFF BT CROSS SEX: M AGE: 18mnths 13.4kg

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
FULL BLOOD COUNT					
> HAEMOGLOBIN			18.2	H	12.0-18.0 g/dl
> RBC		8.27			5.5-8.5 x10^12/l
> HCT		0.520			0.37-0.55 l/l
> MCV		63.4			60.0-77.0 fl
> MCH		22.0			19.5-24.5 pg
> MCHC		34.7			32.0-36.0 g/dl
> RDW		13.8			11.5-14.0
> PLATELETS		498			175-500 x10^9/l
> LEUCOCYTE COUNT		8.50			6.0-17.0 x10^9/l
> Neutrophils %		53.2			%
> Neutrophils		4.52			3.0-11.5 x10^9/l
> Lymphocytes %		32.6			%
> Lymphocytes		2.77			1.0-4.8 x10^9/l
> Monocytes %		6.8			%
> Monocytes		0.58			0.1-1.4 x10^9/l
> Eosinophils %		4.3			%
> Eosinophils		0.37			0.1-1.75 x10^9/l
> Basophils %		2.7			%
> Basophils			0.23	H	0.0-0.1 x10^9/l
> LUC %		0.4			0.0-4.0 %
> LUC		0.03			0.0-0.4 x10^9/l
RETICULOCYTE PROFILE					
> Reticulocyte %		0.7			0.0-1.5 %
> Retics (Absolute)		58			10-100 x10^9/l
> Sodium		149			126-157 mmol/l
> Potassium			5.5	H	3.6-5.1 mmol/l

Specimen haemolysed, please treat result with reserve.

> Chloride		110		100-125 mmol/l

> Urea					3.6-8.9 mmol/l
> Creatinine					70-130 umol/l
> Alkaline phosphatase		76			56-113 U/l
> AST		55			15-62 U/l
> ALT		14			14-66 U/l
> Total Protein		63			53-75 g/l
> Albumin			36	H	25-35 g/l

PATIENT : SPCA,DOG 14 PRE

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids			14.33	H	< 15 umol/l

Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.

PATIENT : SPCA,DOG 14 POST BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids			102.43	H	< 25 umol/l

Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.

PATIENT : SPCA,DOG 15 STAFF-BT CROSS SEX: F AGE: 18mnths 15.6kg

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
FULL BLOOD COUNT					
> HAEMOGLOBIN		15.0			12.0-18.0 g/dl
> RBC		6.89			5.5-8.5 x10^12/l
> HCT		0.460			0.37-0.55 l/l
> MCV		67.2			60.0-77.0 fl
> MCH		21.7			19.5-24.5 pg
> MCHC		32.3			32.0-36.0 g/dl
> RDW			14.8	H	11.5-14.0
> PLATELETS		218			175-500 x10^9/l
> LEUCOCYTE COUNT		8.74			6.0-17.0 x10^9/l
> Neutrophils %		40.2			%
> Neutrophils		3.51			3.0-11.5 x10^9/l
> Lymphocytes %		48.4			%
> Lymphocytes		4.23			1.0-4.8 x10^9/l
> Monocytes %		7.1			%
> Monocytes		0.62			0.1-1.4 x10^9/l
> Eosinophils %		2.0			%
> Eosinophils		0.18			0.1-1.75 x10^9/l
> Basophils %		1.4			%
> Basophils			0.12	H	0.0-0.1 x10^9/l
> LUC %		0.8			0.0-4.0 %
> LUC		0.07			0.0-0.4 x10^9/l
RETICULOCYTE PROFILE					
> Reticulocyte %			1.6	H	0.0-1.5 %
> Retics (Absolute)			110	H	10-100 x10^9/l
> Sodium		150			126-157 mmol/l
> Potassium		4.9			3.6-5.1 mmol/l
> Urea		7.2			3.6-8.9 mmol/l
> Creatinine		89			70-130 umol/l
> Alkaline phosphatase	12			L	56-113 U/l
> ALT		60			14-66 U/l
> Total Protein		70			53-75 g/l
> Albumin			42	H	25-35 g/l

PATIENT : SPCA,DOG 15 PRE

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		6.47			< 15 umol/l

Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.

PATIENT : SPCA,DOG 15 POST

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		12.78			< 25 umol/l

Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.

PATIENT : SPCA,DOGS 8 SEX: m AGE: 18mnths DOG 8 17.5kg

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> HAEMOGLOBIN		14.8			12.0-18.0 g/dl
> RBC		6.85			5.5-8.5 x10^12/l
> HCT		0.470			0.37-0.55 l/l
> MCV		68.1			60.0-77.0 fl
> MCH		21.6			19.5-24.5 pg
> MCHC	31.7			L	32.0-36.0 g/dl
> RDW			14.3	H	11.5-14.0
> PLATELETS			539	H	175-500 x10^9/l
> LEUCOCYTE COUNT		11.45			6.0-17.0 x10^9/l
> Neutrophils %		55.6			%
> Neutrophils		6.37			3.0-11.5 x10^9/l
> Lymphocytes %		32.0			%
> Lymphocytes		3.66			1.0-4.8 x10^9/l
> Monocytes %		5.9			%
> Monocytes		0.67			0.1-1.4 x10^9/l
> Eosinophils %		5.2			%
> Eosinophils		0.60			0.1-1.75 x10^9/l
> Basophils %		0.8			%
> Basophils		0.10			0.0-0.1 x10^9/l
> LUC %		0.5			0.0-4.0 %
> LUC		0.05			0.0-0.4 x10^9/l
> Reticulocyte %		1.4			0.0-1.5 %
> Retics (Absolute)		96			10-100 x10^9/l
> Sodium		150			126-157 mmol/l
> Potassium			5.6	H	3.6-5.1 mmol/l
> Chloride		111			100-125 mmol/l
> Urea		7.3			3.6-8.9 mmol/l
> Creatinine		72			70-130 umol/l
> Alkaline phosphatase		82			56-113 U/l
> ALT		42			14-66 U/l
> Total Protein		63			53-75 g/l
> Albumin			39	H	25-35 g/l
> Calcium		2.57			2.00-3.00 mmol/l
> Inorganic Phosphate			2.25	H	0.9-1.9 mmol/l
> Lipase		50			16-188 U/l
> Total Cholesterol		6.6			3.0-8.0 mmol/l

PATIENT : SPCA, DOG 8 PRE PRANDIAL BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		6.38			< 15 umol/l

Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

PATIENT : SPCA,8 SEX: F AGE: 120 : POST PRANDIAL BILE ACID DOG 9

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		18.11			< 25 umol/l

Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

**** COPY REPORT ****

PATIENT : SPCA,DOGS 7 SEX: M AGE: 24mnths 18kg : DOG 7

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> HAEMOGLOBIN			18.4	H	12.0-18.0 g/dl
> RBC			9.71	H	5.5-8.5 x10^12/l
> HCT			0.570	H	0.37-0.55 l/l
> MCV	58.7			L	60.0-77.0 fl
> MCH	19.0			L	19.5-24.5 pg
> MCHC		32.4			32.0-36.0 g/dl
> RDW			20.5	H	11.5-14.0
> PLATELETS		193			175-500 x10^9/l
> LEUCOCYTE COUNT		12.03			6.0-17.0 x10^9/l
> Neutrophils %		65.4			%
> Neutrophils		7.87			3.0-11.5 x10^9/l
> Lymphocytes %		19.3			%
> Lymphocytes		2.33			1.0-4.8 x10^9/l
> Monocytes %		5.0			%
> Monocytes		0.61			0.1-1.4 x10^9/l
> Eosinophils %		8.7			%
> Eosinophils		1.05			0.1-1.75 x10^9/l
> Basophils %		1.2			%
> Basophils			0.14	H	0.0-0.1 x10^9/l

> LUC %					0.0-4.0 %
> LUC					0.0-0.4 x10^9/l
> Comment					
	Anisocytosis ++.Platelets clumped on blood film.				
> Reticulocyte %		1.1			0.0-1.5 %
> Retics (Absolute)			107	H	10-100 x10^9/l
> Sodium		150			126-157 mmol/l
> Potassium		5.3			3.6-5.1 mmol/l
> Chloride		110			100-125 mmol/l
> Urea		8.3			3.6-8.9 mmol/l
> Creatinine		88			70-130 umol/l
> Alkaline phosphatase	42			L	56-113 U/l
> ALT		26			14-66 U/l
> Total Protein		74			53-75 g/l
> Albumin		35			25-35 g/l
> Calcium		2.10			2.00-3.00 mmol/l
> Inorganic Phosphate		1.33			0.9-1.9 mmol/l
> Total Cholesterol		3.7			3.0-6.0 mmol/l
> Lipase		79			16-188 U/l

CLINICAL INFO : DOG 7 PRE PRANDIAL BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		0.10			< 15 umol/l
	Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.				

CLINICAL INFO : DOG 7 POST PRANDIAL BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		11.62			< 25 umol/l
	Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis				

PATIENT : SPCA,DOG 6 SEX: M AGE: 5mnths 13.5kg

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> HAEMOGLOBIN		16.1			12.0-18.0 g/dl
> RBC		6.51			5.5-8.5 x10^12/l
> HCT		0.450			0.37-0.55 l/l
> MCV		69.7			60.0-77.0 fl
> MCH			24.7	H	19.5-24.5 pg
> MCHC		35.4			32.0-36.0 g/dl
> RDW		13.2			11.5-14.0
> PLATELETS		328			175-500 x10^9/l
> LEUCOCYTE COUNT		6.93			6.0-17.0 x10^9/l
> Neutrophils %		44.6			%
> Neutrophils		3.09			3.0-11.5 x10^9/l
> Lymphocytes %		38.8			%
> Lymphocytes		2.69			1.0-4.8 x10^9/l
> Monocytes %		4.6			%
> Monocytes		0.32			0.1-1.4 x10^9/l
> Eosinophils %		10.8			%
> Eosinophils		0.75			0.1-1.75 x10^9/l
> Basophils %		0.9			%
> Basophils		0.06			0.0-0.0 x10^9/l
> LUC %		0.3			0.0-4.0 %
> LUC		0.02			0.0-0.4 x10^9/l
> Reticulocyte %		0.4			0.0-1.5 %
> Retics (Absolute)		26			10-100 x10^9/l
> Sodium		144			126-157 mmol/l
> Potassium		4.6			3.6-5.1 mmol/l
> Urea		4.2			3.6-8.9 mmol/l
> Creatinine		84			70-130 umol/l
> Alkaline phosphatase	37			L	56-113 U/l
> ALT		37			14-66 U/l
> Total Bilirubin		0			0-7 umol/l
> Total Protein			81	H	53-75 g/l
> Albumin		35			25-35 g/l
> Creatine Kinase			234	H	46-94 U/l

PATIENT : SPCA,DOGS SEX: F AGE:: DOG 6 PRE PRANDIAL BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		14.42			< 15 umol/l
	Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.				

SPCA DOG 6 POST PRANDIAL BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids			26.45	H	< 25 umol/l

Specimen haemolysed. Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.

PATIENT : SPCA,DOG 5 SEX: F AGE: 9mnths DOG 5 14kg

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> HAEMOGLOBIN		15.8			12.0-18.0 g/dl
> RBC		6.65			5.5-8.5 x10^12/l
> HCT		0.470			0.37-0.55 l/l
> MCV		71.1			60.0-77.0 fl
> MCH		23.8			19.5-24.5 pg
> MCHC		33.4			32.0-36.0 g/dl
> RDW		12.9			11.5-14.0
> PLATELETS		317			175-500 x10^9/l
> LEUCOCYTE COUNT		12.32			6.0-17.0 x10^9/l
> Neutrophils %		65.3			%
> Neutrophils		8.05			3.0-11.5 x10^9/l
> Lymphocytes %		26.3			%
> Lymphocytes		3.24			1.0-4.8 x10^9/l
> Monocytes %		4.9			%
> Monocytes		0.60			0.1-1.4 x10^9/l
> Eosinophils %		1.8			%
> Eosinophils		0.22			0.1-1.75 x10^9/l
> Basophils %		1.1			%
> Basophils			0.14	H	0.0-0.10 x10^9/l
> LUC %		0.5			0.0-4.0 %
> LUC		0.06			0.0-0.4 x10^9/l
> Reticulocyte %		1.2			0.0-1.5 %
> Retics (Absolute)		80			10-100 x10^9/l
> Sodium		150			126-157 mmol/l
> Potassium			5.4	H	3.6-5.1 mmol/l
> Chloride		109			100-125 mmol/l
> Urea		6.8			3.6-8.9 mmol/l
> Creatinine		74			70-130 umol/l
> Alkaline phosphatase		82			56-113 U/l
> ALT		29			14-66 U/l
> Total Protein		59			53-75 g/l
> Albumin			40	H	25-35 g/l
> Calcium		2.62			2.00-3.00 mmol/l
> Inorganic Phosphate			2.08	H	0.9-1.9 mmol/l
> Total Cholesterol		5.3			3.0-8.0 mmol/l
> Lipase		32			16-188 U/l

DOG 5 PRE PRANDIAL BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		7.01			< 15 umol/l

Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.

DOG 5 POST PRANDIAL BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		11.40			< 25 umol/l

Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.

PATIENT : SPCA,DOGS4 SEX: F AGE: 9mnths DOG 4 15kg

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> HAEMOGLOBIN		16.5			12.0-18.0 g/dl
> RBC		6.79			5.5-8.5 x10^12/l
> HCT		0.510			0.37-0.55 l/l
> MCV		75.7			60.0-77.0 fl
> MCH		24.3			19.5-24.5 pg
> MCHC		32.0			32.0-36.0 g/dl
> RDW		12.6			11.5-14.0
> PLATELETS		396			175-500 x10^9/l
> LEUCOCYTE COUNT			18.73	H	6.0-17.0 x10^9/l
> Neutrophils %		56.3			%

> Neutrophils				3.0-11.5 x10^9/l
> Lymphocytes %				%
> Lymphocytes		5.54	H	1.0-4.8 x10^9/l
> Monocytes %	5.8			%
> Monocytes	1.09			0.1-1.4 x10^9/l
> Eosinophils %	6.6			%
> Eosinophils	1.24			0.1-1.75 x10^9/l
> Basophils %	1.2			%
> Basophils		0.22	H	0.0-0.1 x10^9/l
> LUC %	0.5			0.0-4.0 %
> LUC	0.10			0.0-0.4 x10^9/l
> Reticulocyte %	1.3			0.0-1.5 %
> Retics (Absolute)	88			10-100 x10^9/l
> Sodium	150			126-157 mmol/l
> Potassium	4.9			3.6-5.1 mmol/l
> Chloride	108			100-125 mmol/l
> Urea	5.2			3.6-8.9 mmol/l
> Creatinine	73			70-130 umol/l
> Alkaline phosphatase		119	H	56-113 U/l
> ALT	24			14-66 U/l
> Total Protein	64			53-75 g/l
> Albumin		38	H	25-35 g/l
> Total Cholesterol	4.3			3.0-8.0 mmol/l
> Calcium	2.59			2.00-3.00 mmol/l
> Inorganic Phosphate		1.95	H	0.9-1.9 mmol/l

PATIENT : SPCA,4 SEX: F AGE: 9mnths : DOG 4 PRE PRANDIAL BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		5.91			< 15 umol/l

Specimen haemolysed.
Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.

PATIENT : SPCA,DOGS4 SEX: F AGE: 9mnths DOG 4 POST PRANDIAL BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids			51.23	H	< 25 umol/l

Sample submitted to The Faculty of Veterinary Science, Onderstepoort for analysis.

PATIENT : SPCA,3 SEX: M AGE: 12mnths 20kg : DOG 3

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> HAEMOGLOBIN		17.1			12.0-18.0 g/dl
> RBC		8.05			5.5-8.5 x10^12/l
> HCT		0.510			0.37-0.55 l/l
> MCV		63.0			60.0-77.0 fl
> MCH		21.3			19.5-24.5 pg
> MCHC		33.8			32.0-36.0 g/dl
> RDW			16.3	H	11.5-14.0
> PLATELETS		212			175-500 x10^9/l
> LEUCOCYTE COUNT		12.38			6.0-17.0 x10^9/l
> Neutrophils %		48.8			%
> Neutrophils		6.04			3.0-11.5 x10^9/l
> Lymphocytes %		32.2			%
> Lymphocytes		3.99			1.0-4.8 x10^9/l
> Monocytes %		5.8			%
> Monocytes		0.72			0.1-1.4 x10^9/l
> Eosinophils %		8.9			%
> Eosinophils		1.10			0.1-1.75 x10^9/l
> Basophils %		2.7			%
> Basophils			0.34	*H	0.0-0.1 x10^9/l
> LUC %		1.5			0.0-4.0 %
> LUC		0.19			0.0-0.4 x10^9/l
> Reticulocyte %		0.9			0.0-1.5 %
> Retics (Absolute)		72			10-100 x10^9/l
> Sodium		151			126-157 mmol/l
> Potassium		5.1			3.6-5.1 mmol/l
> Chloride		110			100-125 mmol/l
> Urea		6.3			3.6-8.9 mmol/l
> Creatinine		89			70-130 umol/l
> Alkaline phosphatase		81			56-113 U/l
> ALT		31			14-66 U/l
> Total Protein		70			53-75 g/l
> Albumin			37	H	25-35 g/l
> Calcium		2.31			2.00-3.00 mmol/l

> Inorganic Phosphate		1.9			0.9-1.9 mmol/l
> Total Cholesterol		[illegible]			3.0-8.0 mmol/l
> Lipase		42			16-188 U/l

PATIENT : SPCA,DOGS 3 SEX: F AGE: 120 DOG 3 PRE PRANDIAL BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		8.16			< 15 umol/l

Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

PATIENT : SPCA,DOGS DOG 3 POST PRANDIAL BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		19.08			< 25 umol/l

Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

PATIENT : SPCA,2 STAFF-BT X SEX: F AGE: 24mnths 12kg: DOG 2

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> HAEMOGLOBIN			18.2	H	12.0-18.0 g/dl
> RBC		7.62			5.5-8.5 x10^12/l
> HCT		0.530			0.37-0.55 l/l
> MCV		69.0			60.0-77.0 fl
> MCH		23.9			19.5-24.5 pg
> MCHC		34.7			32.0-36.0 g/dl
> RDW		13.7			11.5-14.0
> PLATELETS		179			175-500 x10^9/l
> LEUCOCYTE COUNT		6.92			6.0-17.0 x10^9/l
> Neutrophils %		47.9			%
> Neutrophils		3.31			3.0-11.5 x10^9/l
> Lymphocytes %		36.4			%
> Lymphocytes		2.52			1.0-4.8 x10^9/l
> Monocytes %		5.5			%
> Monocytes		0.38			0.1-1.4 x10^9/l
> Eosinophils %		8.1			%
> Eosinophils		0.56			0.1-1.75 x10^9/l
> Basophils %		1.6			%
> Basophils			0.11	H	0.0-0.1 x10^9/l
> LUC %		0.5			0.0-4.0 %
> LUC		0.04			0.0-0.4 x10^9/l
> Reticulocyte %		1.5			0.0-1.5 %
> Retics (Absolute)			114	H	10-100 x10^9/l
> Sodium		148			126-157 mmol/l
> Potassium			5.5	H	3.6-5.1 mmol/l
> Chloride		109			100-125 mmol/l
> Urea		5.4			3.6-8.9 mmol/l
> Creatinine		72			70-130 umol/l
> Alkaline phosphatase		62			56-113 U/l
> ALT		25			14-66 U/l
> Total Protein			80	H	53-75 g/l
> Albumin			37	H	25-35 g/l
> Calcium		2.37			2.00-3.00 mmol/l
> Inorganic Phosphate		1.33			0.9-1.9 mmol/l
> Total Cholesterol		5.3			3.0-8.0 mmol/l
> Lipase		67			16-188 U/l

CLINICAL INFO : DOG 2 PRE PRANDIAL BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		5.91			< 15 umol/l

Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

CLINICAL INFO : DOG 2 POST PRANDIAL BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids			38.65	H	< 25 umol/l

SPECIMEN HAEMOLYSED *
Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

PATIENT : SPCA,DOGS 1 SEX: F AGE: 24mnths - 20kg : DOG 1

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE

> HAEMOGLOBIN	[illegible]			12.0-18.0 g/dl
> RBC	5.70			5.5-8.5 x10^12/l
> HCT	0.390			0.37-0.55 l/l
> MCV	67.9			60.0-77.0 fl
> MCH	23.6			19.5-24.5 pg
> MCHC	34.7			32.0-36.0 g/dl
> RDW		14.2	H	11.5-14.0
> PLATELETS	346			175-500 x10^9/l
> LEUCOCYTE COUNT	14.65			6.0-17.0 x10^9/l
> Neutrophils %	60.6			%
> Neutrophils	8.88			3.0-11.5 x10^9/l
> Lymphocytes %	26.7			%
> Lymphocytes	3.91			1.0-4.8 x10^9/l
> Monocytes %	4.4			%
> Monocytes	0.64			0.1-1.4 x10^9/l
> Eosinophils %	6.7			%
> Eosinophils	0.99			0.1-1.75 x10^9/l
> Basophils %	1.2			%
> Basophils		0.18	H	0.0-0.1 x10^9/l
> LUC %	0.4			0.0-4.0 %
> LUC	0.06			0.0-0.4 x10^9/l
> Reticulocyte %		1.9	H	0.0-1.5 %
> Retics (Absolute)		108	H	10-100 x10^9/l
> Sodium	147			126-157 mmol/l
> Potassium		5.4	H	3.6-5.1 mmol/l
> Chloride	108			100-125 mmol/l
> Urea	5.5			3.6-8.9 mmol/l
> Creatinine	85			70-130 umol/l
> Alkaline phosphatase	60			56-113 U/l
> ALT	20			14-66 U/l
> Total Protein	73			53-75 g/l
> Albumin		39	H	25-35 g/l
> Calcium	2.66			2.00-3.00 mmol/l
> Inorganic Phosphate		1.97	H	0.9-1.9 mmol/l
> Total Cholesterol	5.7			3.0-8.0 mmol/l
> Lipase	135			16-188 U/l

CLINICAL INFO : DOG 1 PRE PRANDIAL BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		5.96			< 15 umol/l

Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

CLINICAL INFO : DOG 1 POST PRANDIAL BILE ACID

TEST	LOW	NORMAL	HIGH	FLAG	REFERENCE RANGE
> Total Bile Acids		14.71			< 25 umol/l

Sample submitted to The Faculty of Veterinary Science,
Onderstepoort for analysis.

END OF REPORT *

DOG (Ref.Ran) (SI Units)	BREED	SEX	AGE	MASS	ALT 14 - 66 U/l	56 - 113 U/l	3.6 – 8.9 mmol/l	70 - 130 µmol/l	Pre-BA < 15 µmol/l	Post-BA < 25 µmol/l	DIFF-BA µmol/l	Pre-UA < 0.06 mmol/l	Post-UA <0.06 mmol/l	DIFF-UA mmol/l
1	CROSS	F	24	20	20	60	5.5	85	5.96	14.7	8.74	0.07	0.07	0
2	STAFF BT X	F	24	12	25	62	5.4	72	5.91	38.65	32.74	0.12	0.06	-0.06
3	CROSS	M	12	20	31	81	6.3	89	8.16	19.08	10.92	0.09	0.14	0.05
4	STAFF BT X	F	9	15	24	119	5.2	73	5.91	51.23	45.32	0.11	0.15	0.04
5	STAFF BT X	F	9	14	29	82	6.8	74	7.01	11.4	4.39	0.07	0.09	0.02
6	BULLTERR	M	5	13.5	37	37	4.2	84	14.42	26.45	12.03	0.05	0.06	0.01
7	CROSS	M	24	18	26	42	8.3	88	0.1	11.62	11.52	0.1	0.11	0.01
8	CROSS	M	18	17.5	42	82	7.3	72	6.38	18.11	11.73	0.09	0.11	0.02
9	CROSS	M	18	12.8	28	41	3.7	117	8.3	19.07	10.77	0.1	0.13	0.03
10	LAB X	M	12	24	31	81	6.5	94	8.13	23.82	15.69	0.06	0.07	0.01
11	LAB X	F	24	26	16	78	3.7	69	14.55	8.8	-5.75	0.05	0.05	0
12	CROSS	F	12	9	34	182	7.5	87	9.7	29.74	20.04	0.05	0.06	0.01
13	CROSS	F	24	13	39	158	9.2	82	12.87	18.56	5.69	0.07	0.08	0.01
14	STAFF BT X	M	18	13.4	14	74	8.3	74	14.33	102.3	87.97	0.04	0.14	0.1
15	STAFF BT X	F	18	15.6	60	12	7.2	89	6.47	12.78	6.31	0.04	0.08	0.04
16	GSD X	M	13	17.5	25	87	14.8	89	10.38	32.32	21.94	0.04	0.06	0.02
17	CROSS	M	12	15.5	60	29	7.9	99	8.76	26.2	17.44	0.04	0.08	0.04
18	BEAGLE	F	12	16.5	27	57	5.1	83	8.3	24.74	16.44	0.04	0.08	0.04
19	BOXER X	M	84	18.6	77	155	7.5	83	13.59	32.63	19.04	0.06	0.05	-0.01
20	CROSS	M	72	17.5	22	109	4.5	84	10.43	13.38	2.95	0.05	0.06	0.01
21	AUS CAT D	M	72	14.5	31	94	6	65	9.24	24.81	15.57	0.06	0.06	0
22	CROSS	M	66	11	21	133	4.8	83	13.85	15.13	1.28	0.04	0.05	0.01
23	CROSS	F	60	13.7	18	174	2.7	72	14.49	38.99	24.5	0.03	0.09	0.06
24	POINTER X	M	40	15.2	27	32	5	146	11.93	32.08	20.15	0.12	0.09	-0.03
25	SPAN X	F	48	12	17	170	3.2	71	10.13	22.53	12.4	0.03	0.06	0.03
Descriptive statistics														
N	25	25	25	25	25	25	25	25	25	25	25	25	25	25
Median			18	15.2	27	81	6	83	9.24	23.82	12.4	0.06	0.08	0.01
Mean			29.2	15.83	31.24	89.24	6.26	84.96	9.57	26.76	17.19	0.06	0.083	0.018
Range			5 – 84	9 – 26	14 – 77	12 – 182	2.7 – 14.8	65 – 146	0.1–14.49	8.8-102.3	-5.8 - 88	0.03-0.12	0.05-0.15	-0.06 -0.1
StdDev			23.47	3.9	15.0	49.15	2.48	16.9	3.6	18.75	18.03	0.03	0.03	0.03

Table 1: Complete data set for the control group of dogs designated group 1. Descriptive statistics of median, mean, range and standard deviation (StdDev) given at the bottom of the table. Ref.Ran – Reference Range.

DOG (Ref.Ran) (SI Units)	BREED	SEX	AGE	MASS	ALT 9 - 73 U/l	ALP 65 - 311 U/l	UREA 3.6 – 8.9 mmol/l	CREAT 40 - 133 µmol/l	Pre-BA < 15 µmol/l	Post-BA < 25 µmol/l	DIFF-BA µmol/l	UA < 0.06 mmol/l
1	CROSS	M	11	3	29	27	6.9	97	47.7			0.06
2	GSD	F	9	21	157	104	2.2	64	117.9	374	256.1	0.09
3	ROTTWEILER	F	7	24.5	9	167	1.6	53	140.4	413.6	273.2	0.07
4	BORD COLLIE	F	3	7.8	73	813	2.7	35	186.81			0.07
5	YORK TERR	F	10	2.6	129	141	1.9	37	236.85			0.05
6	BULLDOG X	M	11	16.4	26	37	8		32.6	166.5	133.9	0.08
7	BULLTERR	F	6	13.1	80	640	2.9		239.53			0.09
8	GREYHOUND	F	10	27.7	151	608	1.4	37	37.6	117.8	80.2	0.06
9	BOXER	M	2	1	16	112	3.8	25	18.92			0.07
10	LAB	F	6	19	30	186	1.3	51	192.6	311	118.4	0.09
11	GSD	F	9	15.7	80	130	6.8	62	18.2	199.6	181.4	0.08
12	SPANIEL	M	3	6	22	205	5.1	57	48.2	239	190.8	0.08
13	BULLDOG X	F	8	10.7			2.8	43	36.3	202.5	166.2	0.1
14	DACHSHUND	F	6	3.04	109	213	6.7	36	115			0.11
15	SCHNAUZER	F	10	3.2	53	39	2.1	50	147.95	161.88	13.93	0.08
16	MIN DOB	M	10	2.6	292	251	5.4	58	46.3	454.4	408.1	0.07
17	MIN DACHS	F	7	3.3	105	156	2.3	30	19.1	426	406.9	0.05
18	PUG	F	4	2			1.9		76.08	14.95	-61.13	0.06
19	GSD	M	5	12			4.1	85	17.63	130.09	112.46	0.06
Descriptive statistics												
N	19	19	19	19	16	16	19	16	19	13	13	19
Median			7	7.8	76.5	161.5	2.8	50.5	48.2	202.5	166.2	0.07
Mean			7.21	10.24	85.1	239.31	3.68	51.25	93.46	247.03	175.42	0.074
Range			2 – 11	1 –27.7	9 – 292	27 – 813	1.3 – 8	25 – 97	0.4– 239.53	14.95-454.4	-61.1-408	0.05–0.11
StdDev			2.88	8.42	73.33	234.5	2.15	19.5	76.72	137.01	136.68	0.016

Table 2: Complete data set for the congenital vascular anomaly group of dogs, designated group 2. Descriptive statistics of median, mean, range and standard deviation (StdDev) given at the bottom of the table. Ref.Ran – Reference Range. Not all parameters had data available (blank boxes).

DOG	HISTOPATHOLOGY	ULTRASOUND	ALT	ALP	Pre-BA	Post-BA
1	Biopsy showed Microvascular Dysplasia (MVD)	Microhepatia. Suspected shunt, but scintigraphy did not identify a shunt	29	27	47.7	
2	Intra-hepatic shunt; Biopsy showed features of microvascular dysplasia	Portogram showed Intra-hepatic shunt	157	104	117.9	374
3	Biopsy showed severe microvascular dysplasia	Porto-caval shunt. Scintigraphy- Porto-splenic shunt – 70% shunt fraction.	9	167	140.4	413.6
4	Post mortem did not identify a shunt and histopathology showed diffuse microvascular dysplasia.	Generalised intense hyperechogenicity. Portal vessels poorly demarcated. Severe hepatopathy, suspected shunt	73	813	186.81	
5	Biopsy showed microvascular dysplastic changes. Post mortem confirmed microvascular dysplasia.	Microhepatia	129	141	236.85	
6	Microvascular dysplasia in biopsies.	No shunt seen	26	37	32.6	166.5
7	Not done	Markedly increased echogenicity, portal and hepatic vasculature not identifiable. No shunts seen. Euthenased due to suspected portal vein hypoplasia.	80	640	239.53	
8	Mild microvascular dysplasia associated with Patent Ductus Venosus	Scintigraphy- Porto-caval shunt - 74% shunt fraction	151	608	37.6	117.8
9	Post mortem identified extra-hepatic shunt vessels	Extra-hepatic shunt	16	112	18.92	
10	Not done	Intra-hepatic porto-caval shunt. Scintigraphy- Intra-hepatic shunt - 72% shunt fraction	30	186	192.6	311
11	Extra-hepatic shunt surgically closed; microvascular dysplasia in biopsies	Extra-hepatic shunt	80	130	18.2	199.6
12	Microvascular dysplasia in biopsies	Not done	22	205	48.2	239
13	Extra-hepatic shunt. Microvascular dysplasia in post mortem histopathology	Extra-hepatic shunt			36.3	202.5
14	Not done	Extra-hepatic hilar porto-caval shunt	109	213	115	
15	History of a congenital portosystemic shunt attempting medical management (Original diagnosis at 2 months of age – records lost)	History of a shunt	53	39	147.95	161.88
16	Biopsies showed microvascular dysplasia	Extra-hepatic porto-caval shunt	292	251	46.3	454.4
17	Microvascular dysplasia in biopsies	Scintigraphy- Azygos vein shunt - 63% shunt fraction	105	156	19.1	426
18	Not done	Extra-hepatic shunt			76.08	14.95
19	Microvascular dysplasia in biopsies	Splenic and liver abscesses			17.63	130.09

Table 3: Histopathology findings and ultrasound results for cases in group 2. All cases had either histopathology or ultrasound confirmation of a congenital vascular anomaly, and many cases had suggestive evidence using both modalities. Liver specific biochemistry parameters listed since elevated pre-prandial bile acids was a data-search variable.

DOG (Ref.Ran) (SI Units)	BREED	SEX	AGE	MASS	U/l	U/l	mmol/l	CREAT 40 – 133 µmol/l	Pre-BA < 15 µmol/l	Post-BA < 25 µmol/l	DIFF-BA µmol/l	UA < 0.06 mmol/l
1	DACHSHUND	M	120	8.6			11	103	99.6	111.82	12.22	0.05
2	STAFF BT	M	72	16.2	1479	790			37.02	48.61	11.59	0.06
3	MALTESE	M	72	5.7	238	1642			115.98			0.08
4	MALTESE	F	128	5.8	63	359	5.5	46	20.6	21.68	1.08	0.07
5	GSD	F	120	36		341		84	44.71	53.02	8.31	0.07
6	BULLTERR	M	144	23	27	184	3.8	95	31.56			0.07
7	CROSS	M	60	5.7	64	340	6	69	45.57	44.99	-0.58	0.06
8	CHOW CHOW	F	119	17.5	39	163			19.7	60.8	41.1	0.05
9	RETRIEVER	F	94	32.9	225	1557	11.6	89	84.8			0.17
10	DACHSHUND	F	120	5.5	49	547	14.3	132	52.3	60.67	8.37	0.08
11	FOX TERR	F	148	5.9			9.2	87	28.71			0.07
12	CROSS	M	155	9	43	72	8.4	106	38.34			0.07
13	MALTESE	F	132	4.4	27	135	5	79	29.65	71.28	41.63	0.06
14	SPANIEL	M	144	18	3680	1810	8	80	184.6			0.51
15	MIN DOB	M	16	2.58	18	60	11.4	59	58.72			0.06
16	CROSS	M	35	20	64	1393	4.4	73	32.05			0.08
17	BULLTERR	F	11	19.4	106	115	8.6	100	12.57			0.1
18	BULLTERR	F	216	19.1	209	1356		114	91.6			0.06
19	DACHSHUND	M	24	4.94	16	149	3.4	47	61.54	186.1	124.56	0.07
20	YORK TERR	F	17	1.8	9	117	13.9	71	42.51	95.95	53.44	0.09
21	DACHSHUND	M	60	7.38	652	6865	2.4	66	26.38	78.22	51.84	0.05
22	PUG	M	3	1.72	122	285	3.9		103.42			0.1
23	MALTESE	F	132	4.88	518	1842			130.53			0.07
24	SPANIEL	M	108	13.7			3.2		232.75	257.09	24.34	0.09
25	MALTESE	F	36	4.74	985		4.6	78	123.7	90.74	-32.96	0.05
26	CROSS	M	12	24	5	104	14.6	125	34.6			0.06
27	CROSS	M	48	11.9	60	344	6	79	45.01	53.31	8.3	0.07
Descriptive statistics												
N	27	27	27	27	23	23	21	21	27	14	14	27
Median			94	8.6	64	341	6	80	45.01	66.04	11.91	0.07
Mean			86.89	12.23	378.17	893.35	7.58	84.86	67.72	88.16	25.23	0.092
Range			3 – 216	1.72 – 36	9 - 3680	60- 6865	2.4–14.3	46 – 132	12.57-232.75	21.68-257	-0.6-125	0.03-0.51
StdDev			56.53	9.37	806.36	1444.92	3.92	22.92	53.25	62.6	36.94	0.09

Table 4: **Complete data set for the liver disease group of dogs, designated group 3. Descriptive statistics of median, mean, range and standard deviation (StdDev) given at the bottom of the table. Ref.Ran – Reference Range. Not all parameters had data available (blank boxes).**

DOG	HISTOPATHOLOGY	ULTRASOUND	ALT	ALP	Pre-BA	Post-BA
1	Not done	Diffuse hyperechoic changes typical of fatty liver, adrenal tumour			99.6	111.82
2	Not done	Diffuse marked hyperechogenicity, suspected steroid hepatopathy due to hyperadrenocorticism confirmed with ACTH-stimulation test.	1479	790	37.02	48.61
3	Biopsies showed chronic cholangitis	No shunt vessels identified, cholangitis, suspected choleliths	238	1642	115.98	
4	Not done	Echocardiography – Mitral and tricuspid valve insufficiency and severe pulmonary hypertension	63	359	20.6	21.68
5	Centrilobular necrosis with duplication of central veins probably due to congestive heart failure.	Moderate hepatomegaly, diffuse hypoechogenicity with uneven surface. No shunts identified.		341	44.71	53.02
6	Well-differentiated hepatocellular carcinoma identified in biopsies	Hepatic mass in medial right liver lobe, suspect neoplasia	27	184	31.56	
7	Biopsies showed centrilobular necrosis due to severe hypoglycaemia as result of severe Cryptosporidiosis	Not done	64	340	45.57	44.99
8	Hepatocellular carcinoma identified in biopsies	Cranio-ventral abdominal mass. Splenic or liver tumour suspected.	39	163	19.7	60.8
9	Post mortem histopathology showed severe diffuse chronic cirrhosis, due to suspected aflatoxin poisoning	Nodular liver with loss of normal echo-texture, query hepatic neoplasm	225	1557	84.8	
10	Chronic centrilobular to bridging necrosis with marked periportal extramedullary haemopoiesis, most likely due to chronic IMHA	Diffusely hypoechoic liver, hepatomegaly.	49	547	52.3	60.67
11	Not done	Echocardiography – Severe tricuspid valve regurgitation			28.71	
12	Numerous mast cell tumours identified at post mortem. Unable to determine if primary in the liver or metastasis	Multifocal lesions in liver and spleen of mixed echogenicity, suspected tumours	43	72	38.34	
13	Not done	Nodular liver margins, shrunken liver. Hepatic fibrosis/cirrhosis. No evidence of a shunt seen.	27	135	29.65	71.28
14	Not done	Generalised hyperechogenicity with rounded margins, numerous intra-hepatic tubular structures, probable bile duct distension. Cholangiohepatitis secondary to chronic pancreatitis.	3680	1810	184.6	
15	Not done	Mild hypoechogenicity, no shunt vessels seen, increased CVC flow. Secondary hepatopathy due to chronic IMHA	18	60	58.72	
16	Not done	Mild hepatomegaly, diffuse hyperechogenicity,no shunt vessels seen but prominent portal vasculature	64	1393	32.05	
17	Moderate, diffuse hepatic cirrhosis with severe biliary stasis. Suspected toxic aetiology but toxin not identified.	Referring vet reported microhepatia on ultrasound.	106	115	12.57	
18	Moderate, chronic centrilobular to bridging necrosis diagnosed at post mortem.	Splenic and liver nodular hyperplasia	209	1356	91.6	
19	Not done	Microhepatia, no shunt vessels identified. Suspected chronic toxicity.	16	149	61.54	186.1
20	Not done	Diffusely hyperechoic, diffuse hepatic abnormality	9	117	42.51	95.95
21	Multifocal necrotic hepatitis secondary to granulomas	Distended gall bladder but not bile ducts. Focal hypoechoic lesion	652	6865	26.38	78.22

	diagnosed in biopsies. Suspected Spirocercosis	crar				
22	Not done	Abnormal echogenicity in some lobes, shunt ruled out. Aberrant larval migrans diagnosed.	122	285	103.42	
23	Not done	Microhepatia, with irregular margins and diffuse mild hypoechogenicity consistent with fibrosis/cirrhosis. Shunts ruled out.	518	1842	130.53	
24	Chronic active hepatitis diagnosed in biopsies	Moderately diffuse hypoechogenicity, early cirrhosis			232.75	257.09
25	Not done	Distended bile ducts, cholangitis/cholangiohepatitis, possible pancreatic mass. No shunt seen.	985		123.7	90.74
26	Multiple metastatic lesions found in liver lobes from an oesophageal osteosarcoma at post mortem	Nodules or masses seen on ultrasound in more than one lobe.	5	104	34.6	
27	Not done	Diffuse hypoechogenicity with nodular margin suggesting fibrosis/cirrhosis. No shunts seen.	60	344	45.01	53.31

Table 5: Histopathology findings and ultrasound results for cases in group 3. Thirteen cases had histopathology confirmation of liver pathology. Twenty-four cases had ultrasound findings suggesting a hepatopathy, but no shunt vessels were seen in ultrasound scans. Two cases of hepatic congestion had echocardiographic confirmation of right-sided heart failure. Liver specific biochemistry parameters are listed since elevated pre-prandial bile acids was a data-search variable.

DOG (Ref.Ran) (SI Units)	BREED	SEX	AGE	MASS	ALT 9 - 73 U/l	ALP 66 - 311 U/l	UREA 3.6 – 8.9 mmol/l	CREAT 40 - 133 µmol/l	Pre-BA < 15 µmol/l	UA < 0.06 mmol/l
1	YORK TERR	M	145	2.92	50	23	70	367	13.83	0.08
2	BOERBOEL	M	48	45			23.1	322	10.3	0.17
3	ROTTWEILER	F	48	37.56			65.2	1167	15.51	0.05
4	GSD	M	66	26.4			49.4	345	17.34	0.08
5	ROTTWEILER	M	70	45			71.7	1641	19.12	0.04
6	FOX TERR	F	60	6.26			125.4	1157	18.64	0.07
7	MIN PINSCH	M	60	5.4			60	426	16.7	0.06
8	BOERBOEL	M	18	29.8	70	153	114.8	1749	19.98	0.06
9	RIDGEBACK	F	12	36	124	152	38.7	584	15.78	0.18
10	GSD	M	156	20.1	86	351	26.9	255	19.72	0.06
11	CROSS	F	120	22			78.4	1072	16.54	0.05
12	CROSS	M	30	41			59.7	480	20.0	0.06
Descriptive statistics										
N	12	12	12	12	4	4	12	12	12	12
Median			60	28.10	78	152.5	62.6	532	17.02	0.06
Mean			69.42	26.45	82.5	219.75	64.44	797.08	16.96	0.08
Range			12 – 156	2.92 – 45	50 – 124	23 – 351	23.1–125.4	255 - 1749	10.3 - 20	0.04–0.18
StdDev			47.07	15.35	31.34	229.12	32.24	535.37	2.88	0.05

Table 6: **Complete data set for the renal disease group of dogs, designated group 4. Descriptive statistics of median, mean, range and standard deviation (StdDev) given at the bottom of the table. Ref.Ran – Reference Range. Not all parameters had data available (blank boxes).**

DOG	HISTOPATHOLOGY		UREA	CREATININE	URINE SG	PHOSPHATE
1	Not done	Bilateral chronic degenerative changes (intensely hyperechoic) consistent with chronic renal failure.	70	367	1.015	1.73
2	Not done	Bilateral moderate hydronephrosis, ureteral calculi and urethral calculi	23.1	322	1.014	
3	Not done	Severe bilateral pyelonephritis resulting in acute renal failure	65.2	1167	1.012	4.63
4	Extensive nephrocalcinosis bilaterally and medullary fibrosis of the right kidney found at post mortem	Not done	49.4	345	1.018	
5	Chronic diffuse renal pathology including glomerulonephritis and interstitial fibrosis diagnosed at post mortem	Bilateral hyperechoic cortices	71.7	1641	1.016	4.95
6	Chronic interstitial nephritis found at post mortem.	Not done	125.4	1157		11.35
7	Bladder wall biopsies showed moderate, diffuse epithelial hyperplasia and fibrosis.	Severe hydronephrosis, bilateral hydroureters. Bladder and urethral uroliths	60	426	1.012	3.02
8	Post mortem showed moderate cortical acute tubular necrosis and multifocal, ongoing tubulo-interstitial nephritis.	Prominent medullary rim signs bilaterally indicating chronic renal disease.	114.8	1749	1.015	5.18
9	Not done	Bilateral hyperechogenicity of renal cortices, unilateral ureteral dilation. Suspect acute exacerbation of chronic renal disease	38.7	584	1.022	4.45
10	Not done	Severe cortical hyperechogenicity typical of chronic renal disease	26.9	255	1.012	
11	Not done	Bilateral hyperechoic cortices, right kidney has mild pelvic dilatation. (Acute renal failure – Ethylene glycol toxicity)	78.4	1072	1.008	3.04
12	Not done	Bilateral cortical hyperechogenicity with loss of corticomedullary junction suggesting acute component.	59.7	480	1.018	6.3

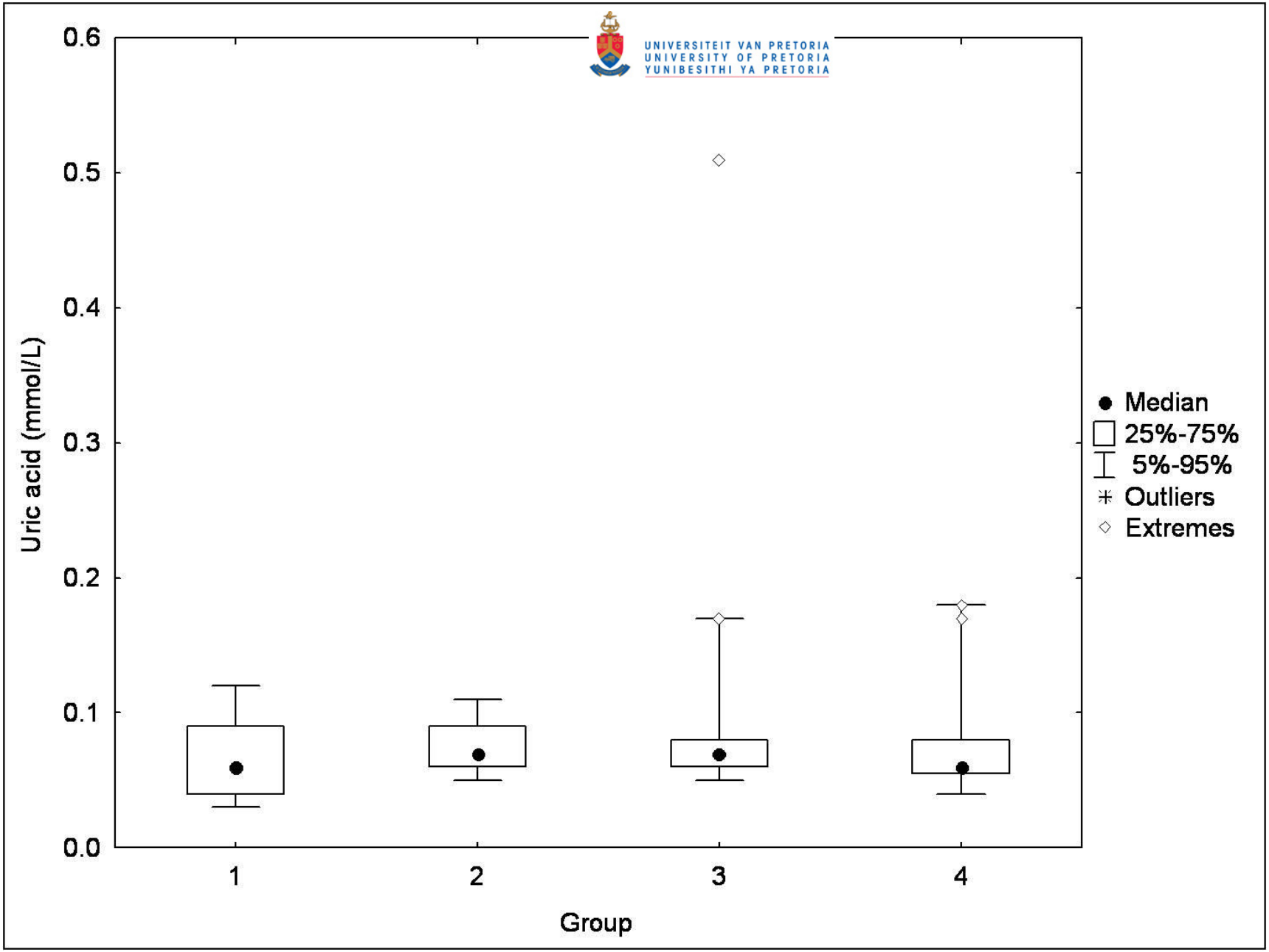
Table 7: Histopathology findings and ultrasound results for cases in group 4. Five cases had histopathology confirmation of renal pathology. Ten cases had ultrasound findings suggesting renal pathology. Supportive clinical pathology data such as hyperphosphataemia (>1.6 mmol/l) and inappropriately-dilute urine specific gravity shown too (where available).

VARIABLE	GROUP 1	GROUP 2	GROUP 3	GROUP 4
Group Size	25	19	27	12
Pre-BA	25	19	27	12
Post-BA	25	13	14	0
Uric acid	25	19	27	12
DIFF-UA	25	0	0	0
DIFF-BA	25	13	14	0

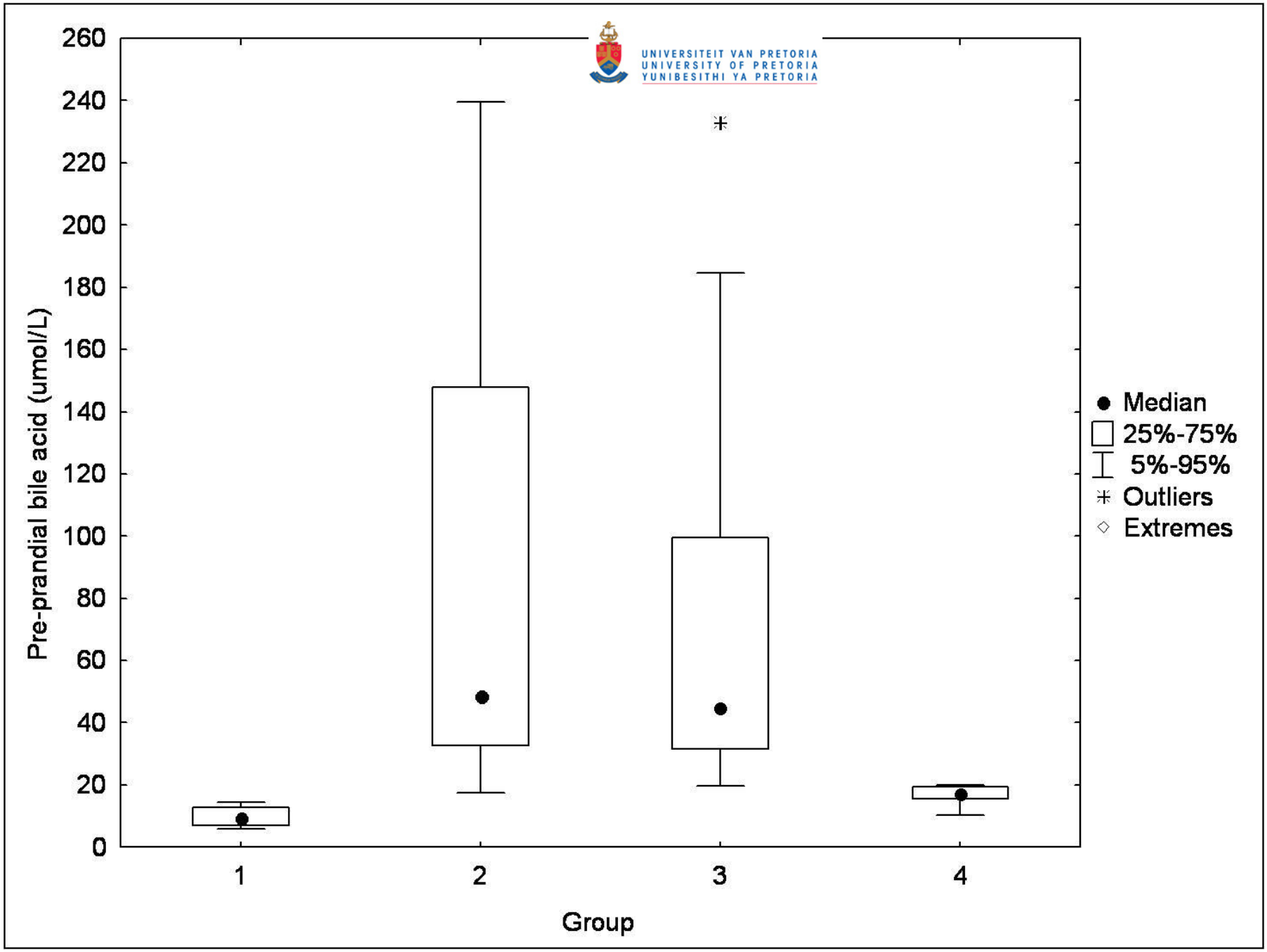
Table 8. The frequency for each variable in each group.

VARIABLE	Grou			Group 4	P-value
URIC ACID					
N	25	19	27	12	
"^" median	0.06[a]	0.07[a]	0.07[a]	0.06[a]	0.1625^
mean	0.06	0.07	0.09	0.08	
std dev	0.03	0.02	0.09	0.05	
Pre-BILE ACID					
N	25	19	27	12	
median	9.24[a]	48.2[b]	45.01[b]	17.02[a]	<0.0001
mean	9.57	93.46	67.72	16.96	
std dev	3.6	76.72	53.25	2.88	
Post-BILE ACID					
N	25	13	14	0	
median	23.82[a]	202.5[b]	66.04[b]		<0.0001
mean	26.76	207.03	88.16		
std dev	18.75	137.01	62.6		
DIFF-BA					
N	25	13	14	0	
median	12.4[a]	166.2[b]	11.91[a]		0.0003
mean	17.19	175.42	25.23		
std dev	18.03	136.68	36.94		

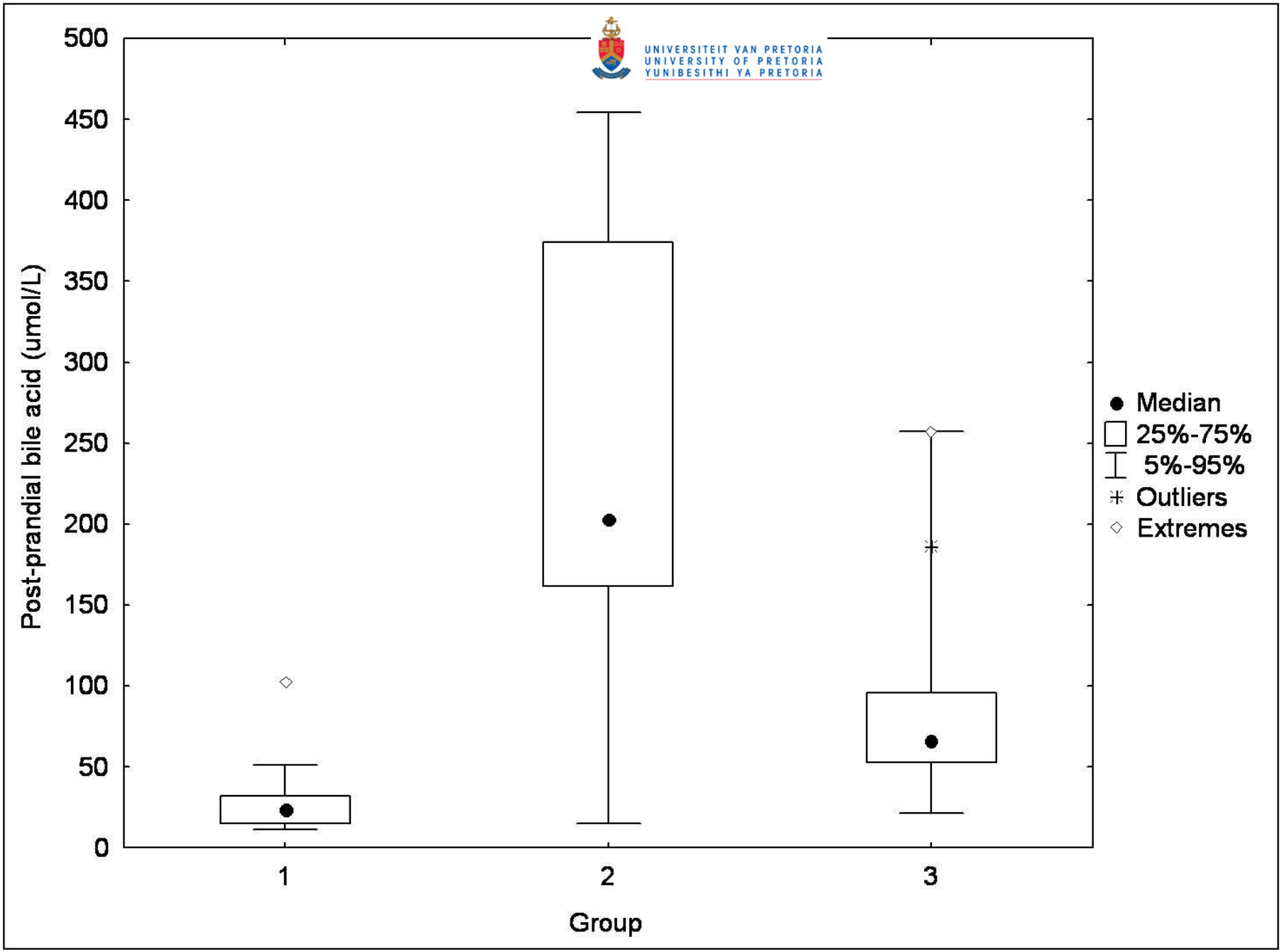
Table 9: Medians and means for uric acid, pre- & post-prandial bile acids and DIFF-bile acids within each group are listed. The medians were compared by the Kruskal-Wallis method. Different superscripts* denote where the median for any variable differs significantly (P < 0.05) between groups according to the Kruskal-Wallis comparison. A different superscript letter (a, b or c) indicates a median value that differs significantly from the others. "^"The uric acid medians for all 4 groups did not differ statistically significantly.



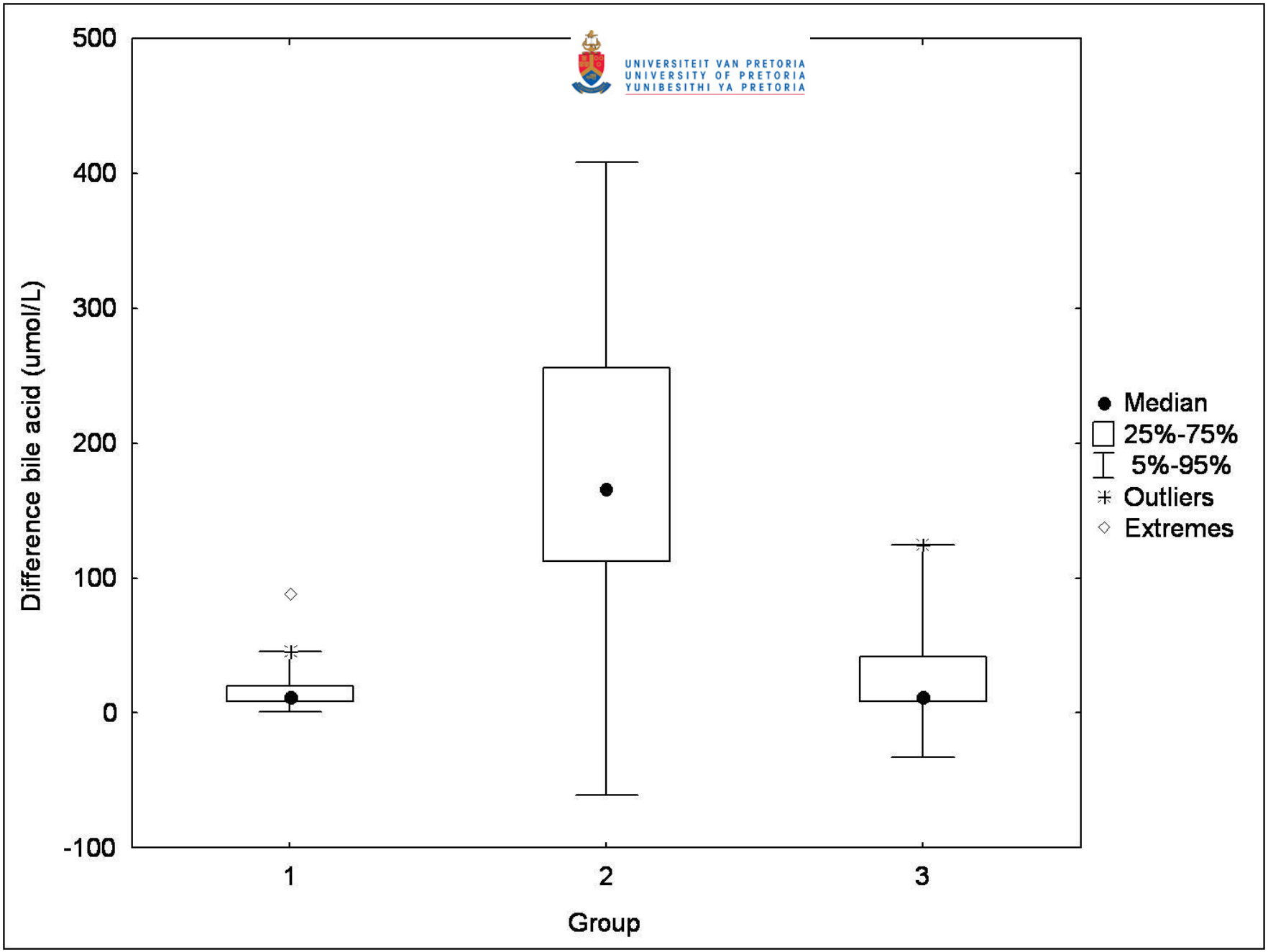
Box Plot 1: The distribution of uric acid for all four groups. The medians are indicated by a dot.



Box Plot 2: The distribution of pre-prandial bile acid for all four groups. The medians are indicated by a dot.



Box Plot 3: The distribution of post-prandial bile acid for groups 1, 2 and 3. The medians are indicated by a dot.



Box Plot 4: The distribution of DIFF-BA (numerical difference between pre- and post-prandial bile acid values) for groups 1, 2 and 3. The medians are indicated by a dot.

Comparing within a group							
Group 1 - Uric Acid							
	Pre-prandial		Post-prandial		difference		p-value
N	25		25		25		
median	0.06		0.08		0.02		0.0007
mean	0.06		0.093		0.02		
std dev	0.037		0.043		0.03		

Table 10: Comparison of the pre-prandial and post-prandial uric acid results for group 1. The Wilcoxon test for paired differences showed a significant difference (P < 0.05).

LIVER DISEASE				
TEST		***True (present)*** *Group 2 + 3*	***False (absent)*** ***Group 1 + 4***	PREDICTIVE VALUES
UA > 0.06 mmol/l	***POSITIVE***	30	15	PPV = 66%
	NEGATIVE	16	22	NPV = 58%
		SENSITIVITY **65%**	SPECIFICITY **59%**	

Table 11: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) calculations for uric acid as a test for liver disease in this population of dogs.

GROUP	1	2	3	4	Total
UA > 0.06mmol/l Frequency Percentage	 10 40.00	 13 68.42	 17 62.96	 5 41.67	 45
UA between 0 – 0.06mmol/l frequency percentage	 15 60.00	 6 31.58	 10 37.04	 7 48.33	 38
Total	25	19	27	12	83

Table 12: Chi-square analysis for uric acid being within or greater than the reference range (0 – 0.06 mmol/l). Chi-Square = 0.1595.

CONGENITAL VASCULAR LIVER DISEASE				
TEST		***True (present) Group 2***	***False (absent) Group 1 + 4***	PREDICTIVE VALUES
UA > 0.06 mmol/l	***POSITIVE***	13	15	PPV = 46%
	NEGATIVE	6	22	NPV = 79%
		SENSITIVITY **68.4%**	SPECIFICITY **59.45%**	

Table 13: Sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) of uric acid for detecting vascular anomalies in this population of dogs (Ref. range 0 – 0.06 mmol/l).

PARENCHYMAL LIVER DISEASE				
TEST		***True (present) Group 3***	***False (absent) Group 1 + 4***	PREDICTIVE VALUES
UA > 0.06 mmol/l	***POSITIVE***	17	15	PPV = 53%
	NEGATIVE	10	22	NPV = 69%
		SENSITIVITY **62.9%**	SPECIFICITY **59.5%**	

Table 14: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of uric acid for detecting primary and secondary parenchymal liver disease in this population of dogs (Ref. range 0 – 0.06 mmol/l).

LIVER DISEASE				
TEST		***True (present) Group 2 + 3***	***False (absent) Group 1 + 4***	PREDICTIVE VALUES
BA > 15 µmol/l	***POSITIVE***	46	10	PPV = 82%
	NEGATIVE	2	25	NPV = 93%
		SENSITIVITY **96%**	SPECIFICITY **71%**	

Table 15: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) calculations for pre-prandial bile acids as a test for liver disease in this population of dogs.

CONGENITAL VASCULAR LIVER DISEASE				
TEST		***True (present)*** ***Group 2***	***False (absent)*** Group 1 + 4	PREDICTIVE VALUES
BA > 15 µmol/l	***POSITIVE***	19	10	PPV = 66%
	NEGATIVE	0	27	NPV = 100%
		SENSITIVITY **100%**	SPECIFICITY **72.9%**	

Table 16: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of pre-prandial bile acids for detecting vascular anomalies in this population of dogs (Ref. Range < 15 µmol/l).

PARENCHYMAL LIVER DISEASE				
TEST		***True (present)*** ***Group 3***	***False (absent)*** ***Group 1 + 4***	PREDICTIVE VALUES
BA > 15 µmol/l	***POSITIVE***	26	10	PPV = 72%
	NEGATIVE	1	27	NPV = 96%
		SENSITIVITY **96.2%**	SPECIFICITY **76.9%**	

Table 17: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV)of pre-prandial bile acids for detecting primary and secondary parenchymal liver disease in this population of dogs (Ref. Range < 15 µmol/l).

TESTS		UA > 0.06mmol/l	UA ≤ 0.06mmol/l	TOTAL
BA ≤ 15umol/l	number	13	15	28
	percent	15.66	18.07	33.73%
BA > 15umol/l	number	32	23	55
	percent	38.55	27.71	66.27%
TOTAL		45	38	N = 83

Table 18: Frequency table for all 4 groups with respect to results for uric acid and pre-prandial bile acids falling within reference range and above reference range.