



Study on Alterations in Urinary and Faecal Parameter in Dogs with Hepatic Dysfunction

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ABSTRACT

The present analysis was conducted from September 2021 to April 2022 at Dr. I.P. Singh Veterinary Clinical Complex and Trauma Centre, College of Veterinary and Animal Sciences, G.B.P.U.A. & T, Pantnagar U.S. Nagar (Uttarakhand) with an aim to investigate variations in urinary and faecal profiles of dogs affected with hepatic dysfunction. Urine samples of 18 dogs affected with hepatic dysfunction were subjected to physical, chemical and microscopic analysis. Analysis resulted with low specific gravity, acidic pH and yellow to dark yellow colour with presence of urobilinogen and bilirubin in the samples. Microscopic crystals for instance ammonium biurate and bilirubin were observed in urine of dogs with hepatic damage. Clay-coloured pasty faeces were observed in dogs with bile-duct blockage, and analysis of faecal samples was useful in identifying parasitic etiology of hepatic dysfunction.

HIGHLIGHTS

- Microscopically crystals of ammonium biurate and bilirubin were observed in urine of dogs with hepatic damage.
- Clay-coloured pasty faeces were observed in dogs with bile-duct blockage.

Keywords: urobilinogen, microscopic, faecal, crystals, hepatic, bile-duct

Liver is a critical organ in the body that accomplishes a number of different activities including metabolism, defence, digestion, detoxification, nutrient repository and many others (Kalra *et al.*, 2022). The liver is segmented into various lobes that are located between the diaphragm and the stomach: the right lateral and medial lobes, the left lateral and medial lobes, the quadrate lobe, and the caudate lobe, which has a papillary process and a caudate process (Larson, 2016). As the liver is engaged in varied metabolic tasks throughout the body, any factor that significantly affects its normal physiology often results in hepatic damage. Dogs with hepatic dysfunction usually are asymptomatic or remain unnoticed until there is about

70 percent loss of functional capacity and signs exhibited are often non-specific (Bexfield and Watson, 2009). In early stages of disease dogs have a variety of symptoms such as anorexia, depression, weight loss, diarrhoea and vomiting. In advanced cases, clinical manifestations may include ascites, icterus, polyuria and polydipsia, as well as nervous signs suggesting hepatic encephalopathy (Rutgers

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and Haywood, 1988). Due to the presence of range of clinical signs, diagnosis of hepatic dysfunction requires a number of procedures. It is only possible to detect functional impairment of the liver once more than 55 % of the functional capacity of hepatocytes are compromised (Elhibu *et al.*, 2015). Various tests that include haematology, serum biochemical analysis, carbohydrate metabolism, fat metabolism, protein metabolism, urine analysis, faecal examination, radiography and ultrasonography are commonly used in diagnosis of hepatic dysfunction (Kozat and Sepehrizadeh, 2017). Urinalysis is a important technique that may uncover numerous illnesses that would otherwise go unreported and untreated due to the lack of obvious signs or symptoms (Parrah *et al.*, 2016). Analysis of urine is of paramount importance in diagnosis of liver dysfunction and dogs may have urine with low specific gravity and have a presence of bilirubin or urobilinogen crystals (Bexfield and Watson, 2009). Moreover, urine analysis can also detect other various underlying disorders such as immune mediated intravascular haemolysis with raised haemoglobin values and diabetes mellitus via glucose and ketones concentrations in urine (Finco, 1997). There is not much documented research work regarding alteration in urine and faecal profile in canine hepatic dysfunction. Thus, the present study was done to evaluate various changes in urine and faeces of dogs that were diagnosed with hepatic dysfunction

MATERIALS AND METHODS

The present study was carried out among dogs presented in outpatient department at Dr. I.P. Singh Veterinary Clinical Complex and Trauma Centre, College of Veterinary and Animal Sciences, G.B.P.U.A. & T., Pantnagar U.S. Nagar (Uttarakhand) during the period of September 2021 to April 2022. Clinical cases of dogs presented with a variety of clinical signs such as fever, vomition, anorexia, diarrhoea, cachexia, lethargy, icterus, ascites, polydipsia, polyuria, changes in colour of urine to dark yellowish and correlating nervous signs were screened on the basis of haematological, biochemical, urinary and faecal analysis, abdominal ultrasonography and radiography. For urinary analysis, samples were collected from 18 dogs affected with hepatic dysfunction on day of presentation from the mid-stream of the voided urine or by passing of urinary catheter. Approximately 10 ml of urine thus collected in a sterile vial and subjected to physical, microscopic and

chemical examination. Urine was preserved toluene as and when required. Physical examination was conducted to look for changes in the urine colour, odour, and turbidity. Microscopic examination involved centrifugation of 5 ml urine sample at 1500 rpm for 5 -6 minutes. The maximum supernatant was discarded and the remaining supernatant (approximately 1ml) was thoroughly mixed with sediment so that they could thoroughly resuspend in urine. A drop of this mixture was then transferred to a clean glass slide and was covered with a cover slip. The slide was then observed under 100X and 400X magnification of microscope to observe urinary crystals and casts respectively. Chemical analysis was carried out using dipstick method to evaluate urobilinogen, bilirubin, glucose, protein, ketones, pH and erythrocytes in urine. Urinary specific gravity was measured with the help of urinometer where approximately 25 ml of urine was placed in a measuring cylinder and urinometer dipped in the urine and reading was taken at eye level and from the lower meniscus of urine (Sink and Feldmann, 2004). Analysis of faecal samples for evaluation of eggs, larvae, oocysts and cysts of various parasites was performed with the help of microscopic examination given by Soulsby (1982). About 1-2 g of faecal sample was collected on day of presentation with the help of gloved finger in a sterile collection vial for further diagnosis. For observation of various parasitic, larvae, cysts, eggs and oocysts in faecal smears, faecal sample was directly viewed under low power of microscope (40 × and 100X); salt flotation technique was followed where collected faecal matter was mixed homogenously in porcelain mortar with addition of saturated salt solution and transferred in a clean glass container. The mixture was then left for 20 minutes so that eggs can float at the top. The top layer was at last placed on a clean microscope slide and observed under microscope.

RESULTS AND DISCUSSION

Physical examination of urine

Physical examination was conducted to evaluate change in colour, odour, turbidity of the urine. Out of 18 dogs included in the study 13 (72.22%) dogs had acidic pH while 5 (27.78%) dog showed alkaline pH. pH is not a specific indicator as it can be altered with various factors such as protein intake, fever, vomition, acidosis, alkalosis

and bacterial infections. Generally, conditions such as fever, extensive vomition, metabolic acidosis causes a shift in pH towards acidic side. Bacterial infection mainly by *Staphylococcus aureus* and *Proteus* spp., metabolic alkalosis, low protein intake in diet are associated with alkaline urine (Yadav *et al.*, 2020). Dark yellow urine was seen in 10 (55.56%) dogs, reddish-brown urine in 2 (11.11%) dogs, brownish-yellow urine in 3 (16.67%) dogs while normal straw-coloured urine was seen in 2 (11.11%) dogs diagnosed with hepatic dysfunction (Fig 1-3).

All dogs presented with hepatic dysfunction showed slight ammoniac smell on urine. Dark yellow urine is seen due to increased urobilinogen excreted in the urine and usually occurs as a result of fever, haemolysis, hepatocellular damage and dehydration (Cornelius, 1958; Yadav *et al.*, 2020). Brownish yellow or greenish yellow urine is due to increased bilirubin in urine and is generally seen in icterus (Cornelius, 1958). Reddish brown urine generally occurs as a result of haemoglobin in urine (Kelly, 1967). Turbidity in urine was seen in 4 (22.22%) cases while in 10 (55.56%) cases turbidity was absent. Turbidity often results due to blood cells, bacteria, urinary crystals, mucus, lipids in the urine (Brobst and Duane, 1989). In this study turbidity in urine was attributed to presence of crystals, blood cells in the urine. Specific gravity of urine was < 1.25 in 12 (66.67%) dogs, specific gravity was between 1.25-1.35 in 4 (22.22%) dogs while specific gravity was between 1.35-1.45 in 2 (11.11%) dogs affected with hepatic dysfunction. Specific gravity measures density of urine and often shows degree of dilution or concentration of urine sample (Kandula and Karlapudi, 2015). The animals with hepatic dysfunction have reduced ability to

concentrate urine which results in low specific gravity of urine. (Lawrence and Steiner, 2017). Specific gravity <1.25 is frequently associated with canine chronic hepatitis and portosystemic shunt and specific gravity <1.20 is seen in dogs with polyuria (Bexfield and Watson, 2006; Feldman *et al.*, 2014). Low specific gravity is also associated with renal disorder (Sink and Weinstein, 2012) thus it cannot be termed as specific for liver disease. The findings of physical examination of urine have been mentioned in Table 1.

Table 1: Physical examination of urine in canines with hepatic dysfunction (n=18)

Physical examination of urine		Total
pH	Acidic	13 (72.22%)
	Alkaline	5 (27.78%)
Colour	Straw yellow	4 (22.22%)
	Dark yellow	10 (55.56%)
	Brownish yellow	3 (16.67%)
	Reddish/Brownish	2 (11.11%)
Odour	Nil/ Slight Ammoniac	18 (100.00%)
Turbidity	Absent	10 (55.56%)
	Present	4 (22.22%)
Specific gravity	<1.25	12 (66.67%)
	1.25-1.35	4 (22.22%)
	1.35-1.45	2 (11.11%)

Chemical analysis of urine

Chemical analysis was conducted in all 18 dogs to find



Fig. 1: Yellow coloured urine of a Spitz dog affected with hepatic dysfunction



Fig. 2: Dark yellow urine of a dog affected with hepatic dysfunction



Fig. 3: Coffee coloured in a dog affected with Babesiosis

any abnormality of urine in hepatic dysfunction. The dogs presented with hepatic dysfunction did not show any abnormality of ketone bodies and glucose in urine. Major abnormalities that were found in urine of dogs included abnormal bilirubin, blood/haemoglobin and urobilinogen. Increased bilirubin (+++) levels as colour changes were seen in 9 (50.00%) dogs, 6 (33.33%) showed moderate (++) while 3 (16.67%) dogs showed mild increase (+) in bilirubin during dipstick test. Bilirubinuria or presence of bilirubin in urine occurs in liver disease when levels of conjugated bilirubin surpasses the renal threshold (Callens and Bartges, 2015). Dogs have less renal threshold for bilirubin thus dipstick test often gives a positive result when urine is concentrated. Dipstick test with (++) or more can suspected as an underlying hepatic condition when urine is diluted (Villers and Ristic, 2016). Blood/haemoglobin findings on dipstick test revealed (+++) in 2 (11.11%) cases while (++) in 1 (5.56%) case. Haemoglobin can be found in urine as a result of intravascular haemolysis and haematuria often results secondary to diseases that cause injury to urogenital tract (Barger and MacNeill, 2015). In this study reddish brown urine or haemoglobinuria observed might be due to babesiosis. Finding of haemoglobinuria in babesiosis infected dog was also reported by Mathe *et al.* (2006). Proteinuria was seen in 4 cases, among them 2 (11.11%) cases had protein concentration as per dipstick test in urine in the range of 30 mg/dl while 5 (11.11%) in the range of 15 mg/dl. In this study proteinuria might be attributed to kidney dysfunction following a liver disorder or due to infections such as babesiosis and trypanosomiasis. Findings of proteinuria in kidney disorder, babesiosis, trypanosomiasis was also reported by Vaden and Elliott. (2016); Mathe *et al.* (2006); Defontis *et al.* (2012). Proteinuria pathologically arises as a result of chronic kidney disease, glomerulonephritis, fever haemoglobinuria, seizures and high environment temperature (Villers and Ristic, 2016; Callens and Bartges, 2015). Results of dipstick test for urobilinogen revealed increased urobilinogen (4) in 8 (44.44%) dogs, urobilinogen (2) in 5 (27.78%) dogs and urobilinogen (1) in 3 (16.67%) dogs while there was absence of urobilinogen in 2 (11.11%) dogs with hepatic dysfunction. Increased urobilinogen in urine results due to hepatic disorder and increased haemolysis of RBCs (Erik *et al.*, 2021). Non-existence of urobilinogen can indicate bile duct obstruction but it not reliable as urobilinogen is unstable and has variations daily (Brobst and Duane,

1989). The findings of chemical examination of urine have been mentioned in Table 2.

Table 2: Chemical examination of urine in canines with hepatic dysfunction (n=18)

Parameter	Results	Total
Bilirubin	+	3 (16.67%)
	++	6 (33.33%)
	+++	9 (50.00%)
Blood/haemoglobin	Negative	15 (83.33%)
	+2	1 (5.56%)
	+3	2 (11.11%)
Protein	Absent	14 (77.78%)
	15 mg / dl	2 (11.11%)
	30 mg/ dl	2 (11.11%)
Urobilinogen	Absent	2 (11.11%)
	1	3 (16.67%)
	2	5 (27.78%)
	4	8 (44.44%)

Microscopic examination of urine

Microscopic examination of urine revealed blood cells, crystals and casts in urine. Findings of blood cells in microscope revealed RBCs < 5HPF (high power field) in 4 (22.22%) cases, WBC'S < 5 HPF in 6 (33.33%) cases. Findings did not reveal any significant abnormalities in the urine as in terms of cells. During microscopic examination of urine RBCs up to 5 / HPF and WBCs up to 5/HPF is often considered normal (Yadav *et al.*, 2020). Crystals like ammonium biurrate crystals were seen in 2 (11.11%) cases, and bilirubin crystals were seen in 1(5.56%) case (Fig. 4, 5).

Ammonium biurrate crystals are formed as a result of decreased conversion of uric acid to allantoin and decreased conversion of ammonia to urea due to disorders of liver (Bartges *et al.*, 1999). Mostly these crystals are associated with chronic hepatitis, portosystemic shunts, microvascular dysplasia of liver (Grauer, 2014). Findings of ammonium crystals was also observed by James *et al.*, (2008) in dogs with chronic hepatitis. Bilirubin crystals are attributed to increased clearance of urobilinogen and bilirubin in urine. Casts like hyaline casts were > 2 / LPF (Low power field) in 3 (16.67%) urine sample and granular casts were > 2 / LPF in 1 (5.56%) urine sample. Less than 2 casts / LPF are often present in a healthy animal (Villers and Ristic, 2016) (Fig. 6).

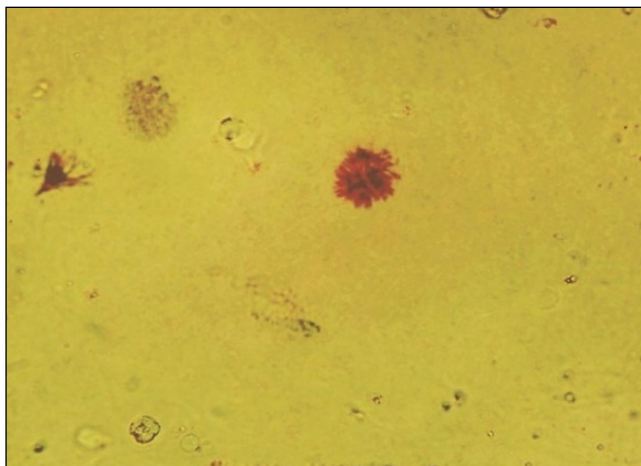


Fig. 4: Bilirubin crystal under microscope in urine of a dog affected with hepatic dysfunction

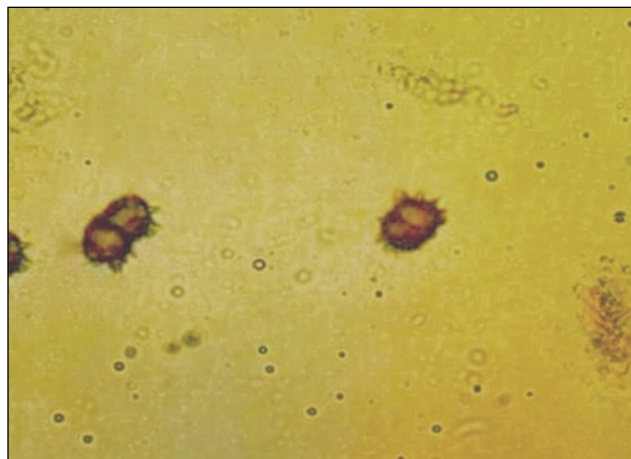


Fig. 5: Ammonium Biurate crystal under microscope in urine of a dog affected with hepatic dysfunction

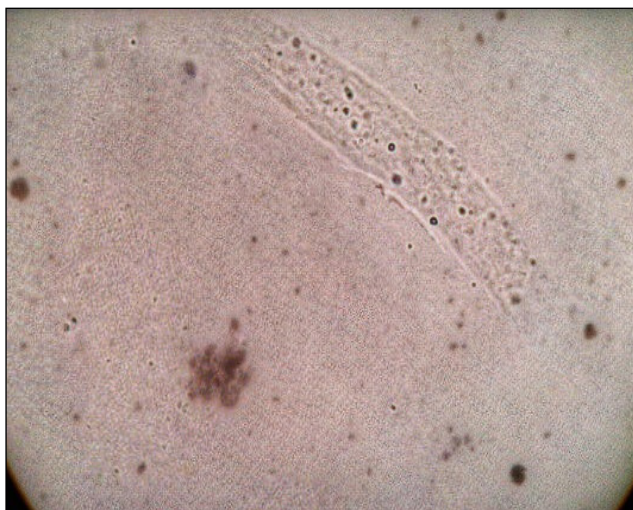


Fig. 6: Hyaline cast under microscope in urine of dog affected with hepatic dysfunction

Hyaline casts are protein in nature and usually are seen in dogs with proteinuria or acute renal impairment (Mary, 2004). Finding of hyaline casts in urine was also observed by Masumoto and Masuoka, (1980) in dogs with severe jaundice. Granular casts constitute cell debris associated with tubular necrosis and usually are seen in tubulointerstitial disease (Nyssa and Cathy, 2005). Finding of granular casts in urine was also reported by De (2006) in dog affected with *Babesiosis*. Presence of casts in this study might be due to the renal involvement in chronic hepatic dysfunction.

Coproscopical examination

In the present study, findings of faecal examination revealed nematode *Ascaris spp.* in 2 (11.11%) dogs and bilirubin pigment on microscopic examination in faeces of 1 (5.56%) dog. Presence of bilirubin in faeces might be attributed to increased bilirubin in circulation due to liver disorder (Fig. 7). Findings of faecal colour examination revealed normal faeces in 10 (55.56%) dogs, red coloured faeces in 2 (11.11%) dogs, tarry coloured faeces in 3 (11.11%) dogs and clay-coloured faeces in 3 (11.11%) dogs (Fig. 8).

Normally brown colour faeces are found in dogs due to pigment called stercobilin (Allerton, 2020). Reddish faeces may be due to haemorrhages in intestine as a result of hepatic dysfunction (Boysen, 2015). Tarry coloured faeces could be due to gastrointestinal bleeding and is seen in conditions such as portal hypertension (Nwoha, 2019). Finding of tarry coloured faeces was also observed by Grenn and Bulmer (1972) in a dog with cirrhosis. Clay coloured faeces occurs due to absence of stercobilin in faeces and is seen commonly in biliary obstruction (Murdoch, 1976). Findings of clay colour faeces was also observed by Gomaa *et al.* (2019) in dogs with experimental induced jaundice. The findings have been mentioned in Table 3.



Fig. 7: Ascaris worm infestation in a dog affected with liver



Fig. 8: Clay coloured faeces of a dog affected with hepatic dysfunction

Table 3: Faecal colour in canines with hepatic dysfunction (n=18)

Faecal colour	No. of animals
Normal	10 (55.55%)
Reddish faeces	3 (16.67%)
Tarry coloured faeces	2 (11.11%)
Clay coloured faeces	3 (16.67%)

CONCLUSION

The present study concludes that urine analysis is a useful diagnostic test for hepatic dysfunction in canines. Dogs with hepatic dysfunction produces urine with low specific gravity, acidic pH and yellow to dark yellow colour with presence of urobilinogen and bilirubin. Crystals such as ammonium biurate and bilirubin are often indicator of hepatic damage if present in urine. Casts such as hyaline and granular in urine often indicate renal involvement in dogs affected with hepatic dysfunction. Coproscopical examination often helps in finding parasitic etiology of hepatic dysfunction and clay-coloured faeces are often a useful indicator of bile duct obstruction.

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