EVALUATION OF URINARY CRYSTALS IN DOG AT DHAKA METROPOLITAN AREA, DHAKA, BANGLADESH

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Roll No: 0119/04

Registration No.: 636

Session: 2019-2020

A thesis submitted in the partial fulfilment of the requirements for the degree of Master of Science in Medicine

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Chattogram -4225, Bangladesh

JUNE 2021

Evaluation of Urinary Crystals in Dog at Dhaka Metropolitan Area, Dhaka, Bangladesh

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This is to certify that, we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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June 2021

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ACKNOWLEDGEMENTS

At the very beginning, I would like to express my ever gratefulness to **The Almighty, Al-Alim Allah SWT** for giving me such strength and opportunity to complete the research and thesis successfully for the degree of Master of Science (MS) in Medicine under the Department of Medicine and Surgery, Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh.

Secondly, I would like to express the first and foremost appreciation, deepest sense of gratitude and best regards to my respected supervisor Prof Dr. Pankaj Chakraborty, Department of Medicine and Surgery (DMS), CVASU. It was my pleasure and great experience to work with him. I feel much pleasure to convey my profound thanks to my co-supervisor, Prof. Dr. Mohammad Mizanur Rahman, Department of Medicine and Surgery (DMS), CVASU for his valuable advice, scholastic guidance, and well thought suggestions during my study. I humbly thank to Dr. FM Yasir Hasib, Phd fellow at University of HongKong, HongKong, Dr. Abdullah Al Sattar, Research Assistant, One Heath Poultry Hub, Dr. Md. Abu Shoieb Mohsin, MS fellow in epidemiology DMS, CVASU and Dr. Ireen Sultana, Senior Veterinarian, AKSACC for their suggestions, encouragement and support during research work.

I would like to express my deep sense of gratitude to AKS Animal Care Centre, Dhaka for the work permission and Ministry of Science and Technology, People's Republic of Bangladesh for providing necessary research fund. I would like to give thanks to Prof. Dr. Azizunnesa Rekha, Head of the Department of Medicine and Surgery, and Prof. Dr. AMAM Zonaed Siddiki, Coordinator of Advanced Studies and Research, CVASU, for their administrative support in relation to the thesis approval.

Finally, I am ever indebted to my respected parents, siblings, seniors and juniors for all of their immense sacrifices, blessings and impulsive encouragement.

The Author

June 2021

List of Abbreviations

Abbreviation and symbols	Elaboration
AKSACC	AKS Animal Care Centre
DMA	Dhaka Metropolitan Area
UTI	Urinary Tract Infection
%	Percent
>	Greater than
<	Less than
≥	Greater than equal
≤	Less than equal
=	Equal to
BCS	Body Condition Score
CVASU	Chattogram Veterinary and Animal Sciences University
et al.,	and others

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Abstract

Stones in the urinary tract, which can originate in the kidney, urinary bladder, ureter, or urethra, are one of the most prevalent urologic illnesses in dogs worldwide. Struvite, calcium oxalate, calcium phosphate, cystine, urate, and calcium oxalate dihydrate crystals are the most common types of crystals found in canine urine. The current study investigates the prevalence of urinary crystals in dogs in Dhaka, Bangladesh, and identifies potential risk factors for the development of canine urinary crystals. From November 2019 to October 2020, a total of 107 urine samples were collected from dogs presenting to the AKS Animal Care Centre, Dhaka for a routine checkup or with symptoms of difficult urination, bloody urine, obstruction, or dribbling. After collection, the urine samples were examined for their physical (color, clarity, specific Gravity), and chemical properties (pH, blood, protein, leukocyte, glucose, ketones, bilirubin, urobilinogen), as well as, microscopic analysis was conducted to identify the type of crystals. Using a questionnaire, data about the dog's owner, animal, diet, clinical history and findings, and urinalysis were also collected. About 23.85% of the dogs tested positive for urine crystals, with the Gulshan region having the highest prevalence. Several clinical signs, including the dog's high body temperature, mucous membrane color, and severe dehydration level, were related to the presence of urine crystals. Urine analysis reveals an increase in a range of chemical components (urine pH, blood, protein, leukocyte, ketones, bilirubin and urobilinogen). Age and diet have a significant contribution ($p \leq 0.05$) to the production of urine crystals in dogs. Elderly dogs fed dry food were more likely to develop urinary crystals than those fed home-cooked meals; likely to be related to the lower water content of dry food and the lower oral water consumption of dry-food-fed dogs. Future researches are needed to explore other factor influencing canine urinary crystals.

CHAPTER-1: INTRODUCTION

Urine is the byproduct of the body metabolism of animal body that is liquid in nature, light yellowish to straw in color and is mild to moderate pungent odor (Yang et al., 2014). Urinary stones are one of the common happening lower urologic diseases in dogs all over the world. Usually stones may found in kidney, urinary bladder, ureteror urethra. Urinalysis is a very basic test to detect the abnormality of urine (Athaley etal., 2018).

Their formation involves multiple pathological and physiological factors. Most of the crystals are located in bladder (Winnie et al., 2010). The urinary crystals generally formed in dogs are struvite, calcium oxalate monohydrate, calcium oxalate dihydrate, calcium phosphate, cystine and urate crystals (Tion et al., 2015). Forming urinary crystals involves many risk factors that can vary in dogs depending on composition. The risk factors includes age, sex, breed, desexing in males specially and sometimes presence of urinary tract infection (UTI) specially struvite crystals. Struvite crystals are much more common in female compared to male dogs; on the other hand, calcium oxalate, calcium phosphate and urate crystals are much more predominant in male dogs (Kopecny et al., 2021)

The urinary crystals are more prevalent in exotic breeds compared to native Bangladeshi or mixed breed (Swieton, 2018). But still native Bangladeshi and mixed breed are also affected (Hesse, 1990). The incidence of crystal formation is much higher in middle age dogs. Male dogs are most affected compared to female dogs and its almost twice (Hesse, 1990). Also the diet act as a key factor for crystals formation (Kopecny et al., 2021)

The feeding of processed dry food contains increased amount of fat, protein, sodium, potassium, calcium, magnesium, phosphorus, chloride and moisture with a little amount of carbohydrate that increase the risk of formation of crystals in dogs. In female excessive feeding of alkaline food like vegetables increase the formation of struvite crystals (Winnie et al., 2010).

The prevalence of urinary crystals reported as 0.5 to 1.0 percent in Germany, 1.5 to 3.0 percent in Norway, 0.1 to 0.3 percent in Czech Republic, 0.24 percent in Sweden and in USA it is around 1.0 to 3.0 percent (Sosnar et al., 2005)

However no research was conducted on urinary crystals of dogs in Dhaka, the capital of Bangladesh, where a significant number of dogs are reared nowadays. That's why a cross sectional surveillance study was performed on all urine test came to AKSAnimal Care Centre, Dhaka situated in Dhaka Metropolitan Area with following objectives

I. To study the prevalence of urinary crystals in dogs.
II. To explore the possible risk factors of forming urinary crystals in dogs.
III. To describe clinical pictures and their association with urinary crystals in dogs.

CHAPTER- II:

REVIEW OF LITERATURE

2.1. Dog- The domestic dog (*Canis familiaris* or *Canis lupus familiaris*) is a domesticated descendant of the wolf (Frantz et al., 2020). The dog derived from an ancient, extinct wolf, and the modern grey wolf is the dogs nearest living relative (Frantz et al., 2020). The dog was the first species to be domesticated, by hunter–gatherers over 15,000 years ago, before the development of agriculture (Frantz et al., 2020). Their long association with humans has led dogs to be uniquely adapted to human behavior, leading to a large number of domestic individuals and the ability to thrive on a starch-rich diet that would be inadequate for other canids (Frantz et al., 2020).

2.2. *Urine*- Urine is a liquid by-product of metabolism in humans and in many other animals (Yang et al., 2014). Urine flows from the kidneys through the ureters to the urinary bladder. Urination results in urine being excreted from the body through the urethra (Rose et al., 2015). Cellular metabolism generates many by-products that are rich in nitrogen and must be cleared from the bloodstream, such as urea, uric acid, and creatinine. These by-products are expelled from the body during urination, which is the primary method for excreting water-soluble chemicals from the body. A urinalysiscan detect nitrogenous wastes of the mammalian body (Carrell and Peterson, 2010). Urine plays an important role in the earth's nitrogen cycle. In balanced ecosystems, urine fertilizes the soil and thus helps plants to grow. Therefore, urine can be used as a fertilizer. Some animals use it to mark their territories (Lison et al., 1980).

2.3. Composition of Urine:

2.3.1. Volume:

A normal adult excretes 100 ml to 150 ml of urine daily. The average is 120 ml containing 2-3 gm. of solids (Yadav et al., 2020). The quantity depends on the water intake, external temperature, the diet and the individual's mental and physical condition (Rudinsky et al., 2019). A high protein diet increases excretion because the urea formed as a result of catabolism of protein has a diuretic action (Norkus andKeir, 2020). The decreased volume of urine in hot weather is due to an increased loss of water by perspiration (Carun et al., 2021). Nervousness or excitement causes increased urinary volume (Rowe et al., 2021). Increased urine volumes are observed in diabetes insipidus, diabetes mellitus and certain types of kidney diseases and decreased volumes are found in acute nephritis, fevers and diseases of the heart, diarrhea and vomiting (Paslawska et al., 2020)

2.3.2. Specific Gravity:

The specific gravity of urine in 24 hours lies between 1.003 and 1.030 and varies according to concentration of solutes in the urine. The specific gravity of the urine varies with the food, water intake, and the activity of the individual (Li et al., 2020). In chronic interstitial nephritis, the specific gravity is lowered (Koenhemsi and Gönül, 2019). The specific gravity is increased in the excretion of abnormal substances such as albumin or glucose (e.g., diabetesmellitus) (Ferlizza et al., 2020).

2.3.3. Colour:

Normal urine is pale yellow or amber. The colour is roughly proportional to the specific gravity. Very dilute urine is colourless (Chacar et al., 2017). Urochrome, composed of a polypeptide and urobilin, is the chief pigment of urine. Traces of coproporphyrtn, urobilinogen and litroerytbrin are also found in urine (Kunz et al., 2020). Reddish urine is due to the ingestion of naturally coloured foods (e.g., beetroot, blackberries). In fever, the urine may be dark yellow or brownish because of concentration. In liver disease, the urine may be green, brown, or deep yellow due to bile pigments. Blood or hemoglobin develops smoky to red colour. The urine is dark brown due to methemoglobin and homogentisic acid. Methylene blue gives the urinea green appearance (Kandula et al., 2017). The urine is transparent. A turbidity is de- veloped in alkaline urine by precipitation of calcium phosphate. Strongly acid urine is pink due to the precipitation of uric acid salts (Kim et al., 2020).

2.3.4. Odour:

The odour is modified by the ingestion of certain foods or drugs. This is noticed after eating asparagus; the odour is due to methyl mercaptan (Horowitz, 2017). The ammoniacal smell of urine is due to the action of bacteria on urea (Sigurdarson et al., 2018). In ketosis, the odour of excreted acetone is detected (Da Silva et al., 2019).

2.3.5. pH:

The mixed sample of normal urine in 24 hours has a pH 6.0. Individual samples vary from 4.6 to 8.0. The urine is acid in high protein intake because excess phosphate and sulfate are formed in the catabolism of protein. Acidity is also increased in acidosis and in fever. The urine becomes alkaline on standing due to the conversion of urea to ammonia and loss of CO_2 to air. It may be alkaline in alkalosis such as after excessive vomiting and after meals due to H^+ secretion in the stomach (the ―alkaline tide‖) (Allen et al., 2020). The acidity of urine is increased after strenuous muscular exercise (elimination of lactic acid), by ingestion of ammonium salts of strong acids. An alkaline urine may be produced by ingestion of sufficient $NaHCO_3$. Ammonium carbonate does not produce an alkaline urine because ammonia is rapidly converted into urea (Dossin et al., 2003).

2.4. Constituents of Urine:

2.4.1. Normal Constituents of Urine:

2.4.1.1. Urea: Nitrogenous Constituents:

Urea is the main end product of catabolism of protein in mammals. Its excretion is directly proportional to the protein intake. It consists of 80-90% of the total urinary nitrogen (Yang and Bankir, 2005). In fever, diabetes, or excess adrenocortical activity, urea excretion is increased due to increased protein catabolism. Decreased urea excretion is due to decreased urea production in the last stages of fatal liver disease. In acidosis, there is decreased urea excretion.

2.4.1.2. Ammonia:

Ammonia is formed by the kidney from glutamine or amino acids in acidosis (Shawcross et al., 2005). There is a high ammonia output in the urine in uncontrolled diabetes mellitus in which renal function is unimpaired.

2.4.1.3. Creatinine and Creatine:

Creatine is excreted by children and pregnant women and much smaller amounts in men. Creatinine is formed from creatine. It is excreted in relatively constant amounts regardless of diet (Wang et al., 2007). The creatinine coefficient is the ratio between the amount of creatinine excreted in 24 hours and the body weight in kg. It is usually 20-26 mg/kg/day in normal men and 14-22 mg/kg/day in normal women. Creatinine excretion is decreased in many pathological conditions (Shao and Hathcock, 2006). Creatine excretion is also found in pathological states such as starvation, hyper-thyroidism, impaired carbohydrate metabolism and infections. Creatine excretion is decreased in hypothyroidism (Kemppainen and Behrend, 2001)

2.4.1.4. Uric Acid:

It is the end product of the oxidation of purines in the body. It is not only formed from dietary nucleoprotein but also from the breakdown of cellular nucleoprotein in the body (Friedman and Byers, 1948). It is slightly soluble in water and precipitates readily from acid urine on standing (Maeta et al., 2021). Uric acid excretion isincreased in leukemia, severe liver disease and various stages of gout. The concentrated urine on cooling forms a brick-red deposit which is mainly acid urate. Pure uric acid is colourless. Deposits of uric acid and urates are coloured by absorbed urinary pigments, particularly the red uroervthrin (Wood and Gibson, 2020). The specificity of the analysis of uric acid is increased by treatment with uricase, the enzyme (from hog kidney) which converts uric acid to allantoin.

2.4.1.5. Amino Acids:

About 15-20 mg of amino acid nitrogen is excreted in the urine of adults in 24 hours. The infant at birth excretes about 3 mg amino acid nitrogen per pound of body weight, and up to the age of 6 months the value reaches to 1 mg/pound which is maintained throughout childhood. Premature infants excrete 10 times amino acid nitrogen than that of full-term infant (Moyle et al., 2018). The low excretion of amino acid nitrogen is due to its high renal threshold value. Increased amounts of amino acids are excretedin liver disease and in certain types of poisoning (Benaiges, 2018). In cystinuria, 4 amino acids-arginine, cystine, lysine and ornithine are excreted in urine.

2.4.1.6. Allantoin:

It is the partial oxidative products of uric acid. Small quantities of the allantoin are excreted in human urine. In other sub-primate mammals, allantoin, the principal end product of purine metabolism, is excreted (Wood and Gibson, 2020)

2.4.1.7. Sulphates:

The urine sulphur is derived from sulphur containing amino acids such as methionine and cystine and therefore, its output varies with protein intake.
The urine sulphur exists in 3 forms:

Inorganic (sulfate) sulfur:

This is the completely oxidized sulfur precipitated from urine. It is proportionate to the ingested protein with a ratio of 5:1 between urine nitrogen and inorganic sulfate (Paßlack, et. al, 2021)

Ethereal sulfur (conjugated sulfates):

It is about 10% of the total excreted sulfur. This includes the organic combination of sulfur excreted in the urine. It consists of the sulphuric esters of certain phenols. It forms no precipitate on addition of acidified $BaCl_2$. Some of the phenols are derived from putrefaction of protein in the large intestine (Bai et al., 2020). Clinically, the ethereal sulfate is that of indoxyl indican which is formed from bacterial decomposition of tryptophan in the large intestine. Normally, 5-20 mg of indican are excreted and the amount increases in constipation. In cholera, typhus gangrene of lung, sufficient indican is excreted. Indoxyl liberated from indican is oxidized to indigo blue on exposure to air.

Neutral sulfur:

These are un-oxidized sulfur and contained in cystine, taurine, thiocyanate or sulfides. They do not vary with the diet (Nair et al., 2019). They are mainly the products of endogenous metabolism.

2.4.2. Abnormal Constituents of the Urine:

2.4.2.1. Proteins:

Proteinuria (albuminuria) is the presence of albumin and globulin in the urine in abnormal concentrations. The traces of protein (10-150 mg) present in normal urine cannot be detected by the ordinary simple tests. Pathologically, several proteins, such as serum albumin, serum globulin, hemoglobin, mucus, proteose, Bence-Jones proteins are found in urine (Kendall et al., 2017).

Physiologic proteinuria:

In this condition, less than 0.5% protein is present in urine which occurs after severe exercise, after a high protein meal or as a result of some temporary impairment in renal circulation when a person stands erect. In 30-35% of pregnancy, there is proteinuria (Pasławska et al., 2020)

Pathologic proteinuria:

Proteinuria is marked in glomerulonephritis. In nephrotic syndrome, a marked proteinuria occurs. The proteinuria increases with the increasing severity of the renal lesion. Proteinuria also results in poisoning of the renal tubules by heavy metals like mercury, arsenic or bismuth (Lindaberry et al., 2021). Hemoglobin is also present as a result of hematuria due to hemorrhage from the kidneys or urinary tract, clotting may occur due to sufficient fibrinogen on passing of much blood (Kendall et al., 2017).

Mucus is the term for an unidentified protein precipitated by acetic acid in the cold. It is mucin. The mucus is increased in infection of the bladder (Rey et al., 2020). Proteose may be found which is of little clinical significance. Bence-Jones proteins found in the urine are the peculiar proteins which are light chain fragments of globulins. Most commonly they occur in multiple myeloma and rarely in leukemia. They are precipitated when the urine warmed to 50-60°C and re-dissolved almost completely at 100°C and precipitated again on cooling (Harris et al., 2021).

2.4.2.2. Glucose:

Normal individuals excrete not more than 16-300 mg of sugar per day which is difficult to detect by simple test. It is said to be glycosuria when more than this quantity is found in urine. There are different causes of glycosuira (Behrend et al., 2019). Transient glycosuria is observed after emotional stress such as exciting atheleticcontest. 15% of cases of glycosuria are not due to diabetes glycosuria suggesting dia- betes must be confirmed by blood glucose studies to eliminate the probability of renal glycosuria. The presence of glucose must be tested by Benedict's test. But in case of pregnant women and lactating mother, the Osazone test must be performed for urine glucose to eliminate the lactose present in urine.

Other sugars:

1. Fructosuria:

Fructosuria is due to the disturbance in fructose metabolism but no othercarbohydrates. (Allen et al., 2020)

2. Galactosuria and lactosuria:

These may occur occasionally in infants, pregnant women and lactating mother. Galactosuria may occur in inherited diseases due to the non-conversion of galactose to glucose (Alonso-Fernández and Mejeras, 2019)

3. Pentosuria:

This may occur transiently after intake of food containing large quantities of pentose's, such as grapes, cherries and plums. It may take place in inherited diseasesin which pentose's are not metabolized. To detect these other sugars in urine it is wiseto perform Osazone test.

2.4.2.3. Ketone bodies:

Only less than 1 mg of ketone bodies are excreted in urine normally in 24 hours. Increased amount of ketone bodies are excreted in urine in starvation, diabetes mellitus, pregnancy, ether anesthesia, and some types of alkalosis (Athaley et al., 2018). Excess fat metabolism may induce a ketonuria in many animals. Increased amount of ammonia is excreted in acidosis accompanying ketosis.

2.4.2.4. Bilirubin and Bile salts:

Bilirubin is found in the urine in cases of obstructive or hepatic jaundice. Bilirubinuria is accompanied by the excretion of bile salts (Romano et al., 2021). Bile salts may be excreted in urine without bile pigment in certain stages in liver disease. In excessive hemolysis, traces of bilirubin without bile salts are excreted in urine.

2.4.2.5. Blood:

In the lesion of the kidney or urinary tract blood is excreted in the urine in addition to its presence in nephritis. Free hemoglobin is also found in urine after quick hemolysis, e.g., in black water fever (a complication of malaria) or after severe burns.

2.4.2.6. Urobilinogen:

In excessive hemolysis, e.g., hemolytic jaundice or pernicious anemia, part of the bile pigment formed by breakdown of hemoglobin is excreted in urine as urobilinogen. Urobilin is formed from colourless urobilinogen when the urine is exposed to air. This gives the urine an orange colour. In liver disease or temporarily in constipation, large amounts of urobilin are found in urine. Urobilinogen excreted in urine normally are 1-3.5 mg/24 hrs.

2.4.2.7. Porphyrins:

Coprophyrins excreted in urine normally are 50-250 (μ gm./day. Coproporphyria are excreted more in certain liver diseases. The increased amount of coproporphyrins in the urine is a characteristic of the urine of patients suffering from porphyria.

2.4.2.8. Urinary Deposits:

The commonest deposits are phosphates, oxalates and urates and are frequently seenin normal urine.

2.4.2.9. Phosphates:

They are usually found in alkaline urines. The commonest is ammonium magnesium phosphate which forms a characteristic crystal. A less common form is calcium hydrogen phosphate which forms long prisms. Amorphous calcium and magnesium phosphates may be deposited from alkaline urines. The deposition of phosphates is due to a change in pH after the urine has been passed.

2.4.2.10. Calcium Oxalate:

This is found in acid urine but may be found in alkaline urine. The crystals are of two types—octahedra, dumb-bells. Calcium oxalate is insoluble in acetic acid.

2.4.2.11. Urates:

They are usually found in acid urines. Uric acid separates into different forms including prisms, barrels, hexagons and needles which are always pigmented. Urates are redissolved on warming the urine. The cause of deposition of urates is the cooling of urine after it has been passed.

Cystocentesis- Cystocentesis is the collection of a sterile urine sample directly from the urinary bladder using a needle. This method allows you to collect a urine sample that has not been contaminated by the lower genital tract, and so is it very useful for diagnostic tests such as urinalysis and urine culture.

Decompressive cystocentesis is a method to drain the urinary bladder using a needle. This is very helpful in situations of urethral obstruction. Cystocentesis is best performed using ultrasound guidance. The patient is placed in dorsal recumbency and the skin is aseptically prepped. The patient should be able to relax comfortably in dorsal recumbency without excessive restraint. In very anxious patients, gentle sedation is helpful (Marynissen et al., 2017). When ultrasound guidance is used, ensure that the needle is introduced in the same plane of theultrasound beam, so that the entire needle can be visualized. Urinary tract infections (UTIs) are common in small animals, developing in up to 27% of dogs. Nearly all infections are caused by pathogenic bacteria, although some are caused by fungi or viruses rarely. Most bacterial lower UTIs result from bacteria ascending the external genitalia and urethra. Less commonly, bacteria travel through the bloodstream and colonize the urinary tract. Numerous innate defense mechanismshelp prevent UTIs. Complete and regular voiding, along with the intrinsic propertiesof urine (high osmolality, antimicrobial solutes), help create a urinary tract environment that is hostile for microbes. Anatomic barriers and mucosal defenses further prevent adherence of virulent bacteria to the urothelium.

However, when these defenses are inadequate, pathogenic bacteria increase the permeability of the urothelium, allowing for passage of inflammatory solutes into the subepithelium as well as secretion of inflammatory cytokines. The result is inflammation and pain, which are exhibited as dysuria, pollakiuria, stranguria, and or hematuria.

Elimination of the virulent organism can enable restoration of the normal permeability and integrity of the urothelium. Successful antimicrobial therapy requires an appropriate choice of antibiotic, including dose, frequency, and duration.

2.4.3. Other organic compounds:

2.4.3.1. Chlorides:

These are excreted as NaCl and output varies with intake.

2.4.3.2. Phosphates:

The urine phosphates consist of sodium and potassium phosphates as well as calcium and magnesium phosphates (Stockman et al., 2021). The greater part of the excreted phosphates is derived from ingested food which contains organic phosphates, e.g., nucleoprotein, phosphoprotein and phospholipids. Phosphates of food are not completely absorbed. Some phosphate is also derived from cellular breakdown (Stockman et al., 2021). Phosphate excretion is increased in certain bone diseases such as osteomalacia, wasting diseases of the nervous system and in renal tubular rickets (Parker et al., 2020). Marked increase of phosphate excretion is also observed in hyperparathyroidism and decrease in hypoparathyroidism and in infectious diseases.

2.4.3.3. Oxalates:

The amount of oxalate in the urine is low (20 mg/day) and found as calcium oxalate crystals in urinary deposits (O'Kell et al., 2017). The excretion of oxalate is increased by ingestion of fruits and vegetables containing high oxalates (spinach). Large quantities of oxalate are excreted in urine in inherited metabolic diseases. The oxalates present in urine are composed of partly unchanged ingested acid and partly oxidative products of other compounds (O'Kell et al., 2017).

2.4.3.4. Minerals:

The 4 cations of the extracellular fluid— sodium, potassium, calcium and magnesium—are present in the urine (Heer et al., 2017). Sodium content varies with intake. Urine potassium increases when the intake is increased or in excessive tissue catabolism. The excretion of potassium is affected by alkalosis. Sodium and potassium excretion are also controlled by the activity of the adrenal cortex (Parvathamma et al., 2020). Calcium and magnesium are not completely absorbed andtheir presence in the urine is low. But their presence in the urine varies in certain pathological states, particularly those involving bone metabolism (Alborough et al., 2020)

2.4.3.5. Enzymes:

Traces of many enzymes are excreted in urine including pancreatic amylase, pepsin, trypsin and lipase (Wirobski, G. et al., 2021). The pancreatic amylase excretion is increased in pancreatic disease (Jimenez et al., 2019).

Hormones and vitamins:

Certain hormones (sex hormones) and vitamins (e.g., B, and C) are found in urine (Palmer et al., 2017). The vitamin needs are assessed by studying the urinary output after test doses. The pregnancy test is also performed by the urinary sex hormones (Wirobski et al., 2021).

2.5. Dog Urine Crystals vs. Bladder or Kidney Stones

Detection of urine crystals in dogs is not irrefutable evidence of a stone-forming tendency. However, there is some association of an increased risk for bladder or kidney stones for animals that are afflicted with crystalluria. Crystals in dog urine can also be an indication of bladder or kidney infection (Fathollahi, 2014).

Crystalluria in individuals with anatomically and functionally normal urinary tracts may be harmless because the crystals are eliminated before they grow large enough to interfere with normal urinary function. However, they still represent a risk factor for bladder and kidney stones, and they may cause discomfort or may promote bladder infections (Nagode et al., 1996).

Different types of stones also have different causes and treatments. Some stones can be dissolved through diet change, while others require surgical removal.

2.6. Types of Urine Crystals in Dogs

Proper identification and interpretation of dog urine crystals is important in determining a medical strategy for treating the condition. Different types of crystals require different treatment strategies. Certain crystal types indicate an underlying disease or genetic condition. Breeds that are prone to calcium oxalate crystals in the urine are Miniature Schnauzers, Yorkshire Terriers, Lhasa Apsos and Miniature Poodles (Tvedten et al., 2019).

Dachshunds, English Bulldogs, Mastiffs and Newfoundlands are prone to cystine crystals in the urine (Marynissen et al., 2017). Dalmatians and English bulldogs tend to have ammonium crystals in the urine, and Cavalier King Charles Spaniels are prone to crystallized xanthine stones (Marynissen et al., 2017).

Symptoms of Dog Urine Crystals

- Pain on urination
- Difficulty urinating
- Frequent urination
- Blood in urine
- Lethargy
- Inappetence or anorexia
- Sometimes no symptoms at all

Causes of Urine Crystals in Dogs

- Concentration of crystallogenic substances in urine, partially influenced by:
    - Genetics
    - Diet
    - Kidney function
    - Environment
- Urine concentration of water (Thrall et al., 1985)
- Urine pH is off-balance (acidic or alkaline levels need to be balanced)
- Solubility of crystallogenic substances in urine (Albasan et al., 2003)

CHAPTER-III:

MATERIALS AND METHODS

3.1. Location, duration and selection of animals

The study was conducted at AKS Animal Care Centre, Dhaka from November 2019to October 2020. The animals included dogs of different breeds, age and sex. Healthy dogs coming for regular checkup, dogs with difficult urination, bloody urine, blocked, dribbling etc. were selected and tested for urine crystals. Selected animals were undergone a comprehensive physical examination and urine were collected by free catch or limitedly taking from floor specially extremely aggressive dog breeds or through cystocentesis. Throughout the period, 107 urine samples from dogs were being analyzed.

3.2. Questionnaire preparation

At first a questionnaire was made. Then it was discussed with academicians, veterinarians and administered to the owners to check its suitability, time requirement for necessary modification. The questionnaire was designed to comprise mostly closed ended (categorical) questions to ease data processing, minimize variation and improve precision of responses. It was designed to collect information dividing into 5 categories such as owner's information, animalinformation, diet information, clinical history and findings and information of urinalysis.

3.3. Clinical examination

Selected animal was examined thoroughly in terms of rectal temperature, pulse, respiration rate, dehydration status, color of mucous membrane etc. Histories of other concurrent diseases, diet type and major source of protein were also recorded.

3.4. Urine Collection

Urine was collected from clean floor and mostly from free catch. But in some cases specially blocked one it's done through cystocentesis.

3.5. Urinalyses

After urine collection through free catch or from clean floor or by cystocentesis it wasanalyzed for physical, chemical character and microscopic examination of crystals.

3.5.1. Physical Analyses:

The urine was immediately examined after collection with regard to color, clarity and specific gravity test.

Color:

Color of the urine was checked and different color such as light straw, yellow, orange, red, dark coffee color etc was recorded.

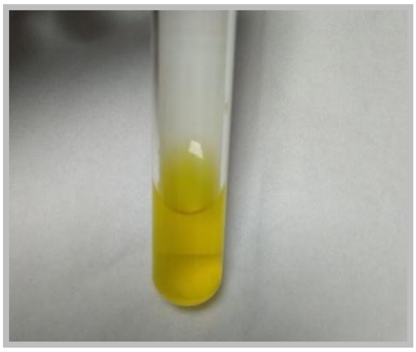

Fig 3.1: Normal Urine Color (Straw)

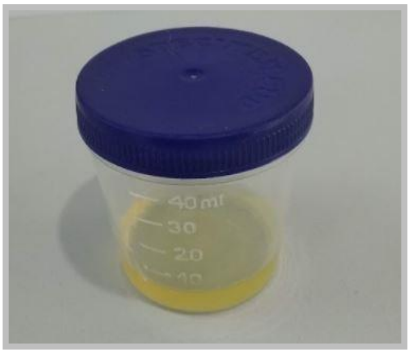

Fig 3.2: Hypostethonic Urine (Light Straw)

Fig 3.3: Hyperstethonic Urine (Orange)

Fig 3.4: Hyperstethonic Urine (Coffee)

Clarity:

Collected urine sample was taken close to the eye and checked for consistency such as clear (no visible particulates), turbid (print can't be seen in urine), cloudy (many particulates and blurred print through urine) etc.

Fig 3.5: Turbid Urine

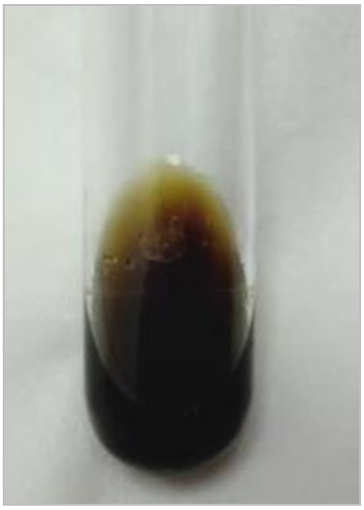

Fig 3.6: Cloudy Urine

Specific Gravity:

Specific gravity was checked through refractometer. At first a drop of urine was given on the glass of Refractometer and slide was pulled over. Then Refractometer was hold against the light and the result from the meter was recorded.

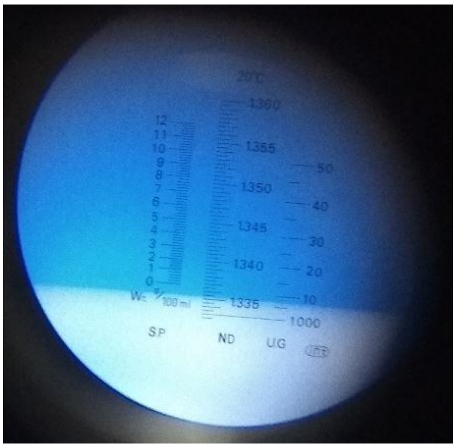

Fig 3.7: Hypostethonic Urine

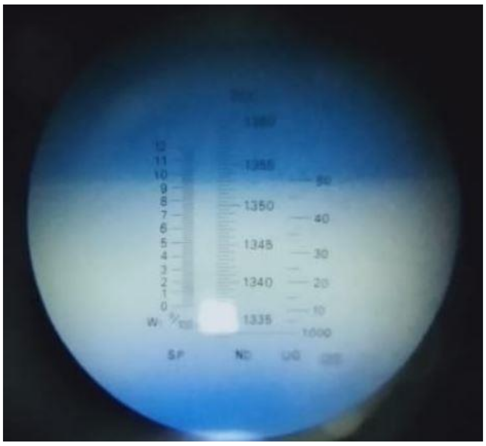

Fig 3.8: Hyperstethonic Urine

3.5.2. Chemical Analyses:

After collection of urine the chemical characters were obtained by checking pH, blood, protein, leukocyte, glucose, ketones, bilirubin, urobilinogen etc. Althrough pH, specific gravity, nitrite can be tested but we did highly specific refractometer for measuring specific gravity and special pH paper for pH determination and nitrite is non-significant in veterinary test.

Fig 3.9: Dip-Stick Test of Urine Sample

3.5.2.1. pH:

The pH of urine was measured pH paper (range 5.5-8 and 0-14 both). For this firstly a small part from pH paper was ripen. Then a drop of urine was added over the paper and waited for few seconds. The color was then compared with color contrast givenon pH paper (Figure 03). The result of pH paper was recorded.

Figure 3.10: pH test by pH paper

3.5.2.2. Glucose:

A drop of urine was given on the pad of glucose, waited for 45 seconds. Then the color contrast was compared with the strip and result was recorded accordingly(Rizzi, Theresa E, 2014)

3.5.2.3. Ketone:

A drop of urine was given on the pad of ketone, waited for 45 seconds. Then the color contrast was compared with the strip and result was recorded accordingly (Rizzi, Theresa E, 2014).

3.5.2.4. Bilirubin:

A drop of urine was given on the pad of bilirubin, waited for 45 seconds. Then the color contrast was compared with the strip and result was recorded accordingly(Rizzi, Theresa E, 2014).

3.5.2.5. Urobilinogen:

A drop of urine was given on the pad of urobilinogen, waited for 45 seconds. Then the color contrast was compared with the strip and result was recorded accordingly(Rizzi, Theresa E, 2014).

3.5.2.6. Protein:

A drop of urine was given on the pad of protein, waited for 60 seconds. Then the color contrast was compared with the strip and result was recorded accordingly(Rizzi, Theresa E, 2014).

3.5.2.7. Blood:

A drop of urine was given on the pad of blood, waited for 90 seconds. Then the color contrast was compared with the strip and result was recorded accordingly (Rizzi, Theresa E, 2014).

3.5.2.8. Leukocyte:

A drop of urine was given on the pad of leukocyte, waited for 120 seconds. Then the color contrast was compared with the strip and result was recorded accordingly(Rizzi, Theresa E, 2014).

3.5.3. Microscopic Analyses:

The collected urine was undergone to microscopic analysis to identify the type of crystals (Rizzi, Theresa E, 2014).

3.5.3.1. Crystals:

2ml urine was taken in a red rubber tube and centrifuged it to 3000 rpm for 5 minutes. Then the crystals were found in the bottom of the red rubber tube. Carefully half to two-third supernatant urine was discarded. Then the rest was shaken well the deposited crystals and with the help of dropper take a drop into the slide and give a cover slip. Then the slide under 4X magnification of microscope and switch to 10X magnification. Identify the type of crystals. For better understanding it was observed on 40X magnification (Rizzi, Theresa E, 2014).

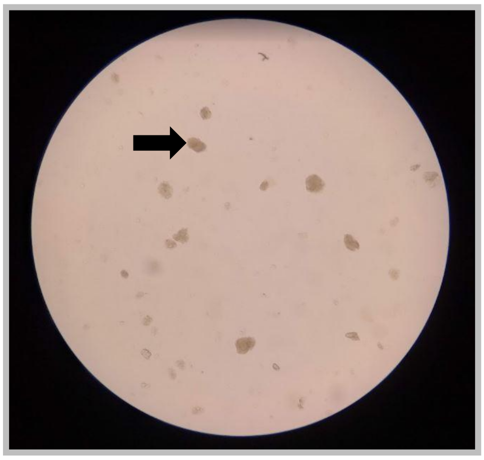

Fig 3.11: Calcium Oxalate Crystal

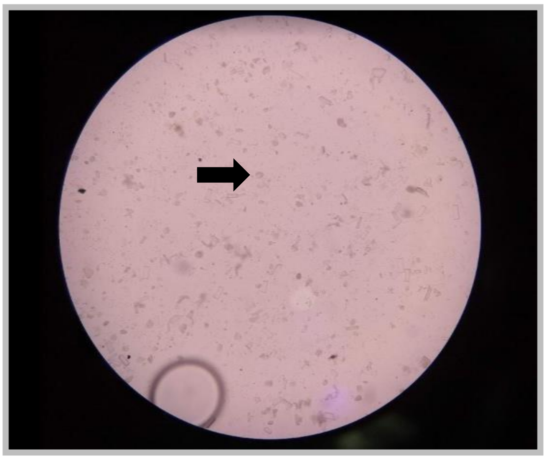

Fig 3.12: Struvite Crystal

Fig 3.13: Urate Stone

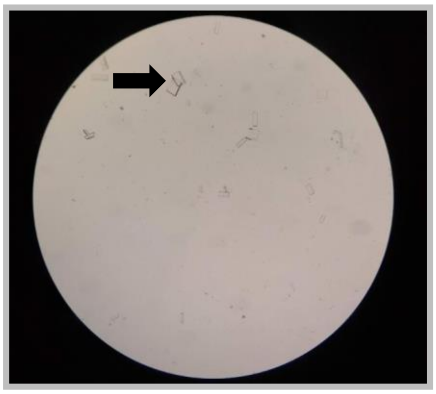

Fig 3.14: Calcium Phosphate Crystal

3.6. Data management and analysis:

The extracted data as required from the hospital paper-based recording system were entered into a Microsoft Excel 2010 spreadsheet. The data were then reviewed for errors and inconsistencies, sorted, and coded using Microsoft Excel 2010. The data were then imported into STATA-IC-13 (Statacorp, 4905 Lakeway Drive, College station, Texas, United States) for statistical analysis.

Chi-square test was carried out to assess associations between the categorized response variable of canine urolithiasis and the selected independent variables of patient information like breed, season, sex, de-sexing, age, diet, major protein source, body condition score, vaccination, deworming, variables related to clinical signs like temperature, mucous membrane, dehydration status, presence of concurrent disease and variable related to urinalysis urine color, clarity, specific gravity, pH, urinary protein, glucose, ketone, urobilinogen, bilirubin, blood, leukocyte etc. The results were expressed in frequency number, percentage, and p-value. The significant difference in the proportion of cases between different categories of independent variables was ascertained by a p-value of ≤ 0.05.

CHAPTER- IV
RESULTS

4.1 Prevalence of Urinary Crystals

The total animal was 109, the overall prevalence of any type of urinary crystals was 23.85%. The prevalence of urinary crystals happens highest in Gulshan area and lowest from Nilkhet area. The prevalence ranges in many areas like Old Dhaka, Bashundhara, Tejgaon, Uttara, Dhanmondi, Mohammadpur of Dhaka Metropolitan Area.

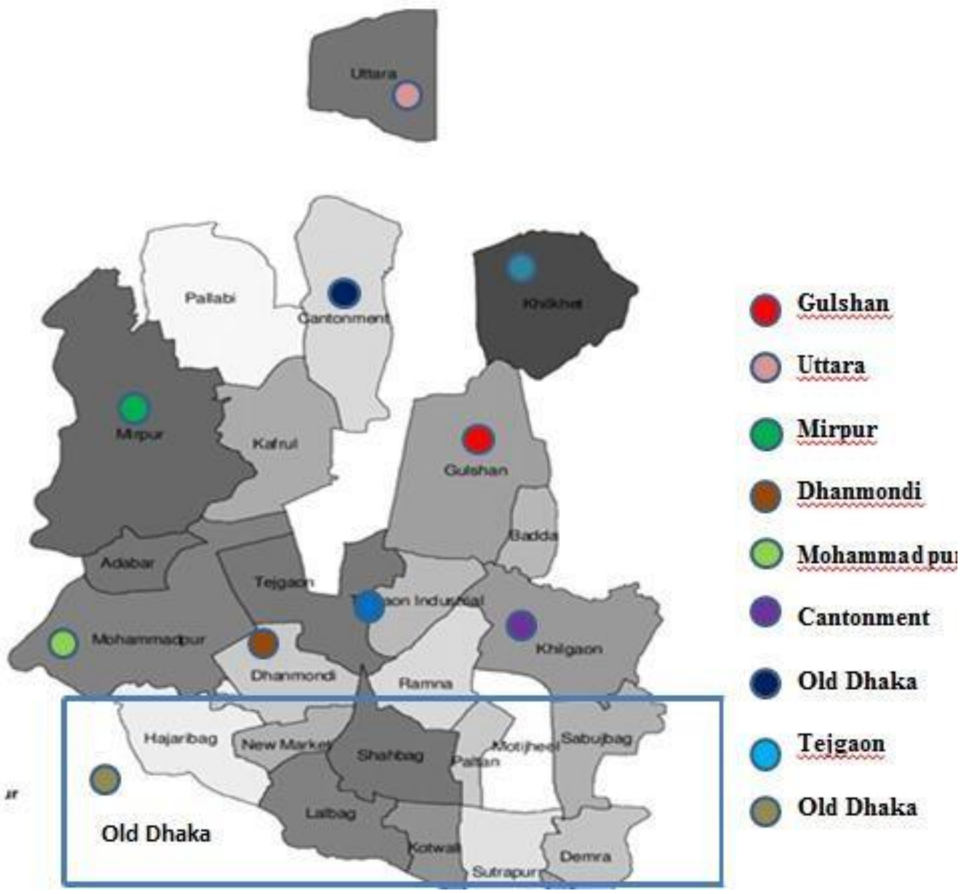

Fig 4.1: Locations showing origin of the affected dogs in Dhaka Metropolitan Area

4.2. Risk factors associated with the occurrence of urinary crystals

Significant associations of urinary crystals in dogs were found with age of animals, and diet type (Table 1 with bold word). The other variables like season, source of dog,sex, breed, de-sexing, vaccination, deworming and body condition score shows no clinical significant in the study.

Table 1. Association of different variables with urinary crystals in dog

Variable	**Level**		**N (%)**	**P value**
Urolith	Non-crystal		83 (76.15)	
	Crystal		26 (23.85)	
Season	Winter,1	Non-crystal	27 (77.14)	0.623
		Crystal	8 (22.86)	
	Summer, 2	Non-crystal	29 (80.56)	
		Crystal	7(19.44)	
	Rainy, 3	Non-crystal	27 (71.05)	
		Crystal	11 (28.95)	
Source of Dog	House hold	Non-crystal	79 (75.96)	0.836
		Crystal	25 (24.04)	
	Street	Non-crystal	4 (80)	
		Crystal	1 (20)	
Age	<2 Years	Non-crystal	24 (92.31)	**0.05**
		Crystal	2 (7.69)	
	2-5 Years	Non-crystal	27 (65.85)	

		Crystal	14 (34.15)	
	6-10 Years	Non-crystal	19 (67.86)	
		Crystal	9 (32.14)	
	>10 years	Non-crystal	12 (92.31)	
		Crystal	1 (7.69)	
Breed	Native	Non-crystal	20 (80)	0.912
		Crystal	5 (20)	
	Mixed	Non-crystal	6 (75)	
		Crystal	2 (25)	
	Exotic	Non-crystal	57 (76)	
		Crystal	18 (24)	
Diet Type	Dry food	Non-crystal	27 (62.79)	**0.008**
		Crystal	16 (37.21)	
	Home cooked food	Non-crystal	56 (84.85)	
		Crystal	10 (15.15)	
Sex	Male	Non-crystal	48 (72.73)	0.299
		Crystal	18 (27.27)	
	Female	Non-crystal	35 (81.40)	
		Crystal	8 (18.60)	
De-sexing	Desexed	Non-crystal	53 (75.71)	0.704
		Crystal	17 (24.79)	
	Intact	Non-crystal	30 (78.95)	

		Crystal	8 (21.05)	
Body Condition Score (BCS)	Very thin, 1	Non-crystal	3 (75)	0.961
		Crystal	1 (25)	
	Thin, 2	Non-crystal	9 (75)	
		Crystal	3 (25)	
	Good, 3	Non-crystal	40 (75.47)	
		Crystal	13 (24.53)	
	Fat, 4	Non-crystal	24 (75)	
		Crystal	8 (25)	
	Obese, 5	Non-crystal	7 (87.50)	
		Crystal	1 (12.50)	
Vaccination	Yes	Non-crystal	74 (75.51)	0.642
		Crystal	24 (24.49)	
	No	Non-crystal	9 (81.82)	
		Crystal	2 (23.85)	
Deworming	Yes	Non-crystal	60 (74.07)	0.388
		Crystal	21 (25.93)	
	No	Non-crystal	23 (82.14)	
		Crystal	5 (17.86)	
		Crystal	14	

4.3. Clinical signs associated with occurrence of urinary crystals

The clinical signs recorded during the study were found significantly associated with the formation of urinary Crystals (Table 2). Changes in temperature, mucous membrane and dehydration status was relevant with cases. But presence of concurrentdisease was not found to be significant to make crystals.

Table 2: Relationship of clinical signs with urinary Crystals in dogs

Variable	**Level**		**N (%)**	**P value**
Temperature	Normal	Non-crystal	72 (81.82)	**0.017**
		Crystal	16 (50)	
	Hypothermic	Non-crystal	1 (50.00)	
		Crystal	1 (50.00)	
	Hyper thermic	Non-crystal	10 (52.63)	
		Crystal	9 (47.37)	
Mucous Membrane	Pink	Non-crystal	75 (88.24)	**0.000**
		Crystal	10 (11.76)	
	Pale	Non-crystal	7 (30.43)	
		Crystal	16 (69.57)	
	Icteric	Non-crystal	0 (0)	
		Crystal	0 (0)	
	Congested	Non-crystal	1 (100)	
		Crystal	0 (0)	
	Cyanotic	Non-crystal	0 (0)	
		Crystal	0 (0)	

Dehydration	Severe	Non-crystal	8 (66.67)	**0.000**
		Crystal	4 (33.33)	
	Moderate	Non-crystal	9 (45.00)	
		Crystal	11 (55.00)	
	Mild	Non-crystal	11 (57.89)	
		Crystal	8 (42.11)	
Presence of concurrent disease	Normal	Non-crystal	55 (94.83)	0.168
		Crystal	3 (5.17)	
	Present	Non-crystal	31 (83.78)	
		Crystal	6 (16.22)	
	Absent	Non-crystal	51 (71.83)	
		Crystal	20 (28.17)	

4.4. Urinalysis results associated with occurrence of urinary crystals

Laboratory analysis of urine was done in terms of physical character; dip sticks urine test, checking urinary specific gravity by refractometer and microscopic evaluation to identify the type of crystals (Table 3). The Physical characteristics were changes in color, clarity, specific gravity significantly whereas in dip stick urine test urinary pH, protein, ketone, urobilinogen, bilirubin, blood and leukocyte were found significant in relation to produce crystals. The urinary glucose were not found to be significant in urolith formation.

Table 3. Relationship of urinalysis with urinary Crystals in dogs

Variable	**Level**		**N (%)**	**P value**
Urine Color	Yellowish	Non-crystal	67 (98.53)	**0.000**
		Crystal	1 (1.47)	
	Orange	Non-crystal	9 (50.00)	
		Crystal	9 (50.00)	
	Black Coffee	Non-crystal	1 (14.29)	
		Crystal	6 (85.71)	
	Red	Non-crystal	4 (28.57)	
		Crystal	10 (71.43)	
	Others	Non-crystal	2 (100.00)	
		Crystal	0 (0.00)	
Urine Clarity	Clear	Non-crystal	78 (96.30)	**0.000**
		Crystal	3 (3.70)	
	Turbid	Non-crystal	3 (20.00)	
		Crystal	12 (80.00)	
	Cloudy	Non-crystal	2 (16.67)	
		Crystal	10 (83.33)	
Urine Specific Gravity	Isothenuria	Non-crystal	44 (100.00)	**0.000**
		Crystal	0 (0.00)	
	Hyposthenuria	Non-crystal	4 (100.00)	

		Crystal	0 (0.00)	
	Hypersthenuria	Non-crystal	35 (76.15)	
		Crystal	26 (23.85)	
Urine pH	Acidic	Non-crystal	59 (88.06)	**0.000**
		Crystal	8 (11.94)	
	Alkaline	Non-crystal	3 (17.65)	
		Crystal	14 (82.35)	
	Neutral	Non-crystal	21 (84.00)	
		Crystal	4 (16.00)	
Urine Protein	Negative	Non-crystal	56 (86.15)	**0.000**
		Crystal	9 (13.85)	
	Trace	Non-crystal	25 (78.13)	
		Crystal	7 (21.88)	
	30mg/dl	Non-crystal	1 (16.67)	
		Crystal	5 (83.33)	
	100mg/dl	Non-crystal	1 (50.00)	
		Crystal	1 (50.00)	
	300mg/dl	Non-crystal	0 (0.00)	
		Crystal	4 (100.00)	
Urine Glucose	Negative	Non-crystal	78 (77.23)	
		Crystal	23 (22.77)	
	Trace	Non-crystal	2 (50.00)	

		Crystal	2 (50.00)	0.566
	250mg/dl	Non-crystal	1 (50.00)	
		Crystal	1 (50.00)	
	500 mg/dl	Non-crystal	1 (100.00)	
		Crystal	0 (0.00)	
	1000 mg/dl	Non-crystal	1 (100.00)	
		Crystal	0 (0.00)	
	2000 mg/dl	Non-crystal	0 (0.00)	
		Crystal	0 (0.00)	
Ketone	Negative	Non-crystal	82 (80.39)	**0.000**
		Crystal	20 (19.61)	
	5 mg/dl	Non-crystal	0 (0.00)	
		Crystal	3 (100.00)	
	15 mg/dl	Non-crystal	1 (25.00)	
		Crystal	3 (75.00)	
	40 mg/dl	Non-crystal	0 (0.00)	
		Crystal	0 (0.00)	
	80mg/dl	Non-crystal	0 (0.00)	
		Crystal	0 (0.00)	
Urobilinogen	Negative	Non-crystal	80 (82.47)	**0.000**
		Crystal	17 (17.53)	
	1 mg/dl	Non-crystal	3 (27.27)	

		Crystal	8 (72.73)	
	4 mg/dl	Non-crystal	0 (0.00)	
		Crystal	1 (100.00)	
	8 mg/dl	Non-crystal	0 (0.00)	
		Crystal	0 (0.00)	
	12 mg/dl	Non-crystal	0 (0.00)	
		Crystal	0 (0.00)	
Bilirubin	Negative	Non-crystal	72 (83.72)	**0.001**
		Crystal	14 (16.28)	
	1 mg/dl	Non-crystal	11 (50.00)	
		Crystal	11 (50.00)	
	4 mg/dl	Non-crystal	0 (0.00)	
		Crystal	1 (100.00)	
	10 mg/dl	Non-crystal	0 (0.00)	
		Crystal	0 (0.00)	
Blood	Negative	Non-crystal	69 (100.00)	**0.000**
		Crystal	0 (0.00)	
	Trace	Non-crystal	6 (50.00)	
		Crystal	6 (50.00)	
	Small 25cell/Micl	Non-crystal	6 (31.25)	
		Crystal	11 (68.75)	
	Moderate	Non-crystal	3 (50.00)	

	80 cell/Micl	Crystal	3 (50.00)	
	Large 200 cell/Micl	Non-crystal	0 (50.00)	
		Crystal	6 (100.00)	
Leukocyte	Negative	Non-crystal	62 (96.88)	**0.000**
		Crystal	2 (3.13)	
	Trace (15 cell/Micl)	Non-crystal	18 (60.00)	
		Crystal	12 (40.00)	
	Small(70 cell/Micl)	Non-crystal	3 (25.00)	
		Crystal	9 (75.00)	
	Moderate (125 cell/Micl)	Non-crystal	0 (0.00)	
		Crystal	3 (100.00)	
	Large (500 cell/Micl)	Non-crystal	0 (0.00)	
		Crystal	0 (0.00)	

CHAPTER-V
DISCUSSION

Urinary crystals are considered one of the major urinary bladder diseases for dogs and cats (Parvathamma et al., 2020). If untreated, it might be turned into a fatal disease leading to severe conditions. Although a frequent disease, no previous study was found about urinary crystals in Bangladesh. This study investigates the overall prevalence of the disease and some epidemiological factors related to this condition.

In this study it was found that around 23% of the hospitalized patients were positive for urinary crystals. Previously, a lower percentage of prevalence was recorded which might be due to selection of hospitalized patients (Nururrozi et al., 2020).

The season was not identified as a significant factor for urinary crystals that might be due to indiscriminate flow of patients in the hospital due to COVID-19 situation. But the previous author finds winter month as a risk factor in crystals formation. Some authors comment that pets might take a lower amount of water in the winter season, which may enhance concentrated urine and form urinary crystals due to inadequate glomerular filtration (Sharun et al., 2020).

Both the household and street dogs don't significantly impact the crystals. Lack of sampling should be the cause of insignificant results.

The breed was not identified as a risk factor for the crystals, but the previous author recognized a specific breed more prone to crystals. Different breeds were recorded in the study with limited sample size. This might have some genetic or autonomic reason (Swieton, 2018; Parvathamma et al., 2020).

It was observed that the type of diet had a high significance in formation of urinary crystals. The dogs taking dry food showed more clinical evidence urinary crystals over home cooked food that might be due presence of lower percentage of water contents in dry food and also lower intake of water by mouth by dogs taking dry food (Parvathamma et al., 2020).

No significant relationship was found according to sex. It was previously reported authors found that male animals were affected mainly by the crystals due to lengthy urinary tracts with winding patterns of the urinary tract (Kovaříková et al., 2021).

Moreover, castrated animals were not commonly affected compared to non-castrated (Parmar et al., 2021). But, the previous author stated that castrated animals were more frequently affected by this condition. Lack of testosterone might be responsible forthe high prevalence of crystals in the operated animals.

In this study impact of body condition score and vaccination status were not found significant (Taylor and Van Veggel, 2018).

On the other hand, deworming was not an important factor to form canine crystals. But previous drug administration would significantly impact the urinary crystals. Previous studies also suggested similar findings (Parmar et al., 2021).

It was sorted out that most of the clinical signs impacted significantly the urinary crystals. Temperature, mucous membrane, dehydration, and concurrent diseases significantly affected the condition (Dobre et al., 2019; Taylor and Smeak, 2021; Thiel et al., 2019).

Besides that, almost all parameter of urine analysis like urine color, clarity, specific gravity, pH, urinary protein, ketone, bilirubin, urobilinogen, and leukocyte was highly significant in terms of disease. But urinary glucose didn’t show any clinical significance. Previous articles have found similar findings (Taylor and Smeak, 2021).

Limitations of the study:

1. This study was hospital-based and urinalysis of dogs were performed at the AKSACC, Dhaka
2. The sample size was small there as the study period was affected with on and off COVID-19 lockdown and shut down by the government.
3. Sometimes it takes more than 30 minutes from the time or urination that might affect the result.

CHAPTER-VI
CONCLUSION

This study shows major clinicalsigns and most urine test result are clinically significant in producing the crystals. This study suggests a plausible source of the prevalence based on limited clinical data.As there is no previous clinical study before this study the data generated in this studywould be beneficial to pet practitioners in Bangladesh as well as may aid in taking appropriate measures to prevent or reduce the prevalence of this condition in future.

Appendix

Evaluation of Urinary Crystals in Dog at Dhaka Metropolitan Area, Dhaka, Bangladesh

OWNER'S PARTICULAR:

Name : Date of reg: Phone no:

Name of the owner: Owner's Address:

ANIMAL'S PARTICULAR:

Source of dog: 1. Indoor 2. Street

Breed:

Sex: 1. Male 2. Female

De-sexed: 1. Yes 2. No

Age: Year/Months……..

BCS: 1 (very thin) 2 (Thin) 3 Good 4 Fat 5 Obese

Vaccination: Yes No,

Deworming: Yes No, if yes, name …………………and when …………

Drugs: Any drugs are used in last one month?

1. No 2. Yes , if yes, which drugs are used and when………..

DIET INFORMATION:

Diet type: 1. dry food 2.. Home cooked food 3. Others…….

CLINICAL HISTORY AND FINDINGS:

Temperature: ^{0}F

Mucous membrane: 1. Pink 2. Pale 3. Icteric 4. Congested 5. Cyanotic

Degree of dehydration: 1. Severe 2. Moderate 3. Mild

Presence of any concurrent disease: 1. Yes ....2. No

UA:

Physical Parameters:

Color: 1. Yellowish 2. Orange 3. Black coffee 4. Orange 5. Others….

Clarity: 1. Clear 2. Turbid 3. Cloudy

Specific Gravity 1. Isosthenuria (1.008-1.012) 2. Hyposthenuria (>1.008)

3. Hypersthenuria (>1.012)

Chemical Parameters:

pH: 1. Acidic 2. Alkaline 3. Neutral

Protein: 1. Negative 2. Trace 3. 30mg/dl 4. 100mg/dl 5. 300mg/dl

Glucose: 1. Negative 2. Trace 3. 250mg/dl 4. 500mg/dl 5. 1000mg/dl

6. 2000mg/dl

Ketone: 1. Negative 2. 5+/- 3. 15mg/dl 4. 40mg/dl 5. 80mg/dl

Urobilinogen: 1. Negative 2. 1 3. 4 mg/dl 4. 8 mg/dl 5. 12 mg/dl

Bilirubin: 1. Negative 2. 1mg/dl 3. 4mg/dl 4. 10mg/dl

Blood: 1. Neg 2. Trace 3. Small 25 4. Moderate 80 5. Large 200cells/MicL

Leukocyte: 1. Neg 2. Trace 15 3. Small 70 4. Moderate 125 5. Large 500cells/MicL

Microscopic Parameters:

Crystals: 1. Struvite 2. Ca Phosphate 3. Ca oxalate 4. Urate 5. Others…..

Diagnosis:

CHAPTER-VII
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Biography

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