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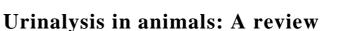
Jove Jacob

B.V.Sc and AH Scholar, Apollo College of Veterinary Medicine, Agra road, Jaipur, Rajasthan, India

Corresponding Author: Jove Jacob B.V.Sc and AH Scholar, Apollo College of Veterinary Medicine, Agra road, Jaipur, Rajasthan, India

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Jove Jacob

Abstract

Urinalysis is one of the underrated, yet remarkable tools in practice. The initial stages of development of several diseases such as diabetes mellitus, urinary tract infection and glomerulonephritis can be easily traced by using urinalysis as an aid. Observing the physical, chemical and microscopical aspects accompanied by microbial culture and sensitivity test reveals majority of the lower tract infection and also determines the overall health status of a patient. Urinalysis being readily available and an inexpensive toolkit is not widely used in practice. This review intends to highlight several beneficial aspects of urinalysis which is an asset and an irreplaceable tool to the veterinary practice.

Keywords: Diabetes mellitus, lower tract infection, urinalysis, urinary tract infection

Introduction

Urinalysis is one of the key tools within the hands of the Veterinarians which play a major role in diagnosis of the disease. From the ancient times till the Victorian era, urine acted as one of the primary diagnostic tool. Laboratory medicine started with urine analysis almost 6000^[1] years ago and then called uroscopy and was later given a new name urinalysis after the 17th century^[2]. Uroscopy is derived from Greek word "uroscopia" meaning scientific examination of urine. Physicians also considered urine as "divine fluid". Hippocrates is credited with being the original Uroscopist.

From an Ancient period, Hindu cultures were aware that some urine tasted sweet and blackants were attracted towards the urine of some patients which compelled them to suspect that individuals might be suffering from a pathological condition which is now termed as Diabetes mellitus. Although uroscopy emerged to be a primary toolkit but overtime, became a more deputized form in the medical field but in veterinary practice, is still a relief to a majority of veterinarians practicing worldwide.

Correct Urine sampling- Why and How?

Urine samples reveal the Health status of the urinary system, majorly kidneys and other systems too. Hence, proper sampling and storing (if necessary) is a prerequisite for any accurate prognosis of an ailment. Sterility while collecting the sample should be maintained at all conditions and a fresh collection container is preferred. Mainly, there are three methods adopted for collection of urine samples. Out of all the three methods available, cystocentesis is the most preferred and accurate one. A sterile needle is inserted into the urinary bladder via the abdominal wall and with the help of a syringe, and urine is withdrawn directly. Collecting a contamination free sample is the major advantage. This method is useful for determination of bacterial infection in the urinary bladder and kidneys. This method is only useful if the bladder of the patient is full and cooperates with the vet [3]. Another method used in practice is catheterization; This is a clean process but not a sterile process. Generally patient is preferred for lateral recumbency (both male, female dogs and cats alike) and a narrow sterile catheter is introduced into the urethral passage till it reaches the bladder and urine starts to drip from the end of the catheter. This technique is usually practiced when a voluntary sample is not available and also has the potential to introduce new bacterial infection via the urethral passage to the urinary bladder. Another most widely practiced method is mid-stream freeflow, a common method used in bovines and easily done non-invasively. But the sample may be contaminated with debris from the urethra or environment.

Ideally a fresh sample collected within 30 minutes of examination is considered. But if it's not possible, urine should be refrigerated as soon as possible and should not be stored for more than 6-12hrs. Refrigerated samples should be thoroughly mixed prior to examination.

Physical Examination of Urine Color and Transparency

Normal color of urine ranges from pale yellow to amber color and it depends on the amount of urochrome present. However dark yellow color in dogs and cats is often associated with dehydration due to vomiting or diarrhea and very pale yellow color may be a clinical sign of polydipsia or polyuria which may be an indication to the underlying kidney disease or the inability to concentrate the urine ^[4]. Brown colored urine is suggestive of haemoglobinuria or nephritis. Faint pink color of urine is an indication of congenital porphyria or urolithiasis ^[5]. The transparency of urine is hindered by the presence of epithelial cells, crystals of calcium carbonate and amorphous urates. But in the case of horses, it is normal to find cloudy urine due to a slight increase in the amount of mucous membrane and calcium carbonates in the urine ^[6]. Interestingly in bovines, urine collected from an obstructive urolithiasis animal is still transparent and clear.

Odour and Foam

Odour of urine is usually ammoniacal in nature, primarily due to decomposition of volatile fatty acids (acetic acid, butyric and propionic acid). Sweet odour leads to primary diagnosis of diabetes mellitus and acetone like smell is found in cases of ketonuria. Foamy urine is a common finding in patients of Proteinuria ^[7]. Common reason for Proteinuria is inflammation and bleeding from the urinary tract. Proteinuria can occur due to a variety of reasons other than kidney dysfunction^[8].

Specific Gravity

Specific gravity is the relative amount of solids in urine and also throws light over the ability of the kidney to concentrate or dilute the urine. Specific gravity is inversely proportional to the quantity of urine but exception is found in patients suffering from diabetes mellitus, where large volume of urine with high specific gravity is voided. Generally, urine with specific gravity of less than 1.001 is marked as hyposthenuria in dogs and cats. Urine specific gravity may falsely be increased by 0.003 to 0.005 for every 1g/dL of protein in urine and for every 1g/dL of glucose may be increased by 0.004 to 0.005^[9]. Specific Gravity of urine can be detected with the help of a urinometer or by a refractometer ^[4]. Refracto meters are generally used for human urine samples but refractometers for veterinary use are also available. Usually, refractometers are provided with four measurement scales which includes Canine Urine Specific Gravity, Feline Urine Specific Gravity, Large Animal Urine Specific Gravity and Serum Protein concentrations for all animals^[10].

Chemical Examination pH of Urine

Urine pH gives a rough estimate of the ability of kidneys to concentrate hydrogen ions and thus, is an aid to veterinarians to determine the rough acid- base status of the body. Herbivores generally produce alkaline urine and carnivores produce acidic urine ^[11]. The normal pH of urine in dogs and cats ranges from 5.5-7.0 and 5.0-7.0 respectively ^[9]. Diet has the potential to contribute to acid base balance via the supply of acid and base from diet. Hepatic oxidation of sulphur containing amino acids such as methionine and cysteine generates hydrogen ion. This is balanced by carbonates from green plants/vegetables which supply more amounts of Magnesium and Potassium, hence equilibrium is maintained.

Kidneys are the main sources of excretion of dietary sources for hydrogen ions. Hence, it is directly reflected into the urine. Alkaline urine is common in dog patients suffering from urinary tract infections ^[12]. Urine pH plays an important role in the formation of uroliths. Struvite and calcium apatite are commonly found in alkaline pH while cystine stones are found in the acidic pH ^[13, 14].

Proteins in urine

Excess amount of proteins present in urine is called Proteinuria. Glomeruli prevents large protein particles such as albumin from getting voided in urine but in case of physical oxidative stress particularly in the renal system, may lead to albuminuria. Along with this various other types of stress either physical or emotional may lead to increased glomerular permeability which ultimately result in Proteinuria ^[15]. The proteins can also originate from any part of the reproductive tract which are directly connected to the urinary tract (prostate gland, uterus and vagina). However in these cases, cystocentesis is recommended. Protein in urine can be detected by use of dipstick which primarily assess albumin in urine. Dipstick detects protein by production of color with an indicator dye. However Bromophenol blue indicator is a popular test for protein, as this test is very sensitive to albumin but can also detect globulin and Bence- Jones, poorly. The Sulfosalicylic acid test is another test which is very efficient in detection of albumin, globulin and Bence-Jones even at low concentrations. The most accurate method of diagnosis of Proteinuria is done by determining the protein: creatinine ratio. Tubular concentration of urine increases the urinary protein and urinary creatinine ratio equally so that the ratio remains constant whether the urine is concentrated or diluted. This ratio is normally less than one [16]. However while using a dipstick for detection of proteinuria, the specific gravity of that urine sample must always be considered as the degree of proteinuria corroborates with the result of specific gravity values. So performing a specific gravity technique along with a dipstick method can provide a more accurate proteinuria result^[17].

Glucose in urine

Presence of glucose in urine is called Glucosuria. Glucose is freely filtered and then reabsorbed in the proximal tubules, hence preserving glucose for being utilized as energy. If blood glucose level is too high (hyperglycemia), it exceeds the renal threshold to be reabsorbed and the glucose is excreted in urine. The renal threshold in dogs is 10mmol/L and is slightly higher in cats (14-17mmol/L) ^[18]. Glucosuria in combination with hyperglycemia reflects a tubular resorption defect in which the renal tubules fail to resorb glucose from the glomerular filtrate ^[19, 20]. Non pathological glucosuria is found in various emotional states. Pathological glucosuria is found in diabetes mellitus, hyperthyroidism, acute renal failure, hyperactivity of adrenal cortex and urinary obstruction in dogs and cats ^[21].

Ketones in urine

Ketones are the product of fat metabolism and their normal reference range is zero to negative ^[22]. Presence of ketone bodies in urine is termed ketonuria. Ketones are produced from fatty acid metabolism and include acetoacetic acid, acetone and beta hydroxybutyrate. Acetones are usually expired by lungs. Dipstick method can be used for detection of ketone bodies. Dipsticks can detect acetoacetate to a huge

extent and to some extent acetone, but are unable to detect beta hydroxybutyrate ^[23]. Ketonuria is a common pathologic condition; especially in ruminants, it may be an indication of diabetic ketoacidosis. Animals in late pregnancy and early post parturition can develop ketonuria. Diets rich in fats, excessive starvation may also lead to ketonuria ^[24].

Bilirubin in urine

When haemoglobin is degraded, the heme part is converted into bilirubin, which is conjugated in the liver and excreted in bile ^[25]. Some conjugated bilirubin is filtered by the glomerulus and excreted in urine. Normal reference range for bilirubin is zero to negative [26]. The threshold range of bilirubin is low, hence, even a minor change in plasma bilirubin may lead to bilirubinuria. Bilirubinuria is occasionally found in dogs partly due to low renal threshold and partly because canine renal tubular cells are able to catabolize heamoglobin to unconjugated bilirubin and then secrete it into urine.^[20] Unlike dogs, bilirubinuria in cats are associated with several underlying diseases. It is associated with feline infectious peritonitis, primary hepatic disorders, diabetes mellitus and feline leukemia related disorders. Bilirubin is unstable and decreased by exposure to sunlight and high levels of vitamin C.

Microscopic examination

Microscopic examination of urine is one of the most integrated parts of urinalysis and also has a great clinical importance. Urine sediments settle down at the bottom of the tube when spun in a centrifuge machine. Usually casts and erythrocytes disappear or disintegrate when urine is kept for a prolonged time. Hence, freshly collected samples are recommended for examination.

Cellular components

Often it becomes difficult to trace the origin of a particular cellular component as they may originate from several areas such as urogenital tract, urothelium or vascular system. Erythrocytes are usually found in increased numbers in the urine samples collected via cystocentesis or catheterization, but excessive numbers of erythrocytes indicate haemorrhage ^[27]. This may be an indication of urolithiasis, trauma, cancer or infection. Excessive numbers of leukocytes indicate pyelonephritis and cystitis. Pyuria indicates suppuration at some point in the urinary tract especially urethritis or cystitis ^[28].

Crystals in urine

Crystals are commonly found in urine. Their formation is dependent on oversaturation of the mineral substrate, urine pH and length of time between urine collection and examination. Crystals in the urine of herbivores animals have no special significance unless they occur in very large numbers and are accompanied by other clinical signs such as irritation of the urinary tract ^[29]. Calcium oxalate and calcium carbonate crystalluria is commonly found in healthy horses and cattle. Bilirubin crystals may be normal in small amounts in dogs. Some crystals are also formed on administration of certain types of drugs such as sulphadiazine, trisilicate, ciprofloxacin, indinavir, etc. ^[30] Different crystals have different shapes. Struvite crystals have coffin lid appearance; calcium oxalate monohydrate crystals have picket fence appearance, envelope ditetragonal pyramids or bipyramidal shape. While calcium oxalate dihydrate crystals have a maltese cross or square envelope shape. ^[31] Urate crystals are thorn apple or fine needle shaped and cystine crystals are of hexagonal shape. Struvite and calcium phosphate crystals may be observed in neutral to alkaline urine of dogs and cats. Acidic urine may contain amorphous urates, cystine urates, calcium oxalate and hippuric acid ^[32].

Pregnancy Diagnosis

The earliest detection of pregnancy relied on chemical test performed in urinalysis. Pregnancy diagnosis can be easily done with the help of urine. In case of mares, cuboni test is commonly used which detects the conjugated estrogen in the urine. While in bovines and camels, barium chloride test is performed. Accuracy of the test is described to be 70-95% from 15 to 210 days of pregnancy. In camels, the test is considered to be 85% accurate between 50-90 days of pregnancy ^[33].

Cancer detection by urinalysis

This provides an exciting opportunity to utilize a true screening test with the help of a simple urinalysis method. Transitional cell carcinoma is the most common tumour of the urogenital system in dogs. The test helps in the detection of mutation in BRAF gene which has been identified as a mutation in 85% of canine urogenital cancers and is available through sentinel biomedical ^[34]. Clinically this test can diagnose the condition, even before exhibition of sizable mass effect or any clinical sign by the patient due to the cancer ^[35].

Conclusion

The fame of ancient technique uroscopy had once kept pace with the modernization almost till the 19th century. But in the new era of modernization, uroscopy has been left behind with its old time consuming method. Though new advancements have been made in this method but still lag the pace. Advance technology like PCR are now being used to detect a wide variety of bacteria from urine, providing a faster diagnosis of urinary tract infection. Even diseases like Tuberculosis are being diagnosed using urine samples. As urinalysis is a safe and simple method with the only requirement of urine samples from the patient causing no discomfort or pain to the patient, emphasis should be given in manufacturing miniaturized point-of-care of urinalysis instruments which would be convenient to carry by the field practitioners and also by the patients who can perform the test and seek appropriate advice from their doctors via telecommunications. In the end the author would conclude by saying that parameters obtained by proper urinalysis are still extensively used to guide even the minor underlying ailments that are often subdued. Though the process of urinalysis has evolved a great deal but yet in near future with science and technology guided by human minds more improvement in this field is anticipated.

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