URINALYSIS IN TORTOISES

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Abstract: In contrast to the conventional view, urinalysis in tortoises is an excellent diagnostic tool for supplying information on kidney and liver disease.

Key words: tortoises, urinalysis, kidney disease, liver disease.

In tortoises, elimination of nitrogenous waste is mainly performed by excretion of urea and urate via urine (Dantzler and Schmidt-Nielsen, 1965; Fox, 1977; Harless and Morlock, 1989). Unlike mammals, tortoises produce hypotonic urine, as they lack the loop of Henle in the nephron of the kidney (Fox, 1977). The urine can be modified in the cloaca and the urinary bladder. Due to the secretory and absorptive capabilities of the bladder wall of tortoises (Bentley, 1962, 1979; Harless and Morlock, 1989) and the fecal contamination when the urine passes the cloaca, the use of urinalysis as a diagnostic tool has conventionally been rated as having little usefulness for diagnosis (Frye, 1991; Campbell, 1996; Gabrisch and Zwart, 1998).

Urine samples of 25 tortoises (Hermann's, *Testudo hermanni*, Mediterranean spur-thighed, *Testudo graeca,* and Horsfield's, *Testudo horsfieldi*) with renal disease were examined. Renal disease had regularly been diagnosed by blood tests and ultrasonography. In 11 cases renal disease was confirmed by necropsy. The urine samples of 50 clinically healthy tortoises of the same three species, which had been fed a strictly vegetarian diet were examined and served as controls.

Urine samples were obtained by spontaneous voiding of urine, by digital emptying the urinary bladder, or by cystocentesis. Macroscopic characteristics of the urine, including color, appearance, consistency, and relative amount of urate were recorded. Urine samples were immediately evaluated for glucose, bilirubin, ketone, occult blood/erythrocytes, pH, protein and urobilinogen using reagent strips. The specific gravity of the liquid portion was measured using a standard clinical refractometer. Microscopic examination of native urine, and of the sediment of centrifugated urine, was performed within 2hr of collection. The urine sediment was stained with Löffler's solution (30.0ml alcoholic methylenblue solution with 100ml 0.015% potassium hydroxide) for better differentiation of cells, especially in samples containing large amounts of urates.

Normal urine of tortoises consists of a liquid clear portion and of small to moderate amounts of white urates. The color of the liquid part varies from colorless to pale yellow. Tortoises with liver disease may show a yellow-brown to yellow-green urine due to biliverdin, and to a lesser extent to bilirubin. The physiological value of the specific gravity ranged from 1003-1014 (average: 1008) in clinically healthy tortoises. In tortoises with renal disease the specific gravity can increase up to 1034 (average: 1013). The pH of the urine of healthy tortoises fed on vegetarian diet is usually alkaline and the urine pH in anorexic tortoises (and in tortoises with high protein intake) is often acidic.

A few leukocytes can normally be found in the sediment of all urine samples. An increase of leukocyte number was established in spontaneously voided urine or urine gained by digital emptying of the bladder and in 16% of the samples of tortoises with renal disease. Although the number of leukocytes in the sediment was increased in some samples, the "dipstick" did not show positive staining for leukocytes in all cases. The nitrite test was negative in 94% of urine samples of healthy tortoises and positive in 20% of the tortoises with renal damage. Protein was detected in 10% of the urine specimens of healthy animals and in 52% of tortoises with renal disease. Glucose was present in 90% of the samples of healthy tortoises, below 30.0mg/dl, but in 48% of the ill tortoises, was higher than 50.0mg/dl.

Bilirubin and ketone tests never proved to be positive in any urine sample. Red blood cells were detected in urine samples of clinically healthy tortoises by "dipstick", and by examination of the sediment when the urine had been obtained by cystocentesis or digital emptying of the bladder. Samples of clinically healthy tortoises, which had been obtained by spontaneous voiding of the urine, never showed any positive reaction for blood.

Renal tubular epithelial cells, transitional epithelial cells, and squamous epithelial cells can normally be found in the urine as a result of the normal sloughing off of old cells. In the urine of tortoises with renal damage, however, the number of these cells was significantly increased.

Renal casts were not detected in urine samples of healthy tortoises. In the urine samples of 25 tortoises with renal damage there were granular casts (4x), hyaline casts (1x) and casts composed of bacteria (4x) or crystals of ammoniumbiurate (3x).

Bacteria were present in every specimen, including ones gained by cystocentesis. Samples voided or gained by digital emptying the bladder contained much more bacteria than those obtained by cystocentesis. In tortoises with renal disease, there was a much higher amount of bacteria in the urine than in clinically healthy ones. Yeast was detected in three samples of tortoises with renal disease.

Amorphus urates and ammonium biurates were seen in nearly all urine samples of clinically healthy tortoises. In tortoises with renal disease many other crystals were found including: calcium oxalates, cholesterol, cystine, hippuric acid, leucine, sodium urate, tyrosine, bilirubine, triple phosphates and especially uric acid in varying formations.

Spermatozoa may be present in urine samples of male and female tortoises. Parasites such as *Hexamita* spp., ciliates and helminths, especially helminthic eggs, and/or cells of plants, were occasionally found in the urine samples, even if the samples were obtained by cystocentesis.

Urinalysis is an underestimated diagnostic tool in tortoise medicine, previously used only for diagnosis of hexamitiasis (Gabrisch and Zwart, 1998). However, present results prove that urinalysis can deliver valuable information on renal and other diseases in tortoises. Possible contamination of samples, even those obtained by cystocentesis, must be taken into account, as the ureters end in the cloaca and not in the bladder (Perschmann, 1956), and the urine may be contaminated when reaching the bladder.

In tortoises, that are too small for taking blood or performing ultrasonography, the physical and chemical properties of urine are important indicators of health. The "dipstick" procedure, which is easily performed, can be used as a screening test. The pH of tortoise urine seems to be an excellent indicator of the overall metabolic status, and an acidic pH can indicate anorexia or improper diet.

Indicators for renal disease are often an acidic pH, glucose higher than 50.0mg/dl, the presence of renal casts, and a higher number of cells (leukocytes, renal tubular epithelial cells, transitional epithelial cells, squamous epithelial cells), and increased bacteria in the urine. Uric acid crystals in varying formations are often detectable in the urine of tortoises suffering from gout. In tortoises with liver disease, bilirubin and tyrosine crystals may be detected.

Urinalysis in tortoises should be a regular part of a health assessment and in the author's experience, has proved as a valuable diagnostic tool.

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