# URINALYSIS IN BOX TURTLES, *Terrapene* spp.

**Paul M. Gibbons[1], DVM*, Susan J. Horton[1], DVM, Shannon R. W. Brandl[2], CVT**

*1. Niles Animal Hospital and Bird Medical Center,*
*7278 North Milwaukee Avenue, Niles, Illinois 60714, USA*
*2. Fox Animal Hospital, 2107 Crawford Avenue, Evanston, Illinois 60201, USA*

*Abstract:* Urinalysis is an underused diagnostic test that provides useful information in evaluating the chelonian patient. Spontaneously voided urine of 31 box turtles, *Terrapene* spp., presented to a private clinical practice was studied. Standard urine "dipsticks" were used to assay glucose, bilirubin, ketones, blood, pH, and protein. Sediment was also evaluated microscopically. Results include a mean urine pH of 6.6 in *T. carolina* spp., and 6.4 in *T. ornata.* Mean urine specific gravity was 1.005 in *T. carolina* spp., and 1.007 in *T. ornata.* Microscopy revealed bacteria and epithelial cells in almost all samples. Urinalysis results cannot be interpreted in the same manner as in mammals. Various unique aspects of the reptilian urinary system must be accounted for in using urinalysis for clinical judgements. When properly interpreted, urinalysis is a useful indicator of the hydration status, urinary system, and general health of box turtles.

*Key words:* box turtle, *Terrapene carolina*, *Terrapene ornata*, urine, urinalysis, chelonia, bladder, reptiles.

## INTRODUCTION

Urinalysis is one of the simplest and most underused diagnostic tests in reptile medicine. Many authorities discount the value of urinalysis because of perceived difficulty in interpretation and the potential for contamination. The authors are convinced, however, that when a patient provides a sample, it should be examined. Box turtles, *Terrapene* spp., often spontaneously provide urine during the physical examination. This sample might be mixed with material from the reproductive or digestive system, but can provide much useful information to the willing clinician.

Chelonian urine cannot be considered equal to that of a mammal. Many factors must be considered in the collection and interpretation of turtle urine. Innis offered an insightful review of the literature and a useful perspective on urinalysis and urinary mechanisms in Chelonia (Innis, 1997). The samples obtained from the examination room table (*i.e.*, "free catch") are simply not a straightforward assessment of renal function or renal pathology. They are, however, a reflection of electrolyte fluid balance and overall urinary system function in the chelonian patient. If the urinary system is viewed as a unit, the urine sample can provide valuable information about urinary tract health, hydration, and disease in these patients.

In mammals, water and solutes pass from the glomerular blood into Bowman's capsule. This filtrate is then modified as it passes through the proximal convoluted tubule into the loop of Henle to the distal convoluted tubule and into the system of collecting ducts. Beyond this point, it is generally thought that little change occurs in the urine. Therefore the renal pelvis, ureters, and bladder serve only for urine transport and storage in mammals.

In contrast, reptiles may utilize both the caudal intestinal and complete urinary tracts to control the final composition of urine that passes through the vent. Because they lack a loop of Henle, reptiles are unable to produce hyperosmotic urine in the kidneys, and in fact, ureteral urine is expected to be hypo-osmotic to plasma (Dantzler and Schmidt-Nielsen, 1965). The final voided urine, however, may have solute concentrations approaching that of plasma by action of extrarenal mechanisms (Frye, 1991; Innis, 1997). Chelonians have been shown to reabsorb water, electrolytes, and nitrogenous wastes across the bladder mucosa, which allows for elevation of urine osmolarity to that of circulating plasma (Innis, 1997). Reptiles also reabsorb water and possibly electrolytes through mucosal membranes in the cloaca, distal colon, and rectum where urine can be stored in species lacking a urinary bladder. Fresh-water turtles, *Pseudemys scripta*, have been shown to increase renal tubular resorption of water and may shut down glomerular filtration in response to dehydration (Dantzler and Schmidt-Nielsen, 1965). The whole of these renal and extrarenal mechanisms can be initially evaluated via urinalysis, which could strengthen the indications for further diagnostic testing.

## MATERIALS AND METHODS

This study evaluates urine that was spontaneously voided from box turtle patients presented by clients to a private clinical practice in the upper midwestern United States. Whenever possible age, weight, sex, and date of collection were recorded. Patients were not excluded from the study due to illness. This allowed for sample interpretation, although inclusion of clinically ill patients prevents data from being used as a clinical reference. Standard urine "dipsticks" (Multistix7, Bayer Co., Elkhart, IN, USA) were used to evaluate glucose, bilirubin, ketones, occult blood, pH, and protein. Unstained and stained (Sedistain7, Bekton-Dickinson, Franklin Lakes, NJ, USA) samples of urine sediment were evaluated under light microscopy within 30min of centrifugation at 2000RPM for 3.0min. Gross appearance of urine samples was noted, and specific gravity was recorded using a temperature compensated refractometer.

## RESULTS

Raw data are listed in Tables 1 and 2. A total of 31 urine samples were evaluated: 20 were *T. carolina* spp. and 11 were *T. ornata*. Of the *T. carolina* spp., 7 were male, 11 were female, and the sex of one individual was not recorded. The *T. ornata* sample group contained 4 males, 6 females, and one unsexed individual. Age can be very difficult to estimate in these animals, but was recorded when possible. It ranged from about 3yr to over 26yr. Weights varied among animals from 86.0-667.0g at the time of sample collection, but body condition was usually not recorded. Most of the samples obtained (25/31) were either clear or light yellow in color. One sample was cloudy white, and two were cloudy yellow. One sample was yellow in color, and one, the sample with the highest specific gravity (1.019), was dark yellow. Urine specific gravity ranged from 1.001-1.019, with a mean of 1.007 in the *T. ornata* group, and 1.005 in the *T. carolina* group. Discarding the 1.019 specific gravity value from the *T. carolina* calculation, the mean drops to 1.004.

Only 3 samples tested positive for glucose. One *T. carolina* had high (500.0mg/dl) urine glucose concurrently with an elevated plasma glucose level (372.0mg/dl). The two *T. ornata* individuals with urine glucose had low (100.0mg/dl) and very high (1000.0mg/dl) levels respectively, and unfortunately in neither case would the client allow further diagnostic testing in light of the urine results. The *T. ornata* with very high urine glucose was considered to be markedly underweight as a full adult (over 18yr of age) at 264.0g.

In none of the urine samples were bilirubin or ketones identified using the Multistix7 reagent strips. Occult blood was positive ("small") in only one of the *T. ornata* samples, but varied from "trace" to "moderate" in 6 of the *T. carolina* samples. The pH ranged from 5.0-8.0, with a mean of 6.6 in the *T. carolina* group, and a mean of 6.4 in the *T. ornata* group.

Trace amounts of protein were found in most of the samples, but was higher in four samples, 2 of them *T. carolina* and 2 *T. ornata.* All of the patients with more than trace amounts of urine protein were clinically ill, and 2 of them were also positive for urine glucose and had relatively high urine specific gravity. One of them died within 2wk after testing.

Microscopic analysis of urine sediment revealed some level of bacteria in almost all (28/31) samples. Likewise, epithelial cells were present in nearly all (29/31) of the samples. Live sperm was present in samples from 2 of the male *T. carolina.* Flagellated protozoa were seen in 3 of the *T. ornata* samples and 3 of the *T. carolina* samples. An occasional granular cast was seen in 1 of the *T. carolina* samples, and 1 *T. ornata* sample contained waxy casts and course granular casts. Urate crystals were identified in 2 samples from *T. ornata*; 1 of them was considered clinically ill.

## DISCUSSION

Although interpretation is quite different than in mammals, urinalysis can be a useful, economical, rapid diagnostic aid in box turtles. The study group represents patients that are often seen by clinical veterinarians in private practice. These patients may be kept in a variety of conditions with different diets, thermogradients, humidity levels, lighting, and access to water. This group is not a controlled study group, rather it provides data relevant to the general veterinary practice setting. The data obtained in this study must be interpreted with this in mind so the findings can be useful as a baseline for interpreting urinalysis in similar practice situations. Of course, it is imperative that each reptile case be interpreted in light of all relevant husbandry, physical examination, and diagnostic findings.

Box turtle urine specific gravity (USG) is a useful tool for assessing the patient's overall hydration and urine solute status. It is not useful in evaluating reptile kidney function. Urine specific gravity is defined as the weight of a solution compared with an equal volume of distilled water (Chew and DiBartola, 1989). It varies with the molecular weight of the solutes and how much of them is present. Solutes in turtle urine include, but are not limited to, glucose, electrolytes, urates, ammonia, urea, mucus, and other proteins. In theory, a well-hydrated box turtle should void urine with a low specific gravity. Many hobbyists, however, do not provide adequate water or humidity for their box turtles, leading to a state of chronic dehydration. The authors use urine specific gravity to help explain to the pet owner how a turtle is responding to chronic water deprivation, and considers USG's above 1.005 to be elevated. These patients should be placed into a shallow pan of tepid water for at least 15.0min daily.

It is difficult to explain the case with a USG of 1.019. The chelonian urinary system has not been found to increase the osmolarity above that of plasma in previous reports (Chew and DiBartola, 1989). It is not likely that this animal actually produced this highly concentrated product, but rather some solute was added or mixed with the sample. The chelonian urinary bladder mucosa is secretory, and could have produced excessive mucus in response to some irritant. It is also possible that the urine was contaminated with reproductive or intestinal material. Ideally, this sample would be retested after collection via cystocentesis.

Although renal thresholds for blood glucose have not been evaluated in reptiles, any glucose in the urine of box turtles is cause for concern. Normal healthy turtles do not have glucose in the urine. Keep in mind that the urine glucose reagent pad can show false positive results from contamination with substances such as peroxide and hypochlorite. False negatives might occur if the urine is refrigerated, or contains vitamin C or formaldehyde (Chew and DiBartola, 1989). Hyperglycemia does occur in chelonians, and may be due to any condition known to cause it in other species (Frye, 1991). Diabetes mellitus has been reported in turtles, but the authors have also found hyperglycemia associated with inflammatory diseases of the coelomic cavity, hepatic lipidosis, and severe wasting in chelonians (Frye, 1991). It can be transient, resolving without insulin therapy, but it can also indicate a grave prognosis. Patients with glucosuria should be thoroughly evaluated for other diseases and offered supportive care which is often necessary.

Interestingly, ketones do not appear in the urine of hyperglycemic, glucosuric chelonians. It may be surmised, then, that ketones are not produced in significant amounts as seen in mammals. Ketones may be found in the urine of mammals suffering from diabetes mellitus with ketoacidosis, during starvation, and possibly associated with liver disease (Chew and DiBartola, 1989). Bilirubin is not expected in the urine of reptiles because they produce it only in very small amounts (Frye, 1991). A more useful test might be one for biliverdin, the major bile pigment in reptiles.

A positive result on the occult blood reagent pad indicates hemorrhage, hemoglobinuria, or myoglobinuria. False positives can occur with contamination from hypochlorite, iodides, and bromides. False negatives might occur in the presence of vitamin C and formaldehyde. Clinically, it is important to consider the possibility of contamination from the reproductive or gastrointestinal tract. Bleeding from within the urinary system can come from nephritis, ureteral calculi, cystic calculi, trauma, or neoplasia. Myoglobinuria could result from cardiac disease, muscle wasting, trauma, or injections of irritating medications such as enrofloxacin. Evaluate the "dipstick" results in light of microscopy when diagnosing hemorrhage.

Urine pH can be influenced by a variety of factors including nutritional status, diet, mixing with fecal matter, metabolic or respiratory acidosis, and possibly urinary tract infection with urease-producing bacteria. Keep in mind that it is normal for urine to be mixed with bacteria from the digestive tract and sterile urine is probably only found in the kidneys and ureters of reptiles. Urinary tract infections can only be diagnosed in light of other findings such as pyuria, hematuria, and possibly biopsy of kidney or bladder wall. Elevated pH is most likely due to a diet rich in plant material, similar to other herbivores such as the horse (Rose and Hodgson, 1993). Decreases in pH could be due to increased muscle catabolism in chronic starvation or possibly a high protein diet. Innis reported that tortoise urine is quite acidic immediately after emerging from hibernation, although it is quite alkaline during the rest of the year (Innis, 1997). Box turtles can have a wide range of urine pH values, and further research is necessary to provide a better understanding of what is considered normal.

Protein is present in small amounts in the urine of healthy domestic mammals (Chew and DiBartola, 1989). Healthy chelonians, however, appear to have only traces of protein in the urine. In this study, only clinically ill patients were found to show more than trace amounts on the protein "dipstick". This assay is sensitive to a variety of other factors and must be interpreted carefully. First, the color change that occurs can be challenging to differentiate, and may be read differently by different technicians (Chew and DiBartola, 1989). Second, high urine pH will cause a false positive result by interference with the buffering mechanism on the test pad. Finally, contamination with fecal matter, semen, or egg material could introduce proteinaceous material into the urine. If these factors are accounted for, then elevations in urine protein could indicate glomerular damage and plasma protein leakage into the urine. Retesting is indicated, and consistently high proteinuria along with other signs of renal failure could indicate the need for renal biopsy to accurately diagnose the disease process.

Microscopic analysis is undeniably one of the most important aspects of routine urinalysis. In this report, flagellated protozoan parasites were identified in many of the samples. These probably came from the box turtle's intestinal tract, but urinary bladder parasite infestations have been reported in turtles (Henke, *et al*, 1990). Sperm present in the urine indicates that these male box turtles are reproductively active, and for breeding situations it can be positive indicator. The uniform presence of epithelial cells and bacteria across most of the samples in this study indicate that these are a normal finding in box turtle urine. At this point, the casts that were identified in this study are of questionable value. Urate crystals can take many different forms, and could be confused with cellular casts (Innis, 1997). When large numbers of waxy and other cellular casts are found, there is a strong indication for further diagnostic testing including plasma biochemistry, laparoscopy, and renal biopsy. Urate crystals in the urine of tortoises are common, but they are abnormal in water turtles (Dantzler and Schmidt-Nielsen, 1965). The presence of urate crystals in the voided urine of box turtles may be a sign of increased water resorption from the bladder. If box turtles are well hydrated, they should not have urate crystals in the urine.

Although reptile urinalysis provides information different from that in mammals. it does provide valuable data that can begin the baseline needed to evaluate the overall health of the reptile patient. When chelonians spontaneously provide a sample of urine, it should be examined. The information will help to guide the clinician in making diagnostic and treatment decisions and in educating the client about captive husbandry issues.

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**Table 1.** Urinalysis in *Terrapene carolina* spp.

| Patient | Sex | Age | Weight g | Urine Color | S.G. | Gluc mg/dl | Bili | Keto mg/dl | Blood | pH | Protein mg/dl | WBC/hpf | RBC/hpf | Casts | Crystals | Bacteria | Cells/hpf | Other |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | u | 290 | yel | 1.09 | n | n | n | n | 6 | tr | n | n | n | n | 4+ cocci | occ eps | ill, 4+ flag prot |
| 2 | F | u | 398 | clear | 1.001 | n | n | n | hem tr | 7.5 | tr | n | n | n | n | occ. cocci | occ. epi | flag prot |
| 3 | F | u | 390 | clear | 1.004 | n | n | n | sm-mod | 8 | tr | n | n | n | n | 4+cocci,bac | 2-4epi | n |
| 4 | F | u | 352 | yel | 1.011 | n | n | n | n | 6 | tr | n | n | n | n | 4+cocci | occ epi | flag prot |
| 5 | F | u | nr | sl yel | 1.003 | n | n | n | hem tr | 7.5 | tr | n | n | occ.gran | n | 2+ cocci | 4+ eps | debris |
| 6 | M | u | 346 | dk yel | 1.019 | n | n | n | n | 6.5 | 100 | n | n | n | n | 3+ cocci | occ epi,sperm | ill |
| 7 | M | 3 | 264 | lt yel | 1.005 | n | n | n | n | 6 | tr | n | n | n | n | 4+ cocci | n | n |
| 8 | F | 26+ | 667 | clear | 1.001 | 500 | n | n | n | 7 | n | n | n | n | n | 1+ cocci | occ epi | bid gluc=372 |
| 9 | nr | 20+ | nr | clear | 1.001 | n | n | n | n | 7 | n | n | n | n | n | n | rare epi | Vit A def |
| 10 | F | u | 496 | clear | 1.002 | n | n | n | hem tr | 6 | tr | n | n | n | n | 2+ cocci | 2-4 epi | n |
| 11 | F | u | nr | clear | 1.004 | n | n | n | hem tr | 5 | 30 | n | n | n | n | 1+ cocci | occ epi | ill |
| 12 | M | u | 327 | clear | 1.002 | n | n | n | n | 6.5 | tr | n | n | n | n | occ cocci | occ epi | n |
| 13 | F | u | nr | clear | 1.003 | n | n | n | sm | 7 | n | n | n | n | n | 1+ cocci | 0-1 epi | n |
| 14 | M | u | nr | sl yel | 1.007 | n | n | n | n | 7 | tr | n | n | n | n | 2+ cocci | 0-2 epi, sperm | debris |
| 15 | M | u | 500 | clear | 1.001 | n | n | n | n | 7 | tr | n | n | n | n | 2+ cocci | 0-1 epi | n |
| 16 | M | ~10 | 431 | lt yel | 1.007 | n | n | n | n | 5 | n | n | n | n | n | 4+ cocci | occ epi | n |
| 17 | F | 5 | 240 | lt yel | 1.002 | n | n | n | n | 7.5 | tr | n | n | n | n | 2+ cocci | rare epi | n |
| 18 | F | 15+ | 539 | lt yel | 1.003 | n | n | n | n | .6 | n | n | n | n | n | 2+ cocci | rare epi | n |
| 19 | M | 15+ | 314 | lt yel | 1.003 | n | n | n | n | 8 | tr | n | n | n | n | 3+ cocci | occ epi | n |
| 20 | F | u | 478 | sl yel | 1.007 | n | n | n | n | 6 | tr | n | n | n | n | 1+ cocci | occ epi | n |
| **Mean** | | | | | **1.005** | | | | | **6.6** | | | | | | | | |

**Table 2.** Urinalysis in *Terrapene ornata.*

| Patient | Sex | Age | Weight g | Urine Color | S.G. | Gluc mg/dl | Bili | Keto mg/dl | Blood | pH | Protein mg/dl | WBC/hpf | RBC/hpf | Casts /hpf | Crystals /hpf | Bacteria | Cells/hpf | Other |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 21 | M | 7-9 | 297 | clear | 1.009 | n | n | n | n | 6 | n | n | n | n | n | 1+ cocci | occ epi/trans | mucus |
| 22 | F | u | nr | lt yel | 1.005 | n | n | n | n | 5 | tr | n | n | n | uric | n | n | ill |
| 23 | F | u | 260 | lt yel | 1.003 | n | n | n | n | 7.5 | tr | n | n | n | n | 4+ cocci | occ epi | flag prot |
| 24 | M | u | 200 | yel-cloudy | 1.005 | n | n | n | n | 5.5 | tr | n | n | n | n | 3+ cocci | occ epi | flag prot, mu |
| 25 | F | u | 240 | cloudy wht | 1/007 | n | n | n | n | 7.5 | n | n | n | n | n | 1+ cocci | occ epi | debris |
| 26 | F | u | 334 | clear | 1.007 | n | n | n | n | 6.5 | tr | n | n | 0-1 way/1-2 c gm | n | n | 0-1 epi | n |
| 27 | F | u | 302 | clear | 1.007 | n | n | n | n | 6 | tr | n | n | n | n | 4+ cocci | 0-1 epi | Vit A def |
| 28 | M | 8-10 | 285 | lt yel | 1.007 | n | n | n | n | 6 | tr | n | n | n | uric | 2+ coc, 1+ bac | 0-1 epi | n |
| 29 | M | 18+ | 264 | cloudy yel | 1.011 | 1000 | n | n | n | 8 | 30 | n | n | n | n | 4+ cocci | 0-1 epi | underwt, dek |
| 30 | nr | u | 86 | lt yel | 1.011 | 100 | n | n | sm | 5 | 100 | n | n | n | n | 4+ coc, 1+bac | 0-1 epi | flag prot, die |
| 31 | F | u | 123 | clear | 1.001 | n | n | n | n | 7 | tr | n | n | n | n | 2+ cocci | 0-2 epi | dehyd, unde |
| **Mean** | | | | | **1.007** | | | | | **6.4** | | | | | | | | |