Urinalysis in chinchillas (Chinchilla lanigera)

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OBJECTIVE
To evaluate urine variables in chinchillas (Chinchilla lanigera).

DESIGN
Evaluation study.

SAMPLE
Urine samples from 41 chinchillas.

PROCEDURES
Voided urine samples were collected from clinically normal chinchillas that were exhibited during a breeder exposition. Urinalysis was performed within 1 hour after collection. Urine specific gravity (USG) was measured before and after centrifugation with a handheld veterinary refractometer. Urine dipstick analysis and microscopic sedimentation examination were performed on all samples. Additionally, a urine sulfosalicylic acid (SSA) precipitation test and quantitative protein analysis were performed on samples with sufficient volume.

RESULTS
17 of 41 (41%) samples had a USG ≥ 1.050, and USG ranged from 1.014 to > 1.060. The USG before centrifugation did not differ significantly from that after centrifugation. Protein was detected in all urine samples on dipstick analysis. The SSA precipitation test yielded negative results for all samples tested. Results of the quantitative protein analyses were not correlated with the results of the dipstick analyses or SSA tests. The recorded pH for all samples was 8.5, which was the upper limit of detection for the reagent strip. Glucose and ketones were detected in 5 and 6 samples, respectively. Crystals were observed in 28 of 41 (68%) samples; 27 of those samples contained amorphous crystals.

CONCLUSIONS AND CLINICAL RELEVANCE
Urinalysis results for clinically normal chinchillas were provided. For chinchilla urine samples, measurement of USG by refractometry prior to centrifugation is acceptable and protein concentration should be determined by quantitative protein analysis rather than dipstick analysis or the SSA test. (J Am Vet Med Assoc 2016;248:901–907)

In veterinary medicine, urinalysis results are part of the minimum database for a patient. Information obtained from a urinalysis can help veterinarians diagnose both urinary tract disorders and systemic diseases. Determination of USG, measurement of chemical analytes by use of a dipstick, and microscopic evaluation of urine sediment are routine components of a urinalysis.

Chinchillas (Chinchilla lanigera) are popular exotic companion animal pets and have a long history of being used in laboratory research because the anatomy of their ears is similar to that of humans. Consequently, chinchillas are frequently brought to veterinarians for both preventative health care and treatment of disease. Acute and chronic renal failure, diabetes mellitus, and hepatic disease are not uncommon in exotic companion mammals, and veterinarians often rely on urinalysis results for guidance in diagnosing those diseases. Except for penile fur rings, urinary tract or urogenital diseases are uncommon in chinchillas. In a retrospective review of necropsy results for 202 chinchillas, only 5 had renal disease (pyelonephritis, n = 2; chronic renal failure, 2; and polycystic kidney disease, 1). Chinchillas can also develop urinary calculi; similar to other species, male chinchillas are more frequently affected with this condition than are females, and hematuria is the most frequently observed clinical sign.

Within the parvorder Caviomorpha, urinalysis results derived from systematic investigations have been reported only for plains viscachas (Lagostomus maximus) and guinea pigs (Cavia porcellus). To our
knowledge, only anecdotal information exists regarding urinalysis in chinchillas.15,16 The objective of the study reported here was to evaluate urine variables in an apparently healthy population of chinchillas to contribute to and enhance the minimal clinicopathologic database available for this species.

Materials and Methods

Animals

Voided urine samples were obtained from 41 clinically normal chinchillas that were exhibited at a local chinchilla breeder exposition and show.4 Owner consent was obtained for all chinchillas before study enrollment. The chinchillas sampled for the study were owned by 5 separate breeders. Information for each chinchilla was obtained from its owner and included signalment, distance traveled prior to the show, diet, and water access. After consultation with the University of Wisconsin Research Animal Resource Center, it was determined that the study was exempt from review by the Institutional Animal Care and Use Committee because the urine samples were collected opportunistically during a public breeder show and urine was collected passively without physical or chemical manipulation of the chinchillas.

Urinalysis

Chinchillas were housed individually in enclosures with wire-mesh floors. The surface underneath the enclosures consisted of clean, white, nonabsorbable table paper. Voided urine samples were collected with sterile syringes from the table paper underneath each individual chinchilla enclosure and evaluated within 1 hour after collection.

For each urine sample, color and turbidity were recorded after collection. An aliquot from the sample was then pipetted onto each reagent pad of a urine dipstickb for biochemical analysis. Results for urine pH and protein, blood, ketone, and glucose concentrations were recorded in accordance with the dipstick manufacturer’s instructions. The upper limit of pH measurement for the dipstick used was 8.5. To minimize variability, all dipstick tests were performed by the same individual (JLW) to minimize variability.

Each urine sample was placed in a plastic centrifuge tube and centrifuged at 229 × g for 5 minutes. The same volume (2 mL) of urine from each chinchilla was centrifuged whenever possible to maximize consistency. For urine samples with a volume < 2 mL, the entire remaining sample was centrifuged. For each sample following centrifugation, the resulting pellet was resuspended in 0.5 mL of supernatant, and a drop of that suspension was placed on a glass microscope slide and covered with a cover slip. A light microscope was used to examine the urine sediment. The sediment was examined under low power (10X) first and then under high power (40X) for the presence of casts, epithelial cells, crystals, RBCs, WBCs, bacteria, debris, fat, and other material. A minimum of 10 hpfs were examined for each sample.

Statistical analysis

Descriptive data were generated for each urinalysis variable. The data distribution for each variable was assessed for normality by use of the Shapiro-Wilk test. The Wilcoxon signed rank test was used to compare USG before centrifugation with that after centrifugation. The Mann-Whitney rank sum test or the Fisher exact test was used when appropriate to assess for differences between males and females within each urinalysis variable. The Spearman rank correlation or Pearson product-moment correlation coefficient was used to evaluate linear dependence between variables. All analyses were performed with commercially available software, and values of P < 0.05 were considered significant.

Results

Chinchillas

The study population consisted of 29 sexually intact females and 12 sexually intact males. The age of the chinchillas ranged from 17 to > 156 weeks, although the age for the majority (40/41) of the chinchillas ranged from 17 to 56 weeks. Eighteen of the 41 chinchillas had the standard gray coat color, whereas the remaining 23 chinchillas had some type of coat color mutation. Coat color mutations represented in the study population included sapphire, mosaic, pink white, brown velvet, black velvet, violet, ebony, tan, and beige.

Urinalysis

The volume of urine collected from each chinchilla ranged from 0.4 to 5.8 mL. The urine samples varied in color from light to dark yellow (n = 29) to amber (11) to brown (1). The samples also varied in turbidity. Thirty of the 41 (73%) samples were clear,
whereas the remaining 11 samples were classified as hazy, cloudy, or flocculent. All of the samples that were classified as hazy, cloudy, or flocculent contained large numbers of crystals, and there was a significant ($P < 0.01$) positive correlation ($r_s = 0.73$) between urine turbidity and the extent of crystalluria.

Of the 41 urine samples evaluated before centrifugation, 7 (17%) had a USG between 1.010 and 1.019, 13 (32%) had a USG between 1.020 and 1.059, 4 (10%) had a USG between 1.040 and 1.049, and 17 (41%) had a USG > 1.050. All urine samples that were dark yellow, amber, or brown in color had a USG ≥ 1.060, whereas all samples with a light yellow color had a USG < 1.040. For 5 samples, the USG before centrifugation differed from that after centrifugation, although that difference was only 0.001 for all 5 samples. The upper limit of the canine scale for USG on the refractometer is 1.060. Eight urine samples had a USG > 1.060 both before and after centrifugation; however, because the USG for those samples could not be further quantified, those samples were excluded from the comparison between USG before centrifugation and USG after centrifugation. The USG before centrifugation did not differ significantly ($P = 0.80$) from that after centrifugation. For consistency, only precentrifugation USG values are reported throughout this report.

On the basis of urine dipstick analysis, protein (dipstick protein) was present in all 41 samples. The protein content ranged from trace to $4+$ (Table 1). The median dipstick protein content for males (3.5; interquartile range [25th to 75th percentile], 2 to 4) was present in all 41 samples. The protein concentration was not significantly correlated with dipstick protein ($r_s = 0.195$) from that for interquartile range [25th to 75th percentile], 2 to 4)

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Table 1—Frequency distribution of results for select urine biochemical variables for 41 clinically normal chinchillas.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Result</th>
<th>No. (%) of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>Trace</td>
<td>1 (2)</td>
</tr>
<tr>
<td></td>
<td>1+</td>
<td>12 (29)</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>8 (20)</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>2 (5)</td>
</tr>
<tr>
<td></td>
<td>4+</td>
<td>18 (44)</td>
</tr>
<tr>
<td>Ketones</td>
<td>None</td>
<td>35 (85)</td>
</tr>
<tr>
<td></td>
<td>Trace</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>10 (2)</td>
</tr>
<tr>
<td></td>
<td>Large</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>None</td>
<td>36 (88)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>1 (2)</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>2 (5)</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>2 (5)</td>
</tr>
<tr>
<td></td>
<td>≥ 2,000</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

A voided urine sample was obtained from each chinchilla and analyzed by use of a commercially available urine dipstick. Results were semiquantitative and recorded in accordance with the dipstick manufacturer’s recommendations.

A sufficient volume of urine was available to perform the SSA precipitation test and quantitative protein analysis for 37 and 18 chinchillas, respectively. The SSA precipitation test yielded negative results for all 37 samples. The mean ± SD quantitative protein concentration was 46.2 ± 20.3 mg/dL (range, 6 to 87 mg/dL). Of the 18 samples for which both dipstick protein and quantitative protein concentration were determined, 9 had a $1+$ dipstick protein content, and those samples had quantitative protein concentrations that ranged from 6 to 62 mg/dL and USGs that ranged from 1.014 to 1.039. Three samples had a $2+$ dipstick protein content, and those samples had quantitative protein concentrations that ranged from 44 to 87 mg/dL and USGs that ranged from 1.023 to 1.033. One sample had a $3+$ dipstick protein content, quantitative protein concentration of 49 mg/dL, and USG of 1.030. Five samples had a $4+$ dipstick protein content, and those samples had quantitative protein concentrations that ranged from 17 to 76 mg/dL and USGs that ranged from 1.029 to 1.056 (1 chinchilla had a USG > 1.060). Urine quantitative protein concentration was not significantly correlated with dipstick protein content ($r_s = 0.36; P = 0.13$) or USG ($r = 0; P = 1.00$).

For all 41 urine samples, the recorded pH was 8.5, which is the upper limit of detection for the urine dipsticks used. Blood was detected in trace amounts in only 1 urine sample. The frequency distribution of results for ketones and glucose was summarized (Table 1). The combination of ketonuria and glucosuria was not detected in any sample.

On sediment analysis, 28 of 41 (68%) urine samples contained crystals (Figure 1). The majority (23/41 [56%]) of samples contained amorphous crystals only. One sample that contained many amorphous crystals also contained a moderate number of calcium carbonate crystals. Three samples that contained moderate to many amorphous crystals also contained rare to many calcium phosphate crystals. One sample contained calcium phosphate crystals only.

Urine casts were not observed in any of the samples. Squamous (n = 20) or transitional (11) cells or both were detected in 28 of 41 (68%) samples. Of those 28 samples, only 2 were classified as having few epithelial cells observed; the remaining 26 samples were classified as having only rare epithelial cells observed. Red blood cells were rarely observed in each of 5 urine samples and were not observed in the remaining 36 samples. Similarly, WBCs were rarely observed in each of 10 urine samples and were not observed in the other 31 samples. Both RBCs and WBCs were detected in only 2 samples.

Debris was observed in 23 of 41 (56%) urine samples and was classified as minimal in 19 of those samples. Other findings observed on urine sediment analyses included rare amounts of fat (n = 1 sample), mucus (2), sperm (1), and pollen (3). None of the urine variables assessed differed significantly between males and females.
The present study provided preliminary information regarding urinalysis results for clinically normal chinchillas. Similar to guinea pigs12 and rabbits,15 chinchillas have urine that can vary greatly in color. Pigments in the diet have been associated with urine color in both chinchillas13 and rabbits.16 In the present study, chinchilla urine samples that were dark in color generally had a higher USG than did lighter-colored samples.

Most of the chinchilla urine samples collected in the present study were clear, which was in contrast to the findings of other studies.13,17 In the present study, all of the urine samples that were turbid contained large numbers of crystals, which likely contributed to their hazy or cloudy gross appearance. Other differentials for cloudy urine include the presence of cells, bacteria, contamination, lipids, or mucus.18 In a study12 that involved guinea pigs, all turbid urine samples likewise contained crystals. Guinea pigs12 and rabbits frequently have hazy urine, which is believed to be a consequence of urinary secretion of calcium.1,2,15 Unlike guinea pigs and rabbits, chinchillas excrete calcium primarily through the feces and do not rely on urinary secretion to maintain calcium homeostasis.19 The findings of that study19 were supported by those of the present study in that only 1 urine sample contained calcium carbonate crystals. However, in a retrospective study,20 calcium carbonate uroliths were the most common type of urolith observed in chinchillas.

The USG for the chinchillas of the present study had a wide range (1.010 to > 1.060), although a large proportion (17/41 [41%]) of the chinchillas had a USG > 1.050. This finding was in agreement with anecdotal reports,5,13,14,17 which state that the USG for chinchillas is typically > 1.045. In the present study, the most frequently recorded USG was > 1.060, which was the upper limit of quantification for the canine scale on the refractometer used. It is possible that the actual USG was far > 1.060 for many of the samples in the present study. The fact that the urine samples evaluated in the present study were obtained from chinchillas that were being exhibited at a breeder show and may not have had ready access to water might explain the high proportion of samples with high USG values.

It is unknown whether previously reported values for USG in chinchillas were obtained from urine samples before or after centrifugation. For rabbits, it has been historically advocated that urine samples be centrifuged before determining USG to prevent sediment from interfering with the measurement.15 However, results of a recent study21 involving rabbit urine samples indicate that the USG after centrifugation was almost identical to that before centrifugation. Likewise, centrifugation did not significantly affect measurement of USG in the chinchilla urine samples of the present study, even those with visible sediment. Thus, we concluded that centrifugation of chinchilla urine samples prior to measurement of USG with a refractometer is not necessary.

In the present study, proteinuria was detected in all 41 chinchilla urine samples analyzed on the basis of dipstick analysis. Several exotic mammals including hamsters, mice, rats, gerbils, and rabbits are reported to have small amounts of protein in their urine.4 In a study11 of 44 adult free-ranging plains viscachas, all animals had a urine protein content of 1+ and 2+ as determined by a urine dipstick. A trace amount of protein is considered a normal finding in the urine of chinchillas; however, the majority (40/41 [98%]) of the chinchillas of the present study had more than a trace amount of protein in their urine. The high proportion of chinchillas with urine dipstick protein contents greater than trace in the present study may be a function of the fairly high USGs for this population.

The detection of 2+ to 4+ proteinuria in 28 of the 41 (68%) samples by dipstick analysis was unexpected and prompted us to perform additional tests for...
The results of the SSA precipitation tests and quantitative protein analysis suggested that the dipstick protein results were not accurate. The urine dipsticks used in the present study are marketed for use in human urine samples, and values of 1+, 2+, 3+, and 4+ are approximately equivalent to urine protein concentrations of 30, 100, 300 and ≥ 2,000 mg/dL, respectively. For the 18 urine samples that underwent quantitative protein analysis, the maximum urine protein concentration was 87 mg/dL, which suggested that the maximum dipstick protein result should have been 1+. In the present study, USG was positively correlated with the dipstick protein results, which might have contributed to the unreliability of the dipstick protein results. In small animals, a high USG is associated with false-positive urine dipstick protein results. 

Alkaline urine is also associated with false-positive dipstick protein results in many small animals with the exception of rats. All of the chinchilla urine samples evaluated in the present study had a pH ≥ 8.5. Prolonged contact of urine with the dipstick reagent pads has been implicated as a cause of artificially increased protein readings. All samples evaluated in the present study were read within the manufacturer’s recommended time frame. In a study of guinea pigs, fecal contamination was the suspected cause of proteinuria, but there was no gross or microscopic evidence of fecal contamination in the chinchilla urine samples evaluated in the present study.

The SSA precipitation test is a turbidimetric screening test for proteinuria and is commonly used to verify positive dipstick protein results. It has a higher specificity than does dipstick protein analysis in small animals and can detect urine protein concentrations ≥ 5 mg/dL. The quantitative protein analysis results for the chinchilla urine samples evaluated in the present study made us question the accuracy of the results for the SSA precipitation test, which was run in parallel with the quantitative protein analysis for 18 samples. The SSA precipitation test yielded negative results for all 18 samples, even those with quantitative protein concentrations up to 87 mg/dL. False-negative SSA test results can occur because grading of turbidity is subjective and variation between individual readers is likely. In the present study, all SSA tests were graded by the same investigator, who was experienced with performing the test; therefore, we believe that it was unlikely that reader error of the SSA test results was responsible for the disparity between the results of the SSA precipitation tests and the quantitative protein analyses. Results of another study indicate that the SSA precipitation test should not be used to verify urine dipstick protein results for Sprague-Dawley rats. Highly buffered alkaline urine samples have yielded false-negative SSA precipitation test results, and the alkalinity of the urine samples evaluated in the present study might have likewise affected the SSA precipitation test results.

Urinary tract disease such as glomerulonephritis and renal amyloidosis and reproductive tract disease can lead to pathological proteinuria. In the present study, the chinchillas from which the urine samples were obtained appeared clinically normal but their systemic health status was unknown. Therefore, we cannot rule out subclinical pathology as a potential cause of proteinuria, although it is unlikely that all chinchillas in the present study had proteinuria subsequent to systemic disease. A reference range for the urine protein-to-creatinine ratio for chinchillas is currently unavailable. Compared with the results of the quantitative protein analysis, the urine protein-to-creatinine ratio is a more reliable measure for identification of proteinuria because it is not affected by changes in the USG. Research is necessary to establish a reference range for the urine protein-to-creatinine ratio in chinchillas.

Results of other studies indicate that the urine pH of chinchillas is alkaline and ranges between 8.0 and 9.0 (mean, 8.5), and the results of the present study do not refute that. All of the urine samples evaluated in the present study had a recorded urine pH of 8.5, which was the upper detection limit for pH on the dipstick used. Some samples likely had a urine pH > 8.5. Although there are several methods for measuring pH values > 8.5, none of those were used in the present study. We chose to use the commercially available urine dipsticks to measure the pH of the urine samples because those dipsticks are commonly used for that purpose in clinical veterinary practice. Evaluation of the use of a digital pH meter or litmus paper to measure the urine pH of chinchillas is warranted; however, use of litmus paper to measure urine pH is not currently recommended because it is an unreliable method for determination of urine pH in dogs.

Ketones or glucose, but not both, was detected in several urine samples evaluated in the present study. In chinchillas, ketoacidosis can develop as a sequela to anorexia. Many of the chinchillas from which urine samples were obtained for the present study were likely under some degree of stress from being transported and exhibited, which may have resulted in a decrease in their food intake that in turn could have caused the production of ketones and their subsequent secretion in the urine. Stress could have also caused hyperglycemia that subsequently led to glucosuria, although an association between stress and hyperglycemia has not been clearly established for chinchillas. Ketonuria in conjunction with glucosuria has been reported in chinchillas as well as viscachas with hepatic lipidosis. For animals that are being treated for ketonuria, evaluation of serial urine samples for the presence of ketones is recommended as a noninvasive method for monitoring response to treatment. Diabetes mellitus has been described in 2 chinchillas, and both had severe glucosuria and ketonuria. Unfortunately, subclinical pathological conditions such as ketoacidosis, hepatic lipidosis, and diabetes mellitus could not be ruled out as the cause of ketonuria or glucosuria for the chinchillas of the present study.

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Several sources indicate that calcium carbonate crystals are commonly found in the urine of chinchillas. Calcium carbonate and struvite crystals have also been described in chinchilla urine. Amorphous crystals were the crystal type most frequently observed in the chinchilla urine samples evaluated in the present study. Amorphous crystals were observed in 50% of the guinea pig urine samples evaluated in another study. The importance of amorphous crystals in the urine of chinchillas is unknown and warrants further research.

Many urine samples evaluated in this study contained rare epithelial cells, which are considered a normal finding in chinchillas. Rare RBCs and WBCs were also observed in some samples. Those results likely represent nonpathological findings. Rare epithelial cells (squamous cells and transitional cells), RBCs, and WBCs are considered nonsignificant findings on free-catch urine samples obtained from small animals. A limitation of the present study was that urine samples were collected from chinchillas for which the urinary tract and systemic health were unknown. Although samples were collected only from clinically normal chinchillas, subclinical disease could have biased our results. Unfortunately, it was not possible for us to perform a physical examination on or create a diverse population similar to what would be encountered in a clinical setting.

Results of the present study indicated that the gross appearance, USG, and protein concentration varied substantially in urine samples obtained from clinically normal chinchillas. Measurement of USG by refractometry prior to centrifugation is acceptable for chinchilla urine samples. Urine protein concentration determined by dipstick analysis should be interpreted with caution or measured by some other method such as quantitative protein analysis. Although this study provided preliminary information regarding urine variables in clinically normal chinchillas, further research is necessary to establish urinalysis reference ranges for that species.

Footnotes

References
Evaluation of computed tomographic enterography with an orally administered lactulose solution in clinically normal dogs
Seoyeon Keh et al

OBJECTIVE
To determine optimal techniques for CT enterography in clinically normal dogs and to evaluate luminal distention after oral administration of lactulose solution as a contrast agent.

ANIMALS
15 healthy dogs.

PROCEDURES
CT was performed in a control group (2 dogs that underwent CT to evaluate metastasis and 5 other dogs). In a bolus administration group (5 dogs from the control group), lactulose solution (1.34 g/mL) was administered (60 mL/kg) rapidly via gastric tube to anesthetized dogs, and CT was performed every 10 minutes for 1 hour. In a continuous administration group of 8 other dogs, lactulose solution (60 mL/kg) was administered slowly via nasoesophageal tube over a period of 45 minutes. Then, 15 minutes after anesthetic induction, CT was performed every 10 minutes for 1 hour. Luminal distention of the small intestines was evaluated qualitatively by use of a 3-point scale.

RESULTS
All small intestinal segments had poor luminal distention in the control group. The terminal portion of the ileum had poor luminal distention for the bolus administration group. Nearly all segments had good luminal distention for the continuous administration group with mild adverse effects. Luminal distention scores from 0 to 20 minutes after lactulose administration were significantly higher than scores from 30 to 60 minutes. Interobserver reproducibility was high for all intestinal segments.

CONCLUSIONS AND CLINICAL RELEVANCE
CT performed between 0 and 20 minutes after continuous administration of lactulose solution (60 mL/kg) may reveal adequate luminal distention for examination of small intestinal segments in dogs. (Am J Vet Res 2016;77:367–373)