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URINALYSIS IN THREE SPECIES OF CAPTIVE RHINOCEROS (*RHINOCEROS UNICORNIS*, *DICERORHINUS SUMATRENSIS*, AND *DICEROS BICORNIS*)

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Abstract: This study reports urinalysis values for three species of captive rhinoceros (*Rhinoceros unicornis*, *Dicerorhinus sumatrensis*, and *Diceros bicornis*) and evaluates individual and species differences. Repeated urinalysis was conducted on 11 individuals to establish normal reference ranges. Although no individual or species differences existed in urinary values for pH, all species differed in specific gravity. Rhinoceros urine demonstrated many physical and chemical properties similar to that of the horse, but reliability of this comparison was limited. Urinary pH in the rhinoceros was within range of that established for the horse and other large herbivores. However, all rhinoceros species exhibited urinary specific gravities below the lower limit of the normal equine reference range. Comparative urinalysis using an outside laboratory source confirmed the results of this study and illustrated the value of conducting in-house analysis. These results are the first data available on reference ranges for urine parameters in the greater one-horned, Sumatran, and African black rhinoceros and provide a useful diagnostic tool for the veterinary care of individuals in captivity.

Key words: Urine, pH, specific gravity, *Rhinoceros unicornis*, *Dicerorhinus sumatrensis*, *Diceros bicornis*.

INTRODUCTION

Urinalysis is a useful, noninvasive diagnostic tool that provides a portion of the minimum database needed for the screening and diagnosis of disease. It is an easy and inexpensive in-house test. Free-catch samples can be obtained with minimal stress to the animal and little or no danger to staff. By revealing both renal and systemic information, urinalysis can aid in monitoring the health of captive wildlife. Early disease detection can vastly improve prognosis and may reduce costs associated with treating advanced disease.

The first report on a rhinoceros urine sample occurred in 1817.[37] However, reference values for rhinoceros urine currently do not exist. Because rhinoceros anatomy and physiology are similar to those of the horse, equine reference ranges are often used to interpret urinalysis results for captive rhinoceroses. Unlike horses, two of the four extant captive rhinoceros species are primarily browsers, the African black (*Diceros bicornis*)[4,5,7,14,16] and Sumatran (*Dicerorhinus sumatrensis*) rhinoceros.[8,36] Although the African white rhinoceros (*Ceratotherium simum*)[18] is a grazer species, the greater one-horned rhinoceros (*Rhinoceros unicornis*) is considered a mixed feeder.[3,9] Despite these differences, captive rhinoceros are fed various diets that are often quite similar to that of the domestic horse. These diets may include alfalfa or grass (timothy, coastal Bermuda grass, and sudan) hays, concentrate pellets, produce, and fresh/frozen browse.[5,6] Depending on the rhinoceros species and available flora, browse may consist of bamboo, ficus, honeysuckle, and other locally available herbaceous plants. In the case of Sumatran rhinoceros, fresh browse is the main component of their diet.[8]

This study was designed to amass physiologically pertinent data on rhinoceros free-catch urine samples. It was a preliminary attempt to establish baseline urine values for three of the four captive species of Rhinocertidae. The aim of this study was to compare urine values in the greater one-horned, Sumatran, and African black rhinoceros with normal reference range values in their closest domestic relative, the horse. Urinalysis was conducted in house for all samples to emulate the conditions at most zoological institutions. The following urine parameters were assessed: color, turbidity, specific gravity, protein, sulfosalicylic acid reaction, nitrite, pH, occult blood, ketones, bilirubin, glucose, and microscopic sediment exam. An aliquot from 44% (47 of 107) of the samples was sent to an outside lab for confirmatory analysis.

MATERIALS AND METHODS

Animals

Urine was collected from six greater one-horned, three Sumatran, and two African black rhinoceroses (Table 1). Samples were collected over a 2-yr period (6 March 2003 to 2 April 2003 and 12 January 2005 to 15 February 2005) for three of the animals (Studbook [SB] No. 238, 225, and 29) in the study. Urine samples collected from the male greater one-

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Table 1. Summary of study animals and urine sample collections.

Rhinoceros species	Studbook no.	Sex	Age during study	Urine sample (no.)
Great one-horned	238	F	7–9 yr	25
Great one-horned	147	M	15 yr	2
Great one-horned	189	F	13 yr	2
Great one-horned	087	M	27 yr	4
Great one-horned	264	F	5 yr	2
Great one-horned	239	M	8 yr	3
Sumatran	29	F	<20 yr	11
Sumatran	43	F	6–7 mo	11
Sumatran	28	M	>30 yr	9
African black	308	M	24 yr	15
African black	225	F	35–37 yr	23

horned rhinoceros SB No. 147 occurred only during the 2003 period. All remaining animals had urine samples collected during the 2005 period.

One male (SB No. 147) and one female (SB No. 238) greater one-horned rhinoceros and the African black and Sumatran rhinoceroses were housed at the Cincinnati Zoo and Botanical Garden (CZBG; Cincinnati, Ohio 45220, USA). All remaining animals in the study were housed at the Wilds (Cumberland, Ohio 43732, USA). The greater one-horned rhinoceroses maintained at the CZBG were fed timothy hay, ADF 16 grain (Mazuri; St. Louis, Missouri 63166, USA), and fresh produce. The Sumatran rhinoceroses were fed a diet of fresh browse composed of up to 10 types of ficus, alfalfa, and orchard grass hay, ADF16 grain and fresh produce. The African black rhinoceroses were fed alfalfa hay, ADF 16 grain, and fresh produce. All animals at the CZBG received 6 cc vitamin E supplement (Emcelle Tocopherol; 500 i.u./ml; Stuart Products Inc., Bedford, Texas 76022, USA) orally in a banana each morning. In addition, they had unlimited access to a mineral block and water. Animals maintained at the Wilds were fed a diet consisting of grass hay, commercial herbivore pellets, and free-choice water. The female greater one-horned rhinoceros SB No. 238 at the CZBG was treated once during the course of this study with GnRH (Cystorelin; 50 μg/mL [w/v] GnRH diacetate tetrahydrate, 10 ml i.m.; CEVA Laboratories, Overland Park, Kansas 66210, USA) on 17 January 2005 and underwent general anesthesia on the same day for an artificial insemination procedure. The animal was vaccinated for leptospirosis and West Nile virus at the time of anesthesia. A single male greater one-horned rhinoceros housed at the Wilds (SB No. 087) was treated with a short course of trimethoprim/sulfa (2 February 2005 to 8 February 2005) and one dose of flunixin meglumine (1 February 2005) 1 wk prior to urine sampling.

Urine collection

Urine was collected into sterile specimen cups attached to the end of a wood or metal rod. Efforts were made to collect midstream samples. Because rhinoceros often spray their urine, it was difficult to obtain true "midstream" samples. In this study, a sample was considered midstream if it was caught shortly after an initial urine spray had been passed. The time urine was collected and then analyzed was recorded for each sample. In the interim, samples were kept covered and refrigerated. A total of 107 urine samples were collected during the study period (Table 1).

Urine processing

Initial processing of the urine sample consisted of gentle agitation to suspend denser elements present on the specimen cup floor. A fraction (2–50 ml) of the initial sample was removed by pipette and placed in a separate sterile vial for analysis at the CZBG. All urinalysis was conducted at the Center for Conservation and Research of Endangered Wildlife laboratory, excluding samples collected on 10 February 2005 and 16 February 2005, which were processed at the Wilds.

The original container of urine was tightly sealed, labeled, covered with Parafilm, and placed in a cooler to await pickup by an external laboratory. Of the 107 total urine samples collected throughout the study, 47 (44%) samples were sent to Antech Diagnostics (Fisher, Indiana 46038, USA) for independent urinalysis. Of the urine samples sent for independent analysis, 43% were collected from Sumatran, 36% from African black, and 21% from greater one-horned rhinoceroses.

Urinalysis

The volume of each urine sample was recorded in addition to the color and turbidity. Color was designated as clear, white, light yellow, yellow, golden, gold-brown, brown, bright red, or dark red. The turbidity was noted as transparent, cloudy, or flecked. A 1-ml volume was transferred into a 15-ml conical vial and centrifuged for 5–10 min at 1,000 *g* to prepare sediment for microscopic examination. During centrifugation, a sterile pipette was used to drop urine onto 10-lg parameter urine reagent strips to identify leukocytes, nitrite, urobilinogen, protein, occult blood, ketones, bilirubin, and glucose. Due to greater accuracy and precision, pH paper was used to quantify urine pH (EM Science, Gibbstown, New Jersey 08027, USA). Spe-

cific gravity was determined using a hand-held refractometer (Schuco Clinical Refractometer; ERMA Inc., 2-31-6 Yushima, Bunkyo-ku, Tokyo, Japan). Any reaction on the protein portion of the dipstick was validated using the sulfosalicylic acid turbidity test (SSA 5% [w/v], Labchem Inc., Pittsburgh, Pennsylvania 15238, USA). After centrifugation, the supernatant was poured off and the sediment resuspended in the incidental urine. Two air-dried (one stained and one unstained) and two wet-mount slides (one stained and one unstained) were prepared from the sediment. Wet-mount slides were stained with Sedistain (Becton Dickinson and Company, Sparks, Maryland 21152, USA), and air-dried slides were stained with Diff-quick (Difco Laboratories, Detroit, Michigan 48201, USA). The slides were examined with light microscopy (×10–100).

Crystalline material analysis

Dried crystalline sediment from urine samples (n = 4) were shipped overnight to the Minnesota Urolith Center (MUC; St. Paul, Minnesota 55108, USA) for crystalline material analysis. The urine sediment crystals that were analyzed had been collected from both female Sumatran rhinoceros (n = 1; SB No. 29 and n = 1; SB No. 43), as well as a female greater one-horned rhinoceros (n = 2; SB No. 238). Quantitative crystal analysis was accomplished at the MUC through polarizing light microscopy, infrared spectroscopy, and energy-dispersive X-ray spectroscopy to determine mineral composition.

Statistical analyses

All statistical analyses were performed using SigmaStat/Plot software (SPSS; Chicago, Illinois 60606, USA). Standard descriptive statistics were used to summarize results. Urinalysis results are reported from all samples analyzed at CZBG and the Wilds. To determine if individual or species differences existed, a one-way analysis of variance (ANOVA) was performed. The urinalysis results from the fraction of samples sent for confirmatory analyses by an outside laboratory were statistically compared with results obtained on the same samples analyzed in house using ANOVA. Statistical significance level was established at $P \leq 0.05$.

RESULTS

Greater one-horned rhinoceros

A total of 38 urine samples were collected from three male and three female adult greater one-horned rhinoceroses (Table 1). Urine samples were collected between the hours of 5:35 AM and 10:43 AM and were evaluated on an average of 6 min (range 2–375) after acquisition. Sample volumes ranged from 0.5 ml to 100 ml ($\bar{x}$ = 80 ml). Urine color ranged from light yellow to gold brown, and turbidity was transparent to cloudy. In general, the physical appearance of the greater one-horned rhino urine was analogous to orange juice.

Table 2. Mean ± SD urinary pH and specific gravity in individual greater one-horned rhinoceros. Different superscripts in a given column represent significant differences among individuals.

Studbook no.	pH	Specific gravity
238	8.08 + 0.2249[a]	1.024 + .0064[b]
147	8.20 + 0.1414[a]	1.023 + .0007[ab]
189	8.70 + 0.00[a]	1.025 + .0042[ab]
087	8.10 + 0.2828[a]	1.020 + .0022[b]
264	8.20 + 0.1414[a]	1.019 + .0042[b]
239	8.10 + 0.5292[a]	1.031 + .0023[a]

Dipstick and refractometer: All samples were negative on the dipstick test for nitrite, occult blood, and glucose. A small percentage of samples were positive for ketones (3%; 5 mg/dl) and bilirubin (5%; trace 1+). A positive protein (trace 30 mg/dl) result occurred in 68% of the samples. Only 15% of samples with a positive protein result on dipstick showed a mild (0–1) but positive SSA reaction. Mean urinary pH was 8.12 ± .2609 and specific gravity was 1.024 ± .00598 (Table 2). Although statistically significant differences were observed in the specific gravity ($P \leq 0.05$; Table 2) of urine from individual greater one-horned rhinos, no statistical difference existed for urine pH (P = 0.598).

Crystals: The majority (97%) of urine samples contained calcium carbonate (Fig. 2A), with a smaller percentage of samples exhibiting amorphous phosphates (21%) and ammonium biurate (3%).

Microscopic examination: Red (RBCs; 3%; <1/hpf) and white (WBCs; 11%; <1–5/hpf) blood cells were observed in a small percentage of samples. Urine samples containing WBCs were collected from one individual (SB No. 239) over a 2-wk period (24 January 2005 to 26 January 2005 and 08 February 2005). An unremarkable quantity of bacterial cocci was observed in 29% of samples, and 5% of samples contained traces of fungal hyphae.

Only a very small percentage of samples contained cellular (5%), granular (5%), or hyaline (11%) casts. Casts were excreted from four of six individuals (SB No. 239, 147, 087, and 264). Squamous cells, refractile bodies (fat), and mucus were major findings in the greater one-horned rhinoceros urine. A single urine sample (SB No. 239) contained transitional epithelial cells.

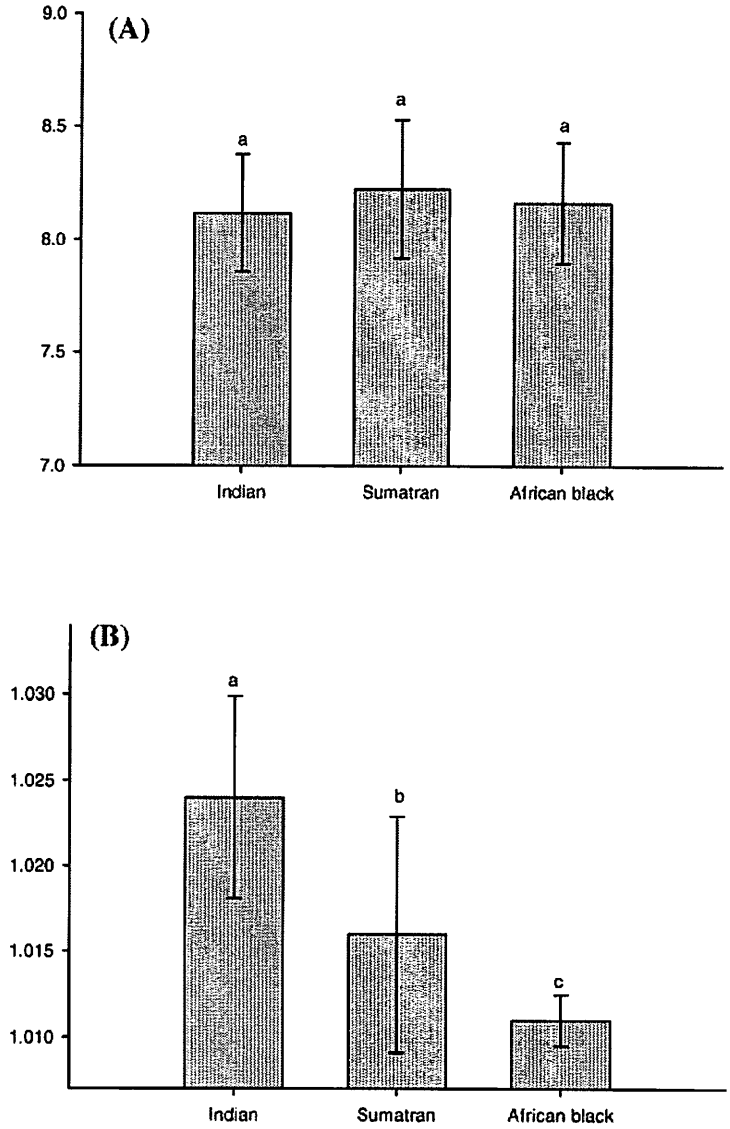

Figure 1. Mean ± SD urinary pH **(A)** and specific gravity **(B)** among captive rhinoceros species. Significant differences among species are represented by different superscripts.

Independent urinalysis: A total of 10 urine samples were sent for independent urinalysis. No significant differences in urinalysis values were detected between the independent laboratory and the CZBG for specific gravity ($P = 0.612$) and pH ($P = 0.067$). Independent urinalysis for protein was in 90% agreement with the CZBG results. A single ketone-positive (5 mg/dl) urine sample detected at the CZBG was negative when analyzed by the independent laboratory. One urine sample positive for bilirubin (1+) at the CZBG was trace bilirubin via the independent lab, and four samples that resulted in negative findings at the CZBG were positive (1+ to 3+) for bilirubin in independent laboratory results. WBCs were detected in four urine samples analyzed at the CZBG, and two of these samples were validated by independent urinalysis results. No RBCs were observed in urinalyses conducted at the independent lab. Although we noted the presence of bacteria and fungi in two urine samples, these were not observed in the urine sediments examined by the independent laboratory. Independent crystal analysis confirmed the presence of the aforementioned crystal types, in addition to calcium oxalate dihydrate.

Sumatran rhinoceros

A total of 31 urine samples were collected from one subadult female and one adult male and female rhinoceros (Table 1). Urine samples were collected

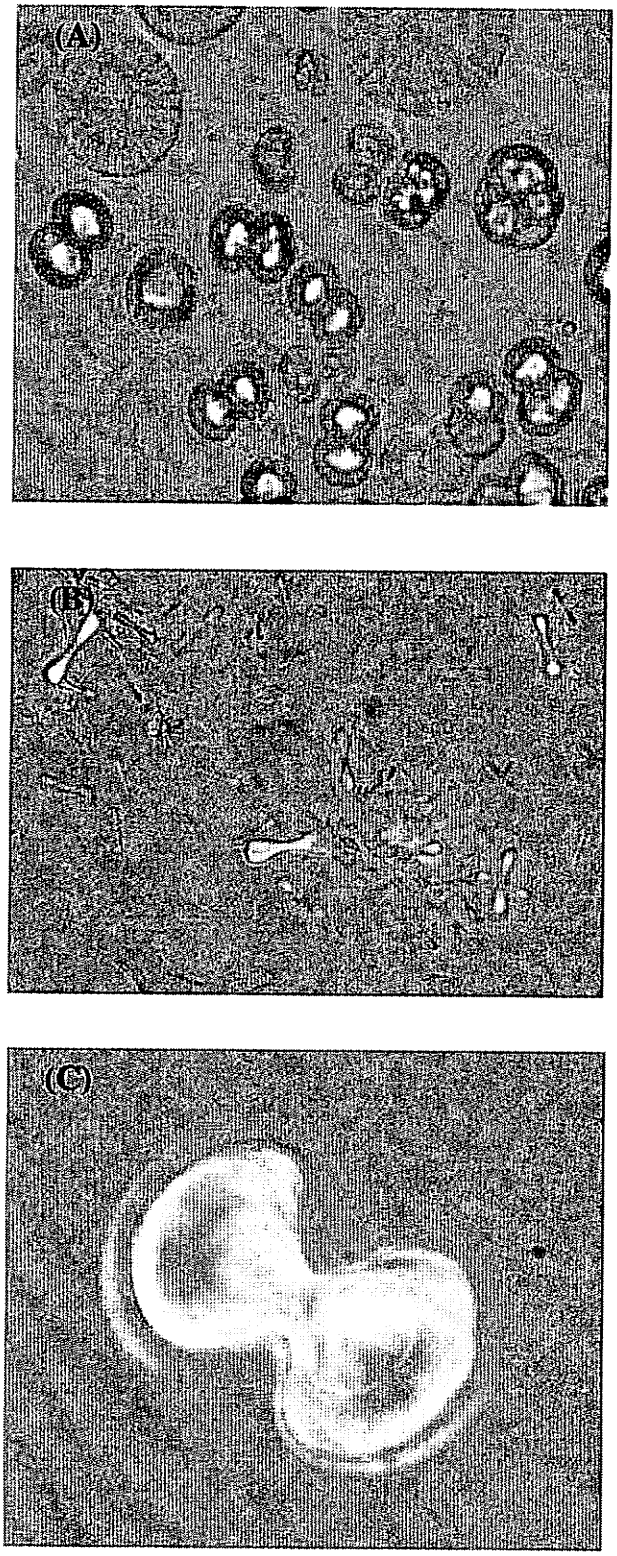

Figure 2. Microscopic appearance of calcium carbonate crystals in the urine of **(A)** a greater one-horned (×40), **(B)** an African black (×10), and **(C)** a Sumatran (×100) rhinoceros.

Table 3. Mean ± SD urinary pH and specific gravity in individual Sumatran rhinoceros. Different superscripts in a given column represent significant differences among individuals.

Studbook no.	pH	Specific gravity
29	8.18 + .3155[a]	1.021 + .0069[a]
43	8.32 + .2601[a]	1.010 + .0025[b]
28	8.16 + .3504[a]	1.015 + .0046[b]

between the hours of 7:20 AM and 1:15 PM and were evaluated on an average of 33 (range 15–90) min after acquisition. Sample volume ranged from .5 ml to 10 0ml ($\bar{x}$ = 46 ml). Urine color ranged from white to light yellow, and turbidity was transparent to cloudy. Sumatran rhinoceros urine samples had the physical appearance of buttermilk.

Dipstick and refractometer: All samples were negative on the dipstick test for nitrite, occult blood, glucose, and ketones. In addition, 13% (n = 4) of dipstick tests were positive for bilirubin ranging from 1+ to 3+. Positive protein (trace 100 mg/dl) results occurred in 40% of urine samples. None of the urine samples positive for protein on dipstick reacted in the SSA turbidity test. Mean urinary pH was 8.22 ± 0.306 and specific gravity 1.015 ± .0069 (Table 3). No statistical differences were observed among individual Sumatran rhinoceros urine pH (P = 0.541), whereas individual differences in specific gravity did reach statistical significance ($P \leq 0.05$; Table 3).

Microscopic examination: Small percentages of urine sediment samples contained WBCs (8%), RBCs (4%; <1/hpf), bacterial cocci (8%), or fungal hyphae (4%). Granular or cellular casts were documented in the sediment of at least one sample collected from each individual. However, the overall percentage of sediment samples with granular (8%) and cellular (4%) casts was relatively low. Squamous cells and refractile bodies (fat) were major findings in Sumatran rhinoceros urine.

Crystals: All sediment samples contained calcium carbonate (Fig. 2B), whereas only 8% contained amorphous phosphates and 4% calcium oxalate dihydrate.

Independent urinalysis: A total of 19 samples were sent for independent urinalysis. There was no statistical difference in urine specific gravity (P = .688) or pH (P = .0534) between urinalyses conducted at the CZBG or the independent laboratory. In agreement with CZBG SSA results, all samples were negative for protein by the independent lab. A single sample that was negative for ketones at the CZBG resulted in trace ketones through inde-

Table 4. Mean ± SD urinary pH and specific gravity in individual African black rhinoceros. Different superscripts in a given column represent significant differences between individuals.

Studbook no.	pH	Specific gravity
308	8.26 + .2640[a]	1.012 + .0010[a]
225	8.10 + .2539[a]	1.010 + .0015[b]

pendent urinalysis. Additionally, the independent lab came up with a positive occult blood on a single sample that was negative at the CZBG. Two of four samples that tested positive for bilirubin (1+) at the CZBG were negative by independent urinalysis. WBCs (0–3/hpf) were noted in two urine samples that had tested negative at the CZBG. Fat droplets, squamous cells, and transitional epithelial cells were major findings in Sumatran rhinoceros urine. Independent laboratory analysis confirmed the crystal types identified by the CZBG, in addition to calcium oxalate monohydrate.

African black rhinoceros

A total of 38 urine samples were collected from one male and one female adult African black rhinoceros (Table 1). Urine samples were collected between the hours of 7:15 AM and 8:45 AM and were evaluated on an average of 46 (range 9–196) min after acquisition. Sample volume ranged from 0.7 ml to 120 ml ($\bar{x}$ = 64 ml). Urine color ranged from light yellow to yellow, and turbidity was transparent to flecked. The physical appearance of African black rhinoceros urine was similar to lemonade.

Dipstick and refractometer: All samples were negative on dipstick for nitrite, occult blood, glucose, ketones, and bilirubin. More than half (55%) of the samples were positive for protein (trace 100 mg/dl). A total of 19% of protein-positive samples demonstrated a mild reaction (0–1) in the SSA turbidity test. Mean urinary pH was 8.16 ± 0.267, and specific gravity was 1.011 ± 0.0015 (Table 4). The female African black rhinoceros excreted statistically lower urine pH and specific gravity than the male (Table 4).

Microscopic examination: A small percentage of samples contained WBCs (8%; <1/hpf), transitional epithelial cells (6%), fungal hyphae (3%), and bacterial cocci (11%). No RBCs were observed in any sediment samples examined. The presence of mucus and yeast was observed in a single examination of sediment from two separate urine samples collected from the female. Squamous cells and refractile bodies (fat) were major findings in the African black rhinoceros urine. A large percentage (53%) of sediment samples from the male contained small numbers of motile sperm cells. In addition, a high percentage (44%) of urine sediment samples contained granular and hyaline casts. However, when taken separately, only 20% of samples collected from the male contained casts versus 62% of the samples collected from the female.

Crystals: The majority (89%) of sediment samples contained calcium carbonate (Fig. 2C) in addition to amorphous phosphates (17%) and calcium oxalate dihydrate (6%).

Independent urinalysis: A total of 17 urine samples were sent for independent urinalysis. Although there was no statistical difference (P = 0.123) in specific gravity, statistically higher ($P \leq 0.05$) pH ($\bar{x}$ = 8.38) was measured in urine samples analyzed by the independent laboratory versus at the CZBG ($\bar{x}$ = 8.13). There was agreement on protein results for 77% of the samples. A positive occult blood on one sample was not in agreement with a negative result obtained at the CZBG. Relatively small percentages of samples contained WBCs (18%) and RBCs (6%). None of the sediment samples that contained bacterial cocci were confirmed through independent urinalysis, and two samples in which no bacteria were seen in house yielded bacteria on independent sediment exams.

Across species

We found no statistical differences (P = 0.2939) in urinary pH across rhinoceros species (Fig. 1A). In contrast, all rhinoceros species differed significantly from each other in urine specific gravity (Fig. 1B).

DISCUSSION

This is the first study presenting reference values for urine parameters in three species of rhinoceros. Among the Perissodactyla family, much data is available on normal urine chemistry in the horse.[11,19] It has been assumed that urinary reference values for the horse can be applied for comparative analysis of rhinoceros urine.

Although there are differences in the digestive anatomy and physiology between grazing equids and browsing rhinoceratids, most captive rhinoceros are fed diets similar to their domestic counterpart.[1,2,4–6,12,13] Renal anatomy and histology of the rhinoceros resemble those of the hippopotamus more than the horse.[21–24] Both rhinoceros and hippopotamus have lobated kidneys, whereas the horse does not. These multipyrimidal kidneys should facilitate greater glomerular filtration rates due to the increase in surface area between cortex and medulla.[38] Despite digestive and renal differences, rhinoceros urine shares many physical properties with that of the horse.

The mean urinary pH for all rhinoceros species was similar to those ranges reported in the horse and other large herbivores.[17,25,27,30,31,39] This present study reports no difference in pH values in relation to species of rhinoceros. However, all species differed from each other with regard to specific gravity. The highest specific gravity was observed in urine from the greater one-horned rhinoceros. However, the mean specific gravity for this species was at the lower limit of variation for the horse.[17,25,27] Specific gravity of urine from the two browsing species (Sumatran and African black) was much lower than the normal reference range for equids. In a healthy animal, specific gravity is influenced by water consumption, urine volume, and dietary protein source and quantity.[17,27] The increased moisture content associated with the fresh browse diet of the Sumatran rhinoceros may account for this species' lower urine specific gravity.[8]

Additionally, the adult male and female in the study typically exhibited spray versus stream urination. Spray urination was associated with territorial marking by the male and scent masking of offspring by the adult female.[28,29] The low volume associated with this type of urination may have additionally influenced specific gravity results. Urine from the female Sumatran calf exhibited the lowest specific gravity for this species. Researchers investigating urine parameters in the horse have shown that specific gravity is lower in foals due to high volume fluid intake associated with nursing.[27] During the course of this study, the female Sumatran rhinoceros calf was nursing at intervals of 1.5 to 2 hr and eating only 25% of the time throughout a 24-hour period (Plair, pers. comm.). The low specific gravity observed in urine from the African black rhinoceros may also be influenced by diet. Although the African black rhinoceros is a browser species, its diet in captivity consists primarily of alfalfa hay. Low specific gravity values have been reported for horses with hypercalcemia.[35] Due to the age of the animals and results of microsediment examinations, specific gravity may have been further affected by clinical findings of early renal impairment in the female of the species. Therefore, additional urinalysis data should be collected on more individual African black rhinoceroses.

Rhinoceros urine is turbid due to the presence of crystals and mucus. The primary crystal formed in the urine of the three species of rhinoceros was calcium carbonate. Urine sediments from each species also contained amorphous phosphate crystals. Crystal formation is common in alkaline urine, and the aforementioned crystal types are common findings in urine sediment of the horse.[20] Calcium carbonate crystals are found in abundance in horse urine, especially if animals are fed alfalfa hay.[10,17] The morphology of calcium carbonate crystals in rhinoceros urine tended toward an elongated dumbbell rather than the spheroid shape more commonly observed in the horse. However, spheroid calcium carbonate crystals were also observed regularly in rhinoceros urine sediments. The cause of the elongated dumbbell crystal morphology is unknown but may result from the lower specific gravity of rhinoceros urine compared with that of the horse. Ammonium biurate was observed only in a single urine sample from a greater one-horned rhinoceros. Calcium oxalate dihydrate was formed in urine sediments from the African black and Sumatran rhinoceros and is also commonly observed in urine from healthy horses and cattle.[34]

As with the horse, normal urine of the rhinoceros contained <1 WBC/hpf and <5 RBCs/hpf. Several urine samples collected from a female greater one-horned rhinoceros did exhibit >3–5 WBCs/hpf over a short time after an artificial insemination procedure. Because the findings were transient and bacteriurea was not associated with the samples, we considered it insignificant. As with the horse,[32] mucus was a common finding in rhinoceros urine.

Microsediment examination also revealed frequent observation of squamous epithelial cells among all species. Transitional epithelial cells were rarely found, and we saw no occurrence of renal epithelial cells. Urine samples containing bacteria were considered nonclinical because they were not associated with increased numbers of WBCs. The presence of bacteria in these samples was attributed to contamination. Urine samples with fungal hyphae were indicative of overgrowth of contaminants and correlated with increased time from collection to urinalysis.

Urinary casts were observed in all species of rhinoceros. However, their number and frequency of occurrence differed among individuals. Relatively few casts were observed in urine sediments collected from individual greater one-horned or Sumatran rhinoceroses. However, the female African black rhinoceros excreted granular and hyaline casts at increased numbers and frequency compared with the male of the species. The findings of this study may indicate early renal impairment, given the age of the female and the degree and excretion pattern of casts. However, this present study consisted of only a single pair of African black rhinoceros. More data should be collected on additional individuals of this species. The absence of cast identification in urine samples analyzed by the outside laboratory was attributed to the breakdown

of these structures in the highly buffered urine of the rhinoceros over time.[10,17]

As with the horse, the alkaline urine of the rhinoceros required confirming dipstick protein results with a more quantitative method. Urinary dipstick tests for protein have produced false-positive/false-negative results due to a lack of specificity.[33] This study documented that rhinoceros urine produced high numbers of false-positive dipstick protein results versus the SSA turbidity test. Research has shown that pH, specific gravity, and turbidity can all influence dipstick protein analysis.[33] In this study, urine of all rhinoceros species frequently produced false-positive dipstick protein results. This occurred more commonly in urine collected from the Sumatran rhinoceroses. However, none of the urine was positive using the SSA turbidity test. Hence, these findings may be attributed to the highly turbid and buffered chemistry of Sumatran rhinoceros urine, in addition to the species' low specific gravity.

To determine the precision of rhinoceros urinalysis at the CZBG, results were compared with those obtained from an independent laboratory. Although no significant differences in urine specific gravity existed between samples analyzed at the CZBG or the independent laboratory, a significant difference did exist in urine pH, but only for the African black rhinoceros. However, urine samples from the greater one-horned and Sumatran rhinoceroses did show a trend of higher alkalinity when analyzed by the outside lab. This difference was likely due to alterations in urine pH that occur over time due to the loss of carbon dioxide.[26] Urinary pH also increases with time as a result of bacterial breakdown of urea.[15] The time of day that urine was collected differed for each species of rhinoceros. In general, African black rhinoceros urine was collected at an earlier time in the day and over a shorter range of time, leading to increased time to urinalysis for the outside lab. The most feasible time to collect urine samples from captive rhinoceroses was during the morning hours, when animals were most likely to have a first void urination and/or before they would go out onto exhibit. In addition to earlier collection times, higher pH values for the African black rhinoceros urine samples could have been due to differences in diet. The African black rhinoceroses were fed a diet consisting primarily of alfalfa hay. Although alfalfa was fed to the other species of rhinoceros, it was not the primary component of their diets. The high protein in alfalfa may have led to increased urea and/or nitrates in the urine and therefore more substrate for bacteria to act upon to raise the alkalinity of the sample over time.

CONCLUSIONS

Identifying urine abnormalities requires a comprehensive understanding of the qualities of normal urine. This study established the first measures of urine chemistry in three species of captive rhinoceros. Although urinary reference values for the horse have served as a model for the rhinoceros, the results of this study indicate differences significant enough to merit reduced dependence. Most important, it was determined that normal urine of the greater one-horned Sumatran and African black rhinoceros has lower specific gravity than that of the horse, particularly the African black rhinoceros. Given its status as the only grazer species, the African white rhinoceros may produce urine more comparable to that of the horse. However, further investigations are warranted to examine urine parameters between strictly grazer and browsing species of rhinoceros.

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