Baseline urinalysis results in 32 healthy Antillean manatees (*Trichechus manatus manatus*)

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OBJECTIVE
To describe results of analysis of free-catch urine samples collected from Antillean manatees (*Trichechus manatus manatus*) under human care in the Caribbean.

ANIMALS
32 Antillean manatees in 5 Caribbean oceanaria and rescue centers.

PROCEDURES
Urine samples were obtained by opportunistic free catch during physical examination or through the use of operant conditioning procedures. Urinalyses consisted of macro- and microscopic evaluations, biochemical analyses with test strips, and refractometry. Results were compared for manatees grouped on the basis of age, sex, and habitat.

RESULTS
Urine samples were typically clear, straw colored, and alkaline (mean pH, 8.0); had a urinoid odor and low specific gravity (mean, 1.010); and had results on qualitative test strips that were consistently negative for the presence of glucose, bilirubin, ketones, proteins, nitrites, RBCs, and WBCs. Microscopically, the mean ± SD number of RBCs and WBCs/hpf was 0.5 ± 0.3 RBCs/hpf and 1.1 ± 1.5 WBCs/hpf. The presence of some epithelial cells and crystals was typical. Spermatozoa were found in urine from 1 of 15 sexually mature males, and parasite larvae and eggs were found in urine from 2 manatees.

CONCLUSIONS AND CLINICAL RELEVANCE
Results of the present study yielded the first compilation of baseline urinalysis values in healthy Antillean manatees under human care, which, when combined with physical examination and other diagnostic procedures, can help in monitoring the health of these animals. We encourage the use of free-catch urine collection methods, as used in the present study, for routine urinalyses of manatees under human care in zoos, aquaria, or rescue centers. (*J Am Vet Med Assoc* 2021;258:416–424)

Antillean manatees (*Trichechus manatus manatus*) are endangered,[a] euryhaline, herbivorous aquatic mammals that live in marine, estuarine, and freshwater ecosystems of the Caribbean.[1] Manatees have been kept under human care since the 1950s,[2] and although considerable attention has been given to their care (including veterinary diagnostic procedures and treatments) during the past 30 years,[3–7] urinalysis is infrequently performed in manatees housed at aquariums and zoos.[8] In previous studies,[8–10,b] urine samples from Florida manatees (*Trichechus manatus latirostris*) and Amazonian manatees (*Trichechus inunguis*) were examined. However, Antillean manatees and Florida manatees are subspecies that differ morphologically, physiologically, and genetically from each other and from Amazonian manatees,[11–14] and there is no published information on results of urinalysis for Antillean manatees. Our aim of the study reported here was to describe the macroscopic, microscopic, and biochemical characteristics of free-catch urine samples collected through the use of noninvasive medical operant conditioning in Antillean manatees under human care in the Caribbean.

ABBREVIATIONS

ppt	Parts per thousand
SG	Specific gravity

Materials and Methods

Animals and facilities

Thirty-two Antillean manatees under human care at 5 oceanaria and rescue center facilities in Colombia, the Dominican Republic, Mexico, and Puerto Rico were enrolled in the study, which was approved by the Inter-American University Institutional Animal Care and Use Committee and conducted in accordance with the US Animal Welfare Act. On the basis of results from physical and hematologic examinations, the manatees were determined to have been clinically normal at the time of urine sampling between January 16, 2016, and November 20, 2019. The manatees were grouped on the basis of sex, age (ie, calf, juvenile, subadult, or adult[15]), and housing environment (ie, freshwater, estuarine, or saltwater). Water salinity at each facility was measured with a salinity refractometer.[c] All facilities were located in the tropical zone, in Caribbean waters with air tempera-

tures between 25°C and 29°C (77°F and 84.2°F) and water temperatures between 26°C and 29°C (78.8°F and 84.2°F). Diet consisted of aquatic plants and vegetables for adult, subadult, and juvenile manatees. For calves, natural or artificial milk formula with small inclusions of vegetables was fed, and depending on each calf's health and age, this diet was supplemented with various multivitamins and with biscuits formulated for folivore primates.[d]

Sample collection

Before a manatee's first feeding of the day, a midstream urine sample was obtained by opportunistic free catch during a veterinary examination or trained medical behaviors[16–18,e] **(Figure 1)**. Urine collection from manatees in Puerto Rico, Colombia, and the Dominican Republic was strictly opportunistic during veterinary evaluations in an empty tank devoid of water or on land along the side of a pond enclosure. Manatees were subject to regular physical examinations on the bottom of the empty tank or on land, during which the animal would typically lie in dorsal recumbency, facilitating urine collection. In Mexico, manatees were trained to urinate on command through operant conditioning, similar to conditioning used in dolphins,[19] except that instead of a hand signal for the urination command, light hand pressure was applied in a sustained manner over the area of the urinary bladder, just cranial to the anus. This pressure would trigger sufficient urination. Because sampling took several minutes in some instances, the trained behavior for urine sample collection included allowing the manatee to breathe without compromising the cleanliness of the genital area, which had been cleaned with isopropyl alcohol and allowed to air-dry before each sample collection.

Urine samples were collected in sterile, 120-mL polypropylene containers[f] or with a sterile 10- to 20-mL plastic syringe. Following collection, the container or syringe was labeled with the manatee's name and the date and time of collection. Urine samples were transported to the laboratory on ice and stored, when needed, at 4°C to 5°C (39.2°F to 41°F) until urinalysis was completed within 1 hour of collection.

Urinalysis

Urinalysis consisted of gross examination followed by biochemical analyses and microscopic evaluation of urine sediment as described previously.[20] A standardized format for marine mammals[g] was used to record data, which were then transferred to a spreadsheet[h] at the end of each sampling and analysis day. Prior to analysis of results, a comparison was made between the recorded information on the urinalysis form versus the computerized spreadsheet to identify and correct any transcription errors.

Gross examination—A sterile, polymethylpentene plastic 50-mL graduated cylinder[i] was used to measure the volume of each urine sample. Physical characteristics of each sample were described, including color (ie, colorless, pale yellow, yellow, dark yellow, dark brown-yellow, pale green-yellow, green-yellow, dark green-yellow, straw, ochre, red-brown, or red), appearance (ie, clear, hazy, or slightly cloudy), and odor (ie, none, urinoid [strong urine smell], fruity or

Figure 1—Representative photograph of the use of operant conditioning in collecting urine from 1 of the 12 Antillean manatees (*Trichechus manatus manatus*) under human care in Mexico between January 19 and July 20, 2017. The manatee in the image is an adult female.

sweet, fruity fishy [mix of sweet and fish smell], fishy, grassy [smell of grass], pungent [having a sharply strong odor], sulfuric [rotten egg smell], aromatic [fragrant smell], fecal [smelling of feces], or ammoniacal [long-standing ammonia smell]). Because physical characteristics of urine are subjective, the evaluators were trained in a veterinary laboratory and practiced gross urine examination on urine collection from domestic mammals and dolphins.

Biochemical examination—Following gross examination, each urine sample was transferred to a sterile, 15-mL conical polypropylene centrifuge tube[j] for biochemical analyses with 3 different types of test strips. One type of test strip[k] was used to assess urine SG, pH, and concentrations of glucose, bilirubin, urobilinogen, ketones, nitrite, protein, RBCs, and WBCs. A second type of test strip[l] was also used to assess urine pH, and a third type of test strip[m] was used to assess urine concentrations of creatinine and microalbumin. Each test strip was immersed in the urine sample, then removed immediately. Excess urine was removed from the strip by touching a paper towel to the edge of the strip. The color change for each urinalysis parameter was compared with a colorimetric scale supplied with the respective test strip kit, and the results were recorded. Qualitative results for whether analytes were present versus absent (negative) were recorded, and if present, semiquantification of results to the extent feasible with the test strips was recorded (eg, urine glucose concentration: negative vs 100, 250, 500, 1,000, or > 2,000 mg/dL). Detection of a semiquantitative trace concentration of urobilinogen (ie, ≤ 0.2 mg/dL) was considered a negative result for the detection of an abnormal concentration of urobilinogen. Because a study[19] of dolphins shows that measurements of urine SG were inaccurate when determined with the same type of test strip[k] used in the present study, we also measured urine SG with a veterinary clinical refractometer[n] that was calibrated with distilled water.

Microscopic examination—To extract urinary sediment from each sample, approximately 3 mL of urine was retained in the conical tube and centrifuged[o] for 2.5 minutes at 1,163 × *g*. Pipette aspiration was used to discard most of the supernatant, leaving a volume of approximately 0.5 to 1 mL of supernatant in which the sediment pellet was resuspended. A drop of the resuspended sediment was placed on a glass slide, covered with a cover slip, and examined with light microscopy at an absolute magnification of 100× and 400× (hpf) to assess for WBCs, RBCs, epithelial cells (transitional, squamous, or tubular), casts (hyaline, granular, waxy, epithelial, or fatty), crystals (calcium oxalate, uric acid, triple phosphate, calcium phosphate, amorphous phosphate, or urate), bacteria, yeast or fungi, spermatozoa, and parasites or parasite eggs. For each urine sample, the mean numbers of microscopy elements of interest (eg, WBCs, RBCs, and epithelial cells)/10 hpfs (400×) were determined, recorded, and then qualitatively categorized as none (mean, 0 elements/hpf), rare (mean, > 0 to ≤ 2.5 elements/hpf), few (mean, > 2.5 to < 5.0 elements/hpf), some (mean, ≥ 5.0 to < 7.5 elements/hpf), or many (mean, ≥ 7.5 elements/hpf). Qualitative results for mucus were defined as none (not observed), rare (occasionally present), few (mildly present), some (moderately present), or many (abundantly present).

Statistical analysis

Descriptive statistics were calculated and analyzed with statistical software.[p] Qualitative results were reported as numbers and percentages, and quantitative results were reported as range and mean ± SD.[21–24] Results were assessed for normality of distribution with the Shapiro-Wilk test, and paired 2-tailed *t* tests were used to evaluate for differences in mean results for manatees grouped on the basis of age, sex, and habitat. Values of $P \leq 0.05$ were considered significant.

Results

Animals

There were 32 manatees (21 males [15 sexually mature] and 11 females [8 sexually mature]) under human care at 5 oceanaria and rescue center facilities in Colombia (n = 9), the Dominican Republic (2), Mexico (12), and Puerto Rico (9). Of the 32 manatees, 6 were calves (age, ≤ 1 year), 3 were juveniles (age, 2 to 4 years), 3 were subadults (age, 5 to 9 years), and 20 were adults (age, ≥ 10 years). Of the 32 manatees, 13 were in open marine systems (salinity range, 31 to 35 ppt), 6 were in open cenote estuarine systems (salinity range, 17 to 22 ppt), and 13 were in freshwater cement tanks or river systems (salinity, 0 ppt).

Urine samples

Free-catch urine samples were obtained by opportunistic free catch during veterinary examinations for 20 manatees and through operant conditioning procedures for 12 manatees. For the manatees with which operant conditioning was used, urination occurred within 5 to 8 minutes after the start of the requesting procedure. Overall, the mean ± SD urine sample volume was 5.4 ± 1.53 mL (range, 3 to 9 mL), and the mean ± SD duration from collection to urinalysis was 19.5 ± 6.8 minutes (range, 8 to 35 minutes).

Gross examination—The color of most urine samples was straw (22/32 [69%]), followed by pale yellow (6/32 [19%]) or colorless (4/32 [13%]; **Table 1**). In addition, the appearance of most samples was clear (21/32 [66%]), and the most common odor of samples was urinoid (strong urine smell; 25/32 [78%]).

Biochemical examination—Qualitative results of urinalyses were predominantly negative for biochemical analytes assessed with test strips; however, semiquantitative trace concentrations were detected for urobilinogen (0.2 mg/dL; n = 32/32), creatinine (0.1 mg/dL; 17/17), and microalbumin (10 mg/dL; 17/17; **Table 2**). The microalbumin-to-creatinine ratio was 100 mg/g for all 17 manatees from which urine samples were evaluated for both analytes.

Overall, the mean ± SD SG measured by refractometry was 1.010 ± 0.007 and did not differ substantially between males (1.011 ± 0.008) and females (1.009 ± 0.005). When considered by manatee age group, the mean ± SD urine SG was 1.009 ± 0.004 for calves (n = 6), 1.010 ± 0.011 for juveniles (3), 1.005 ± 0.001 for subadults (3), and 1.011 ± 0.008 for adults (20). On the basis of manatee habitat, the mean ± SD urine SG was 1.012 ± 0.009 for those in marine habitats (n = 13), 1.011 ± 0.005 for those in estuarine habitats (6), and 1.008 ± 0.006 for those in freshwater habitats (13). The small number of manatees in each of the various categories precluded further statistical analyses regarding urine SG.

Overall, the mean ± SD urine pH was 8.0 ± 0.9 and did not differ substantially between males (7.9 ± 1.1) and females (8.0 ± 0.7). By age group, the mean ± SD urine pH was 7.8 ± 0.7 for calves, 6.7 ± 1.5 for juveniles, 8.0 ± 0.0 for subadults, and 8.2 ± 0.9 for adults. On the basis of manatee habitat, the mean ± SD urine pH was 8.3 ± 0.9, 8.0 ± 0.8, or 7.6 ± 1.0 for those in marine, estuarine, or freshwater habitats, respectively. Similar to findings for SG, the small number of manatees in each category precluded further statistical analyses regarding urine pH.

Table 1—Gross characteristics of free-catch urine samples obtained from 32 Antillean manatees (*Trichechus manatus manatus*) under human care in 5 Caribbean oceanaria and rescue centers between January 16, 2016, and November 20, 2019.

Characteristic	No. (%) of urine samples
Color	
Straw	22 (69)
Pale yellow	6 (19)
Colorless	4 (13)
Yellow	0
Dark yellow	0
Dark brown-yellow	0
Pale green-yellow	0
Green-yellow	0
Dark green-yellow	0
Ochre	0
Red-brown	0
Red	0
Appearance	
Clear	21 (66)
Slightly cloudy	9 (28)
Hazy	2 (6)
Odor	
Urinoid	25 (78)
Grassy	4 (13)
Fruity sweet	2 (6)
Sulfuric	1 (3)
None	0
Fruity fishy	0
Fishy	0
Pungent	0
Aromatic	0
Fecal	0
Ammoniacal	0

Microscopic examination—In urinary sediments of the 32 manatees, the mean ± SD number of RBCs/hpf was 0.5 ± 0.3 (range, 0 to 3 RBCs/hpf), and the mean number of WBCs/hpf was 1.1 ± 1.5 (range, 0 to 8 WBCs/hpf). Qualitatively, epithelial cells were observed in urinary samples from 28 manatees, with transitional cells less commonly present (1/32 [3%]), compared with squamous cells (21/32 [66%]), which were more commonly identified in samples from females (11/11 [100%]) versus males (10/21 [48%]; **Table 3**). Urinary crystals were observed in samples from 13 manatees, with calcium oxalate monohydrate crystals most commonly identified among the affected samples (n = 4). There were rare observations of mucus, erythrocyte casts, hyphae, sperm, or parasite larvae and eggs, and these were found only in samples from adult male manatees. For instance, casts were present in urine samples from only 2 male manatees housed in an estuarine system in Mexico, and both manatees had RBC-type casts. In addition, these 2 males had unidentified nematode-type larvae and eggs observed in their urine. A summary of key urinalysis findings for gross,

Table 2—Summary urinalysis results obtained with urine test strips and refractometry used to evaluate the urine samples from the 32 Antillean manatees described in Table 1.

Parameter	No. of samples			Range	Mean ± SD
	Tested	Negative	Positive		
Glucose (mg/dL)*	32	32	0	—	—
Bilirubin (mg/dL)*	32	32	0	—	—
Urobilinogen (mg/dL)*	32	32	0	0.2†	0.2 ± 0†
Ketone (mg/dL)*	32	32	0	—	—
Nitrite (mg/dL)*	32	32	0	—	—
Protein (mg/dL)*	32	32	0	—	—
RBCs (cells/µL)*	32	32	0	—	—
WBCs (cells/µL)*	32	32	0	—	—
SG‡	32	—	—	1.002–1.030	1.010 ± 0.007
pH§	32	—	—	5.0–9.5	8.0 ± 0.9
Microalbumin (mg/L)‖	17	0	17	10	10.0 ± 0
Creatinine (g/L)‖	17	0	17	0.1	0.1 ± 0
Microalbumin-to-creatinine ratio (mg/g)	17	—	—	100	100 ± 0

*Measured with urine test strips.[k] †The finding was considered a negative result for the detection of abnormal urobilinogen concentration in urine. ‡Measured by refractometry. §Measured with urine test strips.[l] ‖Measured with urine test strips.[m]

— = Not applicable.

Table 3—Qualitative findings from microscopic examination of the sediments in urine samples from the 32 manatees described in Table 1.

Sediment element of interest	No. of samples	No. (%) of samples per qualitative category*				
		None	Rare	Few	Some	Many
Epithelial cells	32	4 (13)	6 (19)	9 (28)	12 (38)	1 (3.1)
Casts	32	30 (94)	2 (6)	0	0	0
Crystals	32	19 (60)	4 (13)	0	9 (28)	0
Bacteria	32	12 (38)	3 (10)	13 (41)	2 (6)	2 (6)
Yeast or fungi	32	30 (94)	0	2 (6)	0	0
Spermatozoa	15	14 (93)	1 (7)	0	0	0
Mucus	32	31 (97)	1 (3)	0	0	0
Parasites or ova	32	30 (94)	2 (6)	0	0	0

*For each urine sample, the mean numbers of each of the urine sediment elements of interest (eg, WBCs, RBCs, and epithelial cells)/10 hpf [400X]) were determined, recorded, and then qualitatively categorized as none (mean, 0 elements/hpf), rare (mean, > 0 to ≤ 2.5 elements/hpf), few (mean, > 2.5 to < 5.0 elements/hpf), some (mean, ≥ 5.0 to < 7.5 elements/hpf), or many (mean, ≥ 7.5 elements/hpf).

Table 4—Summary of key urinalysis parameters with baseline results considered clinically normal in the 32 Antillean manatees described in Table 1.

Parameter	Baseline result
Gross examination	
Color	Straw to colorless
Appearance	Clear
Odor	Urinoid to grassy
SG	1.003–1.017
pH	7.1–8.9
Urine test strip assessments	
Glucose (mg/dL)	Negative
Bilirubin (mg/dL)	Negative
Urobilinogen (mg/dL)	≤ 0.2
Ketone (mg/dL)	Negative
Nitrite (mg/dL)	Negative
Protein (mg/dL)	Negative
RBCs (cells/µL)	Negative
WBCs (cells/µL)	Negative
Microalbumin (mg/L)	≤ 10
Creatinine (g/L)	≤ 0.1
Microalbumin-to-creatinine ratio (mg/g)	100
Microscopy of urine sediment	
WBCs (cells/hpf [400X])	0–2
RBCs (cells/hpf [400X])	0–1
Epithelial cells*	None to some
Casts*	None
Crystals*	None to some
Bacteria*	None to few
Yeast or fungi*	None
Spermatozoa*	None
Mucus†	None
Parasites or ova of parasites*	None

*Reported as the qualitative categories described in Table 3. †Qualitative results for mucus were defined as none (not observed), rare (occasionally present), few (mildly present), some (moderately present), or many (abundantly present).

biochemical, and microscopic characteristics identified in the 32 healthy Antillean manatees under human care was compiled **(Table 4)**.

Discussion

Urinalysis is a basic veterinary diagnostic tool used to evaluate renal function.[25] It is easy, quick, and inexpensive, and we used noninvasive techniques to obtain urine samples from the manatees in the present study. Together with physical examinations, hematologic and serum biochemical analyses, and coprologic tests, urinalysis is a key diagnostic tool for evaluating patients, including routinely evaluating wild animals maintained under human care.[26] Many zoos and aquaria have increasingly relied on training animal behaviors conducive to medical procedures through operant conditioning. Because cystocentesis or urinary catheterization would require patient restraint and could result in patient resistance, injury to the staff and patient, and less cooperation from the patient in future events, the use of operant conditioning to obtain diagnostic samples is less invasive, minimizes stress during sampling, and increases the ease in which basic diagnostic procedures can be performed,[18] including in manatees.[16,17] If renal disfunction is suspected on the basis of results from samples collected with operant conditioning, further tests (eg, assessments of electrolytes and urea waste product in urine[12] and symmetric dimethylarginine in blood[q]) may follow to thoroughly evaluate an animal's renal condition.

Results of the present study indicated that straw-colored urine was most common and considered clinically normal for the Antillean manatees in the present study. The color is attributed to the presence of urochromes, uroerythrin, and urobilin that are formed through bilirubin metabolism and eliminated in the urine.[27] This urine color was consistent with that reported in other herbivorous species, such as cattle, goats, sheep, rabbits, rhinoceros (*Rhinoceros unicornis*, *Dicerorhinus sumatrensis*, and *Diceros bicornis*), and Floridian and Amazonian manatees[8,26,28,29,b] but differed from the ochre or white colors of urine produced by horses and attributed to the presence of mucus and abundant amounts of squamosal epithelial cells.[30] The straw-colored urine of manatees in the present report also differed from the typical yellow to dark-yellow urine of carnivores (including dogs, cats, dolphins, and pinnipeds), reflecting highly concentrated urine and high-protein diets.[19,29,30] In the present study, the small proportion (4/32 [13%]) of manatees with urine that was colorless and had no alteration of other urinary pa-

rameters was similar to findings in Amazonian manatees in which dilute urine is thought to be associated with intake of large quantities of water.[8,31] The urine of Antillean manatees sampled in the present study was predominantly clear, similar to urine from most domestic and wild mammals[19,29,32,33] but unlike turbid urine of horses (owing to the presence of calcium carbonate crystals and mucus[30]) and Amazonian manatees (owing to the presence of cells, crystals, and bacteria[29,34]).

Urine odor varies among animals,[26] and a urinoid odor was most common in the urine of the Antillean manatees in the present study, consistent with findings for most mammals owing to the presence of volatile acids and decomposition products of urea.[27] Some manatees in the present study had urine with a grassy odor that was directly related to their herbivorous diet, and a few manatees from the Dominican Republic and Puerto Rico had urine that smelled fruity sweet, which we presumptively related to their dietary supplementation with fruits and vitamins. Although a fruity-sweet odor in urine could suggest glucosuria, none of the manatees sampled had glucose detected in their urine. Manatees under human care are fed diets rich in fats and carbohydrates,[35] and some animals have high serum fructosamine concentrations and possible hyperglycemia with type 2 diabetes.[r] In addition, previous studies[10,b] indicate that urine glucose concentrations of 3.9 to 6.9 mg/dL and 3.0 to 3.6 mg/dL in Floridian and Amazonian manatees, respectively, possibly reflect the intake of carbohydrate-rich foods, such as beets.[30] Clinicians should pay close attention to urine glucose concentration in manatees.

Urinalysis results with test strips were negative for the detection of glucose, bilirubin, urobilinogen, ketones, nitrite, protein, blood, and leukocytes in the Antillean manatees of the present report. These findings were consistent with those in domestic animals,[29,30] Asian elephants (*Elephas maximus*),[s] rhinoceros,[28] common bottlenose dolphins (*Tursiops truncatus*),[19] and Amazonian manatees.[8,10] Similarly, the Antillean manatees in the present study did not have substantial numbers of RBCs or WBCs in their urine sediment when examined microscopically, as is typical of most domestic animals.[36] A previous study[8] that assessed multiple urine samples from 21 Amazonian manatees with reactive urinalysis strips indicated most urine samples had 10 RBCs/mL and < 25 WBCs/mL; however, the investigators did not speculate on underlying causes, which could have included estrus in females or a urinary tract inflammation.[37] In the present study, microscopic evaluation of urine sediment revealed that the manatees had 0 to 8 WBCs/hpf and 0 to 3 RBCs/hpf, which were findings considered clinically normal because the cells are physiologically eliminated in the presence of mild inflammation, heat, and epithelial desquamation.[38,39] In contrast, Amazonian manatees have been reported[8] to have various numbers of WBCs and RBCs and even suppurative exudate in their urine, suggesting the presence of genitourinary infections.

Marine mammals, such as common bottlenose dolphins, gray seals (*Halichoerus grypus*), and South American sea lions (*Otaria byronia*), tend to have acidic urine,[8,19] and a previous study[5] shows that Florida manatees also have acidic urine. However, our findings indicated that Antillean manatees have alkaline urine, and we hypothesized that this is a consequence of herbivory. Similarly, urine pH is neutral to alkaline (pH, 7.0 to 9.0) in other herbivorous mammals (eg, cattle, sheep, goats, horses, rabbits, rhinoceros, and elephants[28,29,40]), likely owing to ingestion of large amounts of calcium carbonate, which is eliminated by renal excretion.[25]

Our finding that the mean ± SD urine SG was 1.010 ± 0.007 (range, 1.002 to 1.030) was consistent with reports[41,42] that manatees have poorly concentrated urine under normal conditions as part of their physiologic osmoregulation process through semireniculate kidneys. Our findings suggested that the capacity to concentrate urine is similar between Antillean manatees and Amazonian manatees (urine SG, 1.000 to 1.015),[8] Florida manatees (urine SG, 1.007 to 1.008),[12,43,b] various domestic species, and herbivorous great apes[44,45]; however, domestic and wild carnivores, cetaceans, and artiodactyls typically have more concentrated urine.[19,29,37,46]

Urinary creatinine concentrations in the Antillean manatees of the present study were similar to those found in Amazonian and Florida manatees[10,b]; cattle, sheep, and goats[47,48]; domestic dogs[49]; common bottlenose dolphins[19]; and harbor seals.[50] In addition, our findings for urinary microalbumin were also within reference limits for domestic mammals.[37,49] Further, our findings for the mean microalbumin-to-creatinine ratio (100 mg/g) as an evaluation for early detection of renal dysfunction[30,36,51] were similar to results in healthy dogs, cats, and other farm mammals,[20,37,49,52,53] which suggested that the concentrations we observed were the result of physiologic excretion and clinically normal.

Epithelial cells were commonly observed in urinary samples from the Antillean manatees in the present study. This finding is clinically normal in midstream free-catch urine samples, including in Amazonian manatees,[8] and occurs physiologically as a result of cell turnover.[34] Additionally, the finding of higher numbers of squamous epithelial cells in urine samples from females (vs males) may have been attributable to estrus cycles and associated accelerated detachment of vaginal epithelial cells that allowed a large number of these cells to be seen in the sediment of free-catch samples.[8,54] The small numbers of transitional epithelial cells observed seemed to lack diagnostic importance, given that there were no associated abnormalities in other urine parameters.[38] Similarly, rare identification of RBC casts (only in 2 adult males) was considered a normal physiologic finding similar to that in domestic animals.[34,37] In addition, our findings for urinary crystals were consistent with those in herbivores with alkaline urine (eg, horses,[29] Asian elephants,[40] rhinoceros,[28] and Amazonian and

Florida manatees[8,55]) and other marine mammals (eg, common bottlenose dolphins,[56] ringed seals [*Pusa hispida*], northern elephant seals [*Mirounga angustirostris*], Weddell seals [*Leptonychotes weddellii*], and humpback whales [*Megaptera novaeangliae*][8,37]). The small amounts of bacteria and fungi or yeast observed in urine samples of the present study seemed to have represented contamination from free-catch sample collection in aquatic environments where complete sterility is difficult to obtain,[37,38] particularly in male manatees owing to their long (up to approx 45 cm) tubular sheath within a prepuce that can harbor water from their surroundings. In domestic and wild animals, the presence of fungal hyphae without accompanying abnormalities in other urine parameters is typically considered indicative of contamination, and such has been observed in Amazonian manatees.[10] We also identified spermatozoa in the urine sample from 1 adult male and considered the finding clinically normal,[30,34] as was our detection of mucus in urine from a male manatee.[27,36] Although we did not stain slides for urinary sediment assessment because of logistic reasons owing to remote field circumstances in the present study, future studies would benefit from staining urine sediment slides and thereby making it easier to identify the sediment structures of interest.

Our finding of unidentified nematode-type larvae and eggs in the urine samples of 2 adult male Antillean manatees in Mexico was similar to that previously reported.[57] In contrast to mammalian herbivores, urinary sediment findings in carnivores and domestic omnivores more commonly include parasites (ie, giant dog kidney worm [*Dioctophyma renale*], dog heartworm [*Dirofilaria immitis*], bladder worm [*Capillaria plica* and *Capillaria feliscati*], and swine kidney worm [*Sthepanurus dendatus*][33,37]). We suggest that future studies of Antillean manatees should isolate larvae and eggs found in urine samples and investigate their morphological and molecular properties to identify the organisms and ascertain whether they have any clinical impact on manatees.

Although the small number of manatees in each group when considered on the basis of age, sex, and habitat precluded further statistical analyses of results, to our knowledge, our overall findings yielded the first compilation of baseline values for urinalysis in healthy Antillean manatees under human care. We believe that the ease of free-catch urine collection methods (with operant conditioning and by opportunistic collection during physical examination) is encouraging for potential future expanded use in zoos, aquaria, or rescue centers for routine urinalysis in manatees and other wildlife species similarly considered difficult to sample for routine urinalysis. In addition, assessing symmetric dimethylarginine concentration in blood samples from Antillean manatees may serve as a relatively easy, fast, noninvasive, and cost-effective diagnostic tool to augment detection of renal disease or infection[q]; additional research is warranted.

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Footnotes

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i. Nalgene (50 mL), Thermo Fisher Scientific, Waltham, Mass.
j. Greiner Bio-One North America Inc, Monroe, NC.
k. AimStrip 10-SG, Germain Laboratories, San Antonio, Tex.
l. pH-Aware, HealthyWiser LLC, Wyo.
m. Rapid Response Microalbumin 2-1 Combo Strip, BTNX Inc, Markham, ON, Canada.
n. RETK-71, Tekcoplus Ltd, Hong Kong, China.
o. Clay Adams Compact II centrifuge, Becton, Dickinson and Co, Franklin Lakes, NJ.
p. SAS, version 9.1, SAS Institute Inc, Cary, NC.
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From this month's *AJVR*

Time required to achieve maximum amikacin concentration in the synovial fluid of the tarsocrural joint following administration of the drug by intravenous regional limb perfusion in horses

Isabelle Kilcoyne et al

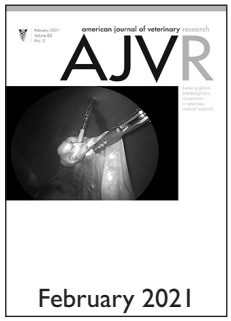
OBJECTIVE
To determine the median time to maximum concentration (t_{max}) of amikacin in the synovial fluid of the tarsocrural joint following IV regional limb perfusion (IVRLP) of the drug in a saphenous vein of horses.

ANIMALS
7 healthy adult horses.

PROCEDURES
With each horse sedated and restrained in a standing position, a 10-cm-wide Esmarch tourniquet was applied to a randomly selected hind limb 10 cm proximal to the point of the tarsus. Amikacin sulfate (2 g diluted with saline [0.9% NaCl] solution to a volume of 60 mL) was instilled in the saphenous vein over 3 minutes with a peristaltic pump. Tarsocrural synovial fluid samples were collected at 5, 10, 15, 20, 25, and 30 minutes after completion of IVRLP. The tourniquet was removed after collection of the last sample. Amikacin concentration was quantified by a fluorescence polarization immunoassay. Median maximum amikacin concentration and t_{max} were determined.

RESULTS
1 horse was excluded from analysis because an insufficient volume of synovial fluid for evaluation was obtained at multiple times. The median maximum synovial fluid amikacin concentration was 450.5 μg/mL (range, 304.7 to 930.7 μg/mL), and median t_{max} was 25 minutes (range, 20 to 30 minutes). All horses had synovial fluid amikacin concentrations ≥ 160 μg/mL (therapeutic concentration for common equine pathogens) at 20 minutes after IVRLP.

CONCLUSIONS AND CLINICAL RELEVANCE
Results suggested that, in healthy horses, maintaining the tourniquet for 20 minutes after IVRLP of amikacin in a saphenous vein was sufficient to achieve therapeutic concentrations of amikacin in the tarsocrural joint. (*Am J Vet Res* 2021;82:99–104)



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