Urinary diagnostic indices in calves

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Objective—To establish reference values for urinary diagnostic indices in healthy calves from birth to 90 days of age.

Design—Prospective field trial.

Animals—12 Holstein heifer calves.

Procedure—Urine and serum samples were collected daily for the first 5 days after birth, then weekly until calves were 90 days old. Urine:serum creatinine ratio, urine:serum urea nitrogen ratio, urine:serum osmolality ratio, fractional clearances of sodium and inorganic phosphate, and urine γ-glutamyltransferase activity were measured. Data were grouped by age of calves at the time of sample collection: 1 to 5 days old (neonatal period), 7 to 27 days old (suckling period), and 28 to 90 days old (weanling period).

Results—Mean urine:serum creatinine, urea nitrogen, and osmolality ratios were significantly higher during the weanling period than during the other 2 periods. There were no significant differences in mean fractional clearances of sodium among age periods.

Clinical Implications—Urinary diagnostic indices calculated for these healthy calves may be used as reference values for early recognition of renal damage or renal failure. (*J Am Vet Med Assoc* 1997;211:212–214)

Renal damage is a frequent sequela to diarrhea, shock, dehydration, and administration or ingestion of nephrotoxic substances. The high incidence of infectious diarrhea, septicemia, and respiratory disease in calves predisposes these animals to abnormalities in fluid balances and places them at high risk of developing renal damage. In animals with renal damage, serum creatinine and urea nitrogen concentrations are insensitive indicators of renal dysfunction and exceed the upper limit of the reference range only after extensive loss of nephron function.[1,2] Increases in serum concentrations of creatinine or urea nitrogen do not allow one to distinguish among prerenal, renal, or postrenal azotemia.[1,3] Urine specific gravity can be used to differentiate prerenal from renal azotemia; unfortunately, results of urinalysis do not reflect the magnitude of the disorder, and they are not specific for particular renal disorders.[2]

Calculation of renal clearance of creatinine, urea nitrogen, and electrolytes, along with measurement of specific enzyme activity in the urine, is a more sensitive means of assessing renal function and may be a more specific indicator of damage to renal tissue than is serum biochemical analysis.[1-8] Urinary diagnostic indices, such as the ratio of urine to serum concentrations of creatinine and urea nitrogen, renal clearance of creatinine and urea nitrogen, ratio of urine to serum osmolality, fractional clearances of electrolytes, and urine enzyme activity, have been used to evaluate renal function and to estimate the extent of renal damage in adult cattle, foals, adult horses, human beings, and other species.[1-8] Early diagnosis of renal damage facilitates initiation of appropriate treatment and reduces the chances of irreversible renal failure. Sequential measurement of these indices can aid in the determination of prognosis and allows clinicians to monitor and evaluate the extent of recovery of renal function.[1,2,6,7] To our knowledge, there currently are no published reference values for these indices of renal function in young calves. The purpose of the study reported here was to establish reference values for common urinary diagnostic indices in healthy calves from birth to 90 days of age. The study was also designed to determine whether there were any age-related changes in these indices as calves matured from birth to 90 days of age.

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Materials and Methods

Calves—Twelve Holstein heifers born at the University of Tennessee Knoxville Experiment Station Dairy Unit were used in the study. A physical examination, CBC, and urinalysis were performed, and urine was submitted for bacterial culture at birth and weekly throughout the study. Calves were housed individually in stalls, and standard calf management procedures were used. Calves were fed milk replacer[a] (22% crude protein) and had free access to water. Hay and grain were added to the diet when calves were 2 weeks old. Calves were weaned at 28 days of age. The study protocol was approved by the University of Tennessee Animal Care and Concerns Committee.

Sample collection—Blood samples were collected by means of jugular venipuncture in plain evacuated tubes for recovery of serum and into EDTA-containing tubes for CBC. Blood in plain glass tubes was allowed to clot for 15 minutes and was then centrifuged; serum was collected and stored at –70 C (–94 F) until analyzed. Urine samples were collected via free catch in specimen cups. Blood and urine samples were obtained daily for the first 5 days after birth, then weekly until calves were 3 months old.

Sample analysis—Automated biochemical analysis procedures were used to measure serum concentrations of creatinine, urea nitrogen, sodium, and inorganic phosphate. Serum and urine osmolality were determined by use of a vapor pressure osmometer.[b] Urine samples were centrifuged at 1,500 X g for 5 minutes, and an automated biochemical analyzer[c] was used to measure concentrations of creatinine, urea nitrogen, sodium, and inorganic phosphate and activity of γ-glutamyltransferase (GGT) in the supernatant.

Data analysis—**Urine:serum creatinine ratio (U_{CR}:S_{CR})** and **urine:serum urea nitrogen ratio (U_{UN}:S_{UN})** were calculated by dividing urine concentration (mg/dl) by serum concentration (mg/dl). **Fractional clearances of sodium (FC_{Na})** and **inorganic phosphate (FC_{PO_4})** were calculated by dividing urine concentration by serum concentration and multiplying by 100/U_{CR}:S_{CR}. **Urine:serum osmolality ratio (U_{OSM}:S_{OSM})** was calculated by dividing urine osmolality by serum osmolality (osm/kg). **Corrected GGT activity (U_{GGT}:U_{CR})** was calculated by dividing urine GGT activity (IU/L) by urine creatinine concentration (mg/dl) and multiplying by 100.

Statistical analysis—Data were grouped on the basis of age of calves at the time of sample collection: 1 to 5 days old (neonatal period), 7 to 27 days old (suckling period), and 28 to 90 days old (weanling period). Descriptive statistics and box plots were produced for each group.[d] In box plots for all calculated variables, 50 data points found to be greater than the 75th percentile by 1.5 times the interquartile range were marked as possible outliers.[9] Fifteen extreme data points were discarded after visual examination of the outliers for consistency across time and considering physiologic limits. The remaining data were still positively skewed, and a square root transformation was used so that data would more closely approximate a normal distribution. An ANOVA with blocking on animal, age-group, and day nested within age-group was performed. Tukey's mean separation was used to identify means that differed significantly. A value of $P < 0.05$ was considered significant.

Results

Results of physical examinations, CBC, and urinalyses performed during the study were normal, and results of bacterial culture of urine were negative, indicating that each calf remained healthy for the duration of the study. Age significantly affected values for all urinary indices except for FC_{Na} (Table 1). Mean values for U_{CR}:S_{CR}, U_{UN}:S_{UN}, and U_{OSM}:S_{OSM} decreased from the neonatal to suckling period, but increased in the weanling period. Mean FC_{PO_4} and U_{GGT}:U_{CR} did not differ significantly between the neonatal and suckling periods, but mean FC_{PO_4} significantly increased during the weanling period and mean U_{GGT}:U_{CR} significantly decreased during the weanling period. Differences among mean values within each age period were small, with mean squares ≤ 7% of the age period mean square, except for FC_{PO_4} for which it was 22%.

Discussion

Detection of renal dysfunction prior to development of substantial renal damage is the primary goal when assessing renal function. Although U_{CR}:S_{CR} and U_{UN}:S_{UN} are not direct measures of renal function, they have been found to be useful in the differentiation of acute prerenal azotemia and acute renal azotemia in human beings and adult horses.[6,7] Patients with acute renal azotemia were found to have low U_{CR}:S_{CR} and U_{UN}:S_{UN}, and patients with acute prerenal azotemia were found to have normal to high U_{CR}:S_{CR} and U_{UN}:S_{UN}.[6,7] The U_{OSM}:S_{OSM} is a measure of the kidney's ability to conserve water.[10] This is a more sensitive and specific test of renal concentrating ability than measurement of urine specific gravity,[11] because urine specific gravity can be affected by other refractory substances in the urine, such as protein and glucose.

Changes in mean values of the U_{CR}:S_{CR}, U_{UN}:S_{UN}, and U_{OSM}:S_{OSM} among age periods may reflect maturation of renal function. However, other studies[12-15] have shown that renal function in newborn calves was similar to that of adult cattle within 2 to 3 days after birth. Additional studies[15,16] have shown that young calves can excrete large volume loads in response to water overload and conserve water in response to water deprivation as efficiently as do adult cattle. As calves age, diet changes from all liquid to partially liquid, and changes in urinary diagnostic indices are probably related to increases in water conservation secondary to decreased water intake. Determination of effective renal blood flow and of glomerular filtration rate as calves age would further elucidate the physiologic basis for the changes in the present study.

There was a wide variation in the values for U_{CR}:S_{CR}, U_{UN}:S_{UN}, and U_{OSM}:S_{OSM} in all age periods as shown by the large SD and wide ranges. Although there were significant differences in mean values between age periods, the ranges of values overlapped extensively, especially between the neonatal and suckling periods. The lower limit of the calculated range for each of these indices is the value of importance, because values less than this limit may indicate a decrease in renal concentrating ability.

Fractional clearance from plasma of a given substance is calculated by comparing the amount of the substance excreted in the urine with the amount filtered through the glomerulus. Sodium and inorganic phosphate are re-

Table 1—Urinary diagnostic indices in 12 calves during the neonatal (1 to 5 days old), suckling (7 to 27 days old), and weanling (28 to 90 days old) periods

	Neonatal period		Suckling period		Weanling period	
Index	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
U_{CR}:S_{CR}	79.71[a] (51.49)	7.14–226.00	50.12[b] (42.15)	2.67–223.33	105.50[c] (44.35)	16.55–233.33
U_{UN}:S_{UN}	38.99[a] (21.77)	4.57–110.00	30.66[b] (18.14)	6.00–72.08	64.44[c] (24.63)	12.50–133.33
U_{OSM}:S_{OSM}	1.77[a] (0.99)	0.03–4.17	1.29[b] (0.89)	0.17–3.49	3.59[c] (1.18)	0.73–9.00
FC_{Na} (%)	0.69[c] (0.52)	0.06–2.73	1.14[c] (0.89)	0.17–3.44	0.84[c] (0.61)	0.04–2.93
FC_{PO_4} (%)	13.04[a] (8.16)	0.15–38.97	11.36[a] (8.26)	0.82–35.67	20.14[c] (11.25)	1.10–49.05
U_{GGT}:U_{CR}	0.58[c] (0.51)	0.10–2.60	0.65[c] (0.57)	0.10–2.50	0.23[a] (0.18)	0.10–1.00

[a,b,c]Mean values in each row with different superscripts are significantly ($P < 0.05$) different.

U_{CR}:S_{CR} = urine:serum creatinine ratio; U_{UN}:S_{UN} = urine:serum urea nitrogen ratio; U_{OSM}:S_{OSM} = urine:serum osmolality ratio; FC_{Na} = fractional clearance of sodium; FC_{PO_4} = fractional clearance of inorganic phosphate; U_{GGT}:U_{CR} = corrected γ-glutamyltransferase activity.

absorbed from the glomerular filtrate by the renal tubules; therefore, FC_{Na} and FC_{PO_4} are indices of tubular function. The FC_{Na} has been used to help differentiate prerenal azotemia from acute tubular necrosis, which is associated with an increase in FC_{Na}.[6,7,10] The FC_{PO_4} was shown to increase early in the development of renal disease in horses and may be a useful diagnostic indicator.[17] There were no significant differences in means for FC_{Na} among age periods; however, there was a significant increase in the mean FC_{PO_4} between the neonatal and suckling periods and the weanling period. This increase in FC_{PO_4} may reflect an increase in phosphate intake associated with an increase in ingestion of grain during the weanling period. Mean values for FC_{PO_4} and FC_{Na} in this study were similar to those reported for nonlactating cattle.[18,19] The upper limit of the calculated range for these 2 indices is the value of importance, because values greater than this limit may reflect renal tubular failure.

Most of the GGT activity in urine originates from the luminal brush border of the proximal tubular epithelial cells of the kidney. Thus, urine GGT activity is believed to be a useful measure of proximal renal tubular epithelial cell function.[20-22] There is normally a low activity of GGT in urine as a result of the normal turnover of proximal renal tubular epithelial cells. High urine GGT activity results from an increase in the rate of proximal tubular epithelial cell destruction. There are 2 advantages to this particular indicator of renal damage: it can be used to differentiate ongoing from previous damage to the kidney tubules, because GGT is only released from the proximal renal tubular cells into the urine during the active phase of tissue destruction, and it can be used to detect proximal renal tubular epithelial cell damage before the onset of renal dysfunction. The decrease in mean $U_{GGT}:U_{CR}$ between the neonatal and suckling periods and the weanling period may reflect a decrease in normal tubular epithelial cell turnover as the kidney matures.

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[a]Vita-lac, Tennessee Farmers Cooperative, La Vergne, Tenn.
[b]Pressure osmometer, Wescor, Logan, Utah.
[c]Gemstar II, Electro-nucleonics Inc, Fairfield, NJ.
[d]Proc Univariate, SAS Institute, Cary, NC.

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