

The Sick Adult Horse

Renal Clinical Pathologic Testing and Urinalysis



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KEYWORDS

- Azotemia • Acute kidney injury • Chronic kidney disease • Urine-specific gravity
- Enzymuria • Symmetric dimethylarginine • Furosemide

KEY POINTS

- Clinicopathologic evaluation of kidney disease includes measures of renal function (glomerular filtration rate, tubular modification of filtrate) and biomarkers of renal injury.
- Acute kidney injury (AKI) is usually a secondary disease process and increases morbidity, length of hospital stay, and mortality of the primary disorder. Early AKI identification is essential for initiating therapies aimed at minimizing renal damage and improving outcome.
- Early recognition of chronic kidney disease is important to allow interventions that may slow progression and prolong life for affected equids.
- Examination findings (eg, urine production) and clinicopathologic testing (eg, serum biochemistry, urinalysis) remain useful in assessing renal status in sick adult horses.

TERMINOLOGY FOR ALTERATIONS IN KIDNEY FUNCTION AND KIDNEY INJURY

The terminology applied to renal dysfunction or injury has changed. Use of the term *prerenal failure* to describe acute, reversible decreases in renal blood flow (RBF), glomerular filtration rate (GFR), and urine output (UO), that may or may not progress to clinical renal failure, has fallen out of favor because the term was not well defined and “reversible” changes in renal function were associated with increased mortality.¹ Consequently, the term *acute kidney injury* (AKI) was introduced to increase awareness of renal injury (subclinical and clinical) that accompanies sudden decreases in RBF, GFR, and UO. Over the past 15 years, the definition of AKI has evolved from the RIFLE (*Risk-Injury-Failure-Loss-End-stage*, introduced in 2004)² and *Acute Kidney Injury Network* (AKIN, introduced in 2007)³ staging systems to the *Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline for Acute Kidney Injury* staging system in 2012.⁴ In all 3 systems, the magnitude of increase in serum

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creatinine (Cr) concentration within 48 hours to 7 days is combined with duration and severity of oliguria to categorize AKI from stage 1 to stage 3. Furthermore, KDIGO guidelines provided consensus recommendations for prevention/limitation of AKI in at-risk patients and a conceptual model for AKI, emphasizing that both decreased GFR and direct injury to renal cells combine to incite AKI (Fig. 1).⁴ Another consensus statement from 2017 further refined AKI to separate reversible AKI (Cr returning to baseline within 48 hours) from persisting AKI.⁵ This group also proposed that *acute kidney disease* (AKD) be defined as azotemia persisting for 7 to 90 days after onset of renal injury. When azotemia persists after 90 days, the term *chronic kidney disease* (CKD) is used. This recent statement emphasized that the intermediate phase of AKD is least understood and provided examples of courses of recovery or disease progression.⁵ Using any of these classification systems, AKI has been documented in 7% to 18% of hospitalized human patients, and approximately 50% of patients admitted to intensive care units.⁶ Furthermore, AKI is nearly always a secondary complication in patients with sepsis or cardiac disease, or receiving intravenous contrast agents and concurrent AKI increases patient morbidity and duration of hospitalization. Unfortunately, mortalities with more severe AKI (AKIN or KDIGO stages 2 or 3) in hospitalized patients remain high (~50%) and have declined little over the past 20 years.^{7,8} Thus, early recognition of AKI and interventions to limit progression and reverse kidney damage are much needed.

ACUTE KIDNEY INJURY AND CHRONIC KIDNEY DISEASE IN EQUIDS

Less data exist to document the prevalence of AKI, AKD, CKD, and outcomes in horses. In the authors' hospital, mild azotemia (arbitrarily defined as Cr >2.5 mg/dL at

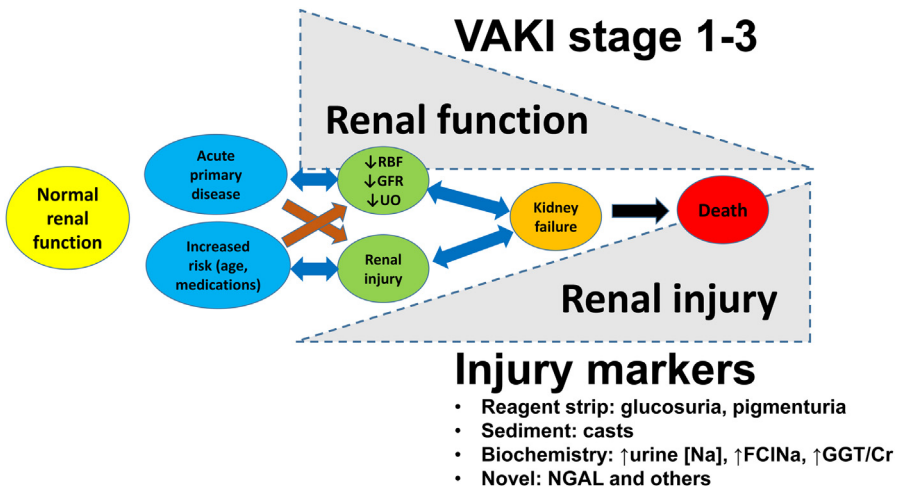


Fig. 1. Conceptual model for AKI: Blue circles represent risk factors for development of AKI, whereas green circles are the 2 primary contributors to AKI; gray triangles indicate the degree of change over time in function and injury, as characterized by the VAKI stage and magnitude of increase in serum and/or urine biomarkers of renal injury. Outcomes can be reversible renal failure or death. FCINa, fractional sodium clearance; GGT/Cr, ratio of urine gamma glutamyl transferase activity to urine creatinine concentration. (Adapted from McCullough PA, Kellum JA, Mehta RL, et al. Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Kidney Int, Suppl* 2012;2:1-138; with permission.)

admission) was detected in ~20% of horses in 1997 and 2000,⁹ translating to an annual incidence of ~5% of the total equine caseload (chemistry profiles were performed in ~20% of cases). As in human patients, AKI was almost always a secondary complication of another disease, usually gastrointestinal (GI) disorders. Horses with moderate azotemia (arbitrarily defined as Cr of 5–10 mg/dL at admission, <1% of caseload) had a mortality of 30% to 45%. However, mortality was 100% for patients with severe azotemia (arbitrarily defined as Cr >10 mg/dL), with the exception of neonates with spurious hypercreatininemia.¹⁰ In another study of 79 horses with GI disease, in which at least 2 Cr measurements were performed 72 hours apart, horses with persisting azotemia (26/79, 33%) were 3 times more likely to die or be euthanized (42% mortality) than horses in which Cr normalized.¹¹ Recently, the Veterinary Acute Kidney Injury (VAKI) staging system (Table 1), initially adapted for dogs¹² from the KDIGO classification, was used to determine prevalence of AKI in hospitalized horses with at least 2 serum Cr measurements (between 24 hours and 7 days of hospitalization).¹³ Of 325 cases (mostly GI emergencies), 4.3% (n = 14) had baseline azotemia (mean Cr 2.4 mg/dL, range 1.9–3.5 mg/dL), which normalized during hospitalization, whereas 14.7% (n = 48) developed AKI (44 VAKI stage 1 and 4 VAKI stage 2) during hospitalization. Mortalities were 29% for horses with baseline azotemia versus 12% for horses developing reversible AKI during hospitalization. During the same time period, 3 horses (<1% of caseload) were admitted for evaluation of renal disease, supporting a low prevalence of primary renal disease (usually CKD). For future investigation of AKI in horses, the timeframe for changes in Cr for AKI staging should be clearly defined (eg, 48 hours to 7 days).

Limited data support a 0.1% to 0.2% prevalence of CKD in horses, which increases to 0.5% in horses older than 15 years of age.¹⁴ These data likely underestimate CKD prevalence because clinicopathologic testing is not routinely performed in healthy older horses. The International Renal Interest Society (IRIS) has developed a CKD staging system to identify dogs and cats in earlier stages of CKD when interventions may be more effective in slowing progression.¹⁵ A similar staging system has not been developed for horses. Unfortunately, many horses have advanced disease (IRIS stage 4 with serum Cr >5.0 mg/dL) when CKD is initially recognized.

ASSESSMENT FOR RENAL DISEASE IN SICK ADULT HORSES

When evaluating adult horses with various medical and surgical disorders, renal function should be considered. Early AKI detection should limit morbidity and improve

Table 1

Proposed veterinary acute kidney injury scoring system for horses, using an increase in serum creatinine concentration from admission value (or baseline value if available) during the initial 7 days of disease process

VAKI Stage	Change in sCr from Baseline
Stage 0	Increase in sCr <150% from baseline
Stage 1	Increase in sCr of 150%–199% or an absolute increase of ≥ 0.3 mg/dL (≥ 26.5 μ mol/L) from baseline
Stage 2	Increase in sCr of 200%–299% from baseline
Stage 3	Increase in sCr of $\geq 300\%$ from baseline or an absolute increase to ≥ 4.0 mg/dL (≥ 354 μ mol/L)

From Savage VL, Marr CM, Bailey M, et al. Prevalence of acute kidney injury in a population of hospitalized horses. *J Vet Intern Med* 2019;330:2294-301; with permission.

outcome in patients with secondary renal disease. If dehydration or compromised hemodynamic status is present, the expected physiologic response would be acute reductions in RBF, GFR, and UO. As an example, when healthy horses were deprived of water for 3 days, UO decreased to 1.25 to 2.5 mL/min (0.15–0.30 mL/kg/h); urine-specific gravity (USG) increased to 1.040 to 1.050, and urine osmolality increased to 1500 to 2000 mOsm/kg during the second and third days of water deprivation.¹⁶ Of interest, UO in these dehydrated horses was less than the accepted definition for oliguria in humans (UO <0.5 mL/kg/h for 6–12 hours or UO <0.3 mL/kg/h for 24 hours).⁵ Finally, a complete history can provide clues as to the duration of decreased renal perfusion and use of potentially nephrotoxic medications. Questioning owners about long-standing excessive drinking and urination habits may provide evidence for pre-existing CKD, especially for patients with weight loss. With hospitalization, observation for urination (and sample collection) when a horse is initially moved into a stall should be considered an essential component of physical examination. Even dehydrated horses may void a small amount of urine when placed into a stall with fresh bedding. Although often overlooked, collection of the first urine sample can provide valuable information and should be done before treatment, because α_2 -agonists or fluid therapy can artifactually decrease urine tonicity.^{17,18} Although not ideal, urine can also be collected from the floor. If urine is not voided spontaneously, a simple collection device can be suspended below the prepuce (Fig. 2) or a bladder catheter can be passed in mares to collect an initial sample.

CLINICOPATHOLOGIC ASSESSMENT FOR RENAL DISEASE

Clinicopathologic testing of kidney function and damage in sick adult horses includes assessment for azotemia, anemia, and inflammation; measurement of blood and urine electrolyte concentrations; urinalysis; and measurement of other urine biomarkers of renal injury.^{19,20} When azotemia is detected, the initial step is to determine whether the patient has AKI, CKD, or an acute exacerbation of CKD (“acute-on-chronic” disease). Unfortunately, iatrogenic, drug-associated nephrotoxicity remains an important contributor to AKI in horses.

Estimates of Glomerular Filtration Rate

Serum/plasma urea nitrogen (UN) and Cr concentrations, as indirect estimates of GFR, are traditionally used to assess renal function. Unfortunately, neither are



Fig. 2. Simple urine collection device for male horses.

sensitive indicators of decreased renal function because values typically do not increase above upper reference interval limits until GFR is reduced by $\geq 75\%$. However, once above the reference interval, small increases become sensitive indicators of further GFR deterioration, with doubling of values indicating a 50% decline in renal function. Because Cr is released as a degradation product of skeletal muscle creatine, breed and sex differences in muscle mass may affect concentrations, as documented in dogs.²¹ The commonly used Jaffe reaction also measures non-Cr chromogens that may account for 45% to 50% of the Cr concentration in dogs.²¹ Finally, a small amount of Cr can be eliminated by tubular secretion; thus, Cr can overestimate GFR.

The UN:Cr ratio can also be used in sick horses. In hypovolemic horses with acute medical and surgical problems, clinical experience finds that Cr increases more rapidly than UN, leading to a UN:Cr ratio less than 10:1. A greater increase in Cr than UN is speculated to be due to greater diffusibility of UN versus Cr. In contrast, with CKD, the UN:Cr ratio often exceeds 10:1 and may exceed 15:1.²² Because a sudden decrease in RBF and GFR typically leads to greater increases in Cr than UN, Cr is the best test to identify acute changes in renal function over short durations in sick horses. With CKD, the UN:Cr ratio may also be useful in assessing dietary protein intake because values greater than 15:1 may suggest excessive dietary protein intake, and increased urea production, or upper GI bleeding.²²

Novel Biomarkers of Glomerular Filtration Rate

To better estimate GFR, novel serum biomarkers have been investigated in horses, including cystatin C (CysC) and symmetric dimethylarginine (SDMA). CysC is a small (13 kDa) cysteine protease inhibitor released by all cells at a fairly constant rate. It is almost exclusively eliminated by glomerular filtration.^{23,24} Thus, as GFR and glomerular filtration of CysC decreases, serum CysC concentration increases. Essentially all CysC is reabsorbed in the proximal tubule, with scant CysC in urine.²³ Increases in serum CysC, and increased urinary CysC excretion, appear to be sensitive indicators of AKI in human patients, but may not be superior to Cr.²⁵⁻²⁷ However, serum CysC yields lower GFR estimates than Cr in CKD, allowing earlier intervention to slow disease progression.^{24,28} Measurement of CysC has been assessed in dogs and cats,²⁴ but initial attempts to measure CysC in equine plasma with a human-based immunoassay were unsuccessful.²⁹

Measurement of SDMA concentration to estimate GFR has gained recent attention, because IDEXX Laboratories now includes this analyte (proprietary enzyme-linked immunosorbent assay [ELISA]) on their equine serum biochemical profile report. SDMA is a stable and continually released end product of protein metabolism within all cells. Similar to inulin, SDMA is freely filtered across glomeruli and neither reabsorbed nor secreted by the tubules.²³ More than 90% of SDMA is excreted in urine, with the remainder degraded by an as yet uncharacterized enzymatic pathway.³⁰ A meta-analysis of 18 human studies showed high correlation between SDMA and other GFR estimates.³¹ Similar to CysC, but unlike Cr, SDMA appears to be minimally affected by muscle mass, breed, age, or sex in humans or small animals.^{23,32} SDMA appears to be most useful as an earlier indicator of CKD in small animals, because serum concentration may increase with loss of only 40% of functional renal mass.³² Furthermore, SDMA increased an average of 10 and 17 months earlier than Cr in dogs and cats, respectively, with spontaneous CKD.^{33,34} Although SDMA concentrations increase in dogs with AKI, there are limited data to support SDMA as a superior measure to Cr in veterinary patients with AKI.³⁵

Recently, Schott and colleagues³⁶ measured serum SDMA concentrations in 165 healthy competition draft horses of different breeds, ages, and sex. They found a

strong correlation ($R = 0.72$, $P < .001$) between liquid chromatography-mass spectrometry and IDEXX ELISA SDMA concentrations. The IDEXX ELISA was further validated by spike and recovery and dilutional parallelism studies. An upper reference limit of 14 $\mu\text{g}/\text{dL}$, as used for dogs and cats, was established for horses, with no apparent age (all animals were >6 months) or sex effects on SDMA concentrations.³⁶ Future evaluation of SDMA, compared with Cr, in horses with AKI and CKD is warranted.

Direct Measurement of Glomerular Filtration Rate

Several clearance techniques have been used experimentally to directly measure GFR in horses.^{37–39} Unfortunately, these tests are cumbersome because they require bolus administration of a compound (classic is inulin) and serial blood sampling (to determine plasma clearance) or continuous rate infusion coupled with timed urine collections. However, determination of endogenous Cr clearance remains a simple and economic method to directly measure GFR. Using a collection device suspended below the prepuce (see Fig. 2) or bladder catheterization in mares, the volume of urine produced over 60 to 180 minutes can be readily determined. Measurement of Cr in a representative aliquot of total urine and a serum sample taken during the urine collection period allows calculation of endogenous Cr clearance:

$$\text{Clearance Cr (GFR, mL / kg / min)} = \left(\frac{[\text{Cr}]_{\text{urine}}}{[\text{Cr}]_{\text{serum}}} \times \text{urine output} \right) / \text{body weight (kg)}$$

Values for GFR in normal horses range from 1.5 to 3.0 mL/kg/min.³⁸

Serum Electrolyte Concentrations

With decreased RBF, GFR, and UO consequent to hypovolemia, electrolyte concentrations should remain normal or increase, whereas hyponatremia and hypochloremia are characteristic findings in horses with AKI leading to acute renal failure.^{40,41} Hyponatremia and hypochloremia are not specific for AKI, because they can be found in horses with GI disorders, for example, colitis. Serum potassium concentrations are variable in AKI, but substantial hyperkalemia (>6 mEq/L) is more common with oliguric to anuric AKI or uroperitoneum. Calcium and phosphate concentrations vary in horses with renal disease. Hypercalcemia and hypophosphatemia are often found in CKD, especially horses fed alfalfa hay, whereas hypocalcemia and hyperphosphatemia may be found with AKI. The combined findings of azotemia and hypercalcemia are essentially pathognomonic for CKD in horses.^{22,42}

Other Serum Chemistry Values

Total protein concentration is often normal in horses with primary renal disease. In horses with AKI secondary to other disorders, total protein concentration is altered more by the primary disease than AKI. Some horses with chronic urinary tract inflammation (eg, pyelonephritis, cystitis) may have high globulin concentrations. With end-stage CKD, intestinal ulceration may result in hypoproteinemia. Mild hypoalbuminemia may develop with protein-losing glomerulopathies, and horses with primary glomerular disease may develop chronic hypoalbuminemia before onset of azotemia.⁴³

Hematology

A minimum database for sick adult horses with suspected renal disease should include a complete blood count. High leukocyte counts and fibrinogen concentrations support an inflammatory process. Mild anemia (packed cell volume $<28\%$) consequent to decreased erythropoietin production and a shortened erythrocyte lifespan

with uremia may be observed in horses with CKD.²² Administration of recombinant human or canine erythropoietin to horses with anemia of CKD is not recommended, because repeated dosing may lead to development of anti-erythropoietin antibodies.⁴⁴

Acid-Base Balance

Venous blood gas analysis in horses with AKI usually reflects the primary disease, rather than the secondary renal insult. In the authors' experience, horses with colic are often mildly alkalotic from pain-induced hyperventilation. With more severe AKI and endotoxemia, a variable degree of lactic acidosis may be present. Horses with primary renal disease may have a mild metabolic acidosis, but acidosis is usually not severe until marked azotemia (Cr >10 mg/dL) develops with oliguric AKI or end-stage CKD.¹⁴

URINALYSIS

Gross Appearance

Color, clarity, odor, and turbidity should be evaluated at the time of collection. Normal equine urine is pale yellow to deep tan and often turbid from calcium carbonate crystals and mucus.¹⁹ Toward the end of micturition or collection, urine may become milky white because of gravitation of crystals in the bladder.

Urine Tonicity

Although determination of USG with a refractometer is quick and easy (reagent strips should not be used to measure USG),²⁰ urine tonicity is more accurately determined by measurement of urine osmolality (U_{osm}). Larger molecules, such as glucose or proteins, can overestimate urine tonicity when assessed by specific gravity. Clinically, this is only a problem in patients with diabetes mellitus or heavy proteinuria, both of which are rare in horses. Use of refractometers with a wide specific gravity scale (1–1.06) is recommended to obviate extrapolating results for more concentrated urine, and the canine scale should be used with refractometers having separate canine and feline scales. The USG or U_{osm} is used to separate urine tonicity into 3 categories: (1) Urine more dilute than serum (hyposthenuria, USG <1.008 and U_{osm} <260 mOsm/kg); (2) Urine and serum of similar osmolality (isosthenuria, USG of 1.008–1.014 and U_{osm} of 260–300 mOsm/kg); and (3) Urine more concentrated than serum (USG >1.014 and U_{osm} >300 mOsm/kg).²⁰ Urine of normal horses consuming dry forage is usually concentrated (2–4 × serum tonicity) with a 1.025 to 1.040 USG and 600 to 1200 mOsm/kg U_{osm} , whereas pastured horses may have more dilute urine from high grass water content. Urine tonicity at hospital admission is an important criterion to determine AKI severity in dehydrated animals. With mild AKI, urinary concentrating ability may be retained with USG greater than 1.020 and U_{osm} greater than 500 mOsm/kg (values can be higher). With more severe AKI, urine concentrating ability is impaired with USG and U_{osm} typically less than 1.020 and 500 mOsm/kg, respectively, in the face of dehydration.⁴⁵

Reagent Strip Analysis

Equine urine is usually alkaline (pH 8–9).⁴⁶ Strenuous exercise, metabolic acidosis, or bacteriuria can result in acidic pH, and urease-producing bacteria can impart a strong ammonia odor. Occasionally, aciduria is detected in anorectic horses with normal blood pH (eg, postoperative ileus with nasogastric reflux or enterocolitis). In these cases, “paradoxical” aciduria likely develops from potassium depletion and increased distal tubular hydrogen excretion (in exchange for potassium reabsorption).⁴⁷

Urine reagent strips can yield false positive results for protein (trace to +) with alkaline samples, especially in concentrated urine.⁴⁸ However, a 2 to 3+ reaction for protein is usually supportive of proteinuria. Proteinuria can be specifically quantified on a chemistry analyzer and is usually less than 100 mg/dL in normal horses, resulting in a urine protein-to-creatinine ratio (UP:UCr) of less than 0.5,⁴⁹ whereas significant proteinuria from glomerulonephritis usually results in a UP:UCr greater than 2. Because proteinuria can accompany bacteriuria, pyuria, and hematuria, or may be found transiently following exercise,⁵⁰ an abnormal UP:UCr result must be interpreted considering these factors.

Normal equine urine should not contain glucose. Although the renal threshold for glucose has not been thoroughly evaluated in horses, early work indicated that it may be lower (160–180 mg/dL) than that of small animals and humans.⁵¹ Glucosuria must always be interpreted with knowledge of serum/plasma glucose concentration. Hyperglycemia-associated glucosuria occurs with physiologic (eg, stress, exercise) and pathologic (eg, sepsis, pituitary pars intermedia dysfunction, diabetes mellitus) conditions or after administration of dextrose-containing fluids, parenteral nutrition, α_2 -agonists, or corticosteroids.¹⁷ When glucosuria is detected without hyperglycemia, proximal tubule dysfunction should be suspected. Glucosuria occurs more often in horses with AKI than CKD. Ketones are rarely detected in equine urine, even with advanced catabolic states or diabetes mellitus.

A positive result for blood on a urine reagent strip does not distinguish between hemoglobin, myoglobin, or intact red blood cells (RBC). Evaluation for hemolytic anemia with intravascular hemolysis (eg, hemoglobinemia), with muscle injury (eg, increased creatine kinase and aspartate aminotransferase activities), and of urine sediment for RBCs (or gross visualization of a centrifuged urine sample) can help differentiate between these pigments. Bilirubin reactions on reagent strips are often false positive reactions (eg, concentrated urine) but can occur with cholestatic liver disease.

Sediment Examination

Sediment examination remains an underutilized tool for evaluating urinary tract disorders in horses. Ideally, sediment should be examined within 30 to 60 minutes after collection. To perform sediment examination, 10 mL of fresh urine is centrifuged (usually in a conical plastic tube) at 300g for 3 to 5 minutes. The supernatant is discarded, and the pellet resuspended in the few drops of remaining urine. A drop of sediment is transferred to a glass slide and cover-slipped. The slide is examined at low magnification to evaluate for casts and crystals and at high magnification to quantify RBC, white blood cells (WBC), and epithelial cells, and to determine if bacteria are present.^{19,20} Granular casts are rare in normal equine urine but can be seen with acute tubular injury. The numbers of granular casts per high-power field (hpf) can help differentiate transient from persistent AKI in people.⁵² Casts are relatively unstable in alkaline urine; thus, sediment should be evaluated as soon as possible after collection. Normal urine contains less than 5 RBC or WBC/hpf and absent to rare bacteria. However, the lack of bacteria on sediment examination does not rule out urinary tract infection, and quantitative bacterial culture should be pursued when infection is suspected (eg, >5 WBC/hpf). Equine urine is rich in crystals, mostly variably sized calcium carbonate crystals, but calcium phosphate and calcium oxalate crystals can also be seen.^{53,54} A few drops of 10% acetic acid solution may be added, if needed, to dissolve crystals if they obscure other sediment constituents.

URINE BIOCHEMICAL TESTS

Urine Electrolyte Clearance

Measurement of urine electrolyte clearances can yield useful information about tubular function/dysfunction with AKI and CKD. Because mammals evolved on a sodium-poor, potassium-rich diet, nephrons are more efficient in reabsorbing filtered sodium as compared with potassium. Horses with normal renal function can reabsorb greater than 99% of filtered sodium but only 85% to 90%, at most, of filtered potassium. Consequently, urine sodium concentration is usually low (<20 mmol/L) in horses fed an all forage diet, unless on salt supplementation, and urine potassium concentration is typically high (100–300 mmol/L).³⁸ With tubular injury and dysfunction, sodium reabsorption decreases and urine sodium concentration increases.⁵⁵ However, an increase in urine sodium concentration is not specific for tubular dysfunction because concentrations also increase with excess dietary salt intake or administration of sodium-rich intravenous or enteral fluids.^{18,38} Thus, it can be challenging to determine whether increases in urine sodium concentration in sick horses are a consequence of AKI and tubular dysfunction or an appropriate response to sodium administration, unless urine assessment is performed before starting treatment.

Fractional electrolyte clearances can be measured to further assess tubular function, specifically, electrolyte resorption.^{56,57} Fractional clearance is defined as the percentage of the filtered electrolyte that is excreted in urine and is calculated by dividing electrolyte clearance by Cr clearance (GFR):

$$\text{Electrolyte clearance (mL/min)} = \frac{[\text{electrolyte}]_{\text{urine}}}{[\text{electrolyte}]_{\text{serum}}} \times \text{urine output}$$

$$\text{Cr clearance (mL/min)} = \frac{[\text{Cr}]_{\text{urine}}}{[\text{Cr}]_{\text{serum}}} \times \text{urine output}$$

which, by rearrangement (canceling out UO) with expression as a percentage, becomes:

$$\text{Fractional electrolyte clearance(\%)} = \frac{[\text{electrolyte}]_{\text{urine}}}{[\text{electrolyte}]_{\text{serum}}} \times \frac{[\text{Cr}]_{\text{serum}}}{[\text{Cr}]_{\text{urine}}} \times 100\%$$

Fractional electrolyte clearances can be determined on a spot urine sample (voided or catheterized), without needing to measure UO with timed urine collection. Reference values for fractional electrolyte clearances in adult horses are provided in **Table 2**. Increased urine sodium concentration and fractional sodium clearance support tubular dysfunction, if measured before administration of sodium-rich solutions. In theory, the magnitude of increase in fractional sodium clearance should be proportional to the degree of tubular injury, although other factors (eg, tubular secretion of Cr, U_{osm}) may also affect calculated values. With AKI, horses with mild injury may continue to reabsorb greater than 99% of filtered

Table 2
Fractional electrolyte clearance (excretion) values for healthy adult horses

Electrolyte	Fractional Clearance, %	References
Sodium	<1	Morris et al, ⁵⁶ 1984; Kohn & Strasser, ⁵⁷ 1986
Chloride	<1.7	Morris et al, ⁵⁶ 1984; Kohn & Strasser, ⁵⁷ 1986
Potassium	24–75	Morris et al, ⁵⁶ 1984; Kohn & Strasser, ⁵⁷ 1986

sodium, and fractional sodium clearance will remain less than 1%, whereas values exceeding 10% may be found in horses with severe AKI.⁴⁵ Although increases in fractional sodium clearance can reflect tubular dysfunction, fractional potassium clearance is of value in assessing potassium balance. When horses are inappetent, fractional potassium clearance decreases, because of increased potassium reabsorption. However, mammalian kidneys do not have the same capacity to retain potassium, as compared with sodium, and ongoing UO results in further obligate losses (and body depletion) of potassium. In the authors' experience, fractional potassium clearance rarely drops to less than 15%, even in inappetent horses. Furthermore, during states of maximal potassium conservation, potassium can be resorbed in exchange with hydrogen in the distal tubule, leading to paradoxical aciduria as a simple proxy measurement supporting total body potassium depletion. With CKD, tubular compensation results in fractional electrolyte clearances remaining near normal values.

Enzymuria

Increased activities of proximal tubular epithelial brush border enzymes, specifically gamma glutamyl transferase (GGT), in urine supports tubular injury in horses (and other species).³⁸ Urine GGT activity is expressed as a ratio to urine Cr (urine GGT/urine Cr) with values >25 U/g considered increased.³⁸ It is a sensitive test, with urine GGT activity increasing 2 to 4 days before serum Cr in horses with gentamicin-induced proximal tubular injury.⁵⁸ Urinary GGT activity measurement is no longer commonly performed, because many clinicians deem the test "too sensitive," because values often increase reversibly in horses being treated with gentamicin for various infections.^{59,60}

Biomarkers of Tubular Injury

There is considerable interest in identifying novel serum and urine markers to detect renal injury, before serum Cr increases or other measures of decreased renal function develop. In humans and small animals, the list of investigated biomarkers is long, but few are commercially available as diagnostic tests.^{23,61–63} Novel biomarkers investigated in horses include neutrophil gelatinase-associated lipocalin (NGAL) and matrix metalloproteases-2 (MMP-2) and -9 (MMP-9).^{29,64} NGAL is a 178-amino-acid protein originally isolated from neutrophils but found in other tissues, including renal tubules. Within hours after kidney injury, NGAL production is upregulated in renal tubules, and serum and urine concentrations rapidly increase.⁶⁵ NGAL appears to be an extremely sensitive marker of renal injury, with serum concentrations increasing in proportion to severity of kidney damage and decreasing in parallel with improvement in renal function after treatment.⁶⁶ Using a commercial porcine-specific NGAL ELISA validated for equine serum, 1 study found concentrations of 7.4 to 103.6 µg/L and 9.8 to 2537 µg/L in normal horses and horses with high serum Cr, respectively.⁶⁴ In 1 small study, serum Cr concentration and urinary MMP-9, but not MMP-2, activity was higher in horses with colic undergoing abdominal surgical exploration versus horses undergoing castration. The investigators postulated that urinary MMP-9 may reflect renal injury.²⁹

Although further work on novel biomarkers of renal function and injury in horses is needed, simple urine reagent strip analysis and measurement of urine enzyme activities remain viable tools for assessing AKI in horses. For example, detection of glucosuria on reagent strip analysis, in the face of normoglycemia, supports proximal tubule injury.

FUROSEMIDE STRESS TEST

In horses with oliguric AKI, intravenous administration of furosemide every 6 hours has been recommended to increase UO. However, anecdotally, this treatment is rarely effective.^{38,67} A continuous rate of infusion of furosemide has been pursued for sustained renal delivery⁶⁸; however, this treatment has not been critically evaluated in horses with AKI. In people, a furosemide stress test was developed to assess the short-term (2 hour) effect on UO. After baseline UO is determined (voiding or bladder catheterization), furosemide (1.0–1.5 mg/kg) is administered intravenously and UO is determined hourly for 2 to 6 hours.^{69,70} In a small cohort of human patients with stage 1 AKI, this test was found to be a strong predictor of the reversibility of AKI (the greater the increase in UO, the more likely AKI was reversible). In fact, the test was a better predictor of AKI progression than several other traditional and novel urine biomarkers.⁶⁸ Evaluation of the furosemide stress test is warranted in horses.

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