UC Davis
UC Davis Previously Published Works

Title

Preliminary evaluation of hepatitis A virus cell receptor 1/kidney injury molecule 1 in healthy horses treated with phenylbutazone

Permalink
https://escholarship.org/uc/item/0nm318rw

Journal
Journal of Veterinary Emergency and Critical Care, 33(4)

ISSN
1534-6935

Authors
Costa, Lais RR
Swiderski, Cyprianna
Palm, Carrie
et al.

Publication Date
2023-07-01

DOI
10.1111/vec.13314

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Received: 14 September 2021 | Revised: 30 May 2022 | Accepted: 2 June 2022

DOI: 10.1111/vec.13314

BRIEF CLINICAL COMMUNICATION

Journal of Veterinary Emergency and Critical Care WILEY

Preliminary evaluation of hepatitis A virus cell receptor 1/kidney injury molecule 1 in healthy horses treated with phenylbutazone

Lais R. R. Costa MedVet, MS, PhD, DACVIM DABVP[1] | Cyprianna Swiderski DVM, PhD, DACVIM[2] | Carrie Palm DVM, MS, DACVIM[1] | Monica Aleman MVZ Cert, PhD, DACVIM[1]

[1]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, Davis, California, USA

[2]Department of Pathobiology and Population Medicine, School of Veterinary Medicine, Mississippi State University, Mississippi State, Mississippi, USA

Correspondence
Monica Aleman, Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, Tupper Hall 2108, One Shields Avenue, Davis, CA 95616, USA.
Email: mraleman@ucdavis.edu

Abstract

Objectives: To investigate if hepatitis A virus cell receptor 1/kidney injury molecule 1 (HAVCR1/KIM1) in urine is detectable concurrently with increases in serum creatinine concentrations in horses receiving a recommended dose of phenylbutazone (PBZ) for 7 days.

Design: Preliminary study.

Methods: Ten clinically healthy horses with normal physical examination and laboratory work were randomly assigned to PBZ or placebo groups (5 each). The PBZ group received PBZ at 4.4 mg/kg mixed with corn syrup orally every 12 hours. The placebo group received corn syrup orally every 12 hours. Both groups were treated for 7 days. Kidney ultrasonography was performed, and venous blood and urine samples were collected prior to commencement and at the end of treatment. Samples from 1 additional healthy horse, 3 horses with acute kidney failure, and 1 horse with chronic kidney failure were also evaluated.

Results: None of the 10 horses had detectable HAVCR1/KIM1 in urine at baseline. Serum creatinine concentrations in placebo group did not increase, and HAVCR1/KIM1 was undetectable in urine. At the end of treatment, 3 of 5 horses receiving PBZ developed increases in serum creatinine of >26.5 μmol/L (>0.3 mg/dL), and HAVCR1/KIM1 was detectable in urine, despite normal findings on kidney ultrasonography in all horses.

Conclusions: HAVCR1/KIM1 is detectable in urine and is associated with increases in serum creatinine concentrations of >26.5 μmol/L in horses following treatment with PBZ for 7 consecutive days. Thus, HAVCR1/KIM1 might aid in the early detection of acute kidney injury in horses.

KEYWORDS
anti-inflammatories, biomarkers, equine, renal

Abbreviations: AKI, acute kidney injury; HAVCR1, hepatitis A virus cell receptor 1; KIM1, kidney injury molecule 1; PBZ, phenylbutazone; USG, urine-specific gravity.

© Veterinary Emergency and Critical Care Society 2023.

1 | INTRODUCTION

Acute kidney injury (AKI) represents a spectrum of kidney damage that ranges from injury without loss of function to fulminant kidney failure, characterized by acute loss of the kidney's excretory function.[1] Kidney dysfunction has been diagnosed by the accumulation of end products of nitrogen metabolism (urea and creatinine), decreased urine output, or a combination of both.[1,2] Biochemical tests commonly used for the detection of kidney disease in veterinary medicine include serum creatinine, BUN, creatinine clearance, fractional excretion of electrolytes, and urinalysis.[2] Although these biomarkers aid in the diagnosis of kidney injury, changes occur late in the disease process, delaying early therapeutic intervention. Serum creatinine concentrations >176.8 μmol/L (2.0 mg/dL) and urine-specific gravity (USG) <1.020 are considered evidence of decreased kidney function, whereas serum creatinine increase ≥26.5 μmol/L (≥0.3 mg/dL) is considered evidence of early kidney injury.[3] Recently, symmetric dimethylarginine and neutrophil gelatinase-associated lipocalin in serum, and *N*-acetyl-*β*-D-glucosaminidase in urine, have been shown to be indicative of kidney injury in horses.[4–6]

Several studies in people and other species have investigated other biomarkers for the early detection of AKI, including kidney injury molecule 1 (KIM1). The gene that encodes KIM1 was recently renamed hepatitis A virus cell receptor 1 (HAVCR1) by the Human Genome Nomenclature Committee (https://www.genenames.org), which is the de facto source for gene nomenclature in animals. HAVCR1 has previously been called T-cell immunoglobulin and mucin domain 1. Under normal conditions, HAVCR1/KIM1 is a type 1 transmembrane glycoprotein minimally expressed in the proximal tubules.[1,7,8] When HAVCR1/KIM1 is expressed, the ectodomain is shed from cells and excreted in urine.[8] Studies in people, monkeys, dogs, and rats have shown that HAVCR1/KIM1 is upregulated in AKI, in postischemic reperfusion injury, and in response to toxins in the proximal tubules.[2,8–11]

Nonsteroidal anti-inflammatory drugs increase the risk of AKI by inhibiting renal vasodilatory prostaglandins, which maintain renal blood flow under conditions of renal hypoperfusion and vasoconstriction.[12,13] Phenylbutazone (PBZ) is a widely used nonsteroidal anti-inflammatory drug in horses, commonly administered in the management of musculoskeletal disorders.[13] Renal papillary necrosis caused by PBZ administration has been documented in horses.[14,15] Although the dosage protocols of PBZ administered to horses vary widely, a commonly used dose of 4.4 mg/kg every 12 hours is often considered to be well tolerated.[13] However, adverse effects have been reported at this dose.[12] Therefore, the goal of this study was to investigate if HAVCR1/KIM-1 was detectable in urine, concurrently with changes in serum creatinine in horses administered PBZ for 7 days at an approved dosage.

2 | METHODS

2.1 | Animals

Five mares and 5 geldings of Quarter Horse and Thoroughbred breeds, all clinically healthy and with ages ranging from 3 to 21 years (mean, 13.8 ± 6.2 years), were included.

Horses were determined to be healthy based upon physical examination and laboratory work consisting of a CBC, serum biochemistry panel, and urinalysis. These horses were housed in a dirt lot and were fed 2% of their body weight as alfalfa and grass hay and had access to water ad libitum.

2.2 | Experimental design

Horses were randomly assigned into 1 of 2 treatment groups of 5 horses each: PBZ or placebo group. Horses in the placebo group received 10 mL of corn syrup orally every 12 hours. Horses treated with PBZ[a] received 4.4 mg/kg of PBZ mixed in corn syrup orally every 12 hours. The treatment period was 7 days for both groups. Physical examinations were performed daily, beginning the day prior to treatment (baseline) and ending the day after termination of the treatment period. After clipping and cleaning the caudodorsal abdomen bilaterally, ultrasound of both kidneys was performed on day 1 prior to (baseline) and 7 days after the last dose of placebo or PBZ was administered. Ultrasonographic findings (morphology, echogenicity, and measurements) were recorded as normal or abnormal. The study protocol was approved by the Institutional Animal Care and Use Committee (UCD IACUC #15140).

2.3 | Clinicopathological evaluation

Venous blood and urine samples were collected at baseline and post-treatment. Venous blood from the jugular vein was placed in vacutainer tubes containing EDTA for a CBC and into tubes with no additive for serum biochemical profile analysis. Detomidine hydrochloride[b] (0.01 mg/kg, IV) was administered for the collection of urine samples via catheterization with a sterile technique. Urine samples were evaluated visually for color and clarity and processed using a commercial urine analyzer[c] for assessment of pH and concentrations of protein, glucose, ketones, bilirubin, and hemoprotein. The urine analyzer used is a highly sensitive dye-binding colorimetric method that changes color under test conditions when complexed with proteins. The increase in absorbance at 600 nm due to the formation of colored complex is directly proportional to the concentration of protein in the reaction. Aliquots of urine from each collection were maintained at −80°C for future HAVCR1/KIM1 analyses. Additional urine samples underwent

centrifugation at 370 × *g* for 6 minutes, and sediment was examined microscopically for epithelial cells, cast, crystals, and bacteria.

2.4 | HAVCR1/KIM1 homology between human and equine

A commercial ELISA[d] was used for the detection of HAVCR1/KIM1 in the urine in accordance with the manufacturer's specifications. The ELISA kit uses goat antihuman HAVCR1/KIM1 polyclonal antibodies. In evaluating the relevance of this kit for identifying equine HAVCR1/KIM1, we initially sought to identify the degree of homology between the respective equine and human proteins. The equine *HAVCR1/KIM1* sequence was not available within the NCBI database (https://www.ncbi.nih.gov/) at the time of this investigation. However, the equine *HAVCR1* gene was identified on chromosome 18 within the Functional Annotation of Animal Genomes (FAANG) database.[e] We aligned the equine, ass, and przewalski genome sequences to the human *HAVCR1/KIM1* gene sequence and identified strong homology across the 3 equid species within the human exons.

2.5 | HAVCR1/KIM1 quantification

In addition to the positive and negative controls included in the kit, urine samples from an additional clinically healthy horse without evidence of kidney disease, 1 horse with chronic renal failure, and 3 horses diagnosed with acute renal failure from our hospital population were tested.

Samples were measured in triplicate. Absorbance at 450 nm with a wavelength correction at 540 nm was measured by use of a spectrophotometer. Concentrations of HAVCR1/KIM1 in samples were determined with computer software using a four-parameter logistic curve derived from known concentrations of recombinant human HAVCR1/KIM1 protein as a standard. The minimum level of detection of HAVCR1/KIM1 for this ELISA kit was 15 pg/mL. The urinary HAVCR1/KIM1 concentrations in 3 horses with confirmed acute kidney failure were 96, 117, and 152 pg/mL, whereas urinary HAVCR1/KIM1 was undetectable in the additional healthy horse and in the 1 horse with chronic kidney failure. This provided presumptive evidence that equine HAVCR1/KIM1 epitopes were recognized by the ELISA polyclonal goat antihuman HAVCR1/KIM1.

2.6 | Data analysis

Incremental creatinine was calculated for each horse by subtracting posttreatment from baseline serum creatinine concentrations. Increases in incremental creatinine ≥26.5 μmol/L (≥0.3 mg/dL) were considered evidence of kidney function loss based on published work from other species and suspected in equids.[2,3] Percent change in creatinine concentration was calculated using the following formula: Posttreatment Cr – Baseline Cr/Baseline Cr × 100.

The effect of treatment groups—placebo versus PBZ treatment—on incremental serum creatinine concentration and urine HAVCR1/KIM1 detection was analyzed using a contingency table and Fisher's exact test. The association between incremental creatinine and HAVCR1/KIM1 was analyzed using logistic regression. Results for HAVCR1/KIM1 were coded as positive if concentrations >56 pg/mL or negative or undetectable if <15 pg/mL (lowest limit of detection). All analyses were performed using commercial software.[f] Statistical significance was set at a *P*-value of <0.05.

3 | RESULTS

All horses were adequately hydrated upon physical examination prior to and at the end of the study. No abnormalities were detected upon ultrasonographic examination of the kidneys at baseline and end of the study. All horses had a serum creatinine ≤176.8 μmol/L (≤2.0 mg/dL), USG > 1.020, and undetectable protein in the urine at baseline (Table 1). At the end of the study, serum creatinine concentrations in all placebo horses were within the reference interval. Three of the 5 horses in the PBZ group had an increase in serum creatinine concentration >26.5 μmol/L (>0.3 mg/dL) from baseline, but only 1 of these horses had a serum creatinine above the reference interval (Table 1). This increase in serum creatinine concentration ranged from 8.8 to 79.6 μmol/L (0.1–0.9 mg/dL), representing an 8.3%–100% increase with a median of 22%. In contrast, after placebo treatment, serum creatinine concentration decreased in 4 horses and increased by 8.8 μmol/L (0.1 mg/dL, 5.8%) in the remaining horse (Table 1). Overall, the increase in serum creatinine concentration was significantly different between the PBZ and placebo groups (Fisher's exact test, $P = 0.05$). After treatment, the USG remained within the reference interval in all horses from the placebo group and in 4 horses in the PBZ group; only 1 horse in the PBZ group developed isosthenuria (Table 1). Two horses from the placebo group and all 5 horses from the PBZ group had detectable protein in the urine at the end of the study (Table 1).

None of the 10 horses had detectable HAVCR1/KIM1 in baseline urine, nor was HAVCR1/KIM1 detectable in urine after placebo treatment. After PBZ treatment in 5 horses, urine HAVCR1/KIM1 was detected (≥56 pg/mL) in the 3 horses whose serum creatinine concentration increased >26.5 μmol/L (>0.3 mg/dL; Tables 1 and 2). Horses 4 and 5 had increases in creatinine of 61.9 and 35.4 μmol/L (0.7 and 0.4 mg/dL), respectively, and an increase in HAVCR1/KIM1 of 56.1 pg/mL. Horse 3 had an increase in creatinine of 79.6 μmol/L (0.9 mg/dL) and an increase in HAVCR1/KIM1 of 104.6 pg/mL. The association between HAVCR1/KIM1 detection and PBZ treatment was significant (Fisher's exact test, $P = 0.05$). Detection of urinary HAVCR1/KIM1 ≥56 pg/mL occurred when the increase in serum creatinine concentration was >26.5 μmol/L (>0.3 mg/dL; Figure 1). There was a strong correlation between HACVR1/KIM1 detection and the increase in serum creatinine concentration (log odds ratio, 16.12). An increase in serum creatinine concentration >26.5 μmol/L (>0.3 m g/dL) was associated with detectable HACVR1/KIM1 at ≥56 pg/mL (240-fold; Figure 1).

TABLE 1 Serum creatinine concentrations, urinary HAVCR1/KIM1, USG, and U-protein.

Group	Animal	Sample	Serum Cr (79.6–176.8 µmol/L)	Serum Cr increase (µmol/L)	Urinary KIM1	USG (1.02–1.05)	U-protein (mg/L)
Placebo	1	Baseline	123.8		–	1.025	0
		Post	70.7	−53.1	–	1.026	0
	2	Baseline	150.3		–	1.030	0
		Post	159.2	8.9	–	1.031	**250**
	5	Baseline	150.3		–	1.038	0
		Post	123.8	−26.5	–	1.041	**250**
	7	Baseline	106.1		–	1.026	0
		Post	88.4	−17.7	–	1.029	0
	8	Baseline	176.8		–	1.040	0
		Post	150.3	−26.5	–	1.041	0
PBZ	6	Baseline	106.1		–	1.025	0
		Post	115.0	8.9	–	1.025	**250**
	3	Baseline	97.3		–	1.028	0
		Post	176.8	**79.5**	+	**1.014**	**250**
	4	Baseline	61.9		–	1.028	0
		Post	123.8	**61.9**	+	1.032	**250**
	9	Baseline	123.8		–	1.029	0
		Post	141.5	17.7	–	1.028	**250**
	10	Baseline	159.2		–	1.025	0
		Post	**194.5**	**35.3**	+	1.025	**750**

Note: The reference interval is presented for serum creatinine concentrations. Results outside the reference interval are shown in bold.
Abbreviations: ↑, increase; –, negative, indicating that HAVCR1/KIM1 <15 pg/mL; +, positive, indicating that HAVCR1/KIM1 ≥56 pg/mL; Cr, creatinine; PBZ, phenylbutazone group; U-protein, urine protein; USG, urine-specific gravity.

TABLE 2 Urinary HAVCR1/KIM1 OD reading in triplicates.

Group	Horse	OD (mean)	OD (SD)	Calculated concentration (pg/dL)
Placebo	1	0.219	0.003	BLD
	2	0.238	0.001	BLD
	5	0.275	0.003	BLD
	7	0.225	0.002	BLD
	8	0.191	0.001	BLD
PBZ	6	0.226	0.003	BLD
	3	0.488	0.030	56.1
	4	0.488	0.030	56.1
	9	0.253	0.011	BLD
	10	0.594	0.019	104.6

Note: The table shows the OD mean and SD and calculated concentration (based on equation generated by standard curve).
Abbreviations: BLD, calculated concentration below level of detection; OD, optical density; PBZ, phenylbutazone group.

4 | DISCUSSION

This study demonstrated that HAVCR1/KIM1 can be detected in horse urine using a commercial ELISA kit originally developed for use in people. Our results demonstrate an increase in serum creatinine concentration of >26.5 µmol/L (>0.3 mg/dL) in 3 horses receiving therapeutic doses of PBZ for a week, although only 1 of these 3 horses had a creatinine increase above the reference interval. Our results

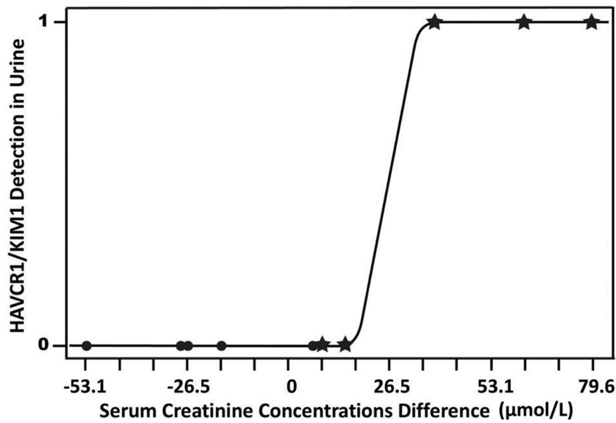

FIGURE 1 Detection of HAVCR1/KIM1 in urine in relation to increase in serum creatinine concentrations (μmol/L) (difference before and after PBZ treatment). Circles represent animals in the placebo group, and stars represent animals in PBZ group. Detection of HAVCR1/KIM1 $\geq$56 pg/mL = 1, and no detection = 0. HAVCR1/KIM1, hepatitis A virus cell receptor 1/kidney injury molecule 1; PBZ, PBZ, phenylbutazone.

suggest that detectable HAVCR1/KIM1 in urine shows promise for early detection of kidney injury. An increase in serum creatinine concentration of >26.5 μmol/L (>0.3 mg/dL) was associated with the detection of HAVCR1/KIM1 in urine and suggested early kidney injury. Furthermore, the USG decreased below the reference interval (isosthenuria) in the horse with the highest increase in serum creatinine (79.6 μmol/L [0.9 mg/dL]) and highest detectable urinary HAVCR1/KIM1 concentration. There were no detectable abnormalities upon renal ultrasonographic examination in horses with increased serum creatinine concentrations, and in the absence of renal histology, confirmation of AKI in this study was not possible. However, if we consider increases in serum creatinine concentration of >26.5 μmol/L (>0.3 mg/dL) as evidence of early kidney injury, urinary HAVCR1/KIM1 deserves further investigation as a potential biomarker of early stages of kidney injury in horses, when serum creatinine concentration is still within the reference interval and no detectable abnormalities are seen on ultrasonograms of the kidneys.

Three horses with acute kidney failure were also evaluated in this study. In these horses, the inducible urinary HAVCR1/KIM1 concentration was higher than that of horses receiving PBZ. In contrast, urinary HAVCR1/KIM1 was not detected in a horse with chronic kidney failure. Although this study includes a small population of horses, our findings are congruent with the information in other species in which upregulation of urinary HAVCR1/KIM1 occurs during AKI.[8,9]

The commercial ELISA assay used in this study has been validated in people, and reference values for healthy individuals have been reported.[16] The equine HAVCR1/KIM1 protein has not been isolated; thus, the human standard curves were used. The concentrations of HAVCR1/KIM1 in urine from horses obtained with the commercial ELISA kit fall within the range given for healthy people and below concentrations reported in people with acute kidney disease.[9] The reasons for the low concentrations obtained in our study might include differences between species and low cross-reactivity of the antibodies between species. Further studies are warranted to investigate if newer commercial ELISA kits used to detect HAVCR1/KIM1 in urine and serum of people will work satisfactorily for the diagnosis of AKI in horses. Considering the amino acid homology between human HAVCR1 and pig and cow is 43% and 50%, respectively, we anticipate at the least a similar homology between the equine and human HAVCR1 proteins that results in limited but conserved epitopes on equine HAVCR1 that were recognized by the goat antihuman HAVCR1 polyclonal antibody in this investigation. This decreased epitope density of equine HAVCR1 would result in a weaker signal than anticipated, with equivalent concentrations of the human protein, and, by extension, antibodies specific to equine HAVCR1 are likely to provide greater HAVCR1 detection sensitivity in urine from horses with renal impairment. Other drugs that might cause AKI, such as flunixin meglumine and aminoglycosides, should also be investigated using HAVCR1/KIM1.[17] Lastly, at the time of our study, the commercial ELISA kit for the detection of HAVCR1/KIM1 was only available for urine. Currently, there are newer ELISA kits for the detection of HAVCR1/KIM1 in human urine and also in serum, and these kits warrant investigation in horses.

Limitations of the study included the small population size available and excluded other common variables often used for the investigation of kidney disease (eg, urine creatinine and gamma-glutamyl transferase concentrations, fractional excretion of electrolytes, and renal biopsy) and the possibility of low cross-reactivity of ELISA for urinary HAVCR1/KIM1 in horses. Furthermore, validation of the use of this commercial kit in horses was not performed. Therefore, caution must be practiced in the interpretation of these results. Another limitation included the lack of daily sampling for serum creatinine concurrently with urinary HAVCR1/KIM1 concentrations to determine if the biomarker under study became detectable before any alterations in creatinine concentrations occurred. Due to gentamicin mechanism of injury at the proximal tubules, detection of urinary HAVCR1/KIM1 in horses warrants investigation. Nonetheless, this protein was detected in urine from horses receiving PBZ, despite a different mechanism of action.

These exploratory data showed that HAVCR1/KIM1 is detectable in horse urine after PBZ treatment and might be a more sensitive biomarker of early stages of kidney injury than a single serum creatinine sample. Further studies are warranted to determine the applicability of the HAVCR1/KIM1 ELISA kit in urine and serum in the clinical setting to confirm early kidney injury in horses.

ORCID

Monica Aleman MVZ Cert, PhD, DACVIM https://orcid.org/0000-0001-5811-9520

ENDNOTES

[a] VET One, Meridian, ID.
[b] Dormosedan, Zoetis Inc., Kalamazoo, MI.
[c] Roche Cobas c311, Roche Diagnostics, Indianapolis, IN.
[d] Human TIM1/KIM1/HAVCR1, R&D Systems, Inc., Minneapolis, MN.
[e] Personal communication from Ted Kalbfleisch (https://gluck.ca.uky.edu).
[f] R statistical software (version 4.0.4).

ACKNOWLEDGMENTS

Funding provided by gifts from anonymous donors #V435804.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

REFERENCES

1. Bellomo R, Kellum JA, Ronco C. Acute kidney injury. *Lancet*. 2012;380:756-766.
2. Schott HC, 2nd, Esser MM. The sick adult horse: renal clinical pathologic testing and urinalysis. *Vet Clin North Am Equine Pract*. 2020;36:121-134.
3. Kopecny L, Palm CA, Skorupski KA, et al. Risk factors associated with progressive increases in serum creatinine concentrations in cats with cancer receiving doxorubicin. *J Vet Intern Med*. 2020;34:2048-2055.
4. Siwinska N, Zak A, Slowikowska M, et al. Serum symmetric dimethylarginine concentration in healthy horses and horses with acute kidney injury. *BMC Vet Res*. 2020;16:396.
5. Bayless RL, Moore AR, Hassel DM, et al. Equine urinary N-acetyl-β-D-glucosaminidase assay validation and correlation with other markers of kidney injury. *J Vet Diagn Invest*. 2019;31:688-695.
6. Jacobsen S, Berg LC, Tvermose E, et al. Validation of an ELISA for detection of neutrophil gelatinase-associated lipocalin (NGAL) in equine serum. *Vet Clin Pathol*. 2018;47:603-607.
7. Siwinska N, Zak A, Paslawska U. Detecting acute kidney injury in horses by measuring the concentration of symmetric dimethylarginine in serum. *Acta Vet Scand*. 2021;63:3.
8. Han WK, Bailly V, Abichandani R, et al. Kidney injury molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. *Kidney Int*. 2002;62:237-244.
9. Chaturvedi S, Farmer T, Kapke GF. Assay validation for KIM-1: human urinary renal dysfunction biomarker. *Int J Biol Sci*. 2009;5:128-134.
10. Waanders F, van Timmeren MM, Stegeman CA, et al. Kidney injury molecule-1 in renal disease. *J Pathol*. 2019;220:7-16.
11. Gu YZ, Vlasakova K, Troth SP, et al. Performance assessment of new urinary translational safety biomarkers of drug-induced renal tubular injury in tenofovir-treated cynomolgus monkeys and beagle dogs. *Toxicol Pathol*. 2018;46:553-563.
12. Fogle C, Davis JL, Yechuri B, et al. Ex vivo COX-1 and COX-2 inhibition in equine blood by phenylbutazone, flunixin meglumine, meloxicam and firocoxib: informing clinical NSAID selection. *Equine Vet Educ*. 2020;33:198-207.
13. MacAllister CG, Morgan SJ, Borne AT, et al. Comparison of adverse effects of phenylbutazone, flunixin meglumine, and ketoprofen in horses. *J Am Vet Med Assoc*. 1993;202:71-77.
14. Gunson DE, Soma LR. Renal papillary necrosis in horses after phenylbutazone and water deprivation. *Vet Pathol*. 1983;20:603-610.
15. Ramirez S, Seahorn TL, Williams J. Renal medullary rim sign in 2 adult quarter horses. *Can Vet J*. 1998;39:647-649.
16. Ichimura T, Bonventre JV, Wei H, et al. Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. *J Biol Chem*. 1998;273:4135-4142.
17. Geor RJ. Acute renal failure in horses. *Vet Clin North Am Equine Pract*. 2007;23:577-591.

How to cite this article: Costa LRR, Swiderski C, Palm C, Aleman M. Preliminary evaluation of hepatitis A virus cell receptor 1/kidney injury molecule 1 in healthy horses treated with phenylbutazone. *J Vet Emerg Crit Care*. 2023;33:481–486. https://doi.org/10.1111/vec.13314