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Short Communication

Evaluation of renal impairment in dogs after envenomation by the common European adder (*Vipera berus berus*)

Mari Palviainen *, Marja Raekallio, Mari Vainionpää, Heini Lahtinen, Outi Vainio

University of Helsinki, Faculty of Veterinary Medicine, Department of Equine and Small Animal Medicine, Pharmacology and Toxicology Section, P.O. Box 57, 00014 University of Helsinki, Finland

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ABSTRACT

Envenomation by the common European adder (*Vipera berus berus*) causes clinical renal injury in dogs. In this study, serum concentrations of albumin, creatinine, total protein and urea were measured in 32 dogs bitten by adders. Urinary creatinine, protein, and retinol binding protein 4 concentrations, and the activities of γ-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP), were measured in 32 affected dogs and 23 healthy controls. Clinical assessment was conducted with a grading scale and a renal function score was applied to classify dogs based on laboratory findings. Urinary protein:creatinine, GGT:creatinine and ALP:creatinine ratios appear to be useful in evaluating renal impairment in dogs with adder envenomation. Increasing kidney function score was correlated with increased urinary ALP:creatinine and GGT:creatinine ratios.

The common European adder (*Vipera berus berus)*, the only venomous snake in Scandinavia, causes intoxications in dogs from April to September (Lervik et al., 2010). Envenomation can produce local and systemic clinical signs in human beings and dogs, including clinical signs referable to the kidneys (Grönlund et al., 2003; Lervik et al., 2010). In human medicine, the Risk-Injury-Failure-Loss-End-stage renal disease (RIFLE) system is used to assess the severity of acute kidney injury (AKI) based on the alteration of serum creatinine concentration from the baseline or measurement of urine output (Hoste et al., 2006). Similar staging systems have been developed for veterinary species (Segev et al., 2008; Thoen and Kerl, 2011).

Urinary enzyme activities are sensitive indicators of renal impairment in dogs (Clemo, 1998). Retinol binding protein 4 (RBP4) is also a potential marker of kidney injury (Smets et al., 2010; Palviainen et al., 2012). In this study, the impact of envenomation by the common European adder on the kidneys in dogs was assessed by measuring serum albumin, creatinine and urea, together with urine protein:creatinine and RBP4:creatinine ratios.

The study included 32 dogs (2–13 years of age, weight 3.1–40.4 kg) bitten by the common European adder (inclusion criteria included clinical evidence of a bite and a history of the owner seeing the dog being bitten or seeing a viper close to the dog) and treated at the Veterinary Teaching Hospital of the University of Helsinki from 2007 to 2009 (see Appendix A: Supplementary Table 1). Twenty-three privately owned dogs (1–13 years of age, weight 3.6–42.5 kg) without a history of renal disease served as controls; urine samples only were collected from these dogs.

The clinical assessment of severity of envenomation was conducted according to the scale of Audebert et al. (1992), with slight modifications (Petite (2005)) (see Appendix A: Supplementary Table 2). The impact of envenomation on renal function was assessed with a three-step grading scale based on laboratory findings (see Appendix A: Supplementary Table 3). Single-void urine samples were collected from all dogs and blood samples were collected into plain tubes (Vacuette, Greiner Bio-One/Mekalasi Oy) from affected dogs; the time between collection of the sample and receipt at the laboratory was variable. Serum was harvested after centrifugation (1300 *g* for 10 min at room temperature) and serum concentrations of albumin, creatinine, total protein and urea, together with urinary creatinine, total protein concentrations and activities of γ-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP), were measured by using a clinical chemistry analyser (Konelab 30i, Thermo Fisher). The remainder of the urine was centrifuged (1300 *g* for 10 min at 4 °C) and stored at −80 °C until analysed. RBP4 was measured from 28 bitten dogs and 18 control dogs using DetectX Urinary RBP immunoassay kit (Arbor Assays).

Statistical analyses were performed using PASW Statistic 18 (IBM SPSS). The normality of each parameter was evaluated with the Shapiro–Wilk test. Logarithmic transformation of urinary protein concentration and activities of creatinine, GGT and ALP was performed before statistical analysis. Back-transformed values (means and confidence intervals) are presented. The Mann–Whitney *U* test was used to compare non-parametric data. Pearson correlation was used for normally distributed data and Spearman

* Corresponding author. Tel.: +358 504155315.
E-mail address: mari.palviainen@helsinki.fi (M. Palviainen).

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Table 1
Urinary creatinine ratios of measured variables in dogs bitten by the common European adder and in healthy controls.

	Creatinine (mg/dL)[a]	U-GGT:creatinine ratio[a]	U-ALP:creatinine ratio[a]	U-Protein:creatinine ratio[a]	U-RBP4:creatinine ratio[b]
Control	175.5 (34.2–393.5)	0.003 (0.0007–0.0068)	0.001 (0.0004–0.0032)	0.001 (0.0002–0.0022)	0.0028 (0.00–0.4)
Case	27.3 (6.3–62.7)	0.007 (0.002–0.017)	0.01 (0.003–0.024)	0.009 (0.003–0.023)	0.0018 (0.00–0.03)
P	<0.001	<0.001	<0.001	<0.001	0.522

GGT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; RBP4, retinol binding protein 4.
[a] Geometric mean (95% confidence interval).
[b] Median (minimum and maximum).

rank correlation for non-parametric data. Significance was set at $P < 0.05$.

Most (20/32; 62.5%) of the affected dogs had received treatment, mainly consisting of fluid therapy and opioid analgesics, from the referring veterinarians, but specific treatment details were not recorded. Serum total protein concentration (mean ± standard deviation) in the affected dogs was 49.0 ± 11.5 g/L, serum albumin was 28.4 ± 6.7 g/L, serum creatinine was 116.3 ± 114.9 μmol/L and serum urea was 9.1 ± 8.6 mmol/L. Urinary GGT, ALP, protein and RBP4 ratios to creatinine are summarised in Table 1 (see Appendix A: Supplementary Tables 4 and 5).

Affected dogs were scored clinically as having minor (grade 1: 4/32), moderate (grade 2: 19/32) and severe (grade 3: 9/32) envenomation. This differs from human beings, who exhibit milder symptoms (Grönlund et al., 2003; Petite, 2005). This can be explained in part because dogs are more likely to be referred to the University of Helsinki Veterinary Teaching Hospital if severely envenomated and therefore such cases may be overrepresented. On routine clinical biochemistry, the impact of envenomation on the kidneys was assessed as no specific findings (grade 1: 12/32, 37.5%), mild deterioration (grade 2: 15/32, 43.0%) and severe deterioration (grade 3: 5/32, 15.6%) of kidney function.

The urinary GGT:creatinine and ALP:creatinine ratios were significantly higher in dogs with adder bites than in unaffected dogs. Urinary RBP4:creatinine ratios did not differ significantly between groups, suggesting that this assay is not useful for evaluating kidney function in dogs bitten by the European common adder.

Both previous scoring systems for AKI in dogs (Segev et al., 2008; Thoen and Kerl, 2011) have disadvantages. The scoring system developed for dogs in haemodialysis by Segev et al. (2008) consists of multiple laboratory variables, which can restrict its use due to the lack of available laboratory analyses in veterinary clinics. The VAKI system (Thoen and Kerl, 2011) is based solely on changes in serum creatinine concentration from the baseline sample. However, a dog bitten by an adder may already have elevated serum creatinine concentration at the time of hospitalisation. We evaluated canine renal function with a three-step grading scale based on serum albumin, urea and creatinine concentrations, and the urine protein:creatinine ratio. The clinical assessment of severity of envenomation was correlated with altered total protein, GGT:creatinine and ALP:creatinine ratios, suggesting that severe envenomation may cause renal impairment (see Appendix A: Supplementary Tables 4 and 5).

In conclusion, urinary total protein, GGT:creatinine ratio and ALP:creatinine ratio can be used to detect renal impairment after adder bites in dogs. Urinary RBP4 did not appear to be useful for assessing renal impairment in dogs bitten by adders. Our scoring system for kidney function warrants further investigation in dogs with kidney impairment due other causes.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tvjl.2013.09.008.

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