Original Article

Assessment of acute kidney injury in canine parvovirus infection: Comparison of kidney injury biomarkers with routine renal functional parameters

M.F. van den Berg [a*], J.P. Schoeman [b], P. Defauw [a], Z. Whitehead [b], A. Breemersch [a], K. Goethals [c], S. Daminet [a, 1], E. Meyer [d, 1]

[a] *Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium*
[b] *Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa*
c Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[d] *Department of Pharmacology, Biochemistry and Toxicology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium*

* Corresponding author. Tel.: +31 61509 8194.
E-mail address: MaritFrederieke.vandenBerg@UGent.be (M.F. van den Berg)

[1] These authors shared senior co-authorship and contributed equally to this research

Highlights

• Dogs with parvoviral infection may be at risk of developing acute kidney injury (AKI).

• Conventional renal functional parameters did not detect AKI, except for urinary protein:creatinine ratio.

• Novel urinary kidney injury biomarkers detected possible AKI in dogs with parvoviral enteritis.

Abstract

Dogs with naturally occurring canine parvovirus (CPV) infection are at risk of developing acute kidney injury (AKI) due to several factors, including severe dehydration, hypotension and sepsis. Serum creatinine (sCr) and serum urea are insensitive markers for the assessment of early kidney injury. Therefore, the aim of this study was to investigate potential kidney injury in dogs with CPV infection using both routine renal functional parameters and several kidney injury biomarkers.

Twenty-two dogs with CPV infection were prospectively enrolled and compared with eight clinically healthy control dogs. Urinary immunoglobulin G (uIgG) and C-reactive protein (uCRP) were measured to document glomerular injury, whereas urinary retinol-binding protein (uRBP) and neutrophil gelatinase-associated lipocalin (uNGAL) served as markers for tubular injury. These biomarkers were compared to routine renal functional parameters, including sCr, serum urea, urinary protein:creatinine ratio (UPC) and urine specific gravity (USG). Dogs with CPV infection had significantly higher concentrations of uIgG, uCRP, uRBP and uNGAL compared to healthy dogs. In contrast, sCr was significantly lower in dogs with CPV infection compared to controls, while serum urea was not significantly different. UPC and USG were both significantly higher in CPV-infected dogs. This study demonstrated that dogs with CPV infection had evidence of AKI, which remained undetected by the routine functional markers sCr and serum urea, but was revealed by UPC, uIgG, uCRP, uRBP and uNGAL. These results emphasize the added value of novel urinary kidney injury biomarkers to detect canine patients at risk of developing AKI.

Keywords: Acute kidney injury; Canine; Parvovirus; Urinary biomarkers.

Introduction

Canine parvovirus type 2 (CPV-2) infection is an important cause of morbidity and mortality in young dogs worldwide. Although dogs of any age may be affected, puppies between 6 weeks and 6 months of age appear to be most susceptible (Prittie, 2004; Goddard and Leisewitz, 2010; Sykes, 2014; Mylonakis et al., 2016). Acute enteritis is the most common manifestation of the disease. Typical clinical signs include anorexia, lethargy, fever, vomiting and small bowel diarrhea, often resulting in dehydration and hypovolemic shock (Prittie, 2004; Goddard and Leisewitz, 2010; Nandi and Kumar, 2010; Sykes, 2014; Mylonakis et al., 2016). Intestinal mucosal barrier disruption increases the risk of bacterial translocation, and subsequent septicemia and endotoxemia, which may result in a systemic inflammatory response syndrome that can progress to multiple organ dysfunction, septic shock and, ultimately, death (Turk et al., 1990; Otto et al., 1997; Prittie, 2004; Kalli et al., 2010, Schoeman et al., 2013, Mylonakis et al., 2016). Several factors, including severe dehydration, hypotension, systemic inflammatory response syndrome and sepsis, put dogs with naturally occurring CPV infection at risk of developing acute kidney injury (AKI).

Current conventional renal parameters, such as serum creatinine (sCr) and serum urea, are insensitive and non-specific markers for early renal damage, because they are only altered when more than 75% of renal mass has been lost (Finco, 1997; Cobrin et al., 2013; De Loor et al., 2013). International Renal Interest Society (IRIS) AKI Grade I represents an early and mild kidney injury with sCr still within the reference range [1], making early identification of AKI challenging with current diagnostic methods. Evaluation of urinary biomarkers, ranging from proteins of low to high molecular weight (MW), has shown to be a promising strategy, not only for detecting the presence and severity of kidney injury, but also for identifying a

[1] See: International Renal Interest Society http://www.iris-kidney.com (accessed 8 August 2018).

pre-injury phase in which the kidneys are under acute stress, which can lead to AKI (Cobrin et al., 2013; De Loor et al., 2013; Kovarikova, 2015; Hokamp and Nabity, 2016; Katz and Ronco, 2016; Ronco, 2016). Immunoglobulin G (IgG, MW 150 kDa), which is involved in the humoral immune system, and C-reactive protein (CRP, MW 115 kDa), an important acute-phase protein, are relatively high MW proteins that are normally not filtered through the glomerular filtration barrier. Detection of urinary IgG (uIgG) and urinary CRP (uCRP) is associated with glomerular damage, as previously reported in multiple studies (D'Amico and Bazzi, 2003; Maddens et al., 2010; Defauw et al., 2012; Nabity et al., 2012; Hrovat et al., 2013). On the contrary, retinol binding protein (RBP) is a protein of low MW (21 kDa), which is freely filtered through the glomerulus and reabsorbed by the proximal tubules under physiological circumstances (Raila, 2010). Its detection in urine reflects tubular damage and has been described in various kidney diseases and naturally occurring AKI (Smets et al., 2010; Defauw et al., 2012; Nabity et al., 2012; Cobrin et al., 2013; De Loor et al., 2013; Hrovat et al., 2013; Hokamp and Nabity, 2016). Neutrophil gelatinase-associated lipocalin (NGAL, MW 25 kDa) is a neutrophil-derived protein that is normally expressed at low concentrations at the level of the kidney, but markedly increases with renal tubular cell injury at an early stage (Cowland and Borregaard, 1997; Mori and Nakao, 2007; Schmidt et al., 2007; Kuwabara et al., 2009; Devarajan, 2010). Plasma NGAL (pNGAL) and urinary NGAL (uNGAL) are used as early markers of AKI in human medicine (Mishra et al., 2005; Mori and Nakao, 2007; Nickolas et al., 2008; Devarajan, 2010, Ennulat and Adler, 2015). Recently, multiple studies have shown the usefulness of -predominantly urinary- NGAL as a sensitive biomarker for detection of AKI in dogs (Lee et al., 2012; Kai et al., 2013; Segev et al., 2013; Steinbach et al., 2014).

Both in human and veterinary medicine, there is a striking lack of studies on the presence of AKI in pediatric patients suffering from acute enteritis, even though these risk factors for AKI are present and even though it is one of the most common diseases in young children and dogs worldwide (Goddard and Leisewitz, 2010; Sykes, 2014; GBD 2013 Mortality and Causes of Death Collaborators, 2015). Early detection of (subclinical) kidney injury and high-risk conditions, that place the kidneys under acute stress, would allow for the identification of patients requiring more intensive treatment and might prevent the development of, still reversible, AKI into more severe stages. Therefore, the main aim of this study was to identify possible incidence of acute kidney injury in dogs with naturally occurring CPV infection using biomarkers for glomerular injury (uCRP, uIgG) and tubular injury (uRBP, uNGAL) compared to routine renal functional parameters, including sCr, serum urea, urinary protein:creatinine ratio (UPC) and urine specific gravity (USG).

Materials and methods

Animals

This prospective study was approved by the Animal Ethics Committee of the University of Pretoria (V092-15, date of approval: 28th September 2015 and 22nd November 2016). Client-owned dogs of various breeds, age and weight presented to the University of Pretoria's Onderstepoort Veterinary Academic Hospital (OVAH) between February 2016 and November 2016 and diagnosed with CPV infection were prospectively enrolled. Dogs between 6 weeks and 9 months of age were included if they demonstrated clinical signs that warranted hospitalization. A preliminary diagnosis of CPV infection was made based on compatible clinical signs (e.g. diarrhea, lethargy, anorexia, vomiting, hypovolemia) and a positive faecal CPV immunoassay (IDEXX Canine Parvovirus Antigen Test kit, IDEXX Laboratories Inc. or Antigen Rapid CPV Ag test Kit, BioNote Inc.). This diagnosis was

confirmed by detection of viral particles in faecal samples using electron microscopy. Electron microscopy was carried out according to standard operating procedures at the Department of Anatomy and Physiology, Faculty of Veterinary Sciences, University of Pretoria using a Philips CM10 transmission electron microscope (Philips Electron Optical Division). Dogs previously treated for CPV infection or dogs that received any recent medication possibly influencing renal functional parameters or kidney injury biomarkers (e.g. antibiotics, glucocorticoids, non-steroidal anti-inflammatory drugs) were excluded. The presence of concurrent systemic diseases was excluded through detailed anamnesis, physical examination, routine haematology (including evaluation of a blood smear to identify *Babesia* spp. and *Ehrlichia* spp.), serum biochemistry and urinalysis including sediment evaluation and aerobic bacterial culture.

Clinically healthy control dogs of comparable age and body weight that presented to the University of Pretoria's OVAH for routine vaccination, sterilization or castration were also prospectively enrolled. They were considered healthy based on unremarkable history and physical examination and the absence of relevant abnormalities on routine haematology, serum biochemistry and urinalysis, including aerobic bacterial culture, UPC (UPC<0.5) and sediment evaluation.

Sample handling and submission

At presentation (T_0), routine haematology, serum biochemistry assays and urinalysis were performed in all dogs (CPV group and control group), whereas 24 h after presentation (T_1), blood and urine samples were collected in the CPV group only. On presentation to the hospital and after collection of samples (T_0), CPV-infected dogs received appropriate symptomatic treatment, which included crystalloid fluid therapy, treatment with antibiotics

(ampicillin 20 mg/kg IV three times daily and metronidazole 15 mg/kg IV two times daily), analgetics (buprenorphine 20 µg/kg IV two to three times daily or a constant rate infusion fentanyl 3 µg/kg/h IV), anti-emetics (maropitant 1 mg/kg SC once daily for up to five consecutive days) and anthelmintics (fenbendazole 50 mg/kg PO once daily for five days), and potassium or glucose supplementation as required. Blood samples were collected for routine haematology and serum biochemistry (including sCr, serum urea, glucose, alanine aminotransferase (ALT), alkaline phosphatase (ALP), total serum protein (TSP), albumin, globulin, electrolytes and total bilirubin) (Cobas-Integra 400 Plus, Roche Diagnostics). Complete blood counts were performed using an automated analyzer (Advia 2120, Siemens Healthineers). All urine samples were collected by cystocentesis. Urinalysis included visual evaluation (clarity, colour), evaluation of USG with a refractometer, routine urine sediment analysis, routine dipstick analysis (Combur 9 Test®, Roche Diagnostics), measurement of UPC and urinary creatinine (uCr) and aerobic bacterial culture. After collection, urine and blood samples were centrifuged (447 g, 5 min). Remaining urine and plasma was stored in several aliquots at -80°C. Frozen samples were transported on dry ice to Ghent University, Faculty of Veterinary Medicine, and were stored at -72°C until analysis. All analyses of urinary markers were performed within eight months of sample collection, except three samples that required reanalysis with another dilution which was performed within 10 months of sample collection.

Evaluation of kidney injury biomarkers

Measurement of uIgG, uCRP, uRBP, uNGAL and pNGAL was performed using commercial canine- or human-specific ELISA kits (Canine IgG ELISA, Canine CRP ELISA, Human RBP ELISA (Immunology Consultants Laboratory), Dog NGAL ELISA (Bioporto Diagnostics)) as previously validated in dogs (Maddens et al., 2010, Steinbach et al., 2014).

Briefly, for each immunoassay, colorimetric measurements were performed at a wavelength of 450 nm using an ELISA plate reader (Multiskan MS, Labsystems Thermo Fisher Scientific). A four-parameter logistic curve fitting program (Deltasoft JV, Biometallics Inc.) was used to generate the standard curve and calculate concentrations of uIgG, uCRP, uRBP, uNGAL and pNGAL. Concentrations of uIgG, uCRP, uRBP and uNGAL were normalized to uCr and expressed as ratios.

Statistical analysis

Data analysis was performed using statistical software program SAS 9.4 (SAS Institute Inc.). Data were assessed for normality of distribution using the Shapiro-Wilk test. To compare results between the CPV-infected dogs and the control group, the non-parametric Mann–Whitney *U*-test was used. The same variables at the time of presentation and at 24 h post-presentation were compared using the non-parametric Wilcoxon signed-rank test for paired samples. Values were considered statistically significant at $P < 0.05$.

Results

Study population

Twenty-six dogs with naturally occurring CPV infection were initially enrolled, and four dogs were later excluded. In two dogs, urine could not be obtained and in two dogs an asymptomatic urinary tract infection was diagnosed. Seventeen control dogs were initially recruited, of which nine were excluded. One had bacterial growth in the urine, one was diagnosed with babesiosis, in one dog parvovirus particles were detected by faecal electron microscopy, and six dogs had an increased UPC (UPC≥0.5). Data of the control dogs and CPV-infected dogs at T_0 and T_1 are presented in Table 1.

Table 1. Haematology, biochemistry, routine serum and urinary renal functional parameters and kidney injury biomarkers for control dogs and canine parvovirus (CPV) infected dogs on presentation (T_0) and for CPV-infected dogs 24 h after presentation (T_1). Values are expressed as median (range).

	Control dogs	Parvovirus (T_0)	*P*	Parvovirus (T_1)	P-value	Reference range
Number of dogs	8	22		22		
Age (months)	5.5 (1.5-7)	4.5 (2-8)	0.49			
Bodyweight (kg)	9.4 (4.9-27.7)	12 (5.1-22.9)	0.96			
Sex	2 M, 6 F	12 M, 10 F				
Haematology						
Packed cell volume (L/L)	0.46 (0.32-0.48)	0.44 (0.27-0.59)	0.85	0.34 (0.18-0.45) [b]	<0.0001	0.37-0.55
Leukocyte count (x10^9/L)	11.44 (7.27-24.43)	4.925 (0.43-22.66) [a]	0.02	3.09 (0.49-14.57) [b]	0.0025	6.0-15
Segmented neutrophils (x 10^9/L)	6.885 (3.56-17.1)	3.45 (0.1-21.3)	0.07	0.65 (0.03-10.64) [b]	<0.0001	3-11.5
Band neutrophils (x10^9/L)	0 (0-0.12)	0.02 (0-1.43)	0.05	0 (0-0.51) [b]	0.008	0-0.5
Lymphocytes (x10^9/L)	3.48 (1.24-5.42)	0.65 (0.28-4.16) [a]	0.0002	1.335 (0.38-3.77) [b]	<0.0001	1-4.8
Monocytes (x10^9/L)	0.8 (0.22-1.52)	0.14 (0.03-1.2) [a]	0.007	0.13 (0.02-0.87) [b]	0.04	0.15-1.35
Eosinophils (x10^9/L)	0.265 (0-0.49)	0.01 (0-0.32) [a]	0.004	0.045 (0-0.38)	0.32	0.1-1.25
Platelet count (x10^9/L)	353.5 (226-457)	320.5 (141-539)	0.98	321 (139-589)	0.66	200-500
Biochemistry						
TSP (g/L)	50.9 (39.2-60.6)	52.75 (42.3-63)	0.50			56-73
Serum albumin (g/L)	35.8 (26.8-43.5)	30 (22.2-43.9) [a]	0.006			28-41
Serum globulin (g/L)	14.8 (12.4-17.8)	20.95 (14.8-31.6) [a]	0.0003			20-41
Glucose (mmol/L)	5.25 (4.4-5.8)	8.75 (6.8-11.7) [a]	0.002			3.3-5.5
ALT (U/L)	30 (17-68)	51.5 (15-267)	0.13			9.0-73
ALP (U/L)	87.5 (21-272)	198.5 (33-313)	0.09			20-165
Sodium (mmol/L)	147 (144-149)	141 (132-145) [a]	0.0002			142-151
Potassium (mmol/L)	4.85 (4.3-5.44)	4.265 (3.67-5.48) [a]	0.008			3.6-5.1
Renal functional parameters						
sCr (µmol/L)	53 (47-78)	34 (<18-97) [a]	0.0025	28.5 (<18-94)	0.053	59-109
Serum urea (mmol/L)	4.9 (3.6-5.4)	5.1 (3.9-15.7)	0.21			2.3-8.9
UPC	0.33 (0.13-0.46)	0.925 (0.15-5.46) [a]	0.0006	0.62 (0.12-1.25) [b]	0.0083	
USG	1.037 (1.021-1.052)	1.055 (1.023->1.060) [a]	0.0041	1.027 (1.013->1.060) [b]	<0.0001	
Kidney injury biomarkers						
uIgG/uCr (mg/g)	2.755 (0.878-13.51)	8.170 (1.597-444.0) [a]	0.0061	4.072 (1.086-24.36) [b]	0.0006	
uCRP/uCr (mg/g)	BDL	0.0484 (BDL-1.066) [a]	<0.0001	0.0321 (BDL-0.4188) [b]	0.043	

uRBP/uCr (mg/g)	0.00438 (BDL-0.0246)	0.0546 (BQL-0.3019) [a]	0.0003	0.0453 (BDL-0.2024)	0.35
uNGAL/uCr (µg/g)	1.314 (0.1841-2.487)	9.613 (0.1340-200.6) [a]	0.014	9.603 (0.0959-357.8)	0.35
pNGAL (µg/L)	6.702 (2.080-21.58)	7.118 (2.575-30.98)	0.90	4.699 (1.654-15.89) [b]	0.0049

M, male intact; F, female intact; TSP, total serum protein; ALT, alanine aminotransferase; ALP, alkaline phosphatase; sCr, serum creatinine; UPC, urinary protein:creatinine ratio; USG, urine specific gravity; uIgG/uCr, urinary immunoglobulin G to creatinine ratio; uCRP/uCr, urinary C-reactive protein to creatinine ratio; uRBP/uCr, urinary retinol binding protein to creatinine ratio; uNGAL/uCr, urinary neutrophil gelatinase-associated lipocalin to creatinine ratio; pNGAL, plasma neutrophil gelatinase-associated lipocalin; BDL, below detection limit; BQL, below quantification limit.

[a] Statistically significant difference ($P < 0.05$) in CPV-infected dogs compared to control dogs at T_0

[b] Statistically significant difference ($P < 0.05$) in CPV-infected dogs between T_0 and T_1

Median age and body weight in the CPV group did not differ significantly from that of control dogs (*P*=0.49 and *P*=0.96, respectively). In the CPV-infected group, four dogs were mixed breed, while pure-bred dogs included six Boerboels, three Rottweilers, two Malinois Belgian shepherds, two Siberian huskies, two American pitbull terriers, and one each of Jack Russell terrier, Bull terrier and Staffordshire bull terrier. The control group consisted of two mixed breeds, two Jack Russell terriers, and one each of Boerboel, American pitbull terrier, Labrador retriever and German shepherd.

In CPV-infected dogs, urine colour at T_0 and T_1 varied from light yellow to dark yellow. Urine dipstick analysis demonstrated hematuria (range 1+ to 4+) in nine dogs out of 22 dogs at T_0 and in eight out of 22 dogs at T_1 (range 1+ to 2+). Median pH was 6.0 (5.0-8.5) at T_0 and 8.0 (5.5-9.0) at T_1. Urinary glucose, ketone and urobilinogen measurements were negative both at T_0 and T_1. On sediment evaluation, 0-1 granular casts per low power field were seen in six CPV-infected dogs at both T_0 and T_1. Hematuria (>5 red blood cells (RBCs) per high power field (HPF)) was present in four out of 22 dogs at T_0 (median 0, range 0-16 RBCs/HPF) and in one out of 22 dogs at T_1 (median 0, range 0-6 RBCs/HPF). In control dogs, hematuria (2+ and 4+) was present in two dogs and in one dog on sediment evaluation (median 0, range 0-75 RBCs/HPF). Urinary casts were absent in the control group.

Routine serum and urinary renal markers

Median sCr was below reference range in all groups, and was significantly lower in CPV-infected dogs compared to control dogs (*P*=0.0025). Serum urea concentrations did not differ significantly from that of control dogs (*P*=0.21). Significantly higher UPC values were found in dogs with CPV infection compared to control dogs (*P*=0.0006; Figure 1F). UPC values in CPV-infected dogs decreased significantly between T_0 and T_1 (*P*=0.0083; Figure

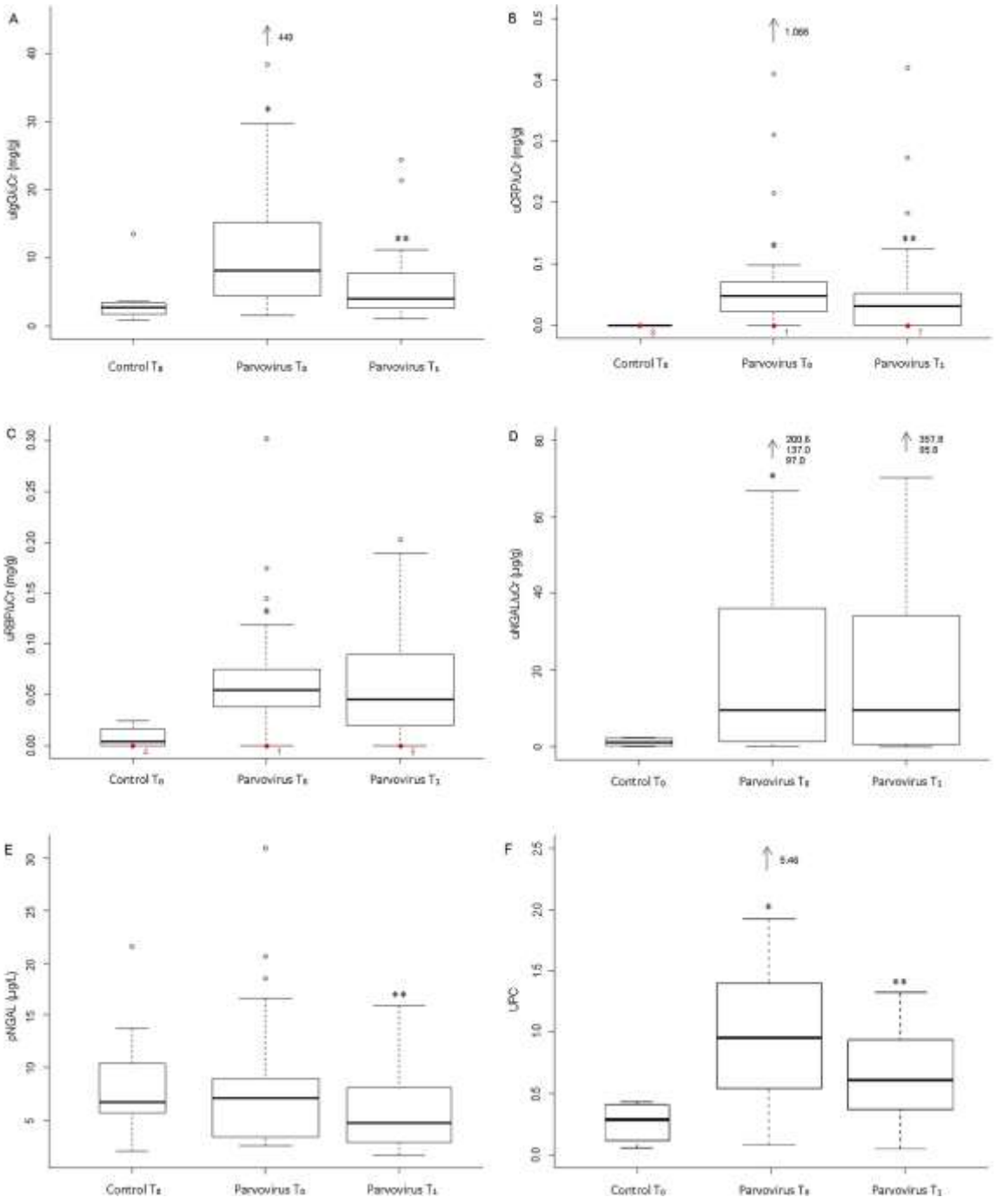

Fig. 1. Comparison of (A) urinary Immunoglobulin G to creatinine ratio (uIgG/uCr); (B) urinary C-reactive protein to creatinine ratio (uCRP/uCr); (C) urinary retinol binding protein to creatinine ratio (uRBP/uCr); (D) urinary neutrophil gelatinase-associated lipocalin to creatinine ratio (uNGAL/uCr); (E) plasma neutrophil gelatinase-associated lipocalin (pNGAL); and (F) urinary protein to creatinine (UPC) ratio between healthy controls and dogs with canine parvovirus (CPV) infection at T_0 and T_1. The box represents the inter quartile range (i.e. the second and third quartiles). The line within the box represents the median. The whiskers represent the range, extending to a maximum of 1.5-times the interquartile range. Outliers are represented by circles above

the whiskers and values next to the arrows. Values that were below detection and quantification limits are represented as diamonds.

* Statistically significant difference ($P < 0.05$) compared to controls at T_0

** Statistically significant difference ($P < 0.05$) in CPV-infected dogs between T_0 and T_1

1F). USG values were significantly higher in CPV-infected dogs compared to control dogs (P=0.0041), and decreased significantly between T_0 and T_1 (P<0.0001).

Biomarkers of kidney injury

Results of uIgG/uCr, uCRP/uCr, uRBP/uCr, uNGAL/uCr and pNGAL are presented in Figure 1 (A-E). Dogs with CPV infection had significantly higher concentrations of uIgG (P=0.0061), uCRP (P<0.0001), uRBP (P=0.0003), and uNGAL (P=0.014) at presentation, whereas there was no significant difference for pNGAL compared to healthy dogs (P=0.90). Between T_0 and T_1, a significant decrease in uIgG (P=0.0006), uCRP (P=0.043) and pNGAL (P=0.0049) was noted in the CPV-infected dogs, while uRBP (P=0.35) and uNGAL (P=0.35) did not differ significantly.

Discussion

This study demonstrated a significantly higher concentration of the four urinary biomarkers uIgG, uCRP, uRBP and uNGAL in dogs with CPV infection compared to healthy control dogs, indicating AKI both at the glomerular and tubular level. This AKI remained undetected by routine renal functional parameters, except for UPC.

Serum urea values in CPV-infected dogs were within the normal reference range and did not differ significantly from healthy controls, whereas sCr concentrations were significantly lower in CPV-infected dogs. USG, which is considered to be a more sensitive indicator of renal damage than renal azotemia (Bagshaw and Gibney, 2008; Hrovat et al., 2013), also failed to detect kidney injury, because values were significantly higher in CPV-infected dogs compared to control dogs, indicating normal urine concentrating ability. Granular casts, possibly indicative of renal tubular injury (Bagshaw and Gibney, 2008), were

only present in small amounts in the urine of a minority of dogs with CPV infection. Thus, of the routine functional renal parameters, only UPC had the capacity to detect AKI. In the majority of cases, proteinuria was mild (UPC<2.0 in 21 out of 22 dogs), so that a distinction between glomerular and tubular proteinuria could not reliably be made based on UPC. However, the proteinuria was most likely of renal origin, because there were no signs of prerenal causes (no significant haemo-/myoglobinuria), nor of postrenal causes (inactive sediment).

Increases in uIgG and uCRP are a reflection of glomerular damage and have been described in different types of kidney diseases (D'Amico and Bazzi, 2003; Maddens et al., 2010; Defauw et al., 2012; Nabity et al., 2012; Hrovat et al., 2013; Hokamp and Nabity, 2016). Elevation of these two markers in the CPV-infected dogs therefore indicates glomerular injury. Increases in uRBP and uNGAL in these dogs reflect tubular injury and have been reported in various kidney diseases and naturally occurring AKI (Smets et al., 2010; Defauw et al., 2012; Nabity et al., 2012; Cobrin et al., 2013; De Loor et al., 2013; Hrovat et al., 2013; Hokamp and Nabity, 2016). In accordance with a previous study in dogs (Lee et al., 2012), pNGAL seems to be less sensitive than uNGAL, because significant differences between CPV-infected dogs and controls were only found for uNGAL. Because of its low MW, increased uNGAL can indicate proximal tubular damage that hinders reabsorption. However, the main source of this increase is considered to be an upregulated NGAL synthesis in the distal tubules in response to injury (Mishra et al., 2003; Mishra et al., 2005; Mori and Nakao, 2007; Schmidt et al., 2007; Devarajan, 2010; Ennulat and Adler, 2015). Multiple studies have shown a rapid increase in renal NGAL mRNA and protein levels in human AKI and animal models for AKI, often preceding a rise in sCr concentrations by several days (Mishra et al., 2003; Mishra et al., 2005; Devarajan, 2010; Lee et al., 2012).

Thus, uNGAL proves to be a very early, highly sensitive and specific indicator for kidney injury. On the contrary, an increase in pNGAL can be explained by multiple mechanisms that are not specific for damage of renal origin. Indeed, NGAL is expressed by several tissues, including the gastrointestinal tract, liver and respiratory tract, where it is produced in response to inflammation, tissue injury or sepsis (Cowland and Borregaard, 1997; Schmidt et al., 2007; Grigoryev et al., 2008; Devarajan 2010). During AKI, increases in circulating NGAL may be derived from extrarenal sources, but can also be caused by a decreased renal clearance of NGAL and the release of NGAL from inflammatory cells during the acute phase response (Schmidt et al., 2007; Grigoryev et al., 2008; Devarajan, 2010, Ennulat and Adler, 2014).

Interestingly, sCr concentrations were significantly lower in CPV-infected dogs compared to control dogs and sCr was below the reference range in both groups in our study. Over the past decades, multiple studies have reported serum biochemical abnormalities in dogs with CPV infection, documenting discrepant findings for sCr, with values that were both higher and lower compared to controls, and values both below and above reference range (Jacobs et al., 1980; Yilmaz and Senturk, 2007; Kalli et al., 2010; Bastan et al., 2013; Sykes, 2014). Serum creatinine can be influenced by several non-renal factors, such as age, body weight and muscle mass (Braun et al., 2003). In our study, body and muscle condition score were not assessed. Also, haemodynamic changes occurring in the course of CPV infection might influence creatinine concentrations. Schetters et al. (2009) evaluated haematological changes in dogs with babesiosis and hypothesized that hypotension-induced volume expansion occurred in these dogs, which resulted in lower sCr. This decrease in these dogs was correlated with a reduction of packed cell volume (PCV), suggesting an increase in plasma volume (Schetters et al., 2009). However, in our study no significant difference in PCV between CPV-infected dogs and controls was found. An increased glomerular filtration

rate (GFR) could theoretically also explain the observed lower sCr concentrations. However, because CPV infection is characterized by severe dehydration, hypotension and sepsis, and thus typically results in a reduced GFR, an increased GFR is an unlikely explanation for this finding. A decrease in creatinine generation rate has been shown to occur in animal models of sepsis and critically ill humans, and is a more likely explanation for the lower sCr concentrations in CPV-infected dogs (Prowle et al, 2014).

A significant decrease in uCRP, uRBP and pNGAL between T_0 and T_1 in the CPV-infected dogs was observed. A possible explanation could be the administration of intravenous fluid therapy to these dogs after T_0. Although the urinary biomarkers were normalized to urinary creatinine concentration to account for variations in urine flow rate, excluding a direct influence of fluid therapy, the latter might have slowed progression of AKI into more severe stages. Moreover, as administration of parenteral fluids will result in increased glomerular perfusion and tubular flow, sCr and USG were expected to decrease significantly between T_0 and T_1.

A recent histopathological study that examined kidneys from two dogs that were experimentally infected with CPV demonstrated renal tubular epithelial degeneration and necrosis and glomerular endothelial and mesenchymal cell proliferation (Zhao et al., 2013). Although it is ideal to correlate histopathological changes to urinary biomarkers, renal biopsies were not performed in our client-owned sick dogs. In addition, the lack of long-term follow-up is a limitation of this study, and future studies that assess long-term follow-up and correlation to clinical outcome are warranted. Human research has shown that AKI is associated with a high risk of long-term renal outcomes in both adults and children, and that a single episode of AKI can cause permanent long-term kidney damage (Coca et al., 2009;

Greenberg et al., 2014). This emphasizes the need for early detection of possible kidney injury and the importance of subsequent follow-up in canine patients.

Conclusions

We demonstrated evidence of acute kidney injury both at the glomerular and tubular level in dogs with naturally occurring CPV infection. This injury remained undetected by the routine renal functional markers sCr and serum urea. Our results emphasize the added value of novel urinary kidney injury biomarkers to detect canine patients at risk of developing AKI.

Conflict of interest statement

None of the authors of this paper have a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

The authors would like to acknowledge Kristel Demeyere, Carien Muller, Sr. Marizelle DeClerque and the staff in the OVAH Clinical Pathology laboratory for their technical assistance, as well as Lizette du Plessis and Antoinette Buys for performing the electron microscopy. Preliminary results were presented as an Oral Abstract at the 27th Congress of the European College of Veterinary Internal Medicine - Companion Animals, Malta, 14-16 September 2017.

References

Bagshaw, S.M., Gibney, R.T.N., 2008. Conventional markers of kidney function. Critical Care Medicine 36, S152-S158

Bastan, I., Kurtdede, A., Özen, D., 2013. Prognostic usefulness of some parameters in dogs with canine parvovirus. Ankara Üniversitesi Veteriner Fakültesi Dergisi 60, 53-58.

Braun, J.P., Lefebvre, H.P., Watson, A.D.J., 2003. Creatinine in the dog: a review. Veterinary Clinical Pathology 32, 162-179.

Cobrin, A.R., Blois, S.L., Kruth, S.A., Abrams-Ogg, A.C., Dewey, C., 2013. Biomarkers in the assessment of acute and chronic kidney diseases in the dog and cat. Journal of Small Animal Practice 54, 647–655

Coca, S.G., Yusuf, B., Shlipak, M.G., Garg, A.X., Parikh, C.R., 2009. Long-term risk of mortality and other adverse outcomes after acute kidney injury: a systematic review and meta-analysis. American Journal of Kidney Diseases 53, 961-973.

Cowland, J.B., Borregaard, N., 1997. Molecular characterization and pattern of tissue expression of the gene neutrophil gelatinase-associated lipocalin from humans. Genomics 45, 17–23

D'Amico, G., Bazzi, C., 2003. Pathophysiology of proteinuria. Kidney International 63, 809–825.

Defauw, P., Schoeman, J.P., Smets, P., Goddard, A., Meyer, E., Liebenberg, C., Daminet, S., 2012. Assessment of renal dysfunction using urinary markers in canine babesiosis caused by Babesia rossi. Veterinary Parasitology, 190, 326–332.

De Loor, J., Daminet, S., Smets, P., Maddens, B., Meyer, E., 2013. Urinary biomarkers for acute kidney injury in dogs. Journal of Veterinary Internal Medicine, 27, 998-1010.

Devarajan, P., 2010. Review: Neutrophil gelatinase-associated lipocalin: A troponin-like biomarker for human acute kidney injury. Nephrology 15, 419–428.

DiBartola, S.P., Chew, D.J., Jacobs, G., 1980. Quantitative urinalysis including 24-hour protein excretion in the dog. Journal of the American Animal Hospital Association 16, 537–547.

Ennulat, D., Adler, S., 2015. Recent successes in the identification, development, and qualification of translational biomarkers: the next generation of kidney injury biomarkers. Toxicologic Pathology 43, 62-69.

Finco, D.R., 1997. Kidney function. In: Clinical Biochemistry of Domestic Animals, fifth edn. cademic Press, San Diego, CA, USA, pp. 441–484.

GBD 2013 Mortality and Causes of Death Collaborators, 2015. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 385, 117-171.

Goddard, A., Leisewitz, A.L., 2010. Canine parvovirus. Veterinary Clinics of North America: Small Animal Practice 40: 1041–1053.

Greenberg, J.H., Coca, S.G., Parikh, C.R., 2014. Long-term risk of chronic kidney disease and mortality in children after acute kidney injury: a systematic review. BMC Nephrology 15:184.

Grigoryev, D.N., Liu, M., Hassoun, H.T., Cheadle, C., Barnes, K.C., Rabb, H., 2008. The local and systemic inflammatory transcriptome after acute kidney injury. Journal of the American Society of Nephrology 19, 547–58.

Hokamp, J.A., Nabity, M.B., 2016. Renal biomarkers in domestic species. Veterinary Clinical Pathology 45, 28-56.

Hrovat, A., Schoeman, J.P., de Laat, B., Meyer, E., Smets, P, Goddard, A., Nagel, S., Daminet, S., 2013. Evaluation of snake envenomation-induced renal dysfunction in dogs using early urinary biomarkers of nephrotoxicity. The Veterinary Journal 198, 239–244.

Jacobs, R.M., Weiser, M.G., Hall, R.L., Kowalski, J.J., 1980. Clinicopathologic features of canine parvoviral enteritis. Journal of the American Animal Hospital Association 16, 809-814.

Kai, K., Yamaguchi, T., Yoshimatsu, Y., Kinoshita, J., Teranishi, M., Takasaki, W., 2013. Neutrophil gelatinase-associated lipocalin, a sensitive urinary biomarker of acute kidney injury in dogs receiving gentamicin. The Journal of Toxicological Sciences 38, 269–277.

Kalli, I., Leontides, L.S., Mylonakis, M.E., Adamama-Moraitou, K., Rallis, T., Koutinas, A.F., 2010. Factors affecting the occurrence, duration of hospitalization and final outcome in canine parvovirus infection. Research in Veterinary Science 89, 174–178.

Katz, N., Ronco, C., 2016. Acute kidney stress - a useful term based on evolution in the understanding of acute kidney injury. Critical Care 20:23.

Kovarikova, S., 2015. Urinary biomarkers of renal function in dogs and cats: a review. Veterinarni Medicina 60, 589–602.

Kuwabara, T., Mori, K., Mukoyama, M., Kasahara, M., Yokoi, H., Saito, Y., Yoshiokai, T., Ogawa, Y., Imamaki, H., Kusakabe, T., et al., 2009. Urinary neutrophil gelatinase-associated lipocalin levels reflect damage to glomeruli, proximal tubules, and distal nephrons. Kidney International 75, 285-294.

Lee, Y.J., Hu, Y.Y., Lin, Y.S., Chang, C.T., Lin, F.Y., Wong, M.L., Kuo-Hsuan, H., Hsu, W.L., 2012. Urine neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute canine kidney injury. BMC Veterinary Research 8, 248–256.

Maddens, B.E., Daminet, S., Demeyere, K., Demon, D., Smets, P., Meyer, E., 2010. Validation of immunoassays for the candidate renal markers C-reactive protein, immunoglobulin G, thromboxane B2 and retinol binding protein in canine urine. Veterinary Immunology and Immunopathology 134, 259–264.

Mishra, J., Ma, Q., Prada, A., Mitsnefes, M., Zahedi, K., Yang, J., Barasch, J., Devarajan, P., 2003. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. Journal of the American Society of Nephrology 14, 2534-2543.

Mishra, J., Dent, C., Tarabishi, R., Mitsnefes, M.M., Ma, Q., Kelly, C., Ruff, S.M., Zahedi, K., Shao, M., Bean, J., et al., 2005. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 365, 1231–1238.

Mori, K., Nakao, K., 2007. Neutrophil gelatinase-associated lipocalin as the real-time indicator of active kidney damage. Kidney International 71, 967-970.

Mylonakis, M.E., Kalli, I., Rallis, T.S., 2016. Canine parvoviral enteritis: an update on the clinical diagnosis, treatment, and prevention. Veterinary Medicine: Research and Reports 7, 91–100.

Nabity, M.B., Lees, G.E., Cianciolo, R., Boggess, M.M., Steiner, J.M., Suchodolski, J.S., 2012. Urinary Biomarkers of Renal Disease in Dogs with X-Linked Hereditary Nephropathy. Journal of Veterinary Internal Medicine 26, 282-293.

Nandi, S., Kumar, M., 2010. Canine parvovirus: current perspective. Indian Journal of Virology 21, 31-44.

Nickolas, T.L., O'Rourke, M.J., Yang, J., Sise, M.E., Canetta, P.A., Barasch, N., Buchen, C., Khan, F., Mori, K., Giglio, J., et al., 2008. Sensitivity and specificity of a single emergency department measurement of urinary neutrophil gelatinase-associated lipocalin for diagnosing acute kidney injury. Annals of Internal Medicine 148, 810–819.

Otto, C.M., Drobatz, K.J., Soter , C., 1997. Endotoxemia and tumor necrosis factor activity in dogs with naturally occurring parvoviral enteritis. Journal of Veterinary Internal Medicine 11, 65–70.

Prittie, J., 2004. Canine parvoviral enteritis: a review of diagnosis, management, and prevention. Journal of Veterinary Emergency and Critical Care 14, 167-176.

Prowle, J.R., Kolic, I., Purdell-Lewis, J., Taylor, R., Pearse, R.M., Kirwan, C., 2014. Serum creatinine changes associated with critical illness and detection of persistent renal dysfunction after AKI. Clinical Journal of the American Society of Nephrology 9, 1015-1023.

Raila, J., Brunnberg, L., Schweigert, F.J., Kohn, B., 2010. Influence of kidney function on urinary excretion of albumin and retinol-binding protein in dogs with naturally occurring renal disease. American Journal of Veterinary Research 71, 1387-1394.

Ronco, C., 2016. Acute kidney injury: from clinical to molecular diagnosis. Critical Care, 20:201.

Schetters, T.P., Kleuskens, J.A.G.M., Van De Crommert, J., De Leeuw, P.W.J., Finizio, A.L., Gorenflot, A., 2009. Systemic inflammatory responses in dogs experimentally infected with Babesia canis; a haematological study. Veterinary Parasitology 162, 7-15.

Schmidt-Ott, K.M., Mori, K., Li, J.Y., Kalandadze, A., Cohen, D.J., Devarajan, P., Barasch, J., 2007. Dual action of neutrophil gelatinase-associated lipocalin. Journal of the American Society of Nephrology 18, 407–413.

Schoeman, J.P., Goddard, A., Leisewitz, A.L., 2013. Biomarkers in canine parvovirus enteritis. The New Zealand Veterinary Journal 61, 217-222.

Segev, G., Palm, C., LeRoy, B., Cowgill, L.D., Westropp, J.L., 2013. Evaluation of neutrophil gelatinase-associated lipocalin as a marker of kidney injury in dogs. Journal of Veterinary Internal Medicine 27, 1362–1367.

Smets, P.M.Y., Meyer, E., Maddens, B.E.J., Duchateau, L., Daminet, S., 2010. Urinary markers in healthy young and aged dogs and dogs with chronic kidney disease. Journal of Veterinary Internal Medicine 24, 65-72.

Steinbach, S., Weis, J., Schweighauser, A., Francey, T., Neiger, R., 2014. Plasma and urine neutrophil gelatinase-associated lipocalin (NGAL) in dogs with acute kidney injury or chronic kidney disease. Journal of Veterinary Internal Medicine 28, 264–269.

Sykes, J.E., 2014. Canine parvovirus infections and other viral enteritides. In: Canine and Feline Infectious Diseases, 1st edn. Elsevier, St. Louis, MO, USA, pp. 141–151.

Turk, J., Miller, M., Brown, T., Fales, W., Fischer, J., Gosser, H., Nelson, S., Shaw, D., Solorzano, R., 1990. Coliform septicemia and pulmonary disease associated with canine parvoviral enteritis: 88 cases (1987–1988). Journal of the American Veterinary Medical Association 196, 771–773

Yilmaz, Z., Senturk, S., 2007. Characterization of lipid profiles in dogs with parvoviral enteritis. Journal of Small Animal Practice 48, 643-650.

Zhao, Y., Lin, Y., Zeng, X., Lu, C., Hou, J., 2013. Genotyping and pathobiologic characterization of canine parvovirus circulating in Nanjing, China. Virology Journal 10:272.