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Changes in Serum Creatinine Concentration and Acute Kidney Injury (AKI) Grade in Dogs Treated with Hydroxyethyl Starch 130/0.4 From 2013 to 2015

N.E. Sigrist, N. Kälin, and A. Dreyfus

Background: Hydroxyethyl starch (HES) solutions may cause acute kidney injury (AKI) in humans.

Objective: To compare AKI grades in 94 dogs exposed and 90 dogs that were unexposed to 6% HES-130/0.4.

Animals: Dogs receiving 6% HES-130/0.4 (HES cohort) or crystalloids (unexposed cohort) between 2013 and 2015.

Methods: Historical cohort study. Diagnosis, total cumulative dose and total mL/kg of HES administered, time frame of HES administration and serum creatinine concentrations up to 90 days after initiation of HES treatment were retrospectively reviewed. The AKI grades were retrospectively determined by IRIS guidelines.

Results: Exposed dogs received a median cumulative dose of 69.4 mL/kg (range, 2–429 mL/kg) HES over a median of 4 (range, 1–16) days, resulting in a median dose of 20.7 (range, 2–87) mL/kg/d. Although the cohorts differed in terms of age and diagnosis, AKI grades were not significantly different at the evaluated short- and long-term time points. Results of ordinal logistic regression identified the number of days of HES administration as significantly associated with an increase in AKI grade within 10 days (P = .038), whereas there was no significant association among HES exposure, HES mL/kg/d, and an increase in AKI grade.

Conclusions and Clinical Importance: HES-130/0.4-treated dogs were not more prone to develop AKI than HES-untreated, but the number of HES days was significantly associated with an increase in AKI grade within 10 days post-HES administration. The time frame of HES treatment should be kept short. Prospective, randomized clinical trials are required to assess the effect of HES on renal function in dogs.

Key words: Acute kidney injury; Canine; HES; Renal damage.

Hydroxyethyl starch (HES) is an artificial colloid solution widely used for resuscitation in veterinary patients. Hydroxyethyl starch increases colloid osmotic pressure,[1,2] systolic blood pressure[2,3,a] , and blood volume.[3,4] Indications for colloid use therefore include hypovolemia, hypotension, perioperative fluid therapy, low colloid osmotic pressure, or hypoalbuminemia.[5,6]

Recently, the safety of HES in dogs has been questioned because several large trials in human patients have shown that HES administration increased the need for renal replacement therapy[7–9] or increased mortality[9], specifically in patients with sepsis.[7,9,10] Based on available data, the Pharmacovigilance Risk Assessment Committee (PRAC) of the European Medicine Agency (EMA) subsequently limited the indications for HES treatment in human patients. Since October 2013, the administration of HES solutions has been restricted to hypovolemic shock in trauma patients and is contraindicated in septic and critically ill human patients as well as burn victims in the European Union (PRAC statement[b]).

In veterinary medicine, the adverse effects of HES administration on platelet function and coagulation are well documented in dogs.[11–13] Studies investigating potential adverse renal effects of HES solutions, however, are rare in dogs. Conflicting results were found regarding the incidence of acute kidney injury (AKI) after HES administration in hospitalized dogs receiving HES solutions.[14,15] The incidence of AKI, defined as a doubling of serum creatinine concentration during hospitalization, was increased in dogs receiving a pentastarch solution (10% HES-250/0.5/5:1).[14] In Europe, pentastarches mostly have been replaced by tetrastarches. The only study evaluating dogs receiving 6%

Abbreviations:

AKI	acute kidney injury
EMA	European Medicine Agency
HES	hydroxyethyl starch
ICD	International Statistical Classification of Diseases and Related Health Problems
ICU	intensive care unit
IRIS	International Renal Interest Society
Lrm	multivariable logistic regression model
Olrm	ordinal logistic regression model
PRAC	Pharmacovigilance Risk Assessment Committee of the European Medicine Agency
pRBC	packed red blood cells

From the Department of Small Animals, Vetsuisse Faculty, University of Zürich, Zürich, Switzerland (Sigrist, Kälin); and the Section of Epidemiology, Vetsuisse Faculty, University of Zürich, Zürich, Switzerland (Dreyfus).

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Corresponding author: Dr. med. vet. N. Sigrist, Department of Small Animals, Vetsuisse Faculty, University of Zürich, Winterthurerstr. 258c, 8057 Zürich, Switzerland; e-mail: nsigrist@vetclinics.uzh.ch

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HES-130/0.4 found no significant increase in serum creatinine concentrations.[15] Studies investigating adverse renal effects, and specifically the incidence of AKI after HES administration, in veterinary patients therefore are needed.

Considering the lack of alternative options, HES solutions remain an important choice of fluid therapy in selected cases. The goal of our historical cohort study was to determine whether administration of the currently available 6% HES-130/0.4 is associated with an increase in serum creatinine concentration and AKI grade as defined by International Renal Interest Society (IRIS) guidelines[c] (Table 1) in dogs. We distinguished between an immediate HES effect (last AKI grade measured within 10 days) and a long-term effect (last AKI grade measured within 11–90 days).

Material and Methods

The dog database of the Small Animal University Hospital of Zürich was retrospectively searched for billing of HES between January 2013 and November 2015. Dogs were eligible to enter the "exposed" cohort if they received ≥1 mL/kg HES 130/0.4[d] between January 2013 and November 2015 and had a pretreatment serum creatinine concentration measured as well as at least 1 additional serum creatinine concentration determined between 2 and 20 days post-HES administration.

The unexposed cohort consisted of all dogs hospitalized in the ICU between January 2014 and November 2015 that received IV isotonic crystalloid fluids, and which had at least 2 serum creatinine concentrations measured, which were at least 2 days apart but determined within 20 days. Dogs that received any other synthetic colloid were excluded.

Data retrieved from the medical records and compiled in a spreadsheet were breed, sex, age, and weight for all patients. The database and patient records were further searched for the number of mL of HES administered per day, the total amount of HES administered, the number of days of HES administration, the final diagnosis, blood product administration, other colloid administration, duration of hospital stay, date of discharge, and any available additional information regarding renal function including urinalysis results, urine output, and ultrasonographic findings. The cumulative amount of HES per kg body weight (mL/kg) administered and the corresponding mL/kg/d were calculated for each patient. The final diagnosis found in the patient record was further classified into 1 of the 21 groups of the International Statistical Classification of Diseases and Related Health Problems (ICD) system.[e] If >1 diagnosis was available in 1 patient, the diagnosis most likely associated with the primary presenting problem (e.g., abdominal for a dog presenting with vomiting caused by an intestinal foreign body and concurrent mitral insufficiency and hyperadrenocorticism) was used for the classification. For statistical analysis, the ICD classification was further narrowed into the following groups: gastrointestinal (ICD XI, including parvovirosis from ICD I), urogenital (ICD XIV), trauma (ICD XIX), and others (all other ICD classifications including neoplasia, infectious disease, respiratory, neurologic, and endocrinologic problems).

The database also was searched for blood analysis results, and results for serum creatinine and albumin concentration were recorded. Day 0 concentration for all blood variables was defined as the last result that was available before HES administration. Serum creatinine concentration then was recorded each available day until day 90 and serum albumin concentration until day 7.

The AKI grade (AKI 1-AKI 5) was determined according to the recently published IRIS guidelines for grading AKI (Table 1). Patients without clinical or laboratory signs of AKI and a serum creatinine concentration <140 μmol/L (1.6 mg/dL) and an increase in serum creatinine concentration of <26.4 μmol/L (0.3 mg/dL) within 48 hours were defined as AKI 0. All available information including serial serum creatinine concentrations before the start of HES administration, urinalysis, and diagnostic imaging findings was used for determination of AKI grade.

The last serum creatinine concentration and AKI grade, which was determined during the time period of day 2–10 after the start of HES or crystalloid administration, was used to define the short-term AKI grade increase. The last serum creatinine concentration and AKI grade determined during the time period of day 11–90 after the start of HES or crystalloid administration were used to define the long-term AKI grade increase.

Statistical Analysis

Data were entered into a spreadsheet and double-checked by 2 of the authors. Descriptive statistical analysis was performed by the statistical software program SPSS.[f] Normality was tested for continuous data by the Shapiro-Wilk test. Normally distributed data are presented as mean ± standard deviation (SD), whereas non-normally distributed data are presented as median and range (minimum–maximum). Categorical data are described as frequencies. Fisher's exact test or chi-square test was used for association of categorical variables, whereas a Mann-Whitney *U*-test was used for continuous variables. The primary outcomes were development

Table 1. Modified acute kidney injury (AKI) grading as defined by IRIS.

AKI grade	AKI description	Serum creatinine level	Creatinine change	History, physical examination
0	No AKI	<140 μmol/L (<1.6 mg/dL)	< 26.4 μmol/L (<0.3 mg/dL)	No clinical, laboratory or diagnostic imaging signs of AKI
1	Nonazotemic AKI	<140 μmol/L (<1.6 mg/dL)	>26.4 μmol/L (>0.3 mg/dL) increase within 48 hour	Any clinical, laboratory or diagnostic imaging sign of AKI, oliguria, or anuria >6 hour
2	Mild AKI	141–220 μmol/L (1.7–2.5 mg/dL)	>26.4 μmol/L (>0.3 mg/dL) within 48 hour	Documented AKI, static or progressive azotemia, oliguria, or anuria >6 hour
3	Moderate	221–439 μmol/L (2.6–5.0 mg/dL)		Documented AKI
4	to severe AKI	440–880 μmol/L (5.1–10.0 mg/dL)		
5		>880 μmol/L (>10 mg/dL)		

AKI, acute kidney injury.

of an AKI grade ≥1 ("new AKI short-term") and an increase in AKI grade ("AKI increase short-term") within 10 days of HES administration. A secondary outcome was an increase in AKI grade within 11–90 days of HES administration ("AKI increase long term").

The primary exposure or risk factor was HES administration, (a) as a categorical variable (yes/no), (b) cumulative amount of HES per kg body weight (mL/kg), (c) mL/kg/d, or (d) number of days HES was given. Other potential exposures or risks or confounding factors were whether natural colloids were given (yes/no), whole blood transfusions administered (yes/no), the diagnosis of the patient (ICD categories were collapsed into 4 categories), age (continuous), sex (4 categories: intact or castrated males or females, Table 2). The tested null hypotheses were that (1) HES administration was not associated with development of a short-term AKI grade ≥1, and HES administration was not associated with a (2) short-term or (3) long-term increase of AKI grade in dogs hospitalized in our intensive care unit, when adjusting for potential confounding factors. The exposure variables were tested for a univariable association with the short- and long-term outcomes by a chi-squared or Fisher's exact test, logistic regression analysis, or both. Exposure variables with $P \leq .3$ and potential confounding variables were included in multivariable regression models, which were built-in Stata 10[g] by a manual stepwise forwards and backwards procedure to test associations between exposure and outcome variables. Exposure variables were included in the model if they significantly improved the model fit assessed by likelihood ratio test statistics with the significance set at $P \leq .05$ and Akaike information criteria. Multivariable logistic regression (lrm) and ordinal logistic regression (olrm) models were used to assess the effect of HES on the AKI grade. In the lrm, the outcome variable "AKI increase" was divided into 2 categories: Category 0 included dogs that remained in the same AKI grade, and category 1 included dogs that changed from 1 AKI grade into a higher AKI grade. Four lrm were built assessing the effect of the 4 primary exposure variables (HES applications a–d) on AKI increase, adjusting for the effect of other confounding variables.

In olrm, the outcome variable included 6 categories, namely the AKI grades 0–5. Hence, in olrm, we tested the odds of changing into a higher AKI grade in HES-exposed and HES-unexposed dogs. Four olrm models were built, given the 4 primary exposure variables (HES applications a–d), adjusting for the effect of other confounding variables. Statistical significance was set at $P < .05$ for all analyses.

The final models were evaluated for goodness of fit by the Hosmer-Lemeshow test. Influential observations were detected using Pearson residuals, hat matrix (leverage), and delta-betas.[16]

Results

A total of 184 dogs met the inclusion criteria. The exposed cohort (HES) included 94 dogs with a mean age of 7.3 ± 3.5 years (range, 0.3–15.7 years) and a median weight of 19.0 kg (range, 1–71 kg). Several breeds were recorded with mixed breed dogs being most frequent (n = 15, 14.1%), followed by Labrador Retriever (n = 6, 6.4%), Chihuahua and Jack Russell Terriers (each n = 4, 4.3%), and Bernese mountain dogs (n = 3, 3.2%). Most dogs (88/94) received HES over >24 hours, with a median time frame of 4 (range, 1–16) days. Dogs in the HES group received a median amount of 69.4 (range, 2–429) mL per kg body weight HES, corresponding to a median dose of 20.7 (range, 2–87) mL per kg body weight per day. Hypoalbuminemia, defined as serum albumin concentration <20 g/L (2 g/dL), was present in 48 of 88 (54.5%) dogs, and median serum albumin concentration at presentation was 19.0 g/L (range, 7–44 g/L; 1.9 g/dL, range, 0.7–4.4 g/dL). The lowest serum albumin concentration within days 1–7 ranged from 8 to 30 g/L (0.8–3.0 g/dL; median, 20 g/L; 2.0 g/dL). Forty-three of 94 (45.7%) of the HES dogs were presented for primary gastrointestinal problems,

Table 2. Frequencies in exposure variables in dogs exposed and unexposed to HES.

Variable (Exposure/Risk factor)	Categories	Unexposed n = 90		HES-exposed n = 94		
		Mean	SD	Mean	SD	*P*-value
Age (years)	Continuous	8.5	4.0	7.3	3.5	.035
		Median	Range	Median	Range	
HES mL/kg total	Continuous	0		69.4	2–429	
HES mL/kg/d	Continuous	0		20.7	2–87	
Number of HES days	Continuous	0		4	1–16	
		n/N	%	n/N	%	
Diagnosis	ICD 19: Trauma	14/90	15.6	14/94	14.9	.004
	ICD 11: Gastrointestinal	20/90	22.2	43/94	45.7	
	ICD 14: Renal	11/90	12.2	11/94	11.7	
	Other ICD classes	45/90	50.0	26/94	27.7	
Whole blood or pRBC transfusion	Yes	4/90	4.4	22/94	23.4	<.001
	No	86/90	95.6	72/94	76.6	
Natural colloid transfusion	Yes	2/90	2.2	35/94	37.2	<.001
	No	88/90	97.8	59/90	62.8	
Other synthetic colloids	Yes	0/90	100	4/94	4.3	.066
	No	90/90	0	90/94	95.7	
Sex	Female intact	10/90	11.1	14/94	14.9	.129
	Female castrated	41/90	45.6	32/94	34.0	
	Male intact	13/90	14.4	25/94	26.6	
	Male castrated	26/90	28.9	23/94	24.5	
HES application	Yes	0/94	0	90/90	100	
	No	94/94	100	0/90	0	

ICD, International Statistical Classification of Diseases and Related Health Problems; pRBC, packed red blood cells.

followed by 27.7% for other diseases, 14.9% for trauma, and 11.7% for urogenital diseases (Table 2). All dogs within the HES group received crystalloid solutions in addition to HES.

In addition to HES solutions, 22 of 94 dogs (23.4%) received red blood cells in the form of packed red blood cells (pRBC) or fresh whole blood, 35 of 94 (37.2%) dogs received natural colloids (whole blood, plasma, human albumin), and 4 dogs (4.3%) received a bolus of 10% HES-200/0.5 at the time of presentation (Table 2).

The unexposed cohort consisted of 90 dogs with a mean age of 8.6 ± 4.0 years (range, 0.3–17.6 years) and a median weight of 17.2 kg (range, 1.6–53.9 kg). Several breeds were represented with mixed breed dogs being most frequent (n = 15, 16.7%), followed by Pugs (n = 5, 5.6%), Chihuahua and Golden Retriever (each n = 4, 4.4%), and Labrador Retriever (n = 3, 3.3%). Most dogs presented with various diseases (45/90, 50%) followed by gastrointestinal causes (20 of 90, 22.2%), trauma (14/90, 15.6%), and renal causes (11/90, 12.2%). Only 4 of 90 (4.4%) and 2 of 90 (2.2%) dogs required red blood cell transfusions or natural colloids, respectively. None of the unexposed dogs received any other synthetic colloids. Five of 74 dogs in the unexposed group presented with a serum albumin concentration <20 g/L (2 g/dL), and the median serum albumin concentration was 29.0 g/L (2.9 g/dL) with a range of 15–46 g/L (1.5–4.6 g/dL), whereas the lowest serum albumin concentration within the first 7 days ranged from 13 to 49 g/L (1.3–4.9 g/dL; median, 26.0 g/L; 2.6 g/dL).

Exposed and unexposed cohorts were comparable in terms of sex ($P = .129$) and weight ($P = .839$), but not age ($P = .035$). Groups also were significantly different in terms of diagnosis (Table 2) and requirement for blood products and natural colloids, with the HES cohort requiring more blood products and natural colloids (Table 2). Dogs receiving HES stayed significantly longer ($P = .041$) in the hospital (median, 8 days; range, 3–43 vs 7 days; range, 2–82 days), and significantly fewer HES-treated dogs left the hospital (77.7% vs 90.0%, $P = .028$).

Serum creatinine concentrations were not significantly different at most time points (Table 3). Results from days 8 to 20 are not reported because <10 measurements per group were available.

The AKI grade was not statistically different between exposed and unexposed cohorts at various time points (Table 4) in the univariable analysis. From exposed and unexposed dogs that presented with no signs of AKI (AKI grade 0) at the beginning of the study, 3 in each cohort developed AKI within 10 days ($P = 1.000$). Of the 3 dogs receiving HES and developing AKI (AKI grade ≥1), 2 dogs received HES over 14 days and 1 dog over 3 days.

Long-term follow-up of the AKI grade increase was possible in 35 HES-exposed (37.2%) and 38 unexposed (42.2%) dogs. None of these dogs showed an increase in AKI grade from presentation to day 11–90. Of the 6 dogs receiving HES that showed an increase in AKI grade during the short-term period, none were available for long-term evaluation because all were euthanized (after 4–17 days of hospitalization, euthanasia was not related to renal disease).

In the subgroup of dogs presented with preexisting AKI (AKI grade 1–5 at day 0), no statistically significant increase in AKI grade was found between dogs exposed and unexposed to HES ($P = .298$). None of the dogs available for the long-term time period showed an increase in AKI grade (Table 4).

Table 3. Comparison of creatinine concentrations (crea) at various time points in dogs exposed (HES) and unexposed to HES.

	Unexposed n = 90				HES exposed n = 94				
Variable	n	Median	min	max	n	Median	min	max	*P*-value
Crea day 0 (μmol/L)	90	85.5	18	1144	94	71.5	17	1240	.077
Crea day 0 (mg/dL)		0.97	0.20	12.94		0.81	0.19	14.03	
Crea day 2 (μmol/L)	35	71.0	39	1056	40	56	26	455	.071
(mg/dL)		0.80	0.44	11.95		0.63	0.29	5.15	
Crea day 3 (μmol/L)	26	76.0	18	1014	35	73.0	20	511	.610
(mg/dL)		0.86	0.20	11.47		0.83	0.23	5.78	
Crea day 4 (μmol/L)	20	81.5	43	739	28	69.0	18	701	.067
(mg/dL)		0.92	0.49	8.36		0.78	0.20	7.93	
Crea day 5 (μmol/L)	12	79.0	37	633	25	68.0	18	564	.142
(mg/dL)		0.89	0.42	7.16		0.77	0.20	6.38	
Crea day 6 (μmol/L)	15	68.0	21	257	13	73.0	20	179	.717
(mg/dL)		0.77	0.24	2.91		0.83	0.23	2.02	
Crea day 7 (μmol/L)	15	85.0	40	556	9	64.0	18	201	.238
(mg/dL)		0.96	0.45	6.29		0.72	0.20	2.27	
Last crea within days 2–10 (μmol/L)	84	78.5	18	1014	39	66.5	19	701	.060
(mg/dL)		0.89	0.20	11.47		0.75	0.21	7.93	
Last crea within days 11–90 (μmol/L)	40	76.5	20	356	39	65.0	18	1768	.136
(mg/dL)		0.87	0.23	4.03		0.74	0.20	20.00	

Crea: serum creatinine concentration.

Table 4. Comparison of AKI grade before HES/crystalloid administration and after HES/crystalloid administration (AKI grade increase/newly developed AKI) in dogs of the exposed and unexposed cohort.

	Unexposed n = 90		HES n = 94		
Variable	n/N	%	n/N	%	*P*-value
AKI grade of dogs at day 0 (before HES administration)					
AKI grade 0 (no AKI)	61/90	67.8	70/94	74.5	.333
AKI grade 1–5 (AKI)	29/90	32.2	24/94	25.5	
AKI grade 1	4/90	4.4	4/94	4.3	.628
AKI grade 2	10/90	11.1	8/94	8.5	
AKI grade 3	9/90	10.0	7/94	7.4	
AKI grade 4	2/90	2.2	4/94	4.3	
AKI grade 5	4/90	4.4	1/94	1.1	
AKI grade increase in dogs after HES/Crystalloid administration (all dogs)					
AKI grade increase (short-term[a])	3/84	3.6	6/86	7.0	.496
AKI grade increase (long term[b])	0/38	0.0	0/35	0.0	NA
AKI grade increase in dogs after HES/Crystalloid administration (only dogs presenting with AKI grade 0 at time point 0)					
Newly developed AKI (short term[a])	3/57	5.3	3/66	4.5	1.000
AKI grade increase in dogs after HES/Crystalloid administration (only dogs with preexisting AKI, AKI grade 1–5 at time point 0)					
AKI grade increase (short-term[a])	1/27	3.7	3/20	15	.298
AKI grade increase (long term[b])	0/13	0	0/8	0	NA

[a]Short-term, AKI grade increase within 10 days.
[b]Long term, AKI grade increase within 11–90 days; NA, not assessed.

Results of the ordinal logistic regression model identified "number of applied HES days" as significantly associated with the short-term increase in AKI grade ($P = .038$). One additional day of HES administration was associated with an 18% increase in odds of having a higher AKI grade than before. Thus, for every day, HES was given, the odds of the patient being classified in the high category of AKI vs the low and middle categories of AKI were 1.18 times greater (Table 5). However, the association between short-term increase in AKI grade and the application of HES (yes/no), HES mL/kg, and HES mL/kg/d was not significant ($P = .341$, $P = .224$ and $P = .529$, respectively).

The multivariable logistic regression model also identified the number of days of HES administration as significantly associated with a short-term change from AKI grade 0 to an AKI grade ≥1 ($P = .042$), whereas exposure to HES (yes/no), HES mL/kg/d, and HES mL/kg was not significantly associated ($P = .329$, $P = .225$ and $P = .504$, respectively). With every additional day of HES application, the odds of developing AKI was 1.17 times higher (Table 5).

When building the lrm and olrm, in a manual stepwise forwards and backwards procedure, the simple models including solely the risk factor "number of days of HES administration" and none of the other risk factors listed in Table 2 had the best model fit.

Table 5. Multivariable analysis: significant effect on short-term[a] AKI grade in dogs exposed (n = 90) or unexposed (n = 94) to HES (January 2013–November 2015).

Variable (Exposure/ Risk factor)	Odds Ratio	95% Confidence Intervals	*P* value
HES days applied[b]	1.17	(1.01–1.37)	.042
HES days applied[c]	1.18	(1.01–1.37)	.038

[a]Short-term, AKI grade increase within 2–10 days.
[b]Logistic regression model.
[c]Ordinal logistic regression model. None of the other included risk factors improved the model fit.

Looking at the long-term effect of HES, none of the dogs showed a persistent increase in AKI grade; therefore, it was not possible to perform a multivariable analysis.

Discussion

Studies in humans indicate an increased need for renal replacement therapy[7–9] and increased mortality[9] in septic and critically ill patients after HES administration. The PRAC therefore restricted HES use in human patients to treatment of acute blood-loss hypovolemia and prohibited its use in critically ill patients or patients with sepsis or burn injury.[b]

In contrast to these studies in humans, we did not find an association between HES administration or HES dose and an increase in AKI grade in dogs receiving HES 130/0.4 in an ICU setting. This finding is in agreement with another veterinary study evaluating the effect of HES 130/0.4 administration on serum creatinine concentrations in ICU patients.[15] The HES population in our study included mainly patients with gastrointestinal or abdominal causes of disease, and although septic patients were not specifically identified, the incidence of severe sepsis patients is expected to be very low. We did not compare dogs with and without signs of sepsis because our study was designed to evaluate a critically ill population of dogs rather than a subgroup of septic dogs. Other recent studies in humans investigating adverse renal effects of HES administration in surgical and trauma patients, but also critically ill patients, did not show an increased risk of AKI after HES administration[17–19], indicating that conclusive studies also are needed in human medicine.

A dose-response relationship of HES administration has been proposed, and the PRAC recommends using HES solutions at the lowest effective dose for the shortest period of time possible, generally for a maximum of 24 hours and avoiding administration as a constant rate infusion (CRI). In contrast to a study that investigated a pentastarch solution and found that higher HES-250/0.5 doses (mL/kg/h) were associated with an increased risk of death and AKI, and a higher HES dosage per kilogram body weight and per kilogram body weight per day were not associated with increased risk of AKI

in this patient population. Whereas exposure to HES itself as well as cumulative HES dose (mL/kg and mL/kg/d) was not associated with an increase in AKI grade, the number of days of HES administration was significantly associated with risk of increased AKI grade within 10 days of HES exposure (short-term effect). Hence, although the use of HES itself does not seem to cause AKI, the duration of HES treatment should be kept as short as possible. To our knowledge, ours is the first veterinary study investigating the time period of HES administration on changes in AKI grade. The median number of days of HES administration in our study was 4 (range, 1–16 days), and HES was administered as a CRI in the majority of cases because HES solutions are used for colloid osmotic support in patients with severe hypoalbuminemia (<16–20 g/L) at our institution. Of the 3 dogs that developed "new AKI", 2 received long-term HES administration (14 days). However, despite long-term use, the patient with the longest duration of HES administration (16 days, total cumulative dose of 429 mL/kg) did not develop AKI.

The mechanism of action of renal impairment by HES is not fully understood. Although newer HES solutions such as HES 130/0.4 have a short half-life and no plasma accumulation occurs over time[20], HES accumulates in the lysosomes of a wide range of tissues and highest concentrations have been identified in the proximal renal tubular cells.[21] In the kidney, cellular uptake of HES leads to osmotic nephrosis, characterized by accumulation of intracellular water, cytoplasmic swelling and cellular disruption.[21] The renal impairment seems to occur independent of inflammation as shown in a rat model of septic AKI, but changes are increased with concurrent sepsis.[22]

In an in vitro model of human proximal tubular cells, various HES solutions (3–200 kDa) induced decreased cell viability.[23] It was concluded that not the type or size of the HES molecule but solely the number of molecules or cumulative dose was responsible for proximal tubular cell injury.[23] Hydroxyethyl starch has been shown to persist in the renal parenchymal cells of dogs up to 18 days.[21,24] This cumulative dose effect is supported by other studies in human patients, and dosages as low as 39 mL/kg HES-130/0.4 may lead to AKI.[25] In contrast to these studies, our results suggest that the time frame of administration had a more pronounced effect than the total cumulative dose. However, the above-mentioned study in rats detected renal tubular cell injury with doses of 50 mL/kg HES administered over 6 hours, whereas our dogs received a median dose of 69.4 mL/kg over a median of 4 days, corresponding to a much lower median daily dose of 20.7 mL/kg. A study that investigated larger median cumulative dosages of 87 mL/kg also failed to show an increase in serum creatinine concentration compared to dogs receiving only crystalloid fluid therapy.[15] No attempt at identification of risk factors was made in this study.

Whereas histopathological changes are seen within 24 hours after exposure[22], increases in serum creatinine concentrations may lag behind the development of AKI.[26] In another study, serum creatinine concentration peaked at day 3[8], yet another study showed an increase in serum creatinine concentration within 24 hours of HES application to septic rats.[22] In our study, peak serum creatinine concentration could not be determined because of a low number of dogs evaluated at each day and presence of measurement bias (dogs with higher serum creatinine concentrations on 1 day were more likely to have additional measurements than dogs with normal results over several days). Because changes in serum creatinine concentrations may lag behind renal injury, only dogs with serum creatinine concentrations and AKI grades determined at least 48 hours after the start of HES administration were included.

In humans, long-term effects caused by tissue accumulation are expected 20 days after initial exposure; therefore, continued monitoring is important and long-term evaluation of renal function may be as important as short-term monitoring.[9] The PRAC recommends monitoring of renal function (serum creatinine concentration) up to 90 days after HES exposure.[b] Whereas the number of dogs available for long-term evaluation in our study was small, comparison of serum creatinine concentration and increases in AKI grade at various time points up to 90 days after HES in dogs exposed and unexposed to HES were not different. None of the dogs that were available for long-term evaluation had an increased AKI grade, but the 6 dogs that showed an increase in AKI grade during the short-term period were not available for long-term evaluation. It therefore remains speculative if those patients may have progressed to more severe AKI or if renal regeneration potentially could have led to a reversal of AKI.

Other studies investigating adverse renal effects of HES solutions in dogs are rare. In a canine hemorrhagic shock model and goal-directed fluid therapy by HES-130/0.4, high dosages did impair renal function (defined as a decrease in creatinine clearance).[27] Dogs in this study received 0.4–0.45 mL/kg/min of HES resulting in a very high mean cumulative dose of 110–122 mL/kg that is not commonly used in clinical situations. Hence, the clinical relevance is questionable. A recently published retrospective study evaluating the effects of HES-250/0.5 on survival and development of AKI in dogs found decreased survival and increased risk of AKI in dogs receiving HES-250/0.5.[14] Acute kidney injury was defined as a doubling of serum creatinine concentration during the hospital stay. The results therefore are not comparable to our study results, because we used a different AKI case definition and another type of HES. The only other study of dogs evaluating HES effects on renal function investigated the same tetrastarch solution (HES-130/0.4) as did our study and did not show any impairment of renal function, as determined by absolute serum creatinine concentrations and changes in serum creatinine concentrations within 2 and 12 weeks, respectively.[15] Their results support our study findings. Although these results suggest that the type of HES may influence the incidence of adverse renal effects, another study concluded that not the type or size of the HES molecule

but solely the number of molecules or cumulative dose was responsible for proximal tubular cell injury.[23] Hydroxyethyl starch solutions can be divided into hetastarches, pentastarches, and tetrastarches depending on their grade of substitution with hydroxyethyl at the C2 and C6 site. The newest generation of HES solution is a tetrastarch (substitution grade, 0.4–0.42) with a lower molecular weight of 130 kd and a low substitution ratio (0.9). The lower molecular weight, lower substitution grade, and high C2:C6 substitution ratio theoretically should decrease adverse effects on coagulation and renal damage. Although reports in human medicine are inconsistent in terms of HES type, and additional factors such as the presence of sepsis affect pathophysiological changes[22], present veterinary studies may suggest that HES-130/0.4 is less likely than pentastarch to induce AKI or increase AKI grade in dogs if used at moderate dosages.

To our knowledge, our study is also the first investigating the impact of HES on patients with preexisting renal disease. The administration of HES 130/0.4 in dogs with preexisting renal disease, defined as AKI grade 1–5 at day 0, did not significantly worsen AKI grade or increase serum creatinine concentrations when compared to dogs with similar AKI grades receiving only isotonic crystalloid solutions. Additionally, none of the dogs available for the evaluation of a long-term effect of HES on AKI showed an increase in AKI grade. This finding is in accordance with the human literature where the 6S study also did not find an increased risk of worsening renal function in a group of patients with preexisting renal disease.[9] However, more dogs in the HES group (15% vs 3.7%, $P = .298$) showed a short-term increase in AKI grade, and although this finding may be a consequence of the potentially sicker group of dogs requiring HES, this finding needs further evaluation before firm conclusions can be drawn.

Dogs receiving HES stayed significantly longer in the hospital and survival to discharge was significantly lower. Whether lower survival was related to HES administration or simply associated with a more severe underlying disease cannot be determined. As in another study[14], the dogs in our HES group seemed to be sicker, presenting with lower admission serum albumin concentrations, prolonged hospital stay and an increased need for blood products. Illness severity scores were not determined because of incomplete information. However, because potentially sicker dogs were not more prone to development of AKI after HES administration, we conclude that administration of HES did not influence the development of AKI and survival was independent of HES administration.

As does any retrospective study, our study has several limitations. First, the 2 groups differed in terms of age, diagnosis, blood product requirements, and serum albumin concentration. Age, blood product requirements, and ICD score were not statistically significant risk factors in the multivariable models and did not improve model fit. Therefore, we assume that our study results were not strongly biased by differences in age or diagnosis in the exposed and unexposed groups. Furthermore, if age or the ICD score had an influence on the outcome, it would have been adjusted for in the multivariable models. However, a prospective randomized clinical control trial would strengthen the causal relationship between HES exposure and AKI grade. Low serum albumin concentration was the reason for HES administration in many of the dogs and was associated with abdominal diseases, making it difficult to find a matching control group. Second, data were collected retrospectively, and serum creatinine concentrations and AKI grades were not available at predetermined time points in all dogs. A bias is expected because dogs with an increase in serum creatinine concentration were probably more prone to have follow-up serum creatinine concentrations measured. However, the median serum creatinine concentrations of both cohorts remained within the reference interval throughout most time points of the study, indicating that enough dogs with serum creatinine concentrations within the reference interval were included.

Third, the different AKI definitions used in the published veterinary studies hinder direct comparisons. We elected to use the IRIS guidelines for AKI grading because these are the most recently published veterinary guidelines, are based on laboratory and diagnostic imaging findings other than merely serum creatinine concentrations and allowed us to account for different grades of AKI. However, because baseline serum creatinine concentrations generally were not available (dogs presented already sick) and not all dogs underwent laboratory and diagnostic imaging evaluation, the "true" AKI grade could have been falsely high (if presented with prerenal azotemia) or falsely low (when an increase in serum creatinine concentration within 48 hours was missed).

Fourth, no attempt was made to evaluate dogs with short-term (<24 hours) administration of HES because only a few dogs were treated for <24 hours. Fifth, concurrent crystalloid fluid administration has not been investigated as a potential risk factor and an effect on study results cannot be completely ruled out even though all dogs receiving HES also received crystalloid fluids.

In conclusion, HES administration to critically ill dogs was not associated with an increase in AKI grade within 10 days and the cumulative HES dose was not a risk factor for the development of AKI. However, the number of HES days significantly increased the odds of an increase in AKI grade ($P = .04$). Although dogs presented without AKI had the same risk to newly acquire AKI ($P = 1.0$), more dogs with preexisting AKI showed an increase in AKI after treatment with HES, but this finding was not statistically significant.

Based on our data, HES can be safely administered to critically ill dogs, but the time period of administration should be kept as short as possible. The effect of HES administration on long-term renal function and in dogs with preexisting renal disease must be further evaluated before additional recommendations can be made.

Footnotes

[a] Garcia AM, Rudloff E, and Kirby R. Efficacy and adverse effects of hetastarch/crystalloid combination in 21 hypotensive cats. J Vet Emerg Crit Care 2002; 12(3):200

[b] European Medicine Agency. Press release: European Medicine Agency, (EMA) PRAC confirms that hydroxyethyl starch solutions (HES) should no longer be used in patients with sepsis or burn injuries or in critically ill patients. *Available at* http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Solutions_for_infusion_containing_hydroxyethyl_starch/Position_provided_by_CMDh/WC500153119.pdf. *Accessed* February 20, 2014

[c] http://www.iris-kidney.com/pdf/grading-of-acute-kidney-injury.pdf

[d] hydroxyethyl starch 130/0.4; Voluven®, Voluven balanced®, Fresenius Kabi, Switzerland

[e] http://apps.who.int/classifications/icd10/browse/2016/en

[f] IBM SPSS v.21 for Mac OS X; IBM Corporation, New York, NY

[g] StataCorp, Data Analysis and Statistical Software, College Station, Texas

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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