European Veterinary Renal Pathology Service: a survey over a 7-year period (2008-2015)

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Abstract: BACKGROUND: The European Veterinary Renal Pathology Service (EVRPS) is the first Web-based registry for canine renal biopsy specimens in Europe. HYPOTHESIS/OBJECTIVES: The aim was to verify whether differences exist between the clinical and laboratory presentation of dogs with nephropathy according to renal pathological findings, as defined by light and electron microscopy of renal biopsy specimens submitted to EVRPS. ANIMALS: Renal biopsy specimens of dogs were collected from the archive of the service (n = 254). Cases were included if both light and electron microscopy were available (n = 162). METHODS: Renal biopsy specimens were classified based on the morphological diagnoses. Thereafter, they were grouped into 3 disease categories, including immune-complex-mediated glomerulonephritis (ICGN), non-immune-complex-mediated GN (non-ICGN), and renal lesions not otherwise specified (RL-NOS). Differences among morphological diagnoses and among disease categories were investigated for clinical and laboratory variables. RESULTS: Serum albumin concentration was lower in dogs with ICGN than in those with non-ICGN (P = 0.006) or RL-NOS (P = 0.000), and the urine protein-to-creatinine ratio (UPC) was significantly higher in ICGN than in the other 2 disease categories. Regarding morphological diagnoses, albumin was significantly lower in amyloidosis (AMY) and membranous (MGN), membranoproliferative (MPGN) or mixed glomerulonephritis (MixGN) than in minimal change disease, primary (FSGS I) or secondary (FSGS II) focal and segmental glomerulosclerosis and juvenile nephropathies (JN). The UPC was higher in MPGN than in FSGS I and FSGS II. CONCLUSIONS AND CLINICAL IMPORTANCE: Dogs with ICGN, in particular MPGN, had higher protein loss than those with non-ICGN or RL-NOS, leading to more severe hypoalbuminemia. Clinical and laboratory differentiation among dogs with the different morphological diagnoses and among dogs with different disease categories was difficult due to overlapping results.

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Methods: Renal biopsy specimens were classified based on the morphological diagnoses. Thereafter, they were grouped into 3 disease categories, including immune-complex-mediated glomerulonephritis (ICGN), non-immune-complex-mediated GN (non-ICGN), and renal lesions not otherwise specified (RL-NOS). Differences among morphological diagnoses and among disease categories were investigated for clinical and laboratory variables.

Results: Serum albumin concentration was lower in dogs with ICGN than in those with non-ICGN ($P = 0.006$) or RL-NOS ($P = 0.000$), and the urine protein-to-creatinine ratio (UPC) was significantly higher in ICGN than in the other 2 disease categories. Regarding morphological diagnoses, albumin was significantly lower in amyloidosis (AMY) and membranous (MGN), membranoproliferative (MPGN) or mixed glomerulonephritis (MixGN) than in minimal change disease, primary (FSGS I) or secondary (FSGS II) focal and segmental glomerulosclerosis and juvenile nephropathies (JN). The UPC was higher in MPGN than in FSGS I and FSGS II.

Conclusions and clinical importance: Dogs with ICGN, in particular MPGN, had higher protein loss than those with non-ICGN or RL-NOS, leading to more severe hypoalbuminemia. Clinical and laboratory differentiation among dogs with the different morphological diagnoses and among dogs with different disease categories was difficult due to overlapping results.

Key words: Diagnosis; Dog; Electron microscopy; Glomerulonephritis; Renal biopsy.

In collaboration with the World Small Animal Veterinary Association-Renal Standardization Study Group (WSAVA-RSSG), the Utrecht Veterinary Nephropathology Service (UVNS) was created in 2008, providing the first Web-based and prospective registry system for canine and feline renal biopsy specimens (RBs) in Europe. In 2014, the service was reorganized and combined with the European Veterinary Renal Pathology Service (EVRPS) and located within the Department of Comparative Biomedicine and Food Science, University of Padova, Italy.

Abbreviations:

AMY	amyloidosis
EVRPS	European Veterinary Renal Pathology Service
FSGS II	secondary focal and segmental glomerulosclerosis)
FSGS I	primary focal and segmental glomerulosclerosis
FWER	family-wise error rate
ICGN	immune-complex-mediated glomerulonephritis
IF	immunofluorescence
JN	juvenile nephropathies
LM	light microscopy
MCD	minimal change disease
MD	miscellaneous diseases
MGN	membranous glomerulonephritis
MixGN	mixed glomerulonephritis
MPGN	membranoproliferative glomerulonephritis
Non-ICGN	non-immune-complex-mediated glomerulonephritis
RBs	renal biopsies
RL-NOS	renal lesions not otherwise specified
TEM	transmission electron microscopy
UPC	urine protein-to-creatinine ratio
UTI	urinary tract infection
UVNS	Utrecht Veterinary Nephropathology Service
WSAVA-RSSG	World Small Animal Veterinary Association-Renal Standardization Study Group

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To date, the EVRPS serves European veterinarians providing renal pathology consultations (LA, AVD) and detailed diagnoses including light microscopy (LM), immunofluorescence (IF), and transmission electron microscopy (TEM) in a short turnaround time. A diagnosis is obtained by the consensus of 3 pathologists with renal pathology experience (LA, SB, and JVL). The EVRPS also represents the first continental registry to record pathological diagnosis, as well as clinicopathologic data of dogs undergoing RBs. Registries represent important tools, providing epidemiological data or characteristic findings of various diseases, and are widely used in human medicine. In 2013, the largest series of RBs in dogs, obtained from 501 dogs living in North America, was reported.[1] A wide array of renal diseases was described in dogs, but clinical and laboratory findings were not provided. A collection of clinical, laboratory, and pathological data from dogs with renal disease is difficult for many reasons. First, RBs are not usually included in the diagnostic evaluation in clinical practice. Furthermore, it is now recognized that complete evaluation of RBs requires LM and TEM examination combined with IF.[2,3] Unfortunately, TEM facilities are not always available in private and academic veterinary diagnostic center and cost may represent a limiting factor. Therefore, comprehensive surveys are difficult to perform and require excessively long time periods and multicenter investigations.

The aims of our report from EVRPS were to characterize the clinical and laboratory presentation of dogs with suspected renal disease that had RBs submitted to the EVRPS, to classify the RBs according to LM and TEM examination, and to identify possible differences between clinical and laboratory findings based on renal morphological diagnoses and renal disease categories. The survey included dogs living in Europe.

Materials and Methods

Case selection

Renal biopsy specimens submitted between 2008 and 2015 were collected from the archive of the UVNS/EVRPS. Although standard RB evaluation consists of LM, IF, and TEM, the presence of LM and TEM reports was the sole inclusion criterion for selection. The IF results were considered to complement the diagnosis but were not included in the data analysis. For all cases, clinical and laboratory variables were retrieved from signalment, history, hematology and serum biochemical profile results, and urinalysis. All data were provided by the referring veterinarians.

Processing of RBs

All RBs were routinely processed for LM and TEM examination.[4] Specimens for LM were sectioned at 3 μm thickness and stained with hematoxylin and eosin, periodic acid-Schiff, Masson's trichrome, and periodic acid-methenamine silver. Congo red staining was performed on 8- to 10-μm sections if amyloid was suspected, to confirm the diagnosis. For TEM, tissues were fixed in chilled 3% buffered glutaraldehyde and processed according to standard procedures.[4]

Evaluation of RBs

The evaluation and classification of renal lesions were based on diagnostic criteria developed by WSAVA-RSSG.[4] Transmission electron microscopy was used as the gold standard method for immune-complex detection. The RBs were analyzed individually and then grouped into 3 disease categories: (1) immune-complex-mediated glomerulonephritis (ICGN) when glomerular immune-complex deposits were identified by TEM, including membranous GN (MGN), membranoproliferative GN (MPGN), mixed GN (MixGN) and focal and segmental glomerulosclerosis pattern secondary to immune-complex deposits (FSGS II); (2) non-immune-complex-mediated GN (non-ICGN) when glomerular immune-complex deposits were not identified by TEM, including minimal change disease (MCD), amyloidosis (AMY), focal and segmental glomerulosclerosis pattern not associated with other glomerular diseases (FSGS I); and, (3) renal lesions not otherwise specified (RL-NOS), including juvenile nephropathies (JN) and miscellaneous diseases (MD). Dogs without evident lesions on TEM were arbitrarily included in the latter group.

Statistical Analysis

Analyses were performed by a commercial software[a] to detect possible associations between clinical or laboratory findings and pathological results. Initially, the analysis was conducted comparing all morphological diagnoses, and it subsequently was reconducted considering the 3 disease categories. The following variables collected from the signalment and history were used in the analysis: age at diagnosis, sex (male, female), disease onset (acute, chronic), body weight and appetite (decreased, increased), anorexia, vomiting, polyuria and polydipsia, lethargy, previous episodes of urinary tract infection (UTI), subcutaneous edema, ascites, hypoalbuminemia, gross hematuria, and proteinuria (present, absent). Furthermore, laboratory results were considered in the analysis if recorded within 1 month before biopsy, including hematocrit; leukocyte count; serum albumin, total protein, creatinine, urea nitrogen, sodium, potassium, total calcium, phosphorus and antithrombin III concentrations; and urinalysis including color, specific gravity, pH, glucosuria, erythrocytes, leukocytes, and UPC.

In particular, contingency tables were prepared for each of the aforementioned variables, and the Pearson chi-square test was performed to assess their possible association with morphological diagnoses and disease categories. When appropriate, Fisher's exact test was used for 2×2 contingency tables.

For continuous variables, a Shapiro-Wilk test was performed to assess whether or not the data were normally distributed.

Kruskal-Wallis or analysis of variance (ANOVA) tests were performed to compare means among the different morphological diagnoses and disease categories. When a significant variation among groups occurred, posthoc analysis was performed with Mann-Whitney, Bonferroni, or Dunnett tests, based on data distribution and homoscedasticity assessment. Significance was set at $P \leq 0.05$ for all tests except for the Mann-Whitney test for which (based on the number of possible paired contrasts) the significance threshold was set at $P \leq 0.016$ for disease categories and at $P \leq 0.001$ for morphological diagnoses to decrease the family-wise error rate (FWER) in multiple comparisons.

When only 2 groups were compared, the independent-samples *t*-test or the Mann-Whitney test was performed according to data distribution. Significance was set at $P \leq 0.05$ for both tests.

Finally, Cohen's kappa was calculated to assess the level of agreement between LM and TEM in the disease categories and morphological diagnoses assignment. Results were evaluated as previously described.[5]

Results

Cases

Over a period of 7 years, 254 RBs were sent to the UVNS/EVRPS. Ninety-two samples were excluded because either LM or TEM was missing. Veterinarians from different European countries used the service; RBs coming from Sweden were most frequent (n = 43), followed by the United Kingdom (n = 41), the Netherlands (n = 33), Belgium (n = 8), Norway (n = 7), Finland (n = 6), France (n = 6), Germany (n = 6), Italy (n = 4), Ireland (n = 3), Hungary (n = 2), Switzerland (n = 2), and Spain (n = 1). The proportion of cases for which the different data were available varied widely within the study population, ranging from 6.8% (for antithrombin III activity) to 100% (for vomiting). Complete details are provided in Tables 2–5 and S1 to S4.

Golden Retrievers (n = 11), Labrador Retrievers (n = 9), and Schapendoes (n = 8) were the most commonly represented breeds. Females (52%) slightly outnumbered males (48%). The median age at presentation was 74 months (6.2 years), with 54.3% of dogs being <7 years old.

Morphological Diagnosis

By LM, 53 (32.7%) renal biopsy specimens were classified as FSGS I (Fig 1A,B), 28 (17.3%) as MGN (Fig 1C, D), 26 (16%) as MPGN (Fig 1E,F), 11 (6.8%) as AMY (Fig 1G, H), 4 (2.5%) as JN, 2 (1.2%) as MixGN, and 1 (0.6%) as MCD; 21 (13%) dogs had MD and 16 (9.9%) showed no abnormalities. By TEM, the definitive diagnosis changed in 71 dogs (Table 1). The final diagnosis did not change for dogs identified by LM as AMY, MCD, MixGN, and JN. Interestingly, none of the dogs had FSGS II based on LM, whereas based on TEM it was diagnosed in 19 cases. Cohen's kappa was 0.494.

Age significantly differed among morphological diagnoses ($P = 0.002$); in particular, dogs with JN were younger than dogs with MPGN ($P = 0.001$) and those with FSGS I ($P = 0.000$; Table 2).

Considering historical data, significant differences in proportions among groups were found for the detection of UTI ($P = 0.040$) and ascites ($P = 0.014$; Table 3). Indeed, UTI was less frequent in dogs with MPGN and MixGN; ascites and abdominal distension were less frequent in dogs with MPGN and FSGS I.

Concerning hematology and serum biochemical profiles, significant differences in proportions among groups were found for alterations of serum concentrations of albumin and total protein ($P = 0.000$ and $P = 0.001$; Table 3). Hypoalbuminemia and hypoproteinemia were more common in dogs with AMY, MGN, MPGN, and MixGN. Significant differences among groups were present for serum concentrations of albumin ($P = 0.000$), total protein ($P = 0.001$), creatinine ($P = 0.013$), urea nitrogen ($P = 0.021$), and phosphorus ($P = 0.012$; Table 2). In particular, serum albumin concentration was lower in dogs with AMY, MPGN, and MixGN than in dogs with FSGS I, FSGS II, and JN (P-values of paired comparisons from 0.000 to 0.001), and in dogs with MGN than in dogs with MCD, MixGN, and JN ($P = 0.000$ for all comparisons). Total protein concentrations were lower in dogs with AMY or MGN than in dogs with FSGS II ($P = 0.040$ and $P = 0.007$, respectively). Serum creatinine concentration was lower in dogs with MCD than in dogs with MPGN ($P = 0.000$). Concerning urea nitrogen concentration, no significant result was obtained for paired comparisons. Serum phosphorus concentration was higher in dogs with MD than in dogs with FSGS I ($P = 0.001$).

Regarding urinalysis, significant differences were found among groups for the presence of erythrocytes ($P = 0.009$) and proteinuria ($P = 0.000$; Table 3). Erythrocytes were less commonly observed in the urine of dogs with FSGS I and more commonly in the urine of dogs with MPGN and MixGN. Proteinuria was slightly less common in dogs with FSGS II and frequently absent in those with JN; in the latter, approximately half had no proteinuria. Mean UPC and urine specific gravity were significantly different among groups ($P = 0.017$ and $P = 0.000$, respectively; Table 2). In particular, UPC was higher in dogs with MPGN than in dogs with FSGS I ($P = 0.035$) and urine specific gravity was higher in dogs with MixGN than in dogs with AMY, FSGS I, and FSGS II ($P = 0.034$, $P = 0.008$, and $P = 0.043$, respectively).

Significant differences among morphological diagnoses were not detected for the remaining variables (Tables S1-S2).

Disease Categories

By LM, 64 (39.5%) RBs were diagnosed as non-ICGN, 57 (35.2%) as ICGN, and 41 (25.3%) as RL-NOS. By TEM, the final diagnosis changed in 46 dogs; overall, 82 (50.6%) dogs had ICGN, 59 (36.4%) non-ICGN, and 21 (13%) RL-NOS. In particular, the final diagnosis changed for 21 (32.8%) dogs diagnosed as non-ICGN, 2 (3.5%) diagnosed as ICGN, and 23 (56.1) diagnosed as RL-NOS based on the LM results, respectively. Cohen's kappa between LM and TEM was 0.560.

The median age in dogs diagnosed with ICGN was 70 months, 90 months for dogs with non-ICGN, and 27 months for dogs with RL-NOS. A significant difference was found among the 3 disease categories ($P = 0.000$). In particular, dogs with RL-NOS were younger than dogs with ICGN ($P = 0.001$) and non-ICGN ($P = 0.000$), whereas dogs with ICGN and non-ICGN were of comparable age (Table 4).

Considering the historical information, significant differences among groups were found for the detection of previous UTI ($P = 0.035$), presence of ascites ($P = 0.040$), and hypoalbuminemia ($P = 0.006$; Table 5). Overall, previous UTI was less frequent in dogs with ICGN. Ascites was more common in dogs with ICGN, whereas hypoalbuminemia was rarely identified in dogs with RL-NOS.

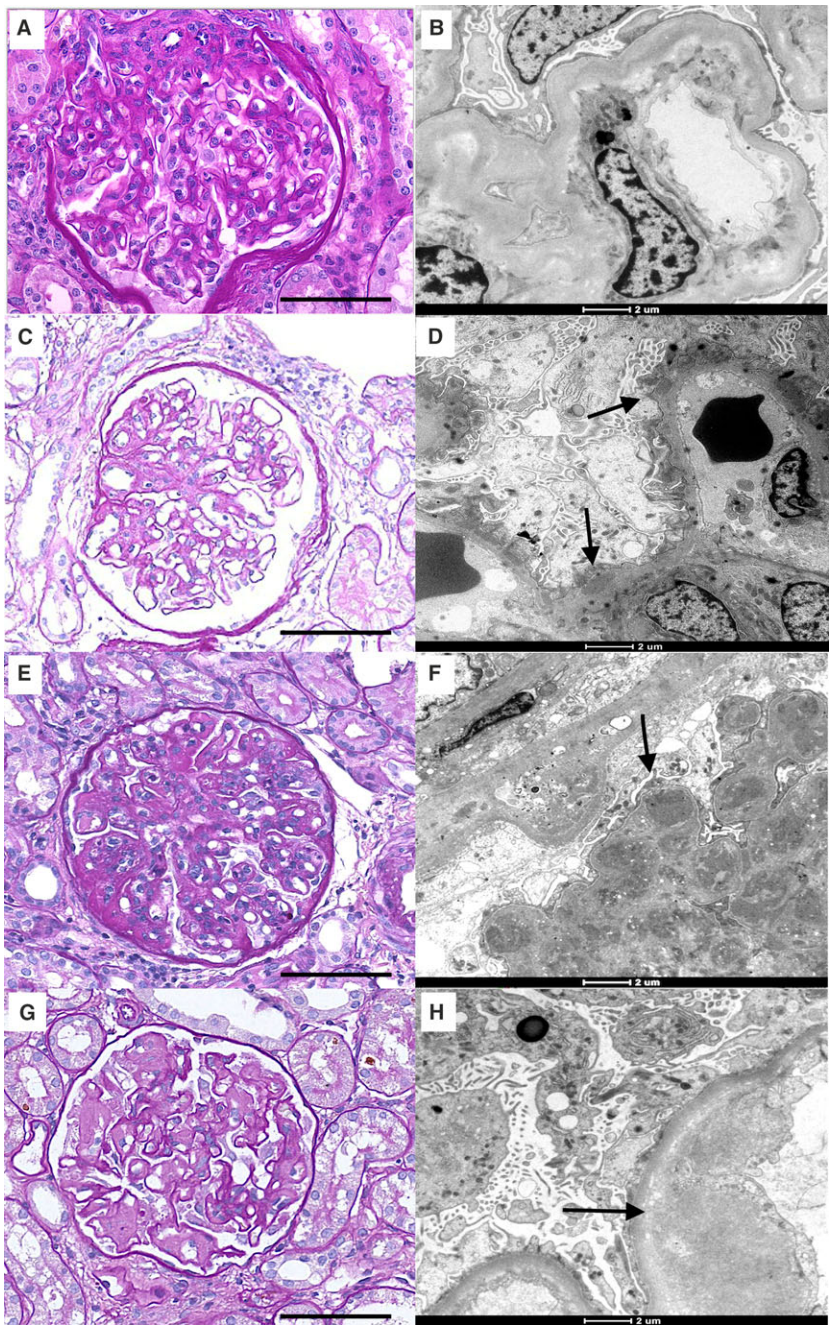

Fig 1. Histological and ultrastructural findings in cases of FSGS I **(A, B)**, MGN **(C, D)**, MPGN **(E, F)**, and AMY **(G, H)**. Periodic acid-Schiff (PAS) section of a glomerulus with a segment of the tuft effaced by mesangial sclerosis **(A)**. Electron microscopy reveals thickened GBM and increased mesangial matrix. Immune deposits are not identified **(B)**. PAS section of a glomerulus shows remodeling of the glomerular basement membrane and podocyte hypertrophy **(C)**. Electron microscopy reveals immune complexes (IC) (arrows) in the subepithelial side of the capillary walls **(D)**. PAS section of a glomerulus characterized by global endocapillary and mesangial hypercellularity. Double contours and thickening of the GBM are observed **(E)**. Electron microscopy reveals IC (arrow) in mesangial and paramesangial regions of the glomerulus **(F)**. PAS section of a glomerulus characterized by pale pink fibrils expanding the mesangial zones **(G)**. Electron microscopy shows expansion of 1 glomerular capillary (arrow) by haphazardly arranged fibrils **(H)**. Scale bar for histological figures = 50 μm.

With regard to recent biochemical data, significant differences among groups were found for alterations in serum concentrations of albumin, total protein, and calcium ($P = 0.003$, $P = 0.019$, and $P = 0.050$, respectively; Table 5). Hypoalbuminemia and hypoproteinemia were more common in dogs with ICGN. Hypocalcemia was more common in dogs with ICGN and hypercalcemia in dogs with RL-NOS. Significant differences in serum concentrations of albumin ($P = 0.000$), urea nitrogen ($P = 0.022$), and phosphorus ($P = 0.003$) were present

Table 1. Morphological diagnosis of 162 canine renal biopsies according to light microscopy and subsequent transmission electron microscopic analysis.

LM Morphological diagnosis (N)	LM Morphological diagnosis confirmed by TEM (%)	LM Morphological diagnosis changed by TEM (%)	List of modified morphological diagnosis (N; %)
MGN (28)	12 (42.9%)	16 (57.1%)	MPGN (1; 3.6%); MixGN (11; 39.3%); FSGS II (2; 7.1%); MCD (1; 3.6%); FSGS I (1; 3.6%)
MPGN (26)	21 (80.8%)	5 (19.2%)	MGN (1; 3.8%); MixGN (4; 15.4%)
MixGN (2)	2 (100%)	0 (0%)	–
MCD (1)	1 (100%)	0 (0%)	–
AMY (11)	11 (100%)	0 (0%)	–
FSGS I (53)	31 (58.5%)	22 (41.5%)	MPGN (7; 13.2%); MixGN (1; 1.9%); FSGS II (11; 20.8%); JN (3; 5.7%)
JN (4)	4 (100%)	0 (0%)	
MD (21)	6 (28.6%)	15 (71.4%)	MPGN (1; 4.8%); MixGN (1; 4.8%); FSGS II (2; 9.5%); MCD (5; 23.8%); FSGS I (4; 19.0%); JN (2; 9.5%)
No lesions (16)	3 (18.8%)	13 (81.2%)	MPGN (1; 6.3%); FSGS II (4; 25.0%); MCD (5; 31.3%); MD (3; 18.8%)

LM, light microscopy; TEM, transmission electron microscopy; MGN, membranous glomerulonephritis; MPGN, membranoproliferative glomerulonephritis; MixGN, mixed glomerulonephritis; FSGS II, secondary focal and segmental glomerulosclerosis; MCD, minimal change disease; AMY, amyloidosis; FSGS I, primary focal and segmental glomerulosclerosis; JN, juvenile nephropathies; MD, miscellaneous diseases.

among the 3 disease categories (Table 4). In particular, serum albumin concentration was lower in dogs with ICGN compared to those with non-ICGN ($P = 0.006$) or RL-NOS ($P = 0.000$), urea nitrogen concentration was significantly higher in dogs with ICGN than in those with non-ICGN ($P = 0.008$), and serum phosphorus concentration was significantly higher in dogs with RL-NOS than in those with non-ICGN ($P = 0.001$).

Considering urinalysis, significant differences in proportions among groups were found for the presence of erythrocytes ($P = 0.015$) and proteinuria ($P = 0.035$; Table 5). A finding of >10 erythrocytes/high-power field was more common in the urine of dogs with ICGN compared with the other 2 disease categories. Proteinuria was slightly less frequent in RL-NOS dogs than in the other 2 disease categories. Mean UPC was higher in ICGN dogs than in non-ICGN ($P = 0.002$) and RL-NOS ($P = 0.000$) dogs (Table 4).

No significant differences among disease categories were detected for the remaining variables (Tables S3-S4).

Because it is perceived by pathologists that AMY and FSGS I are relatively easy to differentiate from any ICGN, further analysis was performed to compare the 2 morphological diagnoses to the whole group of ICGN. In particular, it was found that dogs with AMY were less likely to be anemic than those with ICGN ($P = 0.038$; Tables S1 and S3, respectively), had higher mean hematocrit values ($P = 0.049$; Tables S2 and S4, respectively), and had lower mean urine specific gravity ($P = 0.047$; Tables 2 and 4, respectively). Dogs with FSGS I were more commonly reported to have previous UTI than were those with ICGN ($P = 0.018$; Tables 3 and 5, respectively) and less commonly had decreased serum albumin concentration ($P = 0.002$; Tables 3 and 5, respectively) and total proteins ($P = 0.006$; Tables 3 and 5, respectively). In addition, dogs with FSGS I, compared to those with ICGN, had higher serum concentrations of albumin ($P = 0.000$; Tables 2 and 4, respectively) and total protein ($P = 0.039$; Tables 2 and S4, respectively) and lower UPC ($P = 0.002$; Tables 2 and 4, respectively). Finally, dogs with FSGS I, compared to those with ICGN, less frequently had hematuria ($P = 0.000$; Tables 3 and 5, respectively).

Discussion

We analyzed the clinical, laboratory, and pathological data of 162 canine RBs examined at the EVRPS between 2008 and 2015. The number of RBs submitted has increased consistently over the 7-year period (9% per year), likely as a result of increasing awareness of the service by European veterinarians, the recent publication on RB indications, and new criteria to diagnose renal diseases in dogs.

Interestingly, when examining the geographical origin of the veterinarians using the service, RBs from the North European countries were overrepresented, possibly causing a bias in the distribution of renal diseases. Indeed, northern countries are not endemic for leishmaniasis, which is a major cause of ICGN in dogs from southern Europe.[6,7] However, the proportion of dogs with ICGN in our case series (50.6%) was consistent with that of a recent investigation (48.1%), confirming that approximately half of the renal diseases in dogs are immune-mediated in origin.[1]

In our series, the agreement between LM and TEM diagnosis for either morphological diagnoses or disease categories was only moderate. Hence, our results emphasize the importance of TEM in the diagnostic evaluation of RBs because this tool can identify lesions that would go undetected by LM. Identification of

Table 2. Mean values of different variables in 162 dogs with kidney disease, according to morphological diagnoses based on electron microscopy. Only variables with significant differences among morphological diagnoses are shown.

	Morphological Diagnosis Mean ± standard deviation (median; min–max) [N]									
	MGN	MPGN	MixGN	FSGS II	MCD	AMY	FSGS I	JN	MD	No lesions
Age (months)	65.9 ± 43.5 (48; 15–143) [13]	80.3 ± 35.7 (80; 1–141) [29]	62.6 ± 29.6 (59; 6–128) [19]	76.6 ± 35.0 (87; 17–129) [18]	88.1 ± 42.2 (90; 14–138) [9]	92.5 ± 43.2 (100; 23–165) [11]	85.7 ± 34.4 (88; 22–144) [35]	48.9 ± 44 (40.5; 5–103) [8]	35.3 ± 24.7 (29; 3–79) [9]	26.5 ± 0.7 (26.5; 26–27) [3]
Leukocytes count ($\times 10^9$/L)	11.9 ± 3.3 (11.6; 7.5–16.4) [8]	9.5 ± 4.9 (7.8; 4.8–23.0) [13]	8.8 ± 3.1 (8.1; 5.1–13.2) [9]	10.8 ± 6.8 (8.5; 7.0–26.2) [7]	19.9 ± 3.6 (21.3; 15.8–22.6) [3]	12.4 ± 7.2 (11.4; 4.9–28.9) [8]	9.3 ± 2.1 (8.6; 6.7–13.2) [10]	9.9 ± 1.8 (10.2; 8–11.6) [3]	18.7 ± 6.7 (18.7; 13.9–23.4) [2]	nt
Serum phosphorus conc (mmol/L)	1.86 ± 0.84 (1.47; 1.07–3.62) [10]	2.16 ± 0.94 (1.88; 1.08–4.6) [25]	1.73 ± 0.69 (1.60; 0.86–3.36) [15]	1.43 ± 0.53 (1.40; 0.43–2.71) [13]	1.57 ± 0.43 (1.55; 1.16–2.27) [5]	1.98 ± 1.64 (1.39; 1.10–5.97) [8]	1.49 ± 0.43 (1.40; 0.77–2.57) [21]	2.58 ± 1.99 (1.90; 0.90–6.80) [7]	4.66 ± 4.46 (2.60; 1.86–12.49) [5]	2.03 ± 0.19 (2.03; 1.89–2.16) [2]
Serum albumin conc (g/L)	15.5 ± 5.6 (14.1; 9.9–30.0) [12]	19.3 ± 5.3 (20.3; 10.5–28.0) [26]	18.4 ± 5.9 (16.8; 11.0–32.0) [16]	26.9 ± 6.9 (29.2; 16.0–37.0) [15]	30.0 ± 8.4 (30.0; 15.0–3.2) [6]	15.8 ± 5.2 (16.0; 7.0–21.9) [9]	25.6 ± 5.2 (24.9; 13.0–34.0) [24]	30.0 ± 4.6 (29.0; 23.0–37.0) [7]	26.8 ± 6.5 (29.0; 18.0–35.1) [5]	23.9 ± 3.0 (23.9; 21.8–26.0) [2]
Serum total proteins conc (g/L)	44.7 ± 15.6 (39.5; 29.0–71.0) [12]	53.7 ± 12.4 (54; 30.9–83.0) [25]	50.6 ± 12.1 (51.0; 36.6–78.5) [15]	61.8 ± 12.2 (61.0; 42.2–83.2) [15]	62.4 ± 8.1 (64.5; 49.0–70.0) [5]	45.5 ± 11.2 (49.0; 27.7–65.4) [9]	58.2 ± 7.9 (59.5; 36.0–70.0) [21]	60.0 ± 7.2 (57.0; 53.0–71.9) [7]	60.8 ± 2.5 (60.5; 58.0–64.1) [4]	53.0 ± 12.7 (53.0; 44.0–62.0) [2]
Serum creatinine conc (μmol/L)	117.6 ± 53.7 (99.8; 55–214) [12]	238.2 ± 150 (189.5; 55–570) [27]	157.8 ± 116 (114.5; 60–457) [16]	131.0 ± 74.9 (126.5; 25–260) [16]	70.8 ± 25.6 (65.7; 36–107) [7]	191.0 ± 231 (105.5; 79–756) [8]	149.4 ± 86.7 (132.0; 25–360) [28]	129.9 ± 60.1 (124.5; 24–191) [7]	238.2 ± 249 (156.0; 54–728) [6]	83.3 ± 46.0 (68.0; 47–135) [3]
Serum urea nitrogen conc (mmol/L)	9.66 ± 6.23 (7.65; 3.8–22.7) [10]	24.5 ± 13.75 (23.30; 5.3–43.2) [14]	17.50 ± 13.1 (13.80; 3.1–42.0) [13]	11.56 ± 6.95 (11.70; 1.7–25.0) [11]	5.65 ± 2.73 (5.75; 1.9–9.0) [6]	8.81 ± 6.16 (5.00; 2.9–19.8) [7]	12.02 ± 12.2 (8.10; 1.2–51.8) [15]	12.42 ± 5.56 (13.60; 5.5–19.6) [5]	34.04 ± 47.8 (15.10; 1.0–105.0) [4]	Nt
Urine specific gravity	1.030 ± 0.01 (1.027; 1.018–1.046) [10]	1.020 ± 0.01 (1.018; 1.008–1.049) [22]	1.032 ± 0.01 (1.030; 1.021–1.050) [13]	1.019 ± 0.01 (1.015; 1.005–1.019) [15]	1.034 ± 0.01 (1.030; 1.023–1.050) [3]	1.017 ± 0.00 (1.016; 1.012–1.025) [8]	1.019 ± 0.01 (1.018; 1.007–1.033) [25]	1.019 ± 0.01 (1.019; 1.011–1.028) [8]	1.019 ± 0.02 (1.014; 1.006–1.048) [3]	1.029 ± 0.02 (1.020; 1.017–1.050) [3]
UPC[1]	13.41 ± 8.44 (12.20; 2.60–32.86) [12]	7.52 ± 5.03 (5.68; 0.95–19.2) [27]	10.68 ± 8.88 (8.27; 1.17–33.45) [16]	7.73 ± 16.51 (3.65; 0.20–69.0) [16]	3.76 ± 1.87 (3.00; 1.61–6.16) [7]	7.00 ± 2.20 (6.69; 3.17–10.65) [8]	4.34 ± 2.90 (3.35; 1.05–12.00) [30]	2.20 ± 2.07 (1.77; 0.10–4.85) [6]	2.37 ± 2.52 (1.90; 0.06–6.60) [5]	5.54 ± 5.81 (2.69; 1.70–12.23) [3]

MGN, membranous glomerulonephritis; MPGN, membranoproliferative glomerulonephritis; MixGN, mixed glomerulonephritis; FSGS II, secondary focal and segmental glomerulosclerosis; MCD, minimal change disease; AMY, amyloidosis; FSGS I, primary focal and segmental glomerulosclerosis; JN, juvenile nephropathies; MD, miscellaneous diseases; UPC, urine protein-to-creatinine ratio; conc, concentration; nt, not tested.

Table 3. Distribution of 162 dogs with kidney disease according to morphological diagnoses based on electron microscopy and different categorical variables. Only variables with significantly different proportions among morphological diagnoses are shown.

	Morphological diagnosis [n (%)]									
	MGN	MPGN	MixGN	FSGS II	MCD	AMY	FSGS I	JN	MD	No lesions
History										
Urinary tract infections										
Yes	2 (33.3)	1 (9.1)	1 (9.1)	4 (57.1)	3 (75.0)	2 (28.6)	8 (61.5)	3 (60.0)	1 (25.0)	1 (100.0)
No	4 (66.7)	10 (90.9)	10 (90.9)	3 (42.9)	1 (25.0)	5 (71.4)	5 (38.5)	2 (40.0)	3 (75.0)	0 (0.0)
Ascites										
Yes	6 (75.0)	3 (20.0)	6 (50.0)	0 (0.0)	0 (0.0)	1 (16.7)	1 (9.1)	0 (0.0)	1 (33.3)	0 (0.0)
No	2 (25.0)	12 (80.0)	6 (50.0)	5 (100.0)	4 (100.0)	5 (83.3)	10 (90.9)	4 (100.0)	2 (67.7)	1 (100.0)
Hematology										
Leukocytes count										
Decreased	0 (0.0)	3 (23.1)	3 (33.3)	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Normal	8 (100.0)	9 (69.2)	6 (66.7)	6 (85.7)	1 (33.3)	6 (75.0)	10 (100.0)	3 (100.0)	1 (50.0)	0 (0.0)
Increased	0 (0.0)	1 (7.7)	0 (0.0)	1 (14.3)	2 (66.7)	1 (12.5)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)
Biochemistry										
Albumin concentration										
Decreased	11 (91.7)	18 (69.2)	13 (81.3)	4 (26.7)	1 (16.7)	9 (100.0)	7 (29.2)	0 (0.0)	1 (20.0)	1 (50.0)
Normal	1 (8.3)	8 (30.8)	3 (18.7)	9 (60.0)	3 (50.0)	0 (0.0)	17 (70.8)	6 (85.7)	3 (60.0)	1 (50.0)
Increased	0 (0.0)	0 (0.0)	0 (0.0)	2 (13.3)	2 (33.3)	0 (0.0)	0 (0.0)	1 (14.3)	1 (20.0)	0 (0.0)
Total proteins concentration										
Decreased	9 (75.0)	12 (48.0)	10 (66.7)	3 (20.0)	1 (20.0)	8 (88.9)	4 (19.0)	1 (14.3)	0 (0.0)	1 (50.0)
Normal	3 (25.0)	12 (48.0)	4 (26.7)	9 (60.0)	4 (80.0)	1 (11.1)	17 (81.0)	0 (0.0)	4 (100.0)	1 (50.0)
Increased	0 (0.0)	1 (4.0)	1 (6.6)	3 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (85.7)	0 (0.0)	0 (0.0)
Urinalysis										
Urine color										
Normal	6 (100.0)	16 (100.0)	8 (100.0)	8 (100.0)	4 (100.0)	7 (100.0)	14 (100.0)	3 (100.0)	3 (75.0)	2 (100.0)
Light	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)
Dark	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Glycosuria										
Yes	2 (20.0)	0 (0.0)	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	1 (25.0)	1 (33.3)
No	8 (80.0)	25 (100.0)	11 (91.7)	17 (100.0)	6 (100.0)	8 (100.0)	24 (100.0)	6 (85.7)	3 (75.0)	2 (66.7)
Erythrocytes (n/hpfl)										
No	0 (0.0)	3 (20.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	11 (68.8)	0 (0.0)	0 (0.0)	1 (33.3)
<10	6 (100.0)	9 (60.0)	6 (66.7)	5 (83.3)	1 (33.3)	7 (87.5)	4 (25.0)	2 (66.7)	1 (100)	1 (33.3)
>10	0 (0.0)	3 (20.0)	3 (33.3)	1 (16.7)	1 (33.3)	1 (12.5)	1 (6.2)	1 (33.3)	0 (0.0)	1 (33.3)
Proteinuria										
Yes	13 (100.0)	27 (100.0)	17 (100.0)	14 (82.4)	6 (85.7)	9 (100.0)	32 (100.0)	4 (57.1)	7 (100.0)	3 (100.0)
No	0 (0.0)	0 (0.0)	0 (0.0)	3 (17.6)	1 (14.3)	0 (0.0)	0 (0.0)	3 (42.9)	0 (0.0)	0 (0.0)

MGN, membranous glomerulonephritis; MPGN, membranoproliferative glomerulonephritis; MixGN, mixed glomerulonephritis; FSGS II, secondary focal and segmental glomerulosclerosis; MCD, minimal change disease; AMY, amyloidosis; FSGS I, primary focal and segmental glomerulosclerosis; JN, juvenile nephropathies; MD, miscellaneous diseases; hpf, high-power field.

immune complexes is necessary for proper classification of renal diseases, and the combined use of LM and TEM allowed us to localize them within the glomerulus and to achieve the final morphological diagnosis. Furthermore, our study confirms the necessity of evaluating RBs with both LM and TEM in FSGS cases, because LM alone cannot differentiate between primary podocyte injury (possibly genetic) and deposition of immune complexes.[8,9]

When considering cases divided according to disease categories, dogs with RL-NOS were significantly younger compared to dogs with ICGN and non-ICGN. This finding was mainly related to the high frequency of JN in this disease category, which is a major cause of chronic kidney disease in young pure breed dogs.[10,11] In contrast to age, sex was similarly distributed among disease categories or morphological diagnoses.

Concerning history, differences were documented for UTI, being less frequent in dogs with ICGN than in those with non-ICGN or RL-NOS. Dogs with UTI may develop renal disease due to bacterial invasion of the kidneys. In this setting, the pathogenesis of kidney damage is not expected to be associated with deposition of immune complexes but, depending on the distribution of bacteria or binding affinity of their toxins, with direct tubular or glomerular lesions, possibly leading to non-ICGN.[12] Indeed, the majority of dogs with a morphological diagnosis of MCD and FSGS I (i.e, non-ICGN) had a history of UTI. Conversely, UTI was reported in <10% of dogs with MPGN and MixGN (i.e, ICGN). Whether bacteria triggered the

Table 4. Mean values of different variables in 162 dogs with kidney disease, according to disease category based on electron microscopy. Only variables with significant differences among disease categories are shown.

	Disease Category Mean ± standard deviation (median; min–max) [N]			
	ICGN	Non-ICGN	RL-NOS	Total
Age (months)	72.8 ± 35.7 (70; 1–143) [79]	87.4 ± 36.9 (90; 14–165) [55]	40.1 ± 33.0 (27; 3–103) [19]	74.0 ± 38.4 (74; 1–165) [153]
Serum albumin concentration (g/L)	20.10 ± 6.92 (19.45; 10.0–37.0) [69]	24.01 ± 7.38 (23.30; 7.0–38.0) [39]	27.99 ± 5.36 (28.95; 18.0–37.0) [14]	22.25 ± 7.38 (22.00; 7.0–38.2) [122]
Serum urea nitrogen concentration (mmol/L)	16.54 ± 12.20 (11.90; 1.7–43.2) [48]	9.86 ± 9.67 (7.30; 1.2–51.8) [28]	22.03 ± 31.63 (13.90; 1.0–105.0) [9]	14.92 ± 14.97 (11.30; 1.0–105.0) [85]
Serum phosphorus concentration (mmol/L)	1.86 ± 0.83 (1.60; 0.4–4.6) [63]	1.62 ± 0.86 (1.41; 0.8–6.0) [34]	3.24 ± 3.03 (2.18; 0.9–12.5) [14]	1.96 ± 1.40 (1.56; 0.4–12.5) [111]
UPC	9.28 ± 10.05 (6.47; 0.20–69.00) [71]	4.73 ± 2.82 (4.60; 1.05–12.00) [45]	2.98 ± 3.28 (2.00; 0.06–12.23) [14]	7.02 ± 8.06 (5.04; 0.06–69.00) [130]

ICGN, immune-complex-mediated glomerulonephritis; non-ICGN, non-immune-complex-mediated glomerulonephritis; RL-NOS, renal lesions not otherwise specified; UPC, urine protein-to-creatinine ratio.

development of MCD and FSGS I or were the consequence cannot be answered. The reason why dogs with RL-NOS were more likely to have UTI as compared to those with ICGN is likely a consequence of the fact that some of the dogs within the former disease category had JN, which has been shown to predispose to UTI.[13]

With regard to urinalysis, proteinuria was more frequent in dogs with ICGN or non-ICGN than in those with RL-NOS, although significance was small. However, the overall magnitude of proteinuria in dogs with ICGN was 2-fold and 3-fold higher than in those with non-ICGN and RL-NOS, respectively. The more severe proteinuria observed in dogs with ICGN explained the fact that on the biochemical profile, hypoalbuminemia was more common, in comparison with the other groups. The marked hypoalbuminemia, in turn, accounted for the more frequently documented hypoproteinemia and hypocalcemia as well as the more common ascites reported in dogs with ICGN. Hence, protein-losing nephropathies due to immune-complex deposition may cause larger alterations in the permselectivity of the glomerular capillary wall than those caused either by non-ICGN or by RL-NOS in dogs. Although dogs with non-ICGN had proteinuria slightly more commonly than those with RL-NOS, the degree of severity did not differ. As expected, the biochemical profile showed no differences in serum albumin concentrations between the 2 groups and the frequency of hypoproteinemia, hypocalcemia, and ascites also was similar. The suspected larger permselectivity of glomeruli in dogs with ICGN might also explain the more frequent hematuria observed in this disease category.

Notably, from a clinical standpoint, differentiation of dogs with 1 or the other disease category based on the extent of proteinuria was not possible due to the fact that there was large overlap among the 3 disease categories. However, in our series none with non-ICGN and RL-NOS dogs had UPC >12.5, whereas 21.1% of those with ICGN had UPC >12.5 (data not shown). Although dogs with renal proteinuria above this threshold might be more likely to have a nephropathy with an immune-complex pathogenesis, in our experience dogs with AMY also can have very high UPC. Among morphological diagnoses of ICGN, dogs with MPGN had the most severe proteinuria. However, differentiating dogs with the different forms of ICGN based on the extent of proteinuria was not possible. Unexpectedly, approximately 10% of dogs with FSGS II had no proteinuria. The absence of proteinuria in a disease characterized by glomerular deposition of immune complexes might suggest that in some affected dogs, increased tubular reabsorption capacity was present that compensated for the protein loss. Alternatively, the sclerosis might indicate that an improvement of glomerular lesions has occurred.[14,15] Unfortunately, it was unknown whether dogs were receiving any medication at the time of RB. Similarly to ICGN, it was not feasible to differentiate morphological diagnoses between dogs with non-ICGN and RL-NOS. Also, dogs with AMY (i.e, non-ICGN), which frequently is associated with severe proteinuria, had protein loss that overlapped with dogs affected by other non-ICGN.[16]

The biochemical profile of dogs with ICGN showed higher serum urea nitrogen concentrations, but not serum creatinine concentration. This difference was observed in dogs with non-ICGN and not those with

Table 5. Distribution of 162 dogs with kidney disease according to disease category based on electron microscopy and different categorical variables. Only variables with significantly different proportions among disease categories are shown.

	Disease category [n (%)]			
	ICGN	Non-ICGN	RL-NOS	Total
History				
Urinary tract infections				
Yes	8 (22.9)	13 (54.2)	5 (50.0)	26 (37.7)
No	27 (77.1)	11 (45.8)	5 (50.0)	43 (62.3)
Ascites				
Yes	15 (37.5)	2 (9.5)	1 (12.5)	18 (26.1)
No	25 (62.5)	19 (90.5)	7 (87.5)	51 (73.9)
Hypoalbuminemia				
Yes	23 (100)	16 (94.1)	1 (50.0)	40 (95.2)
No	0 (0.0)	1 (5.9)	1 (50.0)	2 (4.8)
Biochemistry				
Albumin concentration				
Decreased	46 (66.7)	17 (43.6)	2 (14.3)	65 (53.3)
Normal	21 (30.4)	20 (51.3)	10 (71.4)	51 (41.8)
Increased	2 (2.9)	2 (5.1)	2 (14.3)	6 (4.9)
Total proteins concentration				
Decreased	34 (50.7)	13 (37.1)	2 (15.4)	49 (42.6)
Normal	28 (41.8)	22 (62.9)	11 (84.6)	61 (53)
Increased	5 (7.5)	0 (0.0)	0 (0.0)	5 (4.3)
Calcium concentration				
Decreased	22 (36.7)	9 (25.7)	3 (23.1)	34 (31.5)
Normal	35 (58.3)	23 (65.7)	6 (46.2)	64 (59.3)
Increased	3 (5.0)	3 (8.6)	4 (30.8)	10 (9.3)
Urinalysis				
Urine color				
Normal	38 (100)	25 (100)	8 (88.9)	71 (98.6)
Light	0 (0.0)	0 (0.0)	1 (11.1)	1 (1.4)
Dark	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Glycosuria				
Yes	3 (4.7)	0 (0.0)	3 (21.4)	6 (5.2)
No	61 (95.3)	38 (100)	11 (78.6)	110 (94.8)
Erythrocytes (n/hpf)				
No	3 (8.3)	12 (44.4)	1 (14.3)	16 (22.9)
<10	26 (72.2)	12 (44.4)	4 (57.1)	42 (60.0)
>10	7 (19.4)	3 (11.1)	2 (28.6)	12 (17.1)
Proteinuria				
Yes	71 (95.9)	47 (97.9)	14 (82.4)	132 (95.0)
No	3 (4.1)	1 (2.1)	3 (17.6)	7 (5.0)

ICGN, immune-complex-mediated glomerulonephritis; non-ICGN, non-immune-complex-mediated glomerulonephritis; RL-NOS, renal lesions not otherwise specified; hpf, high-power field.

RL-NOS. This observation might be partly explained by an increment in the protein catabolism to counteract the lower oncotic pressure linked to hypoalbuminemia in dogs with ICGN. With regard to serum creatinine concentration, dogs with MCD had lower concentrations than did those with MPGN. In several studies, increases in serum creatinine concentration correlated more with tubulo-interstitial than glomerular damage.[17,18] Minimal change disease is by definition characterized by normal glomeruli on LM, and lesions are detected only by TEM. In contrast, MPGN can have variable involvement of the tubulo-interstitium and associated histological changes. The absence of tubulo-interstitial damage in MCD in this particular case can, at least partially, explain why serum creatinine concentration was lower compared to concentrations in dogs with MPGN.

Furthermore, the biochemical profile showed that dogs with RL-NOS were more likely to have hypercalcemia and hyperphosphatemia. Increased concentrations of phosphorus and, less often, of calcium are observed if glomerular filtration rate is severely impaired. However, serum creatinine concentrations did not differ among the 3 disease categories, suggesting that the estimated extent of renal dysfunction probably was not responsible for these results. Because dogs with RL-NOS included some with JN, which might be diagnosed at a young age, it is possible that hypercalcemia and hyperphosphatemia were associated with physiologic growth. Indeed, among dogs with RL-NOS, 25% were <1 year old (data not shown).

Because AMY is relatively easy to diagnose by LM and Congo red staining and FSGS I still represents a diagnostic conundrum, we decided to compare the 2 morphological diagnoses with the entire group of ICGN. Anemia was less frequent in dogs with AMY, and their urine was less concentrated. The reason for the former observation is elusive, whereas the latter might be explained by the fact that amyloid deposits may be often observed also in the medulla, possibly decreasing the hypertonicity of this compartment and, in turn, water reabsorption.[19] In dogs with FSGS I, hypoalbuminemia and hypoproteinemia as well as proteinuria and hematuria were less marked than in the entire group of ICGN; these results are consistent with the above differences observed between ICGN and the entire group of non-ICGN.

One limitation of our study is the lack of IF data. Indeed, IF integrated with LM and TEM may help better characterize glomerulonephritis in dogs, further refining the morphological diagnosis. Another relevant limitation is represented by the fact that different laboratories performed blood testing and urinalysis, with reference ranges that might have differed. However, the potential bias probably was evenly distributed among disease categories and morphological diagnoses, decreasing its confounding effect.

In conclusion, our study provides useful information about the frequency of renal diseases in dogs across Europe, with ICGN and non-ICGN representing almost 90% of the RBs. From a clinical perspective, dogs with ICGN, in particular those with MPGN, had more severe proteinuria than did those with non-ICGN or RL-NOS, leading to more severe hypoalbuminemia. Clinical and laboratory differentiation among dogs with the different morphological diagnoses and among dogs with different disease categories was difficult due to overlapping results. Based on our results, RBs examined by LM and TEM are recommended to allocate dogs with renal lesions to specific morphological diagnoses and disease categories.

Footnote

[a] SPSS Statistics 20.0; SPSS Company, Wacker Drive, Chicago, IL

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Conflict of Interest Declaration: Eric Zini serves as Associate Editor for the Journal of Veterinary Internal Medicine. He was not involved in review of this manuscript. None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Distribution of 162 dogs with kidney disease according to morphological diagnoses.

Table S2. Mean values of different variables in 162 dogs with kidney disease, according to morphological diagnoses.

Table S3. Distribution of 162 dogs with kidney disease according to disease category.

Table S4. Mean values of different variables in 162 dogs with kidney disease, according to disease category.