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Use of Transmission Electron Microscopy in the Diagnosis of Canine Kidney Disease

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Similar to humans, dogs develop renal disease that result in specific changes in blood work and urine [1]. A major cause of kidney disease is injury to the glomerulus, which is the filtering apparatus. Glomerular injury can be secondary to autoimmune processes or from non-immune mediated mechanisms. Ultrastructural evaluation using transmission electron microscopy (TEM) is one of the main modalities for evaluating the glomeruli [2]. Until recently, it was difficult to correctly diagnose canine glomerular disease in a timely fashion, allowing institution of interventional therapies. As such, evaluation of canine glomeruli with TEM was limited to autopsy specimens with significant tissue degradation and "end-stage renal disease." [3] The International Veterinary Renal Pathology Service (IVRPS) was created in 2005 as a proof of concept model to diagnose renal biopsy materials from dogs and cats. Our hypothesis is that TEM examination of canine glomeruli enhances the diagnostic accuracy in renal biopsy evaluation and some diseases require TEM for diagnosis.

In order to better characterize and classify canine glomerular disease, the IVRPS established tissue collection protocols, including sample submission in 3% glutaraldehyde, Michel's Transport Medium (for immunofluorescence, IF) and formalin. Samples were processed routinely for light microscopy (LM), TEM and IF by the Ohio State University Comparative Mouse Phenotype Shared Resource laboratory. Specifically, TEM samples were embedded in epon blocks, sectioned at 1 μm thickness and stained with Toludine blue to identify regions with glomeruli. Regions of interest were then further sectioned to 55-60nm thickness, placed on copper grids, stained with uranyl acetate and lead citrate, and then examined using a JOEL-JEM-1400 transmission electron microscope at the Nephropathology Core at the Ohio State University, School of Medicine. Given the workflow of the IVRPS, LM diagnoses were rendered earlier than TEM diagnoses. Cases in which LM evaluation was insufficient were included for this study, over a period of 24 months. Pathology reports and tissue specimens were reviewed retrospectively to determine which types of glomerular lesions require TEM for a final diagnosis and to identify cases in which TEM led to reversal of the LM diagnosis.

A total of 318 specimens of canine kidneys were evaluated by the IVRPS over the course of 2 years. LM diagnoses were rendered within 24 to 72 hours after receipt of the renal tissue, whereas TEM evaluation occurred within 1 to 3 weeks (average of 14 days). Final diagnoses required TEM in 41 cases, representing 13% of all canine cases examined by the IVRPS. This fraction requiring TEM is less than the previously reported 25% of cases from a study of 501 renal specimens.[1] Twenty-seven of the 41 cases (66%) were diseases that did not involve immune complex deposition, and the remaining 14 cases (34%) were types of immune complex-mediated glomerulonephritis. The distinction between these 2 categories of disease is vital for deciding whether or not immunosuppression is warranted [4]. TEM led to reversal of the LM diagnosis in 7 cases. In these particular instances, there was a strong level of confidence in the LM diagnosis, and the TEM findings were unexpected. In 13 cases, TEM was useful in the diagnosis of diseases that are considered rare in veterinary medicine (e.g. minimal change disease, mesangioproliferative glomerulonephritis and thrombotic microangiopathy).

Use of TEM is feasible in a veterinary diagnostic laboratory setting and diagnoses are rendered within an average of 2 weeks. TEM evaluation improves diagnostic accuracy: it allows distinction between two major disease categories and impacts the therapeutic plan. Furthermore, it is required for the diagnosis of rare veterinary disease. Lastly, in a small subset of cases, TEM led to the reversal of the LM diagnosis [5].

References:

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[5] The author graciously recognizes the work of the Comparative Mouse Phenotyping Shared Resource at the Ohio State University

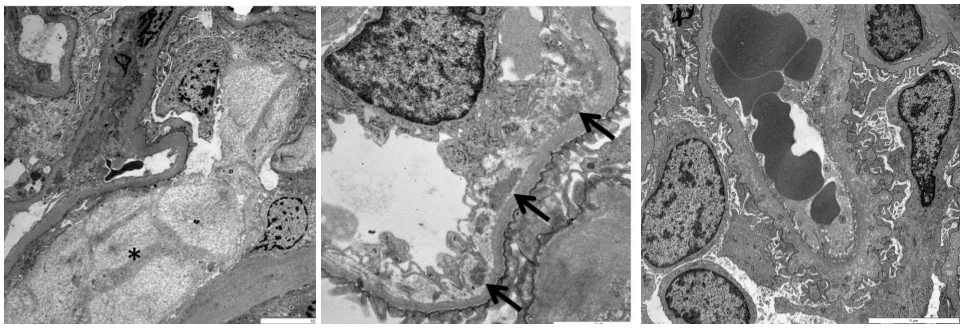

Figure 1. Example of TEM images from canine glomerular biopsies. A) The LM evaluation did not reveal any lesions and TEM was required to diagnose injury to podocytes and parietal epithelial cells (*). B) LM evaluation was reversed when unexpected electron dense deposits (arrows) were identified via TEM. C) TEM led to the identification of a severe glomerular basement membrane abnormality, resulting in the diagnosis of a rare disease.

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