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Case Report

Histopathological Changes in Dogs in Cases of *Ehrlichia canis*

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Abstract

Canine Monocytic Ehrlichiosis (CME), a disease affecting dogs, leads to blood parasitism and damage to multiple organs. A dog in Blitar was diagnosed with CME but could not be saved. Therefore, this study aimed to identify organ damage caused by CME through histopathological examination. A dog from the Blitar district that died due to CME immediately underwent a necropsy. Organ samples were preserved and processed for anatomical pathology examination. Ehrlichia canis detection was performed using rapid tests and PCR. Blood and serum samples were analyzed using an automatic hematology and chemistry analyzer. Histopathological examination of the kidneys revealed mild autolysis in the renal cortex and medulla. Inflammatory cells, predominantly mononuclear cells, especially macrophages, were observed around blood vessels and the glomerular base. The histopathological analysis of the spleen showed an increased diameter of lymphoid follicles of varying sizes. Examination of the liver revealed perivasculitis and dilated sinusoids. Hematological and blood chemistry analyses indicated pancytopenia, non-regenerative anemia, and azotemia. Based on histopathological and clinical pathology findings, the animal died due to organ damage, specifically chronic interstitial nephritis, which resulted in stage-four chronic kidney failure.

Keywords: Ehrlichia canis; histopathology; kidney failure

Introduction

The intracellular parasite Ehrlichia canis from the family Anaplasmataceae and order Rickettsiales causes Canine Monocytic Ehrlichiosis (CME) (Milonakis *et al.,* 2019). Ehrlichiosis is a disease that commonly affects dogs and is transmitted through a vector classified under Canine Vector-Borne Diseases (Sainz *et al.,* 2015). This disease is transmitted by the brown dog tick (Rhipicephalus sanguineus) (Hess *et al.,* 2006). The organism is classified as prokaryotic, Gram-negative (appearing red in Gram staining), and obligate intracellular pleomorphic (Harrus *et al.,* 1997). There are five important species within the genus Ehrlichia: E. canis, E. chaffeensis, E. ewingii, E. muris, and E. ruminantium (Fuente *et al.,* 2006).

Dogs infected with Ehrlichia canis may go through an acute or subclinical phase, which often does not present specific clinical symptoms (Waner & Harrus, 2013). If an animal in the acute phase does not receive appropriate medical treatment, the disease progresses to the chronic phase (Brown *et al.,* 2000). The occurrence of CME is correlated with both acute and chronic kidney failure (Crilliventi *et al.,* 2015). A study by Fuente et al. (2015) reported that 73.3% of dogs infected with Ehrlichia canis developed stage-one kidney failure.

Common clinical symptoms of kidney failure include anorexia (84%), lethargy (77%), vomiting (55%), and diarrhea (37%) (Dunaevich *et al.,* 2020). Other clinical signs include polyuria, polydipsia, anorexia, vomiting, weight loss, pale mucous membranes, oral ulcers, halitosis, and acute blindness (Chan et al., 2015; Fuente *et al.,* 2006). The high levels of urea and creatinine, which are waste products circulating in the blood, cause complications in various tissues (Chandler *et al.,* 2007). In cases of chronic kidney failure, where kidney function declines by more than 75%, treatment is primarily symptomatic and supportive (Brown *et al.,* 2000). The therapy aims to alleviate pain and support the patient's condition due to azotemia (Chew *et al.,* 2015).

A case report on ehrlichiosis in Kintamani dogs in Bali, based on history, clinical symptoms, routine hematology results, and test kit examinations, showed that 20.2% of the tested dogs were positive for E. canis (Erawan *et al.,* 2017). Another case report on the detection of ehrlichiosis in dog patients in Yogyakarta using PCR found a 7.63% positivity rate (Nesti *et al.,* 2018). The incidence of ehrlichiosis in dogs tends to increase each year, yet research on histopathological and clinical pathology changes associated with the disease remains limited (Adrian *et al.,* 2016). The objective of this study is to investigate organ damage and clinical pathology changes in cases of ehrlichiosis in dog patients in Blitar, Indonesia, to aid in diagnosis and determine appropriate therapy for managing ehrlichiosis in dogs.

Materials and Methods

Anamnesis and Physical Examination

Anamnesis was conducted by interviewing the pet owner to obtain information regarding the animal's medical history, particularly tick infestations, use of ectoparasitic treatments, and environmental sanitation. The physical examination included an assessment of the conjunctiva, body condition score, body temperature, dehydration level, appetite, general physical condition, and urinary tract health of the patient (Yanuartono *et al.,* 2017).

Blood and Serum Sample Collection

Blood sample collection was carried out in May 2023 at Sekar Satwa Animal Clinic, Blitar. A 3 ml blood sample was taken from the cephalic vein. Of this, 2 ml was placed into a vacuum tube to separate the serum from the blood, while 1 ml was placed into an EDTA tube.

Blood Smear Examination

Microscopic examination was performed using the thin blood smear staining method, where the smear was stained with 10% Giemsa solution (Merck®) and fixed with methanol for 5 minutes. The stained samples were observed under a light microscope (Olympus, Japan) with 1000x magnification (Saintz *et al.,* 2015).

Hematology and Blood Chemistry Examination

Routine hematology tests were conducted using an automatic hematology analyzer (Mindray DP 10®) to measure total erythrocyte count, hemoglobin concentration, hematocrit value, platelet count, total leukocytes, neutrophils, eosinophils, basophils, lymphocytes, and monocytes (Saintz *et al.,* 2015).

Blood chemistry analysis was performed using a semi-automatic chemical analyzer (Mindray®) to measure BUN (Blood Urea Nitrogen), creatinine, and calcium levels (Corbin *et al.,* 2013).

Histopathological Examination

Following necropsy, anatomical pathology examination was performed, and organ samples were collected and fixed in 10% Buffered Neutral Formalin (BNF). The tissue samples were then processed to prepare histopathological slides and stained using the Hematoxylin-Eosin (HE) staining method.

Antigen Detection

Ehrlichia canis antigen detection is carried out using rapid tests and PCR

Results and Discussion

Detection of Ehrlichia canis

Antigen detection was performed to confirm that the causative agent was responsible

for the animal's morbidity. Detection using antibodies was conducted through a rapid test. The principle of this test involves the use of synthetic peptides that can bind to antibodies produced by E. canis, E. chaffeensis, and E. ewingii (Harrus *et al.,* 1997).

A co-polymer in the synthetic peptide and bovine serum albumin is coated with colloidal gold particles and used in a double antigen sandwich assay to visualize the antibodies formed during the infection process (Adrian *et al.,* 2016). Antibodies binding to the antigen coated with gold particles flow along the test strip and are captured by the antigen present on the T (Test) line, producing a red line visualization (Hess, 2006). The rapid test used is shown in Figure 1.

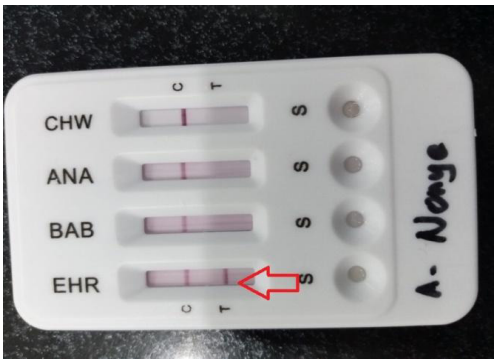

Figure 1. Positive Rapid Test for Ehrlichia canis

Serological testing using a rapid test kit is useful for establishing a diagnosis, as this test can detect antibodies against Ehrlichia sp. and Anaplasma sp. (Erawan *et al.,* 2017). However, it is important to note that the use of rapid test kits may yield false-negative results when the animal is in the acute phase of the disease, which lasts between seven to fourteen days (Fuente *et al.,* 2006; Sainz *et al.,* 2015), as well as in cases of anaplasmosis (Nesti *et al.,* 2018). Serological testing using PCR on the sample confirmed a positive PCR result (sample number 17), indicated by the appearance of a white band in the sample. The PCR test results are presented in Figure 2, marked with an arrow.

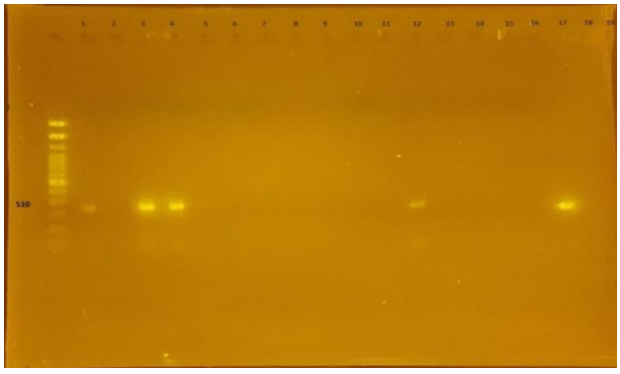

Figure 2. PCR Test Results Showing a White Band

Hematology Profile of the Canine Patient with a Positive PCR Result

Hematology and blood chemistry tests were performed to assess the severity of the condition and to aid in the diagnosis of the pathogenic agent. The hematology test results of the dog infected with CME are presented in Table 1.

Table 1. Results of routine hematology and blood chemistry examinations

Parameter	Reference	results	details
RBC (10^6/L)	5,5-8,5	3,77	Decrease
Haemoglobin (g/L)	110-190	78	Decrease
Hematocrit (%)	39-56	23,9	Decrease
MCV (fL)	62-72	63,5	Normal
MCH (pg)	20-25	20,6	Normal
MCHC (g/L)	300-380	326	Normal
Leukocyte (10^3/L)	6-17	4,6	Decrease
Limphocyte (10^3/L)	0,8-5,1	2,3	Normal
Monosit (10^3/L)	0-1,8	0,4	Normal
Neutrophil (10^3/L)	4-12,6	1,9	Decrease
Eosinophil(10^3/L)	0,4-1,2	0,3	Decrease
Platelet (10^6/L)	117-460	15	Decrease
BUN (mg/dL)	10-25	55	increase
Creatinin (mg/dL)	0,4-1,8	13,2	Increase

Test Results of the Canine Patient

Routine hematology tests on the dog's blood sample indicated thrombocytopenia, anemia, leukopenia, eosinopenia, and neutropenia. These findings align with previous studies stating that anemia and thrombocytopenia are the most common hematological results in CME cases (Adrian *et al.,* 2016). The clinical symptoms observed in the patient included constipation, vomiting, BCS 2 (Body Condition Score), uveitis, edema, and coughing, which are common symptoms similar to those of other diseases. The clinical manifestations and severity of the disease depend on the specific Ehrlichia species involved and the host's immune response (Hess *et al.,* 2006).

The chronic phase is characterized by a decline in hematological values, as Ehrlichia

canis has the ability to infect progenitor cells in the bone marrow (Moritz and Becker, 2010). Anemia is a common complication resulting from chronic kidney failure (Brancaccio *et al.,* 2004). The type of anemia observed is non-regenerative normocytic-normochromic anemia, which occurs due to the loss or limited response of the bone marrow in erythropoiesis (Akmal *et al.,* 1985; Car, 2001).

Thrombocytopenia is marked by vascular collapse and increased secondary infections, caused by several factors, including: Reduced platelet production by the bone marrow, Platelet sequestration in an enlarged spleen, and Platelet destruction (Stockham & Scott, 2008; Moritz & Becker, 2010). Neutropenia and leukopenia occur due to a decrease in precursor cells in the bone marrow as a result of ehrlichiosis (Dunaevich, 2010).

Azotemia is a condition characterized by an increase in urea, creatinine, or other non-protein nitrogen compounds in the blood, plasma, or serum (Triakoso, 2020). Blood chemistry tests indicate an elevated Blood Urea Nitrogen (BUN) level compared to the normal range of 10–20 mg/dL (Vaden *et al.,* 2009). This BUN increase occurs without any indication of urinary tract obstruction (post-renal azotemia), suggesting that the case involves pre-renal azotemia, renal azotemia, or a combination of both (Tilley *et al.,* 2011; Corbin *et al.,* 2013). Pre-renal azotemia is generally caused by reduced renal perfusion due to hypovolemia, heart failure, renal vasoconstriction, increased nitrogenous waste production from tissue catabolism due to infection, high-protein diets, or gastrointestinal bleeding (Triakoso, 2020).

Renal azotemia occurs due to decreased kidney function, especially glomerular function, which can result from primary intrinsic kidney diseases (such as glomerulonephritis) or secondary kidney disorders due to renal ischemia (Bartges, 2012; Codner *et al.,* 1992). The azotemia in this case is classified as both pre-renal and renal, as there is tissue damage, gastrointestinal bleeding, and kidney damage (Crilliventi *et al.,* 2015). These findings align with the anamnesis and clinical symptoms, including melena, non-regenerative anemia, and nephropathy.

Histopathology Examination Results

Suspicion of kidney disorders was confirmed through histopathological examination, revealing glomerulonephritis, and even indications of glomerulopathy and chronic interstitial nephritis (Figure 3). Chronic ehrlichiosis is often accompanied by end-stage renal failure, which is characterized by elevated BUN and creatinine levels (McGavin & Zachary, 2012). The histopathological findings can be observed in Figure 3.

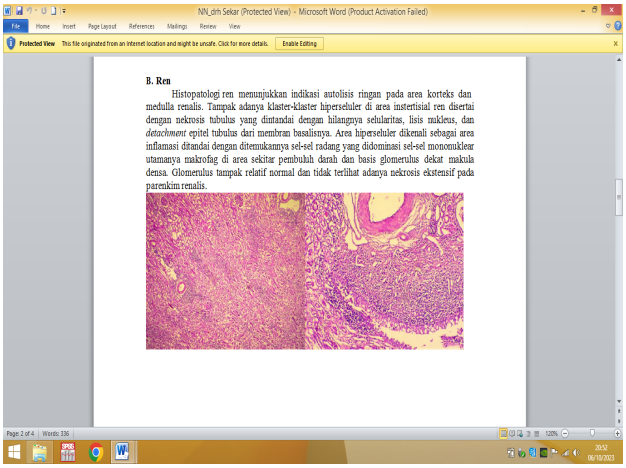

Figure 3. Inflammatory Cell Infiltration Dominated by Macrophages (1000x Magnification)

The renal histopathology indicates mild autolysis in the cortical and medullary regions of the kidney. Clusters of hypercellular areas are observed in the renal interstitial space, accompanied by tubular necrosis, characterized by loss of cellularity, nuclear lysis, and detachment of tubular epithelium from the basement membrane. The hypercellular areas are identified as inflammatory regions, marked by the presence of inflammatory cells, predominantly mononuclear cells, especially macrophages, surrounding blood vessels and the glomerular base near the macula densa. The glomeruli appear relatively normal, with no signs of extensive necrosis in the renal parenchyma.

Kidney failure occurs as a result of a series of immune responses that initially function to combat Ehrlichia canis (Yang *et al.,* 2015). Macrophage infiltration in the kidneys is a common characteristic of chronic kidney disease (Nikolic *et al.,* 2014). Macrophages play a role in phagocytosis, the production of pro-inflammatory cytokines, and the generation

of toxic metabolites to fight pathogens (Rosin & Okusa, 2011). The correlation between the degree of macrophage infiltration and the severity of kidney injury suggests that macrophage effector functions are linked to both tissue damage and repair (Wang & Harris, 2011).

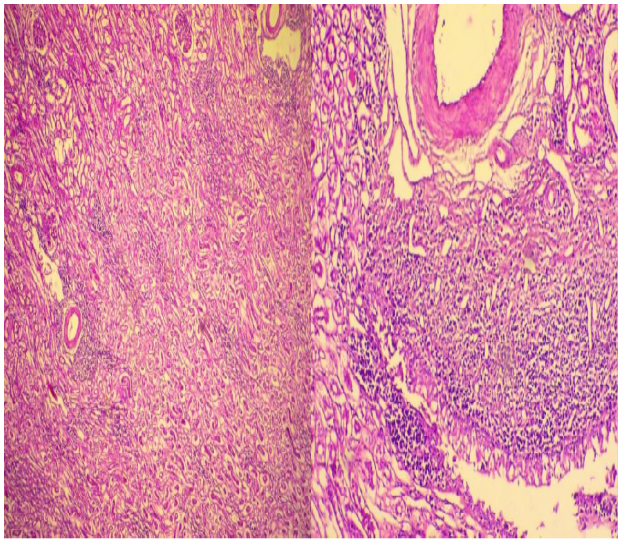

Figure 4. Tubular Necrosis (Arrow Indicated)

Histopathological examination of the kidneys revealed tubular hemorrhage, hydropic degeneration, and necrosis (Figure 4). Tubular necrosis is characterized by eosinophilic cytoplasm, karyopyknosis (nuclear shrinkage), and detachment of tubular epithelial cells from the basement membrane. Hydropic degeneration in renal tubular epithelial cells represents an advanced form of acute cellular swelling, caused by the influx of extracellular fluid into the cytoplasm (Tisher & Brenner, 2008). Another notable finding was protein deposits in the tubular lumen, indicating impaired protein reabsorption by the tubules (Fogo *et al.,* 2006). Tubular epithelial damage may result from bloodborne infections, ascending infections, toxins, and ischemia (McGavin & Zachary, 2012).

The pathogenesis of CME is associated with antibody-dependent cytotoxicity, which leads to severe vasculitis in multiple organs, with perivascular plasma cell inflammatory infiltration being the dominant feature (Mylonakis *et al.,* 2019; Castro *et al.,* 2004). Perivascular lymphohistioplasmacytic infiltration in the renal cortex without damage to the vessel walls suggests that other mechanisms may be involved in the development of CME-related kidney lesions (Crivellenti *et al.,* 2015). Fibrosis with inflammatory cell infiltration is a common feature observed in the chronic phase of Ehrlichia canis infection (Harrus *et al.,* 1997).

Increased serum urea and creatinine levels are due to tubular dilation, atrophy, and glomerulopathy (Grauer, 2011). Renal damage includes membranoproliferative glomerulonephritis and interstitial nephritis (Tisher & Brenner, 2008). Membranoproliferative glomerulonephritis occurs due to immune complex deposition as the body responds to Ehrlichia canis infection (Crivellenti *et al.,* 2015). This condition is often associated with protein loss during urine filtration in the kidneys (Corbin *et al.,* 2013). Interstitial nephritis is caused by lymphocyte infiltration into the nephrons (Fogo *et al.,* 2006). CME can lead to sepsis, which ultimately results in death (Merx & Weber, 2007). Sepsis occurs when bacteria spread throughout the body via the bloodstream, leading to severe systemic infection, multi-organ failure, and ultimately, death (Purnama, 2014).

Conclusion

Based on histopathological and clinical pathology examinations, the animal died due to sepsis and organ damage, specifically chronic interstitial nephritis, which led to end-stage (stage four) chronic kidney failure.

References

Adrian, P.Y., Rochelle, H.D., Ybañez1, R.R., Villavelez, H.P.F., Malingin, D.N., Sharmaine, V.N., 2016, Retrospective analyses of dogs found serologically positive for Ehrlichia canis in Cebu, Philippines from 2003 to 2014, *Vet. World*. 9:43- 47.

Akmal, M., Telfer N., Ansari, A.N., Massry, S,G., 1985, Erythrocyte survival in chronic renal failure, Role of secondary hyperparathyroidism, *J Clin Invest*; 76. 65–72.

Bartges, J.W., 2012, Chronic Kidney Disease in Dogs and Cats, Veterinary Clinics of North America, *Small Animal Practice.* 42: 669-692.

Codner, E. C., Cacei, T., Saunders, G. K., Smith, C. A., Robertson, J. L., Martin, R.A.,Troy,G. C., 1992, Investigation of glomerular lesions in dogs with acute experimentally induced Ehrlichia canis infection*, American Journal of Veterinary Research*, Chicago, 12. 2286-2291.

Chew, D.J., 2015, Chronic Kidney Disease (CKD) in Dogs & Cats - Staging and Management Strategies, A Presentation to the Virginia Veterinary Medical Association, *Virginia Veterinary Conference* : 1 – 22.

Brancaccio, D., Cozzolino, M., Gallieni, M.,2004, Hyperparathyroidism and anemia in uremic subjects: a combined therapeutic approach, *J Am Soc Nephrol*, 15. 21– 41.

Brown, S.A., Brown, C.A., Crowell, W.A., Barsanti, J.A., Kang, C.W., Allen, T., Cowell, C., Finco, D.R., 2000, Effects of dietary polyunsaturated fatty acid supplementation in early renal insufficiency in dogs, *J Lab Clin Med*, 135. 275-286.

Car, B.D., 2001, *Schalm's Veterinary Hematolog,* New York: McGraw-Hill. 271—288.

Castro, M. B., Machado, R. Z., Aquino, L. P., Alessi, A. C., Costa, M. T., 2004, Experimental acute canine monocytic ehrlichiosis: clinicopathological and immunopathological findings. Amsterdam, *Veterinary Parasitol,* 115. 73-86.

Chan, S., Au, K., Francis, R.S., Mudge, D.W,, Johnson, D.W., Pillans, P,I., 2017, Phosphate binders in patients with chronic kidney disease, *J Australian Prescriber*, 40. 9-14.

Chandler, M.L., Elwood. C., Murphy, K.F., Gajanayake, I. and Syme, H.M., 2007, Juvenile nephropathy in 37 boxer dogs, *J Small Anim Pract*. 12. 690-694.

Corbin, A.R., Blois, S.L., Kruth, S.A., Abrams-Ogg, A.C.G., 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.

Crivellenti, L. Z., Silva, G. E. B., Borin , S., Dantas, M., Adin, C. A., Cianciolo, R.,Santana, A. E., 2015, *Glomerulopathies in dogs with erlichiosis - preliminary results*. In: Conference World Small Animal Veterinary Association - WSAVA, 40th, Bangkok.

Dunaevich, A., Chen, H., Musseri, D., 2020, Acute on chronic kidney disease in dogs: Etiology, clinical and clinicopathologic findings, prognostic markers, and survival, *Journal of Veterinary Internal Medicine*, 34. 6.

Erawan, I.G., Sumardika, I.W., Pemayun, I.G.A.G.P., Ardana, I.B., 2017, Laporan kasus: Ehrlichiosis pada anjing kintamani Bali, Indonesia, *Medicus Veterinus*, 6(1). 68-74.

Fogo, A.B., Arthur, H.C., Charles, J., Jan, A.B., 2006, *Fundamentals of Renal Pathology*, New York (US): Springer Science.

Fuente, J., Torina, A., Naranjo, V., Nicosia S., Alongi, A., La Mantia, F., Kocan K.M., 2006, Molecular characterization of Anaplasma platys strains from dogs in Sicily, Italy, *BMCVet Res*, 2. 24-31.

Grauer, G.F., 2011, *Renal Pathology with clinical and functional correlactions*, 2th Ed, JB Lippincoot: Philadelphia.

Harrus, S., Waner, T., Bark, H., 1997, Canine monocytic ehrlichiosis update *Compend ContinEduc Pract Vet*, 19. 431-444.

Harvey, J.W., 2000, Schalm's Veterinary Hematology. 5th ed. Philadephia, Pennsylvania: Lippincott, Williams and Wilkin. 200–204.

Hess, P.R., English, R.V., Hegarty, B.C., Brown, G.D., Breitschwerdt, E.B., 2006, Experimental Ehrlichiacanis infection in the dog does not cause immunosuppression, *Vet Immunopathol,* 109:117-125.

McGavin, M.D., Zachary, J.F., 2012, Pathologic Basic of Veterinary Disease, 5th Ed, St. Louis, Missouri (US): Mosby-Year Book.

Merx, M.W., Weber, C., 2007, Sepsis and the Heart Circulation, J Med, 116: 793- 802.

Moritz, A., Becker, M., 2010, Automated hematology systems, editors. *Schalm's Veterinary Hematology*, 6th ed, Ames, Iowa: Blackwell Publ. 1054–1066.

Mylonakis, M.E., Harrus, S., Breitschwerdi, E.B., 2019, An update on the treatment of canine monocyt ehrlichiosis (Ehrlichia canis), *The Vet J*, 246: 45-53.

Nikolic-Paterson, D.J., Wang, S., Lan, H.Y. 2014. Macrophages promote renal fibrosis through direct and indirect mechanisms. *Kidney Int Suppl* 2014; 4: 34–38.

Nesti, D.R., Baidowi, A., Ariyanti, F., Tjahajati, I,. 2018, Deteksi penyakit zoonosis Ehrlichiosis pada pasien anjing di klinik hewan jogja, *Jurnal Nasional Teknologi Terapan*, 2. 191–197.

Purnama, S., Wilson, L,. 2014, Patofisiologi: Konsep Klinis Proses-Proses Penyakit, 6th Ed, Jakarta (ID): EGC.

Rosin, D.L., Okusa, M.D. 2011. Dangers within: DAMP responses to damage and cell death in kidney disease. *J Am Soc Nephrol*, 22: 416–425.

Sainz. Á., Roura, X., Miró, G., Estrada-peña, A., Kohn, B., Harrus, S., 2015, Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe, *Parasit Vectors*, 8. 75. DOI: 10.1186/s13071015-0649-0.

Stockham, S.L., Scott, M.A., 2008, *Fundamentals of veterinary clinical pathology*, Blackwell Publisher, Iowa, USA.

Tilley, L.P., Smith, F.W.K., 2011, *Wiley-Blackwell's Five-Minute Veterinary Consult, Canine and Feline,* Fifth Edition, USA: Wiley-Blackwell.

Tisher, K., Brenner, B.M., 2006, *Renal Pathology with clinical and functional correlactions*, 2th Ed, JB Lippincoot: Philadelphia.

Triakoso, N., 2020, *Buku Ajar Ilmu Penyakit Dalam Veteriner Anjing dan Kucing*, Surabaya (ID): Airlangga University Press.

Vaden, S.L., Knoll, J.S., Smith, F.W.K., Tilley, L.P., 2009, *Blackwell's Five-Minute Veterinary Consult: Laboratory Test and Diagnostic Procedures: Canine and Feline*, Singapura (SG): Willey-Blackwell.

Wang,Y., Harris, D.C.H., 2011, Macrophages in renal disease. *J Am Soc Nephrol*, 22: 21–27.

Yang, Q., Stevenson, H.L,, Scott, M.J., Ismail, N., 2015, Type I interferon contributes to non canonical inflammasome activation, mediates immunopathology, and impairs protective immunity during fatal infection with lipopolysaccharide-negative ehrlichiae. *Am J Pathol*, 185. 446–61. doi: 10.1016/j.ajpath.10.005

Yanuartono, Nururrozi, A., Indarjulianto, S., 2017, Cronic kidney disease in dog and cat: treatmen and diet management, *J Sain Vet*, 35(1): 16-34.