Research Articles

The concurrence of naturally occurring *Ehrlichia canis* infection and renal dysfunction in dogs: kidney diseases and survival analysis

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Abstract

Canine monocytosis ehrlichiosis (CME) is a common tick-borne infection announced worldwide but the coincident effects on kidney disease in dogs is unclear. This study aims to explore the effect of naturally occurring *Ehrlichia canis* infection on kidney diseases and to evaluate the outcome of CME on survival time in dogs with kidney injury. From the 511 dogs reported death between 2012 to 2017, the 166 dogs with renal insufficiency were included for analysis. The result showed 21.08% (35/166) *E. canis* infection. Among CME dogs, 94.28% (33/35) were simultaneously diagnosed CME together with kidney disease. CME significantly occurred with acute kidney injury (AKI) more than chronic kidney disease (CKD) 5.57 [95%CI: OR (1.99 - 18.45)] times ($p < 0.01$). Also, the *E. canis* infected dogs showed significantly associated with renal death 2.42 [95%CI: OR (1.04 ñ 5.87)] times ($p = 0.04$) compared with non-infected ones. The median survival time of AKI and CKD were 1.5 days [95%CI: (1-3 days)] and 97 days [95%CI: (14-267 days)], respectively. The survival time of both AKI and CKD dogs between with and without CME were not significantly different ($p = 0.71$ and $p = 0.66$, respectively). In conclusion, *E. canis* infection was possibly deteriorated renal dysfunction and was commonly diagnosed with AKI in dogs. The CME should be concerned with kidney injury progression in clinical practice. Further studies are warranted to investigate the pathogenesis of the renal injury caused by CME.

Keywords: acute kidney injury, chronic kidney disease, *Ehrlichia canis*, survival analysis,

Introduction

Canine monocytic ehrlichiosis (CME), caused by *Ehrlichia canis*, is a tick-borne infection from the brown dog tick, *Rhipicephalus sanguineus*, reported worldwide (Neer et al., 2002). The prevalence of this disease determined by molecular technique were 8.6-28% in Asia (Malik et al., 2018, Piratae et al., 2015, Yuasa et al., 2017), 6.7-40.6% in South America (Cicuttin et al., 2016, Rotondano et al., 2017, Vargas-Hernandez et al., 2012), and 6.4-22% in Europe (Sainz et al., 2015). Experimentally exposed to E. canis resulted in acute, subacute and chronic phases with dogs having a variety of clinical signs and laboratory findings, however, as clinical signs were identical, the acute and chronic phases of infection were difficult to distinguish in the practice (Harrus et al., 1999). Without treatment, CME could be a life-threatening condition and cause of death in dogs (Harrus et al., 1999). The infection of *E. canis* might deteriorate many organs include the kidney. *E. canis* DNA was also detected in the renal tissue from naturally infected CME dogs suggesting that this pathogen might contribute to kidney damage (Silva et al., 2016). Therefore, the study aims to 1) explore whether the occurrence of *E. canis* infection associated with the type of kidney diseases and renal death 2) evaluate the outcome of *E. canis* infection on survival time in kidney disease dogs.

Materials and methods

Case selection

The 511 client-own dogs were extracted from the veterinary medical records reported a death in Prasu Arthon Animal Hospital, Mahidol University, Thailand, between March 2012 to June 2017. The 166, out of 511, dogs presenting with clinical signs together with at least 2 characteristics of renal insufficiency were included for our study as follows: (1) blood creatinine 1.4 grams/dl; (2) inappropriate urine concentration or oliguria/ anuria; (3) renal structural abnormality justified by ultrasonography and/or radiology consist of renal calculi, renal calcification, irregular kidney, small size kidney, and hydronephrosis. All dogs with renal insufficiency were then classified as *E. canis* infection and non-infection groups.

CME was carefully identified by the following criteria: (1) demonstration of morulae in buffy coat smear or positive result from the polymerase chain reaction (PCR); (2) presentation of thrombocytopenia and seropositive result from test kit (SNAP® 4Dx Plus®, IDEXX Laboratories, Westbrook, ME, USA) together with clinical signs and responded to the doxycycline treatment. The PCR method of *E. canis* detection was the multiplex PCR which was composed of the detection of *E. canis* and *Hepatozoon canis*. The primers of *E. canis* detection included Ehr1401F 5' CCATAAGCATAGCT GATAACCCTGTTACAA 3í and Ehr1780R 5' TGGATA ATAAAACCGTACTATGTATGCTAG 3' in which a size of PCR product was 380 base pairs (Kledmanee et al., 2009). A total volume of 25 µl PCR reaction contained 12 µl of 2x QIAGEN Multiplex PCR Master Mix (QIAGEN®, Germany), 0.1 µl of each primer, 10.1 µl of Diethyl pyrocarbonate (DEPC) - water, and 2 µl of template DNA. Amplification was performed in a thermocycler (PTC-200, MJ Research, Water Town, MA) and consisted of one step of 15 minutes at 95 °C followed by 35 cycles of 45 seconds at 94 °C, 45 seconds at 65 °C, and 90 seconds at 72 °C with a final extension step of 10 minutes at 72 °C. Positive and negative control were included in each run. The amplicons were separated by electrophoresis in 2% agarose gel.

The dogs with CME were included to our study if the diagnosis of *E. canis* infection occurred: (1) within 21 days (incubation period of *E. canis* infection) (Sainz et al., 2015) after the first detection of renal insufficiency; (2) after 21 days of the first detection of renal insufficiency but *E. canis* infection induced the progression of kidney injury; (3) at the same time of the first detection of renal insufficiency; (4) within the duration of standard CME treatment (1 month) (Sainz et al., 2015) before the first detection of renal insufficiency (Figure 1). The cause of death was defined by veterinary medical records and necropsy report (if available). The exclusion criteria consist of: (1) the dogs that could not be specified the date of death; (2) unclear evidence of *E. canis* infection; (3) CME was unrelated to kidney injury. The excluded dogs with *E. canis* infection that unrelated to kidney were indicated by stable renal function in the period of CME infection and no evidence of reinfection with CME until death (Figure 1).

Animal ethics

Our study did not involve the use of live animals. The animal ethics approval then was not required from the Committee on Animal Care and Use, Faculty of Veterinary Science, Mahidol University, Thailand. All data extraction from the veterinary medical records were permitted by the director of Prasu Arthon Animal Hospital, Mahidol University.

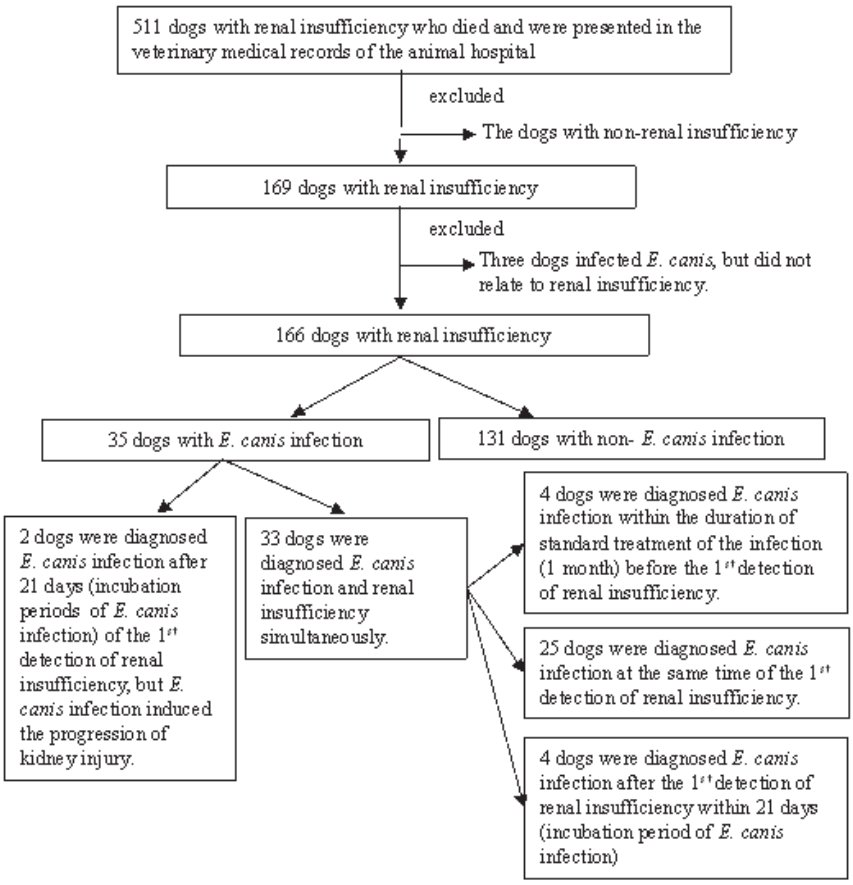

Figure 1. Flowchart of case selection

Statistical analysis

The 166 dogs with renal insufficiency were explored their signalment and laboratory findings by descriptive analysis and the coincident diseases occurring with the dogs infected *E. canis* were classified. All variables were tested the correlation with *E. canis* infection by Fisher's exact test and were tested the association with renal death, and a probability of occurrence in acute kidney injury (AKI) and chronic kidney disease (CKD) by using the binary logistic regression. CME factor was adjusted the association with those significant factors by multiple logistic regression. Survival analysis and Cox proportional hazard regression were tested for survival time and a hazard ratio of AKI and CKD by the CME factors: infection and non-infection. The survival time was counted between the first diagnosis of renal insufficiency and death. R version 3.4.4 (The R Foundation, Vienna, Austria) was used for all analysis.

Results

The dogs with CME were 35 out of 166 dogs (21.08 %). There was an evidence that 33 out of 35 dogs (94.28%) were diagnosed CME simultaneously whereas 2 out of 35 (5.71%) were diagnosed CME infection after 21 days (incubation periods of *E. canis* infection) of the first detection of renal insufficiency but *E. canis* infection induced the progression of kidney injury. Of 35 dogs, there were 28 dogs presented thrombocytopenia significantly whereas the dogs with anemia were not associated with CME ($p = 0.81$). The 7 out of 35 dogs with CME were detected *Anaplasma* spp. together.

From univariate analysis, the dogs with *E. canis* infection have significantly a probability to be detected with AKI more than CKD 3.21 time [95%CI: OR (1.41 - 8.07), $p < 0.01$] comparing with dogs without infection. Also, CME associated with renal death as 2.49 times [95% CI: OR (1.09- 5.91), $p = 0.03$]. The age and hepatobiliary diseases correlated with the type of kidney diseases whereas the breed only related to the renal death (Table 1).

E. canis infection was adjusted the association the type of kidney and renal death by the other significant factors using the multiple logistic regression (Table 1). CME became to increase the association with the type of kidney diseases as 5.57 times [95% CI: OR (1.99-18.45), $p < 0.01$] which the dogs with CME would have a probability to find AKI more than CKD strongly (Table 2). From the multiple logistic regression, CME still associated with the renal death as 2.42 times [95% CI: OR (1.04-5.87), $p = 0.04$] (Table 2).

Table 1. Univariate analysis between the factor variables related and the renal insufficiency in dogs

Factor	*Ehrlichia canis* infection	Type of kidney diseases[a]		Renal death	
	p-value	OR[b] (95% CI)	p-value	OR[b] (95% CI)	p-value
***E. canis* infection**					
Yes	-	3.21 (1.41 - 8.07)	<0.01*	2.49 (1.09- 5.91)	0.03*
No	0.65	referent	referent	referent	referent
Breed					
Poodle	0.65	0.61 (0.24- 1.54)	0.30	0.32 (0.11-0.88)	0.03*
Shih Tzu		0.33 (0.10- 0.97)	0.05	0.31 (0.07-1.09)	0.07
Others		1.23 (0.58-2.62)	0.58	0.53 (0.23-1.19)	0.13
Mixed breed		referent	referent	referent	referent
Age group					
>12	0.61	0.58 (0.23-1.37)	<0.01*	2.35 (0.76-7.68)	0.12
>10-12		0.20 (0.07-0.53)	<0.01*	0.81 (0.29- 2.26)	0.70
>6-10		0.58 (0.23-1.37)	0.22	1.02 (0.42-2.50)	0.96
<= 6		referent	referent	referent	referent
Sex					
Male	0.56	0.97 (0.51- 1.82)	0.93	1.03 (0.51-2.06)	0.92
Female		referent	referent	referent	referent
Neuter status					
Yes	0.63	0.5 (0.08- 2.47)	0.40	1.10 (0.17-6.26)	0.91
No		referent	referent	referent	referent
Cardio-respiratory diseases					
Yes	0.45	0.65 (0.29 - 1.47)	0.30	0.48 (0.18-1.19)	0.12
No		referent	referent	referent	referent
Pancreatitis					
Yes	0.75	1.95 (0.68 - 6.38)	0.23	0.90 (0.30- 2.58)	0.85
No		referent	referent	referent	referent
Hepatobiliary diseases					
Yes	0.24	0.41 (0.19 - 0.89)	0.02*	0.65 (0.27- 1.51)	0.33
No		referent	referent	referent	referent

Type of kidney diseases[a], odds ratio presented a risk to be acute kidney disease compared with chronic kidney disease (referent); OR[b], odds ratio; *the significant association between factor and renal death by renal failure or type of kidney diseases ($p < 0.05$)

Table 2. Adjusted factors relating to the type of kidney diseases and renal death by multiple logistic regression

Factor	Type of kidney diseases[a]		Renal death	
	OR[b] (95% CI)	**p-value**	**OR[b] (95% CI)**	**p-value**
***E. canis* infection**				
Yes	5.57 (1.99-18.45)	<0.01*	2.42 (1.04-5.87)	0.04*
No	referent	referent	referent	referent
Breed				
Poodle	-	-	0.34 (0.11-0.94)	0.04*
Shih Tzu	-	-	0.30 (0.07-1.09)	0.07
Others	-	-	0.54 (0.23-1.23)	0.14
Mixed breed	referent	referent	referent	referent
Age group				
>12	0.18 (0.04-0.63)	<0.01*	-	-
>10-12	0.18 (0.05-0.59)	<0.01*	-	-
>6-10	0.60 (0.20-1.68)	0.34	-	-
<= 6	referent	referent	-	-
Hepatobiliary diseases				
Yes	0.36 (0.12-0.95)	0.04*	-	-
No	referent	referent	-	-

Type of kidney diseases[a], odds ratio presented a risk to be acute kidney disease compared with chronic kidney disease (referent); OR[b], odds ratio; *the significant association between factor and renal death by renal failure or type of kidney diseases ($p < 0.05$)

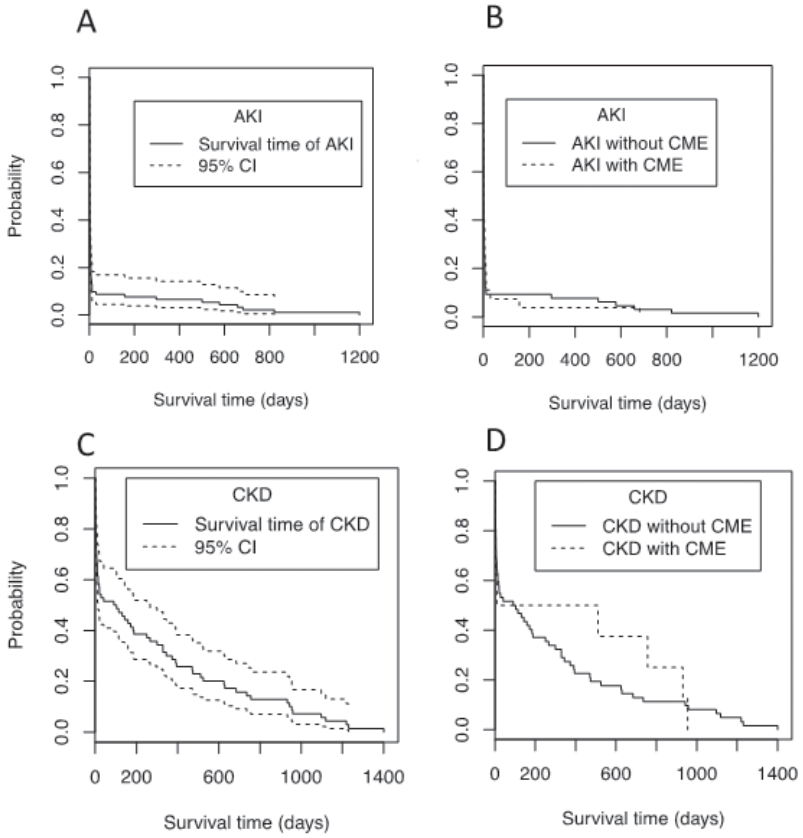

Figure 2. The Kaplan-Meier survival curve of the acute kidney injury (AKI) and chronic kidney disease (CKD) in dogs. (A) the survival time of AKI (B) the comparison of survival time of AKI between with and without CME (C) the survival time of CKD (D) the comparison of survival time of CKD between with and without CME

The median survival time of dogs with AKI was 1.5 days [95%CI: (1-3 days)] after diagnosis of renal insufficiency (Figure 2). The median survival time of dogs with CKD was 97 days [95%CI: (14-267 days)] after diagnosis of renal insufficiency (Figure 2)

The survival time of CKD was significantly different from the survival time of AKI ($p < 0.001$). However, the survival time between AKI on CKD and CKD were not significantly different ($p = 0.22$). Although, the CME associated with renal death, the survival time between the AKI with and without CME was not a difference ($p = 0.71$) and was similar to survival time comparing between CKD with and without CME ($p = 0.66$). By using Cox proportional hazards analysis, CME on both AKI and CKD was not a significant factor in all causes of death at $p = 0.69$ and 0.71, respectively. Therefore, the CME did not associate with the all-cause mortality but it was only a cause of death by renal failure.

Discussion

Our result showed 94% of CME dogs were simultaneously diagnosed together with kidney disease and significantly occurred with AKI more than CKD. From the authors' knowledge, pathogenesis of CME in kidney diseases remains vague. Immune-mediated mechanism has been suggested as a central role for pathologic alterations. In experimental *E. canis* infection model illustrated glomerulonephritis and vasculitis characterized by plasmocytic-lymphocytic cellular infiltration, especially in the periglomerular region (de Castro et al., 2004, Unvera et al., 2009). Also, the perivascular lymphoid and plasma cell accumulation

were prominent in the kidney, brain, and liver (Lakkawar et al., 2003, Nair et al., 2016). In naturally CME, the major renal histopathological changes consist of membranoproliferative glomerulonephritis and interstitial nephritis, implying that these structural changes associated with proteinuria and hypoalbuminemia from a renal origin (Silva et al., 2016).

Acute ehrlichiosis from the experimental studies that showed pathological changed in the kidney suggesting that *E. canis* infection could be a cause of renal damage and then induce AKI in dogs. Chronic infection of *E. canis* with a subclinical stage may also cause kidney injury from the immune response (Langston 2017). Nonetheless, in the naturally occurring CME, there is difficult to identify the acute phase of infection. Prolong damage to the kidney might turn AKI to CKD which permanently loss of renal functioning nephrons. In the clinical practice, to be more confident that the treatment was sufficient and patients did not enter the subclinical phase, PCR testing within several weeks after discontinuation of medication should be performed (Sainz et al., 2015).

The coincident condition between CME and renal insufficiency was a cause of renal death in dogs, although the difference showed the weak association at $p = 0.04$. This finding might affect by the necropsy and histopathology diagnosis were not recorded in all dogs in this study, thus we were unable to determine the kidney lesion and the other organs that might be influenced by naturally *E. canis* infection. Therefore, the histopathological investigation is warranted in further researches.

The survival analysis showed the median survival time of AKI were shorter than CKD and the survival time of both AKI and CKD dogs between with and without CME were not significantly different. In CKD patients, the median survival time was 97 days [95%CI: (14-267 days)] which was comparable to the other study that reported the median survival time 226 days [95% CI: (112 - 326 days)] (O'Neill et al., 2013). The survival rate of AKI at 50% was 1.5 days [95%CI: (1-3 days)] in our analysis. The range of survival rate for dogs with AKI treated with conventional therapy was highly variable from 0-82% depending on the underlying cause (Cowgill and Langston 2011).

In naturally *E. canis* infected dogs, the negative correlation of serum albumin level with a severity of the glomerular lesion, suggesting that protein-losing nephropathy might associate with this disease (Silva et al., 2016). Hypoalbuminemia due to protein-losing nephropathy was reported in 5/10 natural cases of *E. canis* infection (Mylonakis et al., 2004). Another study in dogs also suggested that hypoalbuminemia associated with an acute phase of infection might be attributable primarily to renal proteinuria (Codner et al., 1992). As proteinuria indicates poor prognosis and survival time in dogs with kidney diseases, CME may cause death by protein loss from the kidney. Serum albumin and urine protein to urine creatinine ratio (UPC) had not been measured in all dogs in our study, with this limitation, we were unable to evaluate the relationship between CME and renal proteinuria. Hence, the further analysis of the serum albumin level and UPC value in *E. canis* infected dogs were warranted.

Detection of *E. canis* could be practically performed by microscopic visualization of intracellular morulae, serology detected antibodies and molecular detected nucleic acid sequences by PCR, however, all having limitations regarding clarification of results in relation to the status of infection (Allison and Little 2013, Harrus and Waner 2011). Isolation of Ehrlichia species was used more in research than a clinical

diagnostic tool because of time and labor consuming (Harrus and Waner 2011). Molecular detection of DNA by PCR was suggested to be more sensitive and specific than the other techniques (Harrus and Waner 2011, Vargas-Hernandez et al., 2012), especially in spleen and blood (Baneth et al., 2009). Our research is a retrospective cohort study, hence have a limitation to control the diagnostic method. However, the dogs included in the study were carefully diagnosed *E. canis* using PCR, positive buffy coat smear and positive ELISA antibody together with clinical signs, hematology and response to the treatment. The dogs with unclear evidence of CME were excluded from the analysis.

Conclusion

E. canis infection was possibly deteriorated renal dysfunction and was commonly diagnosed with AKI in dogs. To investigate the renal pathophysiological and histopathological changes from *E. canis* infection, further studies are warranted. Moreover, it must be aware that *E. canis* infection does not produce permanent immunity, therefore, dogs can be reinfected with the same species or with other pathogens after re-exposure to the infected tick (Neer et al., 2002). Prevention of naturally occurring CME is achieved with effective tick control. However, due to the intensity of the brown dog tick population in many geographic areas tick eradicate can be challenging.

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Conflict of interest

The authors declare no conflicts of interest.

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