Clinical observation of dogs serologically positive for the potentially zoonotic *Ehrlichia Canis* in the Philippines

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ABSTRACT

Canine Ehrlichiosis is a disease with worldwide distribution caused by the potentially zoonotic *Ehrlichia canis*, a Gram negative intra-cellular pathogen transmitted by the *Rhipicephalus sanguineus* tick. Most studies have reported the epidemiological distribution of *E. canis*, but information on the clinical signs and hematological values were limited and retrospectively gathered. The present study endeavored to prospectively document the clinical profile of dogs that were serologically positive for *E. canis* in the Philippines. A total of 68 canine patients presented at the Makati Dog and Cat Hospital, Manila, Philippines were investigated. Inclusion criteria were history or presence of tick infestation, anemia, and/or thrombocytopenia. Blood samples were collected in EDTA tubes, and plasma samples were tested for *E. canis* using a commercial serological test kit. Clinical signs were recorded, and complete blood counts (CBC) were performed. Results revealed that 86.7% (59) were found serologically positive for *E. canis*. Most seropositive dogs had tick infestation, inappetence, and lethargy. Thrombocytopenia and anemia accompanied with normal WBC counts were the common hematologic findings. Statistical analyses revealed no significant differences in the hematological values of seropositive and seronegative dogs. Also, from the clinical signs, only lethargy was found to be significantly correlated with seropositivity (p value = 0.026). This study documents the clinical profile of dogs that were serologically positive for *E. canis* infection in the Philippines. Results are very useful to clinical practitioners who usually rely on clinical signs, CBC and commercial test kits for the diagnosis of *Canine Ehrlichiosis*

Keywords: *Ehrlichia canis*, dogs, Philippines

I. INTRODUCTION

Canine Monocytic Ehrlichiosis (CME) is a disease with a global distribution but is commonly reported in tropical and subtropical regions (Waner & Harrus, 2000). It is caused by *Ehrlichia canis*, a Gram negative obligate intracellular pathogen (Higuchi, Kuroda, Hoshi, Kawamura & Yasuda, 1999) that is potentially zoonotic (Perez, Bodor, Zhang, Xiong, & Rikihisa, 2006). It is transmitted by the brown dog tick *Rhipicephalus sanguineus* (Higuchi, Kuroda, Hoshi, Kawamura, & Yasuda, 1999; Ybañez et al., 2012; Groves, Dennis, Amyx, & Huxsoll, 1975), the same tick species that can be a vector for several other pathogens (Dantas-Torres, 2010; Alleman & Sayler, 2010). Clinical signs can be non-specific and can vary depending on the stage of the disease (Ybañez, 2014).

CME is a potentially fatal disease in dogs that requires a rapid and accurate diagnosis to initiate appropriate therapy leading to a favourable prognosis (McBride, Corstvet, Breischwerdt, & Walker, 2001). Its diagnosis may be accomplished using peripheral blood smear examination (PBSE), PCR (Polymerase chain reaction) and commercial test kits. PBSE is the simplest diagnostic test for most veterinarians in detecting *E. canis*. However, technical expertise is required to identify *E.*

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canis from the other pathogens. Moreover, underdiagnosis may occur as it can be undetectable in the blood smears when bacteremia is low (Ybañez, 2014). On the other hand, PCR can be the most reliable method but can be time consuming and expensive. Moreover, accessibility to facilities that would cater molecular testing is limited. Thus, using commercial serological test kits can be practical, and is a common practice in veterinary clinics and hospitals. Although this method can be convenient, it must be interpreted carefully with the presenting clinical signs and hematological results because serology only shows evidence of exposure, and may not be reflective of an active infection (Waner & Harrus, 2000, Ybañez, 2014, Ybañez et al., 2016).

In the Philippines, the presence of *E. canis* has been reported in ticks and dogs (Ybañez et al., 2012, Ybañez , 2013, Ybañez, 2014, Ybañez, Ybanez, Yokohama, & Inokuma, 2015, Ybañez et al., 2016). In several countries, recent studies report on the pathogen detection but without or limited mention on the presenting clinical signs and hematological profile of infected dogs. Except for a case report (Ybañez, 2014) and a retrospective study (Ybañez et al., 2016), there are no recent published researches and prospective studies documenting the clinical signs of CME cases in the Philippines. Hence, the present study aimed to describe the clinical observations in dogs that were diagnosed with CME based on clinical signs, CBC (complete blood count), and serological test results.

II. Methodology

A total of 68 dogs presented for consultation or treatment at the Makati Dog and Cat Hospital, Makati City, Metro Manila, Philippines were the subjects of the study. Dogs were selected regardless of age, sex, and breed following the inclusion criteria: thrombocytopenia, anemia, paleness of mucous membranes, and history or presence of tick infestation. Profile and presenting clinical signs were recorded.

Blood collection and analysis. From each dog, 0.5 to 3 ml of blood was aseptically extracted from the peripheral vein using 1 or 3 ml sterile syringe and placed in sterile EDTA tubes. Collected samples were analyzed for CBC using Mythic 18 Vet Hematology Analyzer (Woodley Equipment Company, United Kingdom), and were tested for *E. canis* using a commercial ELISA-based antibody test kit (Immunocomb®, Biogal, Israel). The test kit has a reported sensitivity and specificity of 86% and 98%, respectively (Harrus, Alleman, Bark, Mahan, & Waner, 2002).

Statistical analysis. Obtained data were inputted in Microsoft Excel® using appropriate variable coding. Coded data were imported into an SPSS IBM version 23 software. Simple frequencies and percentages were obtained. Significant differences were computed using Mann-Whitney U test and correlations were tested using Chi square test or logistic regression.

The study was performed in accordance with the Institutional Animal Care and Use Committee guidelines of Southwestern University, Cebu City and the Animal Welfare Act of the Philippines (RA 8485), and with the approval of the attending veterinarians and proprietor of the veterinary establishment.

III. Results and Discussion

Most of the patients were male (61.8%) and purebred (75%), with a mean age of 4.5 years old. Serological testing revealed that 59 out of 68 dogs (86.8%) where found to be seropositive for *E. canis*. The most common clinical observations include thrombocytopenia, tick infestation, and anemia (Table 1). For the hematology, RBC count, MCV, MCH, MCHC, RCDW, MPV, plateletcrit, PDW, total WBC count, and the differential and absolute WBC counts were mostly within or close to the normal range (Table 2). However, most hematocrit values were found to be sub-normal. Most platelet counts were also lower than the normal range and were highly variable in seropositive dogs. Interestingly, mean RBC count, hematocrit and platelet count of seronegative dogs were lower compared to the seropositive dogs. Statistical analyses revealed no significant differences in the hematological values between seropositive and seronegative dogs. Except for lethargy (p value=0.026), no correlation was found between the profile and clinical signs and the seropositivity.

None of the profile parameters were found to be significantly associated with seropositivity, implying that CME may affect dogs at any age, breed or sex. These findings were similar to that of Waner and Harrus (1997) and Akhtardanesh, Ghanbarpour, and Blourizadeh, (2010). While Baneth, Waner, Koplah, Weintein, and Keysary (1996) observed that CME might occur less frequently in dogs one year or younger, Inokuma, Ohno, and Yamamoto (1999) reported no association on age as well as sex with seropositivity. The latter also noted that some breeds were predisposed to having higher seropositivity. More studies also reported possible breed predisposition to CME (Nyindo et. al., 1980; Buhles, Huxsoll, & Ristic, 1974).

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Table 1
Hematologic profile of dogs serologically tested for Ehrlichia canis exposure (N=68)

Parameter	Seronegative (n=9)		Seropositive (n=59)		Reference values[ab]
	x̄	SD	x̄	SD	
Red blood cell (x10^6)	5.4	1.6	5.6	1.7	5.50-8.50
Hemoglobin (mg/dL)	11.4	3.1	11.9	3.9	8.0-15.0
Hematocrit (%)	31.9	8.7	33.9	10.0	37.0-55.0
Mean corpuscular volume (MCV)	59.5	2.9	61.1	4.6	60.0-77.0
Mean corpuscular hemoglobin (MCH)	21.5	2.7	21.2	2.0	19.5-25.0
Mean corpuscular hemoglobin concentration (MCHC)	36.0	3.1	34.8	2.4	32.0-36.0
Red cell distribution width (RCDW)	16.5	2.5	16.9	1.8	13.8-18
Platelet count	144.9	42.1	196.6	165.9	200-900
Mean platelet volume (MPV)	8.4	0.7	8.1	1.2	7.9-13.5
Platelet crit	0.1	0.0	0.2	0.1	0.16-0.58
Platelet distribution width	14.2	3.1	13.2	3.8	
Total White blood cell (x10^3)	15.1	8.1	12.9	7.5	6.0-17.0
Differential count (%)					
Lymphocyte	16.8	7.0	19.5	7.7	12.0-30.0
Monocyte	6.1	3.5	5.1	2.3	3.0-10.0
Granulocytes	77.1	9.8	75.4	9.1	60.0-77.0
Absolute count (x10^3)					
Lymphocyte	2.2	0.9	2.4	1.7	1.0-5.0
Monocyte	0.8	0.4	0.7	0.5	0.1-1.0
Granulocytes	12.0	7.3	9.9	6.2	2.0-8.0

[a]*Ybañez et al., 2016,*[b]*Žvorc et al., 2010*

Clinical observation	Seronegative (n=9)		Seropositive (n=59)		Total (N=68)	
	f	%	f	%	f	%
Thrombocytopenia	9	100	47	79.7	56	82.4
Tick infestation	4	44.4	42	71.2	46	67.6
Anemia/paleness of mucous membranes	4	44.4	31	52.5	35	51.5
Inappetence	4	44.4	20	33.9	24	35.3
History of Ehrlichia infection	1	11.1	22	37.3	23	33.8
Lethargy	0	0.0	22	37.3	22	32.4
Vomiting	1	11.1	9	15.3	10	14.7
Fever	2	22.2	7	11.9	9	13.2
Seizures	0	0.0	2	3.4	2	2.9

Akhtardanesh, Ghanbarpour, & Blourizadeh, (2010). While Baneth, Waner, Koplah, Weintein, and Keysary (1996) observed that CME might occur less frequently in dogs one year or younger, Inokuma, Ohno, and Yamamoto (1999) reported no association on age as well as sex with seropositivity. The latter also noted that some breeds were predisposed to having higher seropositivity. More studies also reported possible breed predisposition to CME (Nyindo et al., 1980; Buhles, Huxsoll, & Ristic, 1974).

The detection rate (86.8%) in this study is lower than previously reported by Baticados et al. (2011). It will be interesting to compare molecular and serological detection results as a previous study in the Philippines (Corales, Viloria, Venturina, & Mingala, 2014) reported low detection rates of *E. canis* using PCR. The high serological detection rate can also be alarming and as cross-reactions are possible, seropositive dogs might be also infected with the zoonotic Anaplasma phagocytophilum (Breitschwerdt, Hegarty, & Hancock, 1998). On the other hand, not all seropositive dogs were found to have ticks during its presentation. This may imply that actual observation of tick infestation during presentation does not necessarily rule-out infection due to the possibility of previous tick exposure. Similar findings were found by Ybañez et al. (2016).

Thrombocytopenia and anemia, among the commonly observed signs in this study, are considered to be the hallmark of CME (Ybanez et al., 2016, Ozata and Ural, 2014). As platelet count values were obtained from a hematology analyzer, it must be interpreted with caution as they can also be inaccurate especially in abnormal platelet-related conditions (Tvedten, Lilliehook, Hillstrom, & Haggstrom, 2008). Low platelet counts observed in both seronegative and seropositive dogs may imply infections with *E. canis* and/or Anaplasma platys, which have been already reported in the country (Ybañez et al., 2012; Ybañez, 2013; Ybañez, 2014; Ybañez et al., 2015; Ybañez et al., 2016). On the other hand, anemia can be manifested by the paleness of mucous membranes (Harikrishnan, Pazhanivel, & Chellappa, 2005, Ybañez et al., 2016). Most dogs had low to normal RBC counts but were accompanied with low PCV and mostly normal MCHC values. This indicates a normochromic macrocytic anemia. Since the MCHC was not mostly seen to be elevated, the *E. canis*-infected dogs may not be in the sub-clinical phase (Waner et al., 1997). Interpretations may differ based on reference values, which may also prompt local practitioners to establish local reference ranges because populations, instruments, and reagents may differ from published reference ranges (Botma, Mogongoa, Jaftha, & Van Rensburg, 2012).

Although several non-specific clinical signs were observed in the study, only lethargy was found to be correlated with seropositivity. Lethargy is among the common clinical signs observed in CME cases. Bleeding disorders and epistaxis that were reported by other studies were not seen (Frank & Breitschwerdt, 1999). Since observed clinical signs can vary depending on the stage of CME disease (Skotarczak, 2003), veterinary practitioners must be familiar with the pathogenesis of CME to better understand the disease and formulate the necessary interventions according to assessment.

Due to a common tick vector, it is difficult to rule-out co-infection with other pathogens, which may interfere with the observed findings. Anemia may have partly resulted from erythrocyte destruction by B. gibsoni (Yamane, Conrad, & Gardner, 1993). On the other hand, thrombocytopenia may have also been influenced by A. platys and/or *B. canis* co-infection (Zygner, Gojska,

Rapacka, Jaros, & Wedrychowicz, 2007; Ybanez, 2013).

Performing CBC (Ybañez et al., 2016) together with serological tests remains very important in assessing disease status and diagnosis of CME. As there were no significant differences observed in most of the hematological profile, diagnosis of *E. canis* by CBC alone is therefore difficult because of possible co-infection with other tick-borne pathogens (Alleman & Sayler, 2010; Breitschwerdt, Hegarty, & Hancock, 1998; Mylonakis et al., 2004). Co-infection with other pathogens can be observed to produce more severe signs (Gaunt et al., 2010) which can be fatal (Gal et al., 2007). Singling out specific clinical signs or haematological abnormality to a particular canine vector-borne disease maybe difficult due to possibilities of co-infection (de Caprariis et al., 2011). The manifestation of common clinical signs has made diseases more difficult to ascertain as to which pathogen have caused the patho-physiologic disturbance.

IV. Conclusion

E. canis infection remains prevalent in dogs with tick infestation or history. The present study prospectively documented the clinical signs and hematological results of dogs serologically tested for CME exposure. Inappetence and lethargy accompanied by thrombocytopenia and anemia were the common observations in seropositive dogs. Performing CBC remains vital to the diagnosis of CME. Because of the non-specificity of the clinical signs and the possibility of co-infection with other pathogens, diagnosing CME can be challenging.

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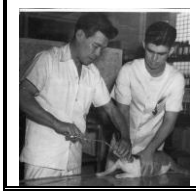

Enrique Rodriguez Carlos, DVM took over the Dog and Cat Hospital in 1958, the same building in 185 Marquez de Comillas and later renamed 839 Romualdez Street. This was the first dog and cat hospital in Manila founded in 1927 by his father Sixto Almeda Carlos y Nepomuceno. The Carlos family have been offering services to dog and cat companion animals. Currently, Dr. Sixto Enrique Miguel Alimudin Carlos y Siapno continues the small animal practice in the Makati Dog and Cat Hospital.