Original Article

Prediction of *Ehrlichia canis* and *Babesia canis* in canine blood sample by Mindray BC-5000 Vet hematology analyzer

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Abstract

Tick-borne diseases caused by the *Babesia canis* (*B. canis*) and *Ehrlichia canis* (*E. canis*), have been increasing recently in clinical veterinary. In veterinary clinics, complete blood counts (CBCs) are commonly analyzed using automated flow cytometric analyzers like the Mindray BC-5000 Vet hematology analyzer. In this study, the predictive significance of the diagnostic blood parasites *B. canis* and *E. canis* obtained from hematological parameter were measured. Red blood cell (RBC) and platelet parameters firstly discriminated the canine blood parasites infection from those healthy dogs. The diagnostic performance of absolute number of monocytes between *B. canis* and *E. canis* infection was sensitivity 67.9%, specificity 64.5% with AUC of 0.650 (p= 0.013) and percentage of monocyte was sensitivity 78%, specificity 58.1% with AUC of 0.653 (p= 0.016). Moreover, abnormal white blood cell (WBC) scattergram patterns were established for the diagnostic efficiency of the hematology analyzer in blood parasite detection. The sensitivity and specificity of *B. canis* were 31.3% and 91.0 %, respectively. Whereas the sensitivity was increased in *E. canis* (79.3 %). In summary, we established the prognostic value of diagnostic blood parasites, *B. canis* and *E. canis*, especially monocyte parameters and WBC scattergram patterns. It may be valuable in diagnosing blood parasites in canine species during routine laboratory hematology.

Keywords: canine blood parasite, *Babesia canis, Ehrlichia canis*, hematology analyzer

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Introduction

Tick-borne pathogens cause significant disease in domestic dogs and humans, and they have potential public health significance.(Ahantarig *et al.* 2008; Yabsley *et al.* 2008; Chomel 2011) Blood parasites; *Babesia* spp. and *Ehrlichia canis* (*E. canis*) are causative agents of infectious tick-borne diseases in dogs, such as babesiosis and ehrlichiosis, respectively.(Baneth *et al.* 1998; Chomel 2011) These diseases are transmitted by the hard tick vector, *Rhipicephalus sanguineus*, commonly called the brown dog tick.(Shaw *et al.* 2001) Babesiosis involves progressive intravascular hemolytic anemia.(Irwin & Hutchinson 1991; Shaw *et al.* 2001) . In Thailand, most canine babesiosis cases are caused by *B. canis*. (Piratae *et al.* 2015; Liu *et al.* 2016) *Ehrlichia canis* also is a commonly blood parasites species reported in Thailand.(Liu *et al.* 2016; Piratae *et al.* 2019; Rucksaken *et al.* 2019) Canine ehrlichiosis has been recognized by morulae of *E. canis* in the cytoplasm in monocytes or occasional granulocytes. (Grindem *et al.* 1999; Shaw *et al.* 2001) Canine monocytotropic ehrlichiosis (CME) is a multisystemic disease exhibiting in acute, subclinical or chronic character such as bleeding diathesis, polysystemic immune complex disease and bone marrow destruction. (Hegarty *et al.* 1997)

Routine diagnosis of tick-borne pathogens is based usually on microscopic examination of pathogens in blood smears, using Wright's or Giemsa staining. However, classical microscopic examination is labor-intensive and time-consuming. Serology testing is performed occasionally, but cross-reactions occur commonly. (Shaw *et al.* 2001; Harrus *et al.* 2002) Moreover, a molecular technique based on polymerase chain reaction (PCR) or real-time PCR is more sensitive and specific for detection. (Mathew *et al.* 2000; Birkenheuer *et al.* 2003; Doyle *et al.* 2005; Kaewkong *et al.* 2014) However, these techniques have certain limitations, by requiring laboratory equipment, facilities and high costs. Limitations in diagnosing blood parasites using these methods have led to development of several new techniques that simplify and speed up diagnosis and increase sensitivity.

Complete blood count (CBC) is one of the most frequently used clinical laboratory tests. The automated hematology analyzer, developed originally for human laboratories, has been established for veterinary species, especially dogs and cats.(Moritz *et al.* 2004; Lilliehook & Tvedten 2009; Bauer *et al.* 2011, 2012; Stirn *et al.* 2014; Thongsahuan *et al.* 2020b) CBC screening has become a rapid and inexpensive source of valuable data in diagnostic investigation, especially on canine blood parasites. Hematological abnormalities data were reported in *B. canis* and *E. canis*. Anemia, thrombocytopenia, eosinopenia and lymphopenia were related with *B. canis*-infected dogs. (Happi *et al.* 2018; Thongsahuan *et al.* 2020a) In *Ehrlichia* infections, thrombocytopenia is a characteristic hematological finding during the acute stage, moderate to severe clinical symptoms. (Harrus & Waner 2011) Anemia, monocytosis, and eosinopenia were also observed in this disease. (Thongsahuan *et al.* 2020a) Thus, hematological parameters were likely useful for examining the prognostic value of diagnostic blood parasites; *B. canis* and *E. canis*.

In malaria infection, automated hematology analyzers are being studied for diagnosis. It has been reported that abnormal depolarizing scattergram patterns detected by a Cell-Dyn hematology analyzer showed high sensitivity for malaria detection. (Mendelow *et al.* 1999; Hanscheid *et al.* 2000a; Hanscheid *et al.* 2001) In previous studies, the Sysmex XE-2100 hematology analyzer reported an abnormal white blood cell (WBC) differential scattergram and pseudoeosinophilia or abnormal scattergrams in a case of malaria infection. (Huh *et al.* 2005; Huh *et al.* 2008; Buoro *et al.* 2018) Abnormality of a WBC differential scattergram results from hemozoin-containing neutrophils or monocytes, and it appeared to misidentify eosinophils. (Hanscheid *et al.* 2000b)

Thus, the aim of this study was to determine the usefulness of automated hematology analyzer data, as detected by the Mindray BC-5000 Vet hematology analyzer. Hematological abnormalities data and the abnormal WBC differential scattergram were examined to establish the prognostic value of different blood parasite to improve clinical decisions in canine.

Materials and Methods

***Samples*:** Blood samples were collected by venipuncture in ethylenediamine tetraacetic acid (EDTA) tubes from 319 dogs. Healthy dogs with no known disease (n=177) and infected dogs diagnosed with tick-borne disease (31 *B. canis* and 111 *E. canis* samples) at the Veterinary Teaching Hospital, Prince of Songkla University from January 2017 to October 2019. All animal protocols were approved by the Institutional Animal Care and Use Committee, Prince of Songkla University (EC 2560-10-041). The inclusion criteria were dogs between 2 - 9 years old, fully vaccinated, dewormed, have a body score of around 3/5, and no obvious clinical abnormality. Dogs that didn't meet the requirements were excluded. The blood samples in the control group were collected from healthy dogs that arrived hospital for a checkup or blood donors and were preliminary physical examined by a veterinarian to ensure that they had normal vital signs (heart rate, respiratory rate, temperature, and mucous membrane color), and free of ectoparasites. Additionally, no blood parasites were discovered, and all blood test results were inside the permitted range. For the *B. canis* and *E. canis* sample groups, only samples that were microscopic analysis proved to be infected were used. However, other types of anemia, such as IMHA or other illnesses, were ruled out in the samples.

The infected groups used in this study were verified using a microscopic examination, which is the gold standard and a very specific procedure.

Laboratory investigation: The Mindray BC-5000 Vet hematology analyzer is for animal blood diagnosis, providing results for both the CBC and 5-part white cell differential. This analyzer calculates the red blood cell (RBC) and platelet count (PLT) data using the electrical impedance method, the hemoglobin (HGB) data using the colorimetric method, and the WBC data

using laser flow cytometry. Other parameter results are obtained from the calculation.

Hematological parameter comprising of RBC count, HGB, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), red blood cell distribution width (RDW), white blood cell (WBC) count, WBC differential count (absolute number and percentage), PLT, plateletcrit (PCT), mean platelet volume (MPV) and platelet size deviation width (PDW) were verified and analyzed. Thin blood films were prepared from each blood sample and stained with 10% Giemsa to examine the blood parasites using a light microscope at high magnification (400× and 1000×) (Nikon, Japan). WBC parameters were determined by using scattered light flow cytometry principle. The 5-part WBC differential detected cell size by low-angle scattered light reflection and intracellular density (nucleus size and density) using high-angle scattered light reflection. Tri-angle laser scatter (LAS) shows cell volume, middle angle scatter (MAS) shows cellular complexity and wide angle scatter (WAS) shows cellular granularity. WBC differential was examined for abnormal WBC differential scattergrams. Atypical characters were categorized as abnormal WBC differential scattergrams.

Statistical analysis: Hematological data were presented as means plus standard deviation (SD). The Mann–Whitney U test was used to calculate statistical significance. (SPSS 23.0, SPSS, Inc., Chicago, Ill., USA) A *p-value* of less than 0.05 was considered statistically significant. A receiver operating characteristic (ROC) analysis was performed to assess the predictive value. ROC analysis was calculated by MedCalc software free trial version. Abnormal WBC differential scattergrams of blood parasite detection was determined sensitivity, specificity and positive and negative predictive values (PPV and NPV).

Results

Microscopic examination, RBC and platelet parameter: Microscopic examination of stained blood smears assessed canine blood parasites. *B. canis* infected the erythrocyte cell (Fig. 1A), while *E. canis* was identified infecting the monocyte (Fig. 1B). The hematology data were examined, and anemia was observed in both blood parasite infection, characterized by a significantly decreasing RBC count, and Hb and HCT value. However, a decreasing value of MCH and MCHC was only observed in *B. canis* infection comparing that of healthy dog (Table 1). MCV value increased the blood count significantly in *E. canis* infection, when compared with that of the normal sample (Table 1). To explore the predictive power of anemia parameter; RBC count, Hb and HCT to predict parasite infection, we performed ROC curve analysis (Table2). RBC count, Hb and HCT value discriminated canine blood parasites infection from those healthy dogs. High sensitivity of anemia parameters was observed in *B. canis* infection. While, high specificity was detected in *E. canis* infection.

Table 1 Hematological data of dogs infected with *Babesia canis* and *Ehrlichia canis* infection compared to healthy dogs.

Parameters	Healthy dogs (n=177)	*Babesia canis* (n=31)	*Ehrlichia canis* (n=111)	Reference Ranges
RBC count (10^6/ µL)	6.3 ± 0.9	4.6 ± 1.3[a]	4.2 ± 1.5[a]	5.10-8.50
Hb (g/dL)	16.2 ± 2.7	11.3 ± 3.3[a]	10.7 ± 4.1[a]	11.0-19.0
HCT (%)	42.0 ± 6.7	31.4 ± 8.6[a]	27.9 ± 10.0[a]	33.0-56.0
MCV (fL)	66.7 ± 3.0	67.5 ± 4.7	67.6 ± 4.9[b]	60.0-76.0
MCH (pg)	25.7 ± 2.0	24.5 ± 2.5[a]	25.7± 2.5	20.0-27.0
MCHC (g/dL)	38.6 ± 2.9	35.9 ± 3.4[a]	37.9 ± 3.4	30.0-38.0
RDW (%)	14.4 ± 1.3	15.7 ± 3.3[b]	15.2 ± 2.7[b]	12.5-17.2
WBC (10^3/µL)	11.5 ± 3.6	11.0 ± 8.2	13.2 ± 8.6[b]	6.00-17.00
Neutrophil (10^3/µL)	7.7 ± 2.5	7.9 ± 7.6	9.3 ± 7.0	3.62-12.30
Neutrophil (%)	67.5 ± 9.1	70.7 ± 18.1[a]	68.0 ± 15.4	52.0-81.0
Lymphocyte (10^3/µL)	2.6 ± 1.9	1.7 ± 1.9[a]	2.1 ± 2.7[a]	0.83-4.91
Lymphocyte (%)	21.6 ± 8.7	17.0 ± 14.4[a]	17.2 ± 13.3[a]	12.0-33.0
Monocyte (10^3/µL)	0.6 ± 0.3	1.0 ± 0.8	1.4 ± 1.0[a]	0.14-1.97
Monocyte (%)	5.4 ± 2.3	10.0 ± 7.3[a]	12.0 ± 5.6 [a]	2.0-13.0
Eosinophil (10^3/µL)	0.6 ± 0.4	0.1 ± 0.1[a]	0.2 ± 0.2[a]	0.04-1.62
Eosinophil (%)	5.3 ± 2.7	1.4 ± 1.0 [a]	1.8 ± 1.3 [a]	0.5-10.0
Basophil (10^3/µL)	0.03 ± 0.02	0.1 ± 0.1[a]	0.1 ± 0.1[a]	0.00-012
Basophil (%)	0.3 ± 0.2	0.9 ± 0.9 [a]	1.0 ± 1.3 [a]	0-1.3
Platelet count (10^3/µL)	245.3 ± 115.8	57.5 ± 41.4[a]	81.1 ± 85.2[a]	117-490
PCT (%)	0.24 ± 0.1	0.09 ± 0.1[a]	0.09 ± 0.09 [a]	0.09-0.58
MPV (fL)	10.2 ± 1.6	11.0 ± 2.4[a]	10.6 ± 3.2	8.0-14.1
PDW (%)	15.7 ± 1.0	18.0 ± 2.2 [a]	18.0 ± 2.0 [a]	12-17.5

RBC: red blood cell; Hb: hemoglobin; HCT: Hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular Hb; MCHC: mean corpuscular Hb concentration; RDW: RBC distribution width; WBC: white blood cell; PCT: plateletcrit, MPV: mean platelet volume; PDW: platelet size deviation width.

[a]Indicates a significant *p* value of <0.01. [b]Indicates a significant *p* value of <0.05 using the independent Student's t-test when compared to normal individuals.

Table 2 Receiver Operating Characteristic (ROC) curve analysis of red blood cell and platelet parameter in *Babesia canis* and *Ehrlichia canis* infection compared to healthy dogs

Parameters	*Babesia canis*				*Ehrlichia canis*			
	Sensitivity (%)	Specificity (%)	AUC	Cut-off	Sensitivity (%)	Specificity (%)	AUC	Cut-off
RBC count	87.1	77.4	0.858	5.63	82.7	80.2	0.881	5.56
Hb (g/dL)	83.9	81.8	0.864	13.7	80.0	81.8	0.863	13.8
HCT (%)	83.9	74.0	0.838	38.5	75.5	86.4	0.873	34.7
Platelet count	93.3	88.7	0.952	124	86.4	85.9	0.896	138
MPV	61.3	74.6	0.658	11.1	34.9	87.6	0.516	11.7
PCT	87.1	88.7	0.884	0.139	83.6	88.7	0.899	0.141
PDW	90.3	85.3	0.898	15.9	85.5	91.0	0.924	16.1

Table 3 Diagnostic efficiency of the Mindray BC-5000 Vet hematology analyzer in *Babesia canis*

		Babesia canis: microscopy examination		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		Positive (n=31)	Negative (n=177)				
Abnormal WBC differential scattergrams	Positive	10	16	31.3	91.0	38.5	88.5
	Negative	21	161				

Table 4 Diagnostic efficiency of the Mindray BC-5000 Vet hematology analyzer in *Ehrlichia canis*

		Ehrlichia canis: microscopy examination		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		Positive (n=111)	Negative (n=177)				
Abnormal WBC differential scattergrams	Positive	88	16	79.3	91.6	84.6	87.5
	Negative	23	161				

In platelet parameter, thrombocytopenia was also observed in both blood parasite infection (Table 1). Platelet count parameter showed high sensitivity (93.3%) and specificity (88.7%) with an area under the curve (AUC) of 0.952 (95%CI: 0.914 to 0.977; p<0.001) in *B. canis* infection. The value of platelet count was also accurate parameters (sensitivity; 86.3%, specificity; 85.9%) with AUC of 0.896 (95%CI: 0.854 to 0.928; p<0.001) in *E. canis* infection. In addition, both PCT and PDW parameters revealed significant potential as the prognostic value in both blood parasite infection (Table 2). However, MPV parameter was good sensitivity with AUC of 0.658 (95%CI: 0.589 to 0.722; p=0.021) only in *B. canis* infection. Our data suggested that RBC and platelet parameter firstly discriminated the canine blood parasites infection from those healthy dogs. However, those parameters cannot distinguish the *B. canis* and *E. canis* infection.

WBC parameter and Abnormal WBC differential scattergrams: To explore diagnostic value of hematological data to discriminate the blood parasites; *B. canis* and *E. canis* infection. WBC differential count and abnormal WBC differential scattergrams obtained from hematology automation were assessed. Neutrophilia, basophilia, lymphopenia and eosinopenia were observed with *B. canis*-infected dogs. While monocytosis, neutrophilia, basophilia, lymphopenia and eosinopenia were detected in *E. canis* infection (Table1). Then, The ROC was analyzed to assess the predictive value of monocyte parameter. The ROC graph was compared to discriminate the blood parasites from those healthy dogs. The diagnostic performance of absolute number of monocytes was sensitivity 32.3%, specificity 96.6% with AUC of 0.601 (95%CI: 0.531 to 0.668; p= 0.139) and percentage of monocyte was sensitivity 64.5%, specificity 69.5% with AUC of 0.727 (95%CI: 0.661 to 0.786; p<0.001) in *B. canis* infection (Figs. 2A and B). Interestingly, absolute number of monocytes showed increased sensitivity (67.9%) and specificity (86.4%) with an area under the curve (AUC) of 0.808 (95%CI: 0.757 to 0.852; p<0.001) and percentage of monocyte was increased sensitivity 75.2%, specificity 89.8% with AUC of 0.879 (95%CI: 0.836 to 0.914; p<0.001) in *E. canis* infection (Figs.2 C and D). Then, to discriminate between *B. canis* and *E. canis* infection, the ROC was assessed the predictive value of monocyte parameter. Our result found that the diagnostic performance of absolute number of monocytes was sensitivity 67.9%, specificity 64.5% with AUC of 0.650 (95%CI: 0.565 to 0.729; p= 0.013) and percentage of monocyte was sensitivity 78%, specificity 58.1% with AUC of 0.653 (95%CI: 0.568 to 0.731; p= 0.016) (Figs.3A and B).

Besides monocyte parameter, we also established abnormal WBC differential scattergram to diagnostic blood parasite infection. Healthy dog samples demonstrated a normal WBC differential scattergram (n=161; Fig. 4A). However, 16 samples from healthy canines showed an abnormal WBC differential scattergram that represented a neutrophil plot extending upward (Fig. 4B). When compared to a normal WBC differential scattergram, *B. canis* infection showed normal features (n=21; Fig. 5A), whereas, 10 samples exhibited an abnormal WBC differential scattergram that represented a neutrophil plot extending upward (n=8; Fig. 5B.) and neutrophils and a monocyte plot extending upward (n=2; Fig. 5C). All samples that represented abnormal WBC differential scattergram showed significantly increased WBC count compared that of normal WBC differential scattergram samples (18.1± 10.0 *vs* 7.1± 3.3, p <0.001).

In the case of *E. canis* infection, abnormal WBC differential scattergrams were found. The most strikingly notable abnormality in a WBC differential scattergram was neutrophils and a monocyte plot extending upward (n=68; Fig. 6B). Other features encountered in *E. canis*-positive samples were neutrophil (n=14; Fig. 6C) and monocyte plots extending upward (n=6; Fig. 6D). However, the 23 samples of *E. canis* infection showed a normal WBC differential scattergram (Fig. 6A).

When using the abnormal WBC differential scattergrams representing neutrophil plots extending upward and neutrophils and a monocyte plot extending upward, for diagnosing *B. canis*, the sensitivity, specificity, PPV and NPV were 31.3 %, 91.0 %, 38.5% and 88.5%, respectively (Table 3). Remarkably, WBC differential scattergrams was assessed for diagnosing *E. canis*, the sensitivity, specificity, PPV and NPV were 79.3 %, 91.0 %, 84.6% and 87.5 % (Table 4).

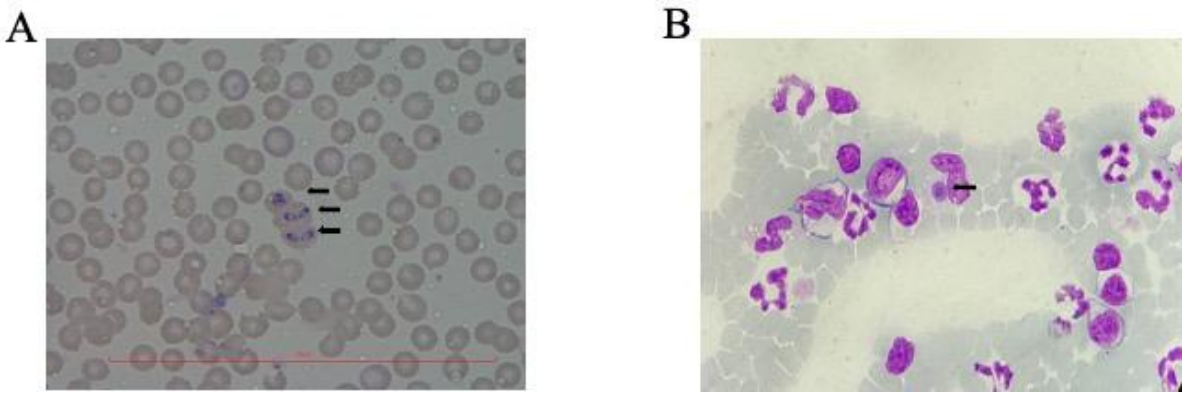

Figure 1 Canine blood smears showing (A) *Babesia canis* in a red blood cell (B) *Ehrlichia canis* in a monocyte (Giemsa stain; ×100 objective).

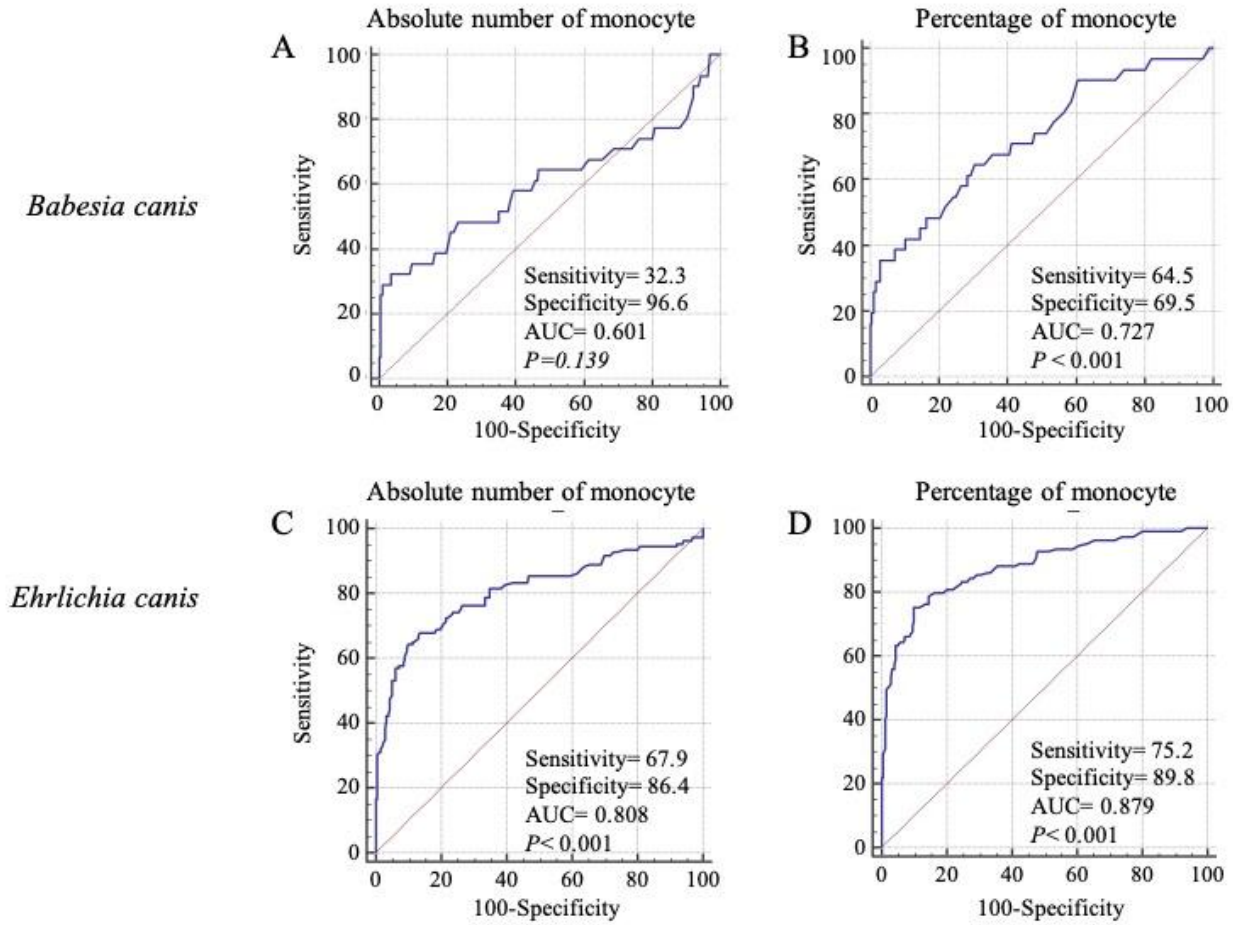

Figure 2 Receiver Operating Characteristic (ROC) curve analysis of monocyte parameter. The ROC graph was compared to discriminate the absolute number of monocytes (A) and percentage of monocyte (B) *Babesia canis* and the absolute number of monocytes (C) and percentage of monocyte (D) *Ehrlichia canis* from those healthy dogs.

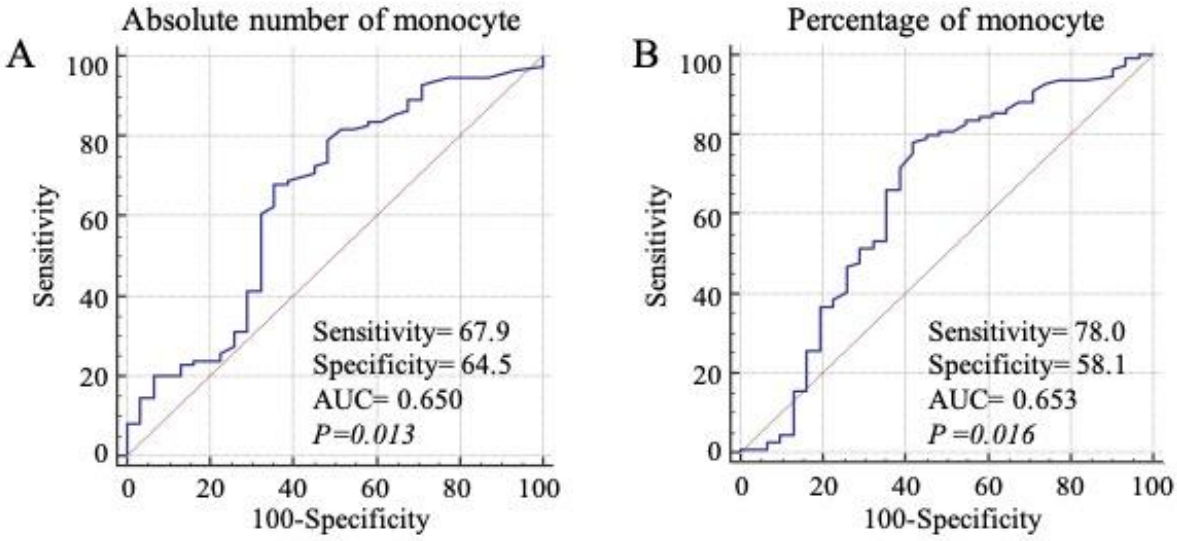

Figure 3 Receiver Operating Characteristic (ROC) curve analysis of monocyte parameter. The ROC graph was compared to discriminate the absolute number of monocytes (A) and percentage of monocyte (B) *Babesia canis* from *Ehrlichia canis*.

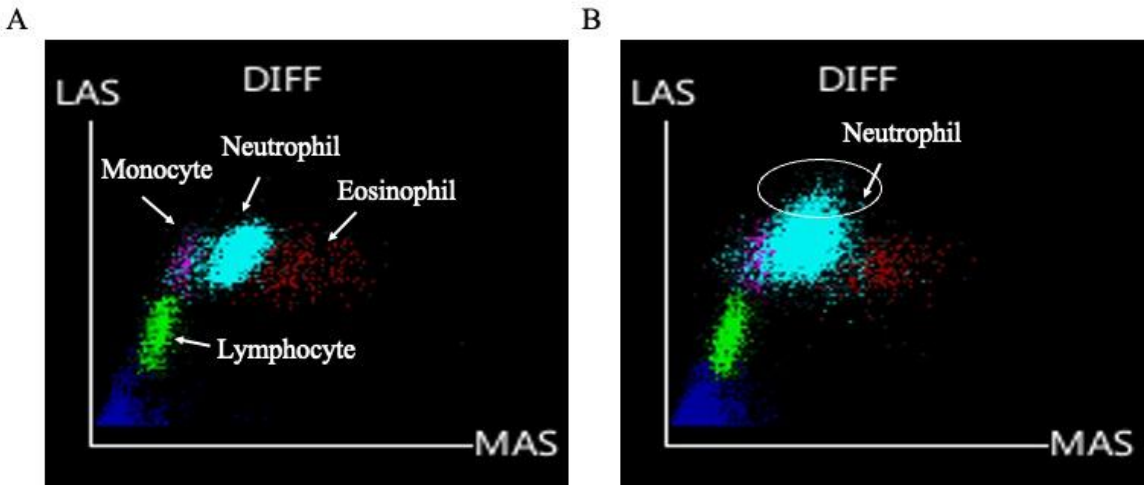

Figure 4 Healthy samples showing a normal WBC differential scattergram (A) and abnormal WBC differential scattergram representing a neutrophil plot extending upward (B).

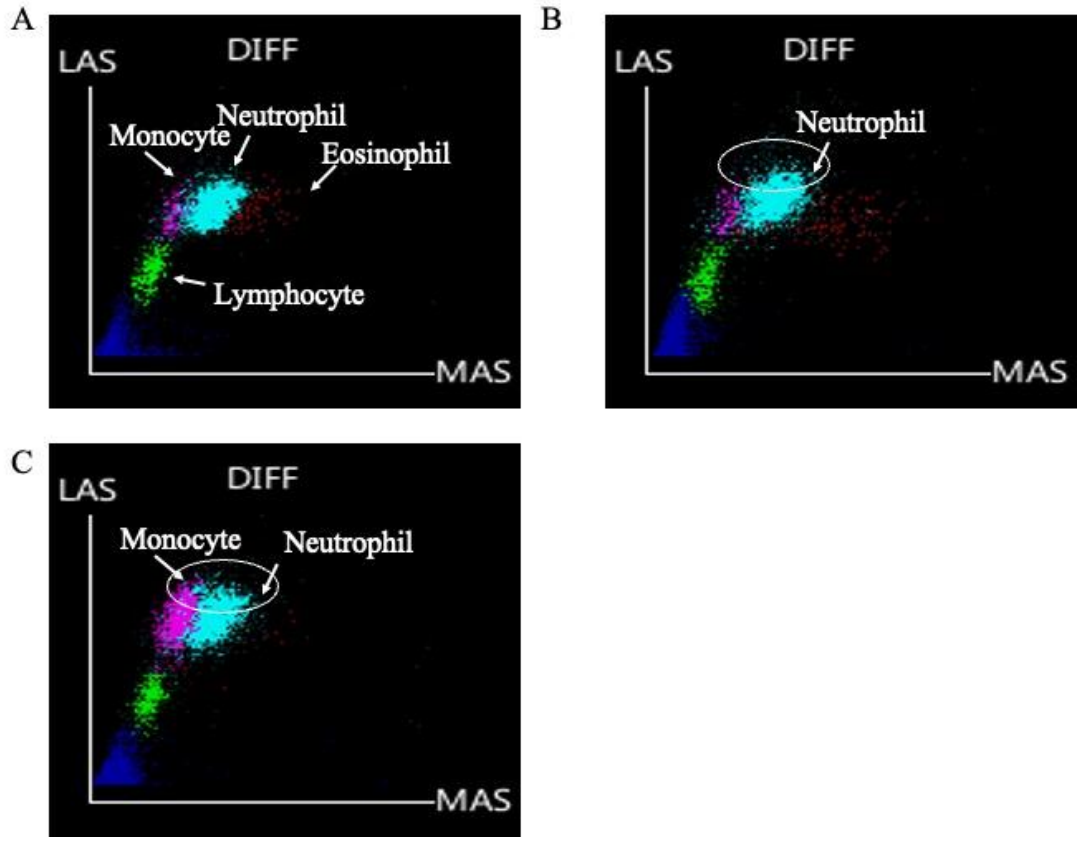

Figure 5 *Babesia canis* showing a normal WBC differential scattergram (A) and abnormal WBC differential scattergram representing neutrophil plot extending upward (B), neutrophils and monocyte plot extending upward (C).

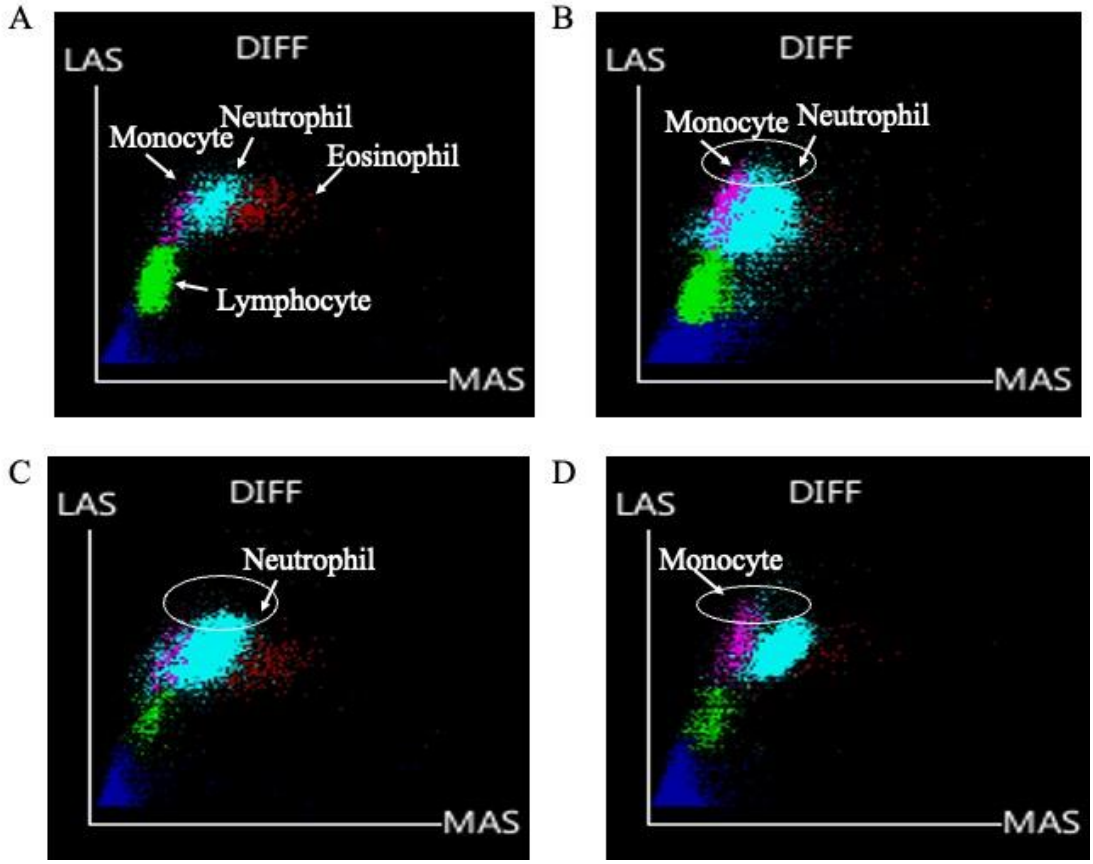

Figure 6 *Ehrlichia canis* showing a normal WBC differential scattergram (A) and abnormal WBC differential scattergram representing neutrophils and monocyte plot extending upward (B), neutrophil plot extending upward (C) and monocyte plot extending upward (D).

Discussion

Tick-borne diseases currently caused by canine blood parasites, e.g. *B. canis* and *E. canis*, are detected commonly in veterinary clinical settings (Dantas-Torres *et al.* 2012). Diagnosis of tick-transmitted diseases requires a combination of compatible clinical and laboratory data. The most common method for blood parasite diagnosis is usually microscopic examination, but this technique requires highly skilled personnel and time consumption.

The automated hematology analyzer is inexpensive and provides rapid and valuable data in diagnostic investigation. By screening CBC data, the results in this study show that the anemia phenotype of canine, *B. canis* and *E. canis*, blood parasites is characterized by significantly decreasing RBC count, and Hb and HCT value. This concurs with previous reports. (Jain *et al.* 2017; Happi *et al.* 2018; Thongsahuan *et al.* 2020a) Likewise, thrombocytopenia has been also reported high incidence in *B. canis* and *E. canis*. (Coralic *et al.* 2018; Happi *et al.* 2018; Thongsahuan *et al.* 2020a) Our report supports that RBC and platelet parameters showed high sensitivity and specificity in *B. canis* and *E. canis* infection. Hence, anemia and thrombocytopenia are being used as screening test for discriminate *B. canis* and *E. canis* infection from those healthy dogs. Because monocytosis was the main WBC abnormality in dogs with ehrlichiosis. (Johansson *et al.* 1995; Harrus *et al.* 1999; Waner *et al.* 2001; Harrus & Waner 2011; Thongsahuan *et al.* 2020a) Consequently, absolute number and percentage of monocyte was determined to assess the predictive value by ROC analysis. The results of this study indicated that absolute number and percentage of monocyte was used to diagnosis ehrlichiosis. Those parameters were significantly increased sensitivity compared that of babesiosis. Furthermore, anemia and thrombocytopenia are the main abnormality in dogs with immune-mediated disease i.e., immune-mediated hemolytic anemia (IMHA) and immune-mediated thrombocytopenia (ITP). Babesiosis and Ehrlichiosis can be screened for and differentially diagnosed from those diseases using our CBC findings, particularly the amount of monocytes. In dogs with IMHA, autoagglutination of erythrocytes in the blood sample might lead to an unintentionally elevated mean cell volume (MCV) and mean cell hemoglobin concentration (MCHC). (Harvey, 2011) Additionally, significant tissue injury-related excessive leukocytosis with a neutrophilic left shift is a typical abnormality in dogs with IMHA and ITP, whereas monocytosis has not been detected. (McManus & Craig, 2001; Smith *et al.*, 2014) In accordance with other published results, the blood values were also identified in 4 IMHA dogs hospitalized to the Veterinary Teaching Hospital. However, the comparative of CBC results between immune-mediated disease and these parasitic infections were further investigated.

Previous studies found that automated hematology analyzers have been used to study malaria blood parasite infection, and abnormal WBC differential scattergram patterns. (Mendelow *et al.* 1999; Hanscheid *et al.* 2000a; Hanscheid *et al.* 2001; Huh *et al.* 2005; Huh *et al.* 2008; Thongsahuan *et al.* 2016) Our study was firstly report that abnormal WBC differential scattergrams are being used as screening test blood parasite infection in canine. Abnormal WBC differential scattergrams were found in 3.1% of *B. canis* infected dogs that related with increased WBC count. High WBC of these case results from increasing the number of neutrophil and/or monocyte. While 97.7% of abnormal scattergrams were detected in *E. canis* infected dogs. Abnormal WBC differential scattergrams represented neutrophils and/or a monocyte plot extended upward in *E. canis* infected dogs results from increasing the number of neutrophil and/or monocyte. In addition, infection of *E. canis* in neutrophils and monocytes can cause increased cell volume in scattergrams. As a result of *E. canis* infected cell and increasing the number of neutrophil and/or monocyte, the abnormal WBC differential scattergrams are being used as screening test for separate *B. canis* and *E. canis*. High sensitivity and specificity of the Mindray BC-5000 Vet hematology analyzer for diagnosing *E. canis* blood parasites by detecting abnormal WBC differential scattergrams were observed. However, blood smear evaluation is necessary to determine co-infections other tick-borne pathogens.

Herein, our studies were to determine the usefulness of automated hematology analyzer data, as detected by the Mindray BC-5000 Vet hematology analyzer. Abnormalities of RBC, platelet, monocyte parameter and abnormal WBC differential scattergram were demonstrated. Hematological abnormalities data was clearly manifested its advantages of simplify, speed up diagnosis and good sensitivity. Automated hematology analyzer should be considered as an alternative to existing methods for diagnosing canine blood parasites during routine laboratory hematology.

Authorship: ST contributed to the study design, collected samples, interpreted data and edited the manuscript. HB and ST contributed to interpreted data and edited the manuscript. SN and SC contributed to sample collection, performed experiments and drafted the manuscript. KS contributed to the study design, performed experiments, interpreted data, and drafted and edited the manuscript. The final version for publication was read and approved by all of the authors.

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