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Clinicopathological profile of *Babesia canis vogeli* infection in dogs*

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Abstract

The clinicopathological profile of Babesia canis infection in dogs presented to University Veterinary hospital, Kokkalai was studied. Eight animals with clinical signs of babesiosis like pyrexia, anorexia, amaemia and lethargy and found positive for Babesia piroplasms in blood smear were included in the study. The organism was identified as Babesia canis vogeli by PCR. Haematological analysis showed highly significant alterations in granulocyte count, monocyte count, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), platelet count and significant alterations in total RBC count. Systemic inflammatory response syndrome (SIRS), as per the classification of Matijatko *et al*. (2010) *was noticed in two out of eight animals. This indicate that B. c. vogeli organisms which are considered to be benign in some parts of the world can cause severe complications in a different geographical location.*

Keywords: *Babesiosis, dogs,* SIRS

Canine babesiosis is considered as the most important haemoprotozoan disease of pet dogs all over the world. The haemoprotozoan *Babesia canis* is a common piroplasm affecting the erythrocytes of the dogs of India and is transmitted by the brown dog tick *Rhipicephalus sanguineus* (Dantas- Torres, 2010). There are mainly three genotytpes viz. *Babesia canis canis, Babesia canis rossi* and *Babesia canis vogeli* (Uilenberg, 2006; Irwin, 2009; Eichenberger *et al.,*2016), among which, *B. c. vogeli* is reported to cause only clinically unapparent infections. Anorexia, lethargy, dyspnoea and haemoglobinuria are the most common clinical signs noticed. Animals that are positive for *Babesia canis* piroplasms and having severe clinical signs are occasionally presented

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to the veterinary hospitals in Kerala. So this study was conducted to identify the genotype of babesia causing this severe form of disease in our state.

Materials and Methods

Animals

Eight animals that were presented to University Veterinary Hospital Kokkalai with severe clinical signs of babesiosis like pyrexia, anorexia, lethargy and dyspnoea, and found positive for *B. canis* piroplasms in blood smears under Field's staining were included in the study. Detailed clinical examination of the animals was done.

Preliminary examination

General examination, physical examination and clinical examination of all animals were done and results recorded. Peripheral blood smears from the tip of ears were collected,stained using Field staining and examined under oil immersion objective of microscope (100x).

Haematological analysis

Two ml of blood was collected from the saphenous or medial cephalic vein of each dog under aseptic techniques. The blood was transferred to a collection tube with EDTA (Hi Media, Mumbai). Complete blood count included the following parameters; Total erythrocyte count (TEC) (10^6/µl), Total leukocyte count(TLC) (10^3/µl),differential leukocyte count (%), Mean corpuscular volume (MCV) (µm^3), Mean corpuscular haemoglobin (MCH) (pg), Mean corpuscular haemoglobin concentration (MCHC (g/dl) and Thrombocyte count (10^3/ µl) were analysed using Automatic Haematology Analyzer (Orphee, Mythic Vet 18) was used.

Molecular identification

Extraction of DNA was done from blood using DNeasy blood and tissue kit (QIAGEN. Hilden, Germany) as per the manufacturer's protocol. The PCR reactions were performed using the MJMini™Personal Thermal cycler, and S1000 thermal cycler (Biorad, USA). The primers used were given in table 1 and followed the protocol recommended by Arthi *et al*., 2017

Analysis of PCR amplicons

Products of PCR were analysed by agarose gel electrophoresis in a submarine gel electrophoresis apparatus (GeNei™, Bangalore). Subsequent to electrophoresis, the gel was transferred to a UV transilluminator (GeNeiTM) for visualization of bands. The gel was analysed in Gel Documentation System (BIO-RAD, USA).

Therapeutic management

Systemic inflammatory response syndrome (SIRS)

Classification of the affected animals into SIRS positive and SIRS negative based on the vital parameters and haematology as per Matijatko *et al*. (2010)

Two standard protocols were employed based on the severity of the clinical signs in the animals affected with canine babesiosis. Diminazineaceturate@ 3.5 – 4 mg/kgbwt intramuscularly to adult animals and pups were treated with Clindamycin, metronidazole and doxycycline at the dose rates of 11 mg/kgbwt, intravenously, 20 mg/kgbwt, intravenously, 10 mg/kgbwt. once daily, per orally respectively.

Statistical analysis

The IBM - SPSS software version 24 was used to analyze numerical data gathered in the present study. The one sampled t test was choosen for analysis.

Results and Discussion

The microscopical examination of blood smear using Field's staining revealed characteristic pear shaped piroplasms of *B. canis* in pairs (Fig 1) from all the eight animals under study.

Description of animals

The age of eight animals under study ranged from 27 days to 8 years. Adaszek *et al*.

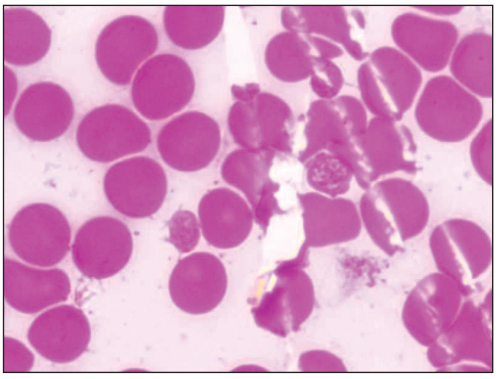

Fig 1: Pear shaped *B. canis* piroplasms **(Field staining X1000)**

(2016) reported *B. c. canis* infection in eight week old puppies. The major clinical signs noticed in eight animals understudy are given in table 1

Haemoglobinuria which was described as one of the most common clinical sign by Aleri *et al*. (2011) was noticed in three out of eight animals affected with *B. c. vogeli* under the present study. The intraerythrocytic multiplication of parasite by binary fission leads to direct erythrocyte injury and haemoglobinuria. Abortion was noticed in one dog on the 48th day of gestation, and cerebral form of babesiosis was noticed in one dog.

Haematological analysis

The statistical analysis of the haematological alterations in dogs affected with and *B. c. vogeli* are given in table3. Significant difference from standard values were noticed in total RBC, granulocyte, monocyte, MCV, MCH and platelets in the affected animals. Significant increase from standard values were noticed in granulocyte percentage, Monocyte percentage, MCV whereas significant reduction in RBC and platelets count. This was in accordance with Solano-Galego and Baneth (2011). The granulocytosis and monocytosis observed in the present study was in contrast to the findings of Schetters (2009) who has described granulocytopenia and monocytopenia.

Furlanello *et al*. (2005) has described increased consumption of thrombocytes associated with immune mediated injury, sequestration and supression of bone marrow are reasons for thromvocytopenia in dogs affected with babesiosis.

Molecular Identification

DNA extracted from the whole blood of all the affected animals was subjected to genus, species and genotype specific PCR for molecular identification of babesia organisms. The genus specific PCR for *Babesia* spp. with primer pair PIRO A and PIRO B yielded indended PCR product of approximately 400 bp, while the species specific PCR for *B. canis* with primer pair Can172F and Can626R yielded indended PCR product of approximately 454 bp and sub-species specific PCR for *B. c. vogeli* with primer pair BAB 1 and BAB 4 yielded indended PCR product of approximately 590 bp from all the eight samples. No bands were observed with negative control. The *B. c. canis* and *B. c. rossi* sub-species specific PCR with primer pair BAB 1 and BAB 3 and BAB 1 and BAB 5 did not yield amplicons from any of the samples.

Systemic inflammatory response syndrome(SIRS)

Occurrence of two or more of clinical signs like tachycardia, hypothermia, tachypnoea, hyperthermia, leukocytosis or leukopenia and neutrophilic shift was suggestive of SIRS (Matijatko *et al*., 2010). Among the eight animals affected with *B. c. vogeli* two animals were SIRS positive as per Matijatko *et al*. (2010) ,which was lower than that reported by Galan *et al*. (2018) for *B. c. rossi* and *B. c. canis* and it could be due to the lesser virulence of *B. c. vogeli*. Table 5 shows SIRS categorisation into SIRS positive and negative in selected eight animals

The two SIRS positive animals and SIRS negative adult animals were treated with Diminazine aceturate injection @ 3.5 mg/kgBwt intramuscularly once (Schoeman 2009). The SIRS negative pup was treated with the standard protocol using Clindamycin @ 11mg/kgbwt and Metronidazole @ 20mg/kgbwt intravenously, and doxycycline@10 mg/kgbwt per orally for 14 days. (Greene, C.E. 2012)

One of the SIRS positive animal was subjected to blood transfusion. But condition

Table 1. Genus, species and sub-species specific primers selected for PCR amplification of blood samples from 25 dogs. (Arthi *et al.*, 2017)

Organism	Primer name		Gene sequence 18s rRNA	Product size
Babesia genus	Forward	**PIRO A**	5'-AATACCCAATCCTGACACAGGG -3'	400bp
	Reverse	**PIRO B**	5'-TTAAATACGAATGCCCCCAAC -3'	
Babesia canis	Forward	**Can172F**	5-'GTTTATTAGTTTGAAACCCGC- 3'	454 bp
	Reverse	**Can626R**	5'-GAACTCGAAAAAGCCAAACGA- 3'	
Babesia canis vogeli	Forward	**BAB1**	5'-GTG-AAC-CTT-ATC-ACT-TAA- AGG-3'	590 bp
	Reverse	**BAB4**	5'-CAA-CTC-CTC-CAC-GCA-ATC G-3'	
Babesia canis canis	Forward	**BAB1**	5'-GTG-AAC-CTT-ATC-ACT-TAA-AGG-3'	746 bp
	Reverse	**BAB3**	5'-CTA-CAC-AGA-GCA-CAC-AGC C-3'	
Babesia canis rossi	Forward	**BAB1**	5'-GTG-AAC-CTT-ATC-ACT-TAA-AGG-3'	342 bp
	Reverse	**BAB5**	5'-AGG-AGT-TGC-TTA-CGC-ACT CA-3'	

Table 2: Clinical signs noticed for dogs affected with *B. c. vogeli*

Animal number	Anorexia	lethargy	Haemo-globinuria	pyrexia	Anaemia	Nasal discharge	Abortion	seizures
1	+	-	-	+	-	+	-	-
2	+	+	-	+	+	-	+	-
3	+	-	-	+	-	-	-	-
4	-	+	-	+	+	-	-	-
5	-	-	+	+	-	-	-	-
6	-	-	+	+	-	-	-	-
7	+	-	+	+	+	-	-	-
8	+	-	-	-	-	+	-	+

Table 3: General parameters of dogs affected with *B. c. vogeli*

Animal no	Temperature (°F)	Mucous membranes	Lymphnode	Heartrate (beats/min)	Respiration (breaths/min)
1	105.3	Pink	Enlarged	88	45
2	103.3	Pale	Enlarged	87	23
3	102.6	pale roseate	Enlarged	79	12
4	102.9	pale roseate	Enlarged	92	32
5	103.8	Pale	Enlarged	112	38
6	103.6	Congested	Enlarged	85	36
7	103.2	pale roseate	Enlarged	80	28
8	97	Pale	Enlarged	70	42

Table 4: Statistical analysis of haematological alterations in dogs affected with *B. c. vogeli*

Sl no	Variable	Mean±SE	Test value	t value	P value
1	Total RBC ($x10^6/mm^3$)	3.9475*± 0.60484	5.5	2.567	0.037
2	Total WBC ($x10^3/mm^3$)	6.550^{ns}± 0.5695	6	.966	0.366
3	Granulocyte (%)	71.050**± 2.9406	51.6	6.614	<0.001
4	Monocyte (%)	6.100** ± 0.7597	2.5	4.739	0.002
5	MCV (μm^3)	66.338**± 1.6391	60	3.866	0.006
6	MCH (pg)	22.713 **±0.8391	26	3.918	0.006
7	MCHC (g/dL)	34.450^{ns}± 1.7786	36	0.871	0.412
8	Platelets ($x10^3$/ µl)	58.63** ± 11.503	200	12.290	<0.001

** - Highly Significant (P < 0.01), * - Significant at (P < 0.05), ns - Non - Significant

Table 5: SIRS categorisation into sirs positive and negative in selected eight animals

Sl no	Temperature (°F)	Heartrate (beats/min)	Respiration (breaths/min)	Total WBC ($x10^3/mm^3$)	SIRS Positive or Negative
1	105.3	88	45	**7**	Negative
2	103.3*	87	23	4*	Positive
3	102.6	79	12	6.8	Negative
4	102.9	92	32	6.1	Negative
5	103.8	112	38	9.2	Negative
6	103.6	85	36	7.5	Negative
7	103.2*	80	28	4.8*	Positive
8	97	70	42	7	Negative

D- Diminazineaceturate CMD- Clindamycin, Metronidazole and Doxycycline

Table 6: Response to therapy in dogs affected with *B. c. vogeli*

Sl no	No of animals	Clinical status	Therapeutic protocol	Progression of disease	End result
1	1	SIRS positive	D	Deterioration in body condition, complete, anorexia, lethargy, recumbency and death	Death
2	1			Gradual alleviation of clinical signs, slow restoration of appetite, complete recovery within one month	Recovery
3	5	SIRS negative Adults	D CMD	Rapid alleviation of clinical signs, regaintment of appetite, complete recovery within five days	Recovery
4	1	SIRS negative pup			

D: Diminazineaceturate, C: clindamycin, M: Metronidazole, D: Doxycycline

of animal deteriorated leading into abortion and death after two days. Table 6 shows the response to therapy in dogs affected with *B. c. vogeli.* The response to therapy was assessed with alleviation of clinical signs, becoming smear negative and with improvement in haematology.

The recovery rate in all the eight *B. c. vogeli* affected animals was 87.5 per cent and this is in agreement with Solano-Gallego and Baneth. (2011). *B. c. vogeli* is considered to be the least virulent sub species among the three causesing all the subclinical infections with low parasitemia in adult dogs. Koster *et al.* (2015a) But the findings of current study, with SIRS being noticed in two out of eight animals resulting in one fatality suggests that *B. c. vogeli* is a pathogen capable of causing severe complicated disease in affected dogs.

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