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## Detection of a co-infection state of anaplasma marginale and Babesia gibsoni in a Pomeranian dog

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### Abstract

A Pomeranian male dog, aged about 11 years admitted in a private veterinary clinic in Jaipur with varying clinical signs of anorexia, lethargy, lameness, weight loss, anemia & temperature. The clinical examination of the dog ruled out the infestation with *Rhipicephalus sanguineus* tick. The hemogram and the biochemical studies reports are as follows. Hemoglobin: 8.4 g/dl; RBC 4.48 mill/mm<sup>3</sup>, PCV: 24.50%, MCV: 54.7 FL, MCH: 18.80. pg. The hemogram values revealed microcytic anemia. Biochemical parameters studies revealed elevated levels of SGOT values i.e. 160.30 u/l as against the values of 9-49. The elevated SGPT value was i.e. 92.10 u/l (8-57) as against the normal range of 10-100u/l. The elevated alkaline phosphatase level was 374.80 u/l as against the normal range of 10-100u/l. The blood urea nitrogen was at 60.47 mg/dl (As against the normal values of 8.89-25.9). (Table 1). The blood smear examination of the dog revealed *A. marginale* in the infected RBCs and *Babesia gibsoni* in the infected RBCs and neutrophils. The tick *Rhipicephalus sanguineus* played a vector role for the coinfection state of *A. marginale* and *Babesia gibsoni* in this infected dog. The infected dog suffered with varying clinical signs which were attributed to the concerned pathogens due to inadequate clinico-pathological patterns.

**Keywords:** Co-infection-A, marginale-B, gibsoni-Pomeranian dog

### Introduction

Tick borne diseases are an important cause of morbidity and mortality in dogs worldwide with the tick *Rhipicephalus sanguineus* implicated as a vector of several disease agents including *A. platys*, *B. canis vogeli*, *B. gibsoni*, *E. canis*, *Rickettsia spp* and *H. canis*. Ehrlichia/Anaplasma and Babesia spp are canine pathogens transmitted by the Rhipicephalus sanguineus tick which can cause several varied clinical signs. This tick species is also a vector for several other pathogens and co-infection in individual ticks has been shown to occur. (Dantas-Torres F. 2010) [1]. Rochelle Haidee D *et al.* (2018) [9] diagnosed Ehrlichia/Anaplasma and Babesia spp. infections by serological, PCR and peripheral blood smear examination.

### Co-infection state of Ehrlichia/Anaplasma and Babesia spp in canines

Babesiosis spp, Ehrlichia spp., and Anaplasma spp have global distribution, primarily in tropical and subtropical regions (Kelly PJ, *et al.* 2013) [6]. Happi A N *et al.* (2017) [4] identified blood-borne infections in dogs in Nigeria. In this study, a single co-infection with Babesia canis and Ehrlichia canis is being reported by light microscopy. By PCR analysis infections with 1, 2 and 3 infectious agents were reported in 55.1%, 40.4% & 4.55% cases respectively. Further, the authors claimed that the dogs aged between 1 and 12 months were the most frequently infected with multiple agents. i.e. 47.2% with double infections and 50.0% with triple infections. Grenada showed 19 % & 7% of dogs infected with *A. platys* and *B. canis* as coinfections (Yabsley M J *et al.* 2008) [17].

Rochelle Haidee D *et al.* (2018) [9] evaluated the presence of Ehrlichia/Anaplasma and Babesia spp in Philippine dogs in which one dog was found to be positive, a co-infection state for both pathogens. Coinfection with *Ehrlichia/Anaplasma* and *Babesia spp* in canines reported in other countries. At least one clinical sign that characterizes the different tick-borne diseases was observed in these cases. (Das M and Konar S, 2013; Santos F, *et al.* 2009; Rojas A, *et al.* 2014) [2, 11]. The possibility of coinfections of Ehrlichia /Anaplasma and Babesia spp or even more than two pathogens is usually higher in thrombocytopenic cases. (Santos F, *et al.* 2009; Rojas A, *et al.* 2014) [11].

The most common clinical signs observed include inappetence, lethargy, thrombocytopenia, and anaemia. Analyses in this study revealed that inappetence and weight loss were found significant with Ehrlichia/Anaplasma infection.

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Ehrlichia/Anaplasma and Babesia spp are canine pathogens transmitted by the Rhipicephalus sanguineus tick which can cause several varied clinical signs. (Rochelle Haidee D *et al.* 2018) [9]. The varying clinical signs may be caused by indefinite clinico-pathological patterns (Solano-Gallego L, *et al.* 2008) [13].

### Hemogram

Rochelle Haidee D *et al.*, (2018) [9] reported diagnosis of Ehrlichia / anaplasma and babesia spp. infections by serological, PCR and peripheral blood smear examination and the complete blood count which revealed 85%,69% and 55% were found positive for anaemia, thrombocytopenia, and for both, respectively. Anemia and thrombocytopenia were the most common haematological observations in Canine babesiosis, canine ehrlichiosis and canine anaplasmosis. The mean PCV and RBCs were lower than the reference values. The haematological and biochemical profile of mixed B. canis and Anaplasma infection among dogs was studied by Himalini *et al.* (2018) [3]. The values were Hb: 5 gm % (as against the normal values of 14.52-15.36 gm %); RBC 2.33 ul (As against the normal values of 7.5-8) PCV: 14 (As against the normal values of 44.95- 48.12%), MCH: 23 FL (As against the normal values of 19.43-19.74), & MCV: 71 pg. (As against the normal values of 60.30 - 60.67). The reasons for low levels of the above haematological changes are attributed to immune-mediated destruction of erythrocytes (Meinkoth *et al.* 2002) [7]. Low levels of MCV and MCH were observed in the infected cases of *B. gibsoni* infections indicating normocytic normochromic anemia due to the acute infection of the bone marrow.

The biochemical profile of mixed B. canis and Anaplasma infection in dogs was studied by Himalini *et al.* (2018) [3]. The studies revealed an increased level of SGOT (AST), and SGPT (ALT) & the alkaline phosphatase (ALP) values were 76 iu/l, 67 iu/l, & 145 iu/l as against the normal range values of 36.15-41.14; 39.83-43.38; 61.58-67.06 respectively. Blood urea nitrogen values were 34.5 (As against the normal range values of 27-31.89 mg/dl) and creatinine was 0.95 mg/dl (As against the normal range values of 0.93 mg/dl). Solano-Gallego L *et al.* 2011 [12] & Irvin PJ (2009) [5] reported that the varying clinical signs and haematological/biochemical parameter changes could be due to the inability of the immune system to eliminate the infection or when the immune system is in abatement.

### A case report

A Pomeranian, the male dog, aged 11 years was admitted in a private veterinary clinic in Jaipur with a varying clinical history of anaemia, fever, in-appetence, lethargy, popliteal lymph node swelling, congested mucus membrane of the eyes and lameness. Examination of the dog for tick infestation and the blood smear examination for blood parasites carried out. The haematological and biochemical parameters were evaluated using the infected dog's blood samples.

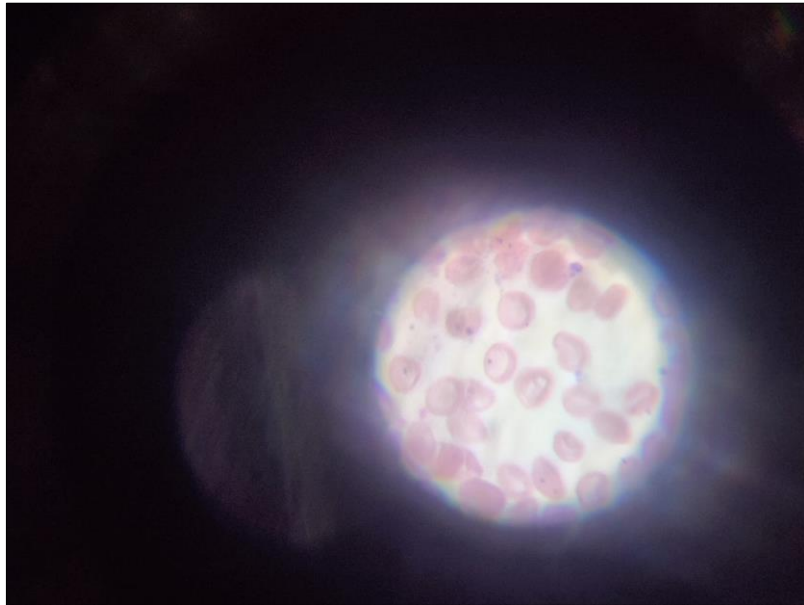
### Material and methods

The dog was examined for tick infestation and for the clinical signs. Blood samples analyzed for blood parasites, hematological and for the biochemical parameters.

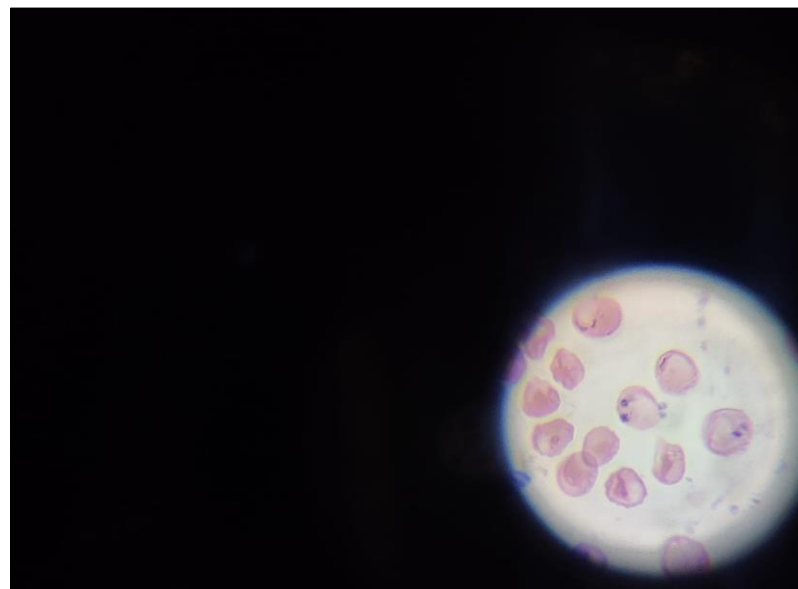
### Results

**Table 1:** Hematological and biochemical profiles of the dog co-infected with *Anaplasma marginale* and *B. gibsoni*

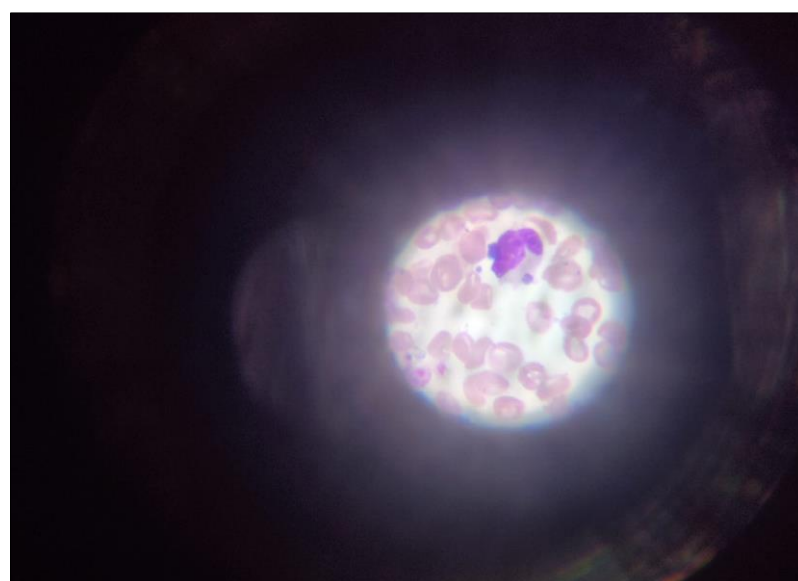
Parameters	Findings	Unit	Normal values	Diagnostic interpretation
<b>Complete blood count</b>				
Hemoglobin	8.4	g/dl	12.0-18.0	Anemia
TLC	9.4	Thou/cu.mm	6.0-17.0	---
<b>Differential leucocyte count</b>				
Neutrophils	66	%	60.0-70.0	
Lymphocytes	27	%	12.0- 30.0	
Eosinophil	3	%	2.0-10.0	
Monocytes	4	%	3.0-10.0	
Basophils	0	%	0.0-1.0	
<b>Absolute leucocyte count</b>				
Neutrophils	6.20	thou/cu.mm	3.0-11.0	
Lymphocytes	2.54	thou/cu.mm	1.0-4.8	
Eosinophils	0.28	thou/cu.mm	0.1-1.3	
Monocytes	0.38	thou/cu.mm	0.1-1.4	
Basophils	0.00	thou/cu.mm	0.0-0.1	
<b>RBC parameters</b>				
RBC	4.48	Mill/mm <sup>3</sup>	5.5-8.5	Anemia
PCV	24.50	%	37.0-55.0	Anemia
MCV	54.70	Fl	60.0-77.0	Microcytic
MCH	18.80	Pg	19.5- 24.5	Low
MCHC	34.30	g/dl	32.0-36.0	
Platelet count	215.00	thou/cu.mm	200.0-900.0	
<b>Biochemical profile</b>				
SGOT/AST	160.30	u/l	9-49	High
SGPT/ALT	92.10	u/l	8-57	High
Alkaline phosphatase (ALP)	374.80	u/l	10- 100	High
Blood urea nitrogen (BUN)	60.47	mg/dl	8.8-25.9	High



**Fig 1:** *A. marginale* in the infected dog's RBCs



**Fig 2:** *B. gibsoni* in the infected dog's RBCs



**Fig 3:** *B. gibsoni* in the infected dog's neutrophil

In this clinical case a Pomeranian, male dog, aged 11 years admitted in a private veterinary clinic, Jaipur had the history of tick infestation with *Rhipicephalus sanguineus*, along with varying clinical signs of anaemia, fever, inappetence, lethargy, weight loss, popliteal lymph-node swelling, lameness, congested mucus membrane of the eyes. Blood smear examination revealed co-infection of *A. marginale* and *Babesia gibsoni*. The hemogram and the biochemical studies reports are as follows. Hemoglobin: 8.4 g/dl; RBC: 4.48 mill/mm<sup>3</sup>; PCV: 24.50%; MCV: 54.7 FL, MCH: 18.80. pg. The hemogram values revealed microcytic anaemia. In this studies, the observed values were Hb: 8.4 gm% (As against the normal values of 14.52-15.36 gm %): RBC4.48 ul. (As against the normal values of 5.5-8.5). PCV: 24.5% (As against the normal values of 37-55%): MCH: 18.8 FL (As against the normal values of 19.5-24.5), & MCV: 71 pg. (As against the normal values of 60-70). Biochemical parameters studies revealed elevated levels of SGOT values i.e.160.30 u/l (As against the values of 9-49). The elevated SGPT value was i.e. 92.10 u/l ( ) as against the normal range of 8-57 u/l). The elevated alkaline phosphatase level was 374.80 u/l (As against the normal range of 10-100u/l). The blood urea nitrogen was at 60.47 mg/dl (As against the normal values of 8.89-25.9). (Table 1).

## Discussion

In this clinical case a Pomeranian, male dog, aged 11 years admitted to a private veterinary clinic, Jaipur. The dog was infested with *Rhipicephalus sanguineus* tick. Stuen S *et al.* 2013 [15]; Uilenberg *et al.* 1989 [16] and Ramos RAN, *et al.* (2014) [8] reported canine babesiosis, canine ehrlichiosis and canine anaplasmosis transmission by *Rhipicephalus sanguineus* tick. Dantas-Torres F (2010) [1] reported this tick species as a vector for several other pathogens and for a co-infection state, which is in concurrence with this study.

The blood smear examination of the dog revealed *A. marginale* in the infected RBCs and *Babesia gibsoni* in the infected RBCs and neutrophils. (Fig 1, 2 & 3). Rochelle Haidee D, *et al.* (2018) [9] reported the presence of Ehrlichia/Anaplasma and Babesia spp in Philippine dogs. Das M and Konar S, 2013 [2]; Santos F, *et al.* 2009 [11]; Rojas A, *et al.* (2014) [10] reported the coinfections with *Ehrlichia/Anaplasma* and *Babesia spp* in canines. Yabsley MJ, *et al.* (2008) [17] reported 19% & 7% of dogs in Grenada were infected with *A. platys* and *B. canis* coinfection by PCR assay. In this study also the involvement of this tick is confirmed.

Clinical examination of the dog showed varying clinical signs i.e. anaemia, fever, inappetence, lethargy, popliteal lymph node swelling, lameness, weight loss & congested mucus membrane. Das M and Konar S, 2013 [2]; Santos F, *et al.* 2009 [11]; Rojas A, *et al.* (2014) [10] reported the coinfection with *Ehrlichia/Anaplasma* and *Babesia spp* in canines with at least one clinical sign that characterizes the different tick-borne diseases. Rochelle Haidee D, *et al.* (2018) [9] reported that Ehrlichia/anaplasma and Babesia spp. are canine pathogens transmitted by the *Rhipicephalus sanguineus* tick which can cause several varied clinical signs. Solano-Gallego L, *et al.* (2008) [13] reported that the varying clinical signs expressed by these coinfecting dogs could be caused by indefinite clinico-pathological patterns.

The complete blood count revealed a hemoglobin value of 8.4 g/dl. The RBC value stands at 4.48 mill /mm<sup>3</sup>, and the PCV values were 24.50%, The MCV was 54.7 FL (microcytic anemia), and the MCH values were 18.80 pg. (low). The

hemogram studies revealed a status of microcytic anemia of the infected dog. Himalini *et al.* (2018) [3] evaluated the hematological profile of the mixed infection of *B.canis* and *Anaplasma* infection among dogs. The present studies correlated with the findings of the above author in the assessment on hemoglobin, RBC, PCV values and differ in the assessment of increased values of MCV & MCH. Schoeman *et al.* (2009) observed that the low level of MCV and MCH observed in the infected cases of *B.gibsoni* infections indicated normocytic normochromic anaemia due to the acute infection of the bone marrow. Thrombocytopenia was observed by Santos F, *et al.* 2009 [11]; Rojas A, *et al.* (2014) [10] in the coinfection state of Ehrlichia/Anaplasma and Babesia spp. which was not observed in this case. It could be attributed to the concerned pathogen due to inadequate clinico-pathological patterns as suggested by Solano-Gallego L, *et al.* (2008). Further, the reasons for low levels of the above haematological changes are attributed to immune-mediated destruction of erythrocytes (Meinkoth *et al.* 2002) [7].

Biochemical studies in this case revealed elevated levels of SGOT i.e.160.30 u/l (As against the values of 9-49). The SGPT level was i.e. 92.10 u/l (As against the normal range of 8-57u/l). The elevated alkaline phosphatase level was 374.80u/l (As against the normal range of 10-100 u/l). The blood urea nitrogen was at 60.47 mg/dl (As against the normal values of 8.8-25.9). The biochemical studies on the mixed infection of *B. canis* and *Anaplasma platys/phagocytophila* infection in dogs carried out by Himalini *et al.* (2018) [3] correlated with the findings of these studies. Solano-Gallego L *et al.* 2011 & Irvin PJ (2009) [5] reported that the varying clinical signs and haematological/biochemical parameter changes could be due to the inability of the immune system to eliminate the infection or when the immune system is in abatement which is concurrence with the present observation of these studies.

## Conclusion

The study revealed the coinfection state of *Anaplasma marginale* and *Babesia gibsoni* infection in a Pomeranian dog infested with *Rhipicephalus sanguineus* ticks. The infected dog suffered with varying clinical signs which were attributed to the concerned pathogens due to inadequate clinico-pathological patterns. The study underpins the need for detailed studies on the prevalence of various tick species involved in the development of various tick-borne diseases and their specific control measures.

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