



Case Report

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Mixed *Babesia Canis* and *Anaplasma Platys/ Phagocytophilum* Infection in Dog



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Abstract

A 3 yr old male Non-descript dog presented to Referral Veterinary Hospital, Sher-e-Kashmir University of Agricultural Sciences and Technology Jammu with history of fever, anorexia, tick infestation, vomition, dark yellow urine and epistaxis. On clinical examination temperature 103.6°F and pale mucous membrane were observed. Blood smear was found negative. Blood sample collected for CBC, LFT, KFT, PCR, and oxidative indices. PCR was positive for *Babesia canis* and SNAP4Dx test positive for *Anaplasma platys/ phagocytophilum*. Animal was treated with fluids, Analgin (Injection Vetalgin @ 0.5 – 1 mg /kg b.wt I/M) and Oxytetracycline (Injection terramycin @ 20mg/kg b.wt I/V in NSS). No further therapeutic intervention could be done as the animal collapsed within 24 hrs.

Keywords: Tick infestation; Epistaxis; Dog; CBC; LFT; KFT; Oxidative indices; PCR; *Babesia canis*; SNAP4Dx test; *Anaplasma platys/ phagocytophilum*; Vetalgin; Terramycin; Piroplasms; Anorexia; Tick infestation; Vomition

Introduction

Babesia species are tick-transmitted apicomplexan parasites infecting a wide range of wild and domestic vertebrate hosts [1]. Traditionally, identification of species has been based on host specificity and morphology of the intraerythrocytic piroplasms. Based on these, canine piroplasms have been originally recognised to belong to two distinct species, the large (4–5mm) *Babesia canis* and the small (~2.5mm) *Babesia gibsoni*. The association of *Babesia* species. with other haemoprotozoa can be attributed to the presence of the vector tick *R. sanguineus*. *Babesiosis* can range from a very mild to a hyper acutely fatal disease. Parasites mainly affect erythrocytes, and typically cause haemolytic anaemia, but can also result in multiple organ dysfunctions [2]. It is characterized by erythrocyte destruction, anaemia, hypoxic tissue injury leading to disseminated pigmenturia, thrombocytopenia, γ - hyperglobulinemia, and waxing and waning of fever, pallor, jaundice, extravascular haemolysis, hypoxic injury, splenomegaly and systemic inflammation [3]. *Anaplasma phagocytophilum* is a gram-negative, obligate intracellular bacteria that parasitize neutrophils in dogs, people, cats, cattle, sheep, and goats and horses worldwide. *Anaplasma phagocytophilum* is an obligate, intracytoplasmic coccus that belongs to the family *Anaplasmataceae*. The agent affects mostly neutrophils and rarely eosinophils; it is present within intracytoplasmic vacuoles (morulae) [4]. Canine cyclic thrombocytopenia is caused by *Anaplasma platys*, a gram-negative, obligate intracellular bacterium that infects platelets [5].

Case History and Observation

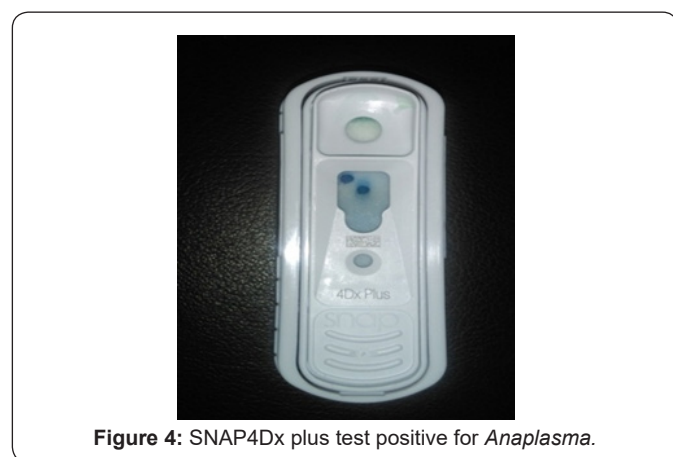
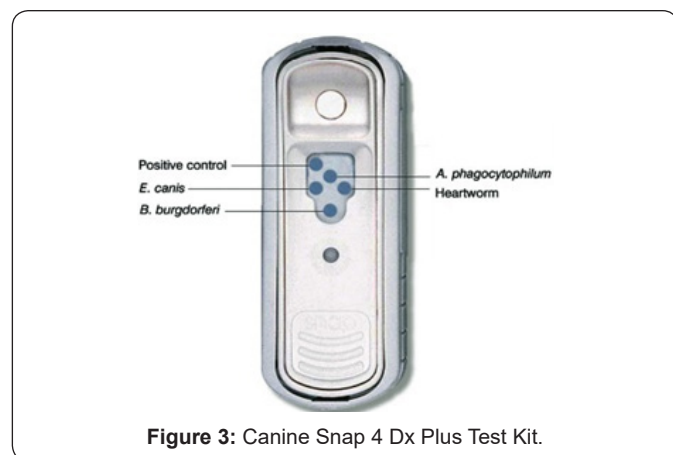
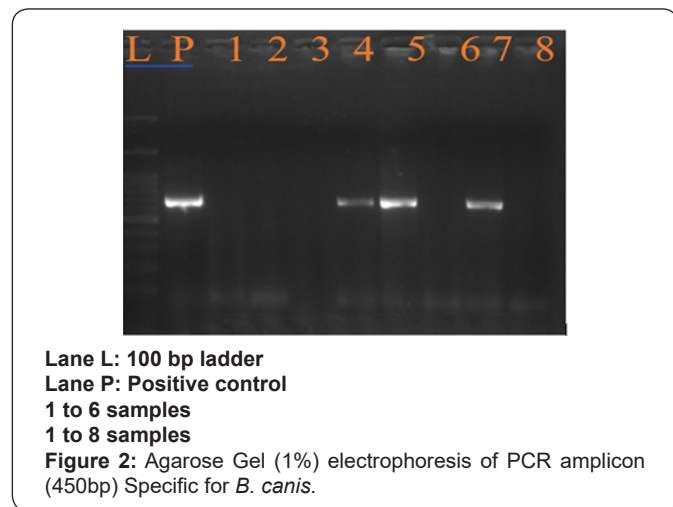
A 3-yr. old male Non-descript dog presented to Referral Veterinary Hospital, Sher-e-Kashmir University of Agricultural Sciences and Technology Jammu with history of fever, anorexia, tick infestation, vomition, dark yellow urine and epistaxis. The owner also reported that vaccination and deworming were regular. On clinical examination animal had pyrexia (103.6 °F) and pale oral and conjunctival mucous membrane. Ticks were present on body with pityriasis (Figures 1-4).



Figure 1: Pale oral mucous membrane in mixed *B. canis* and *Anaplasma* infected dogs.

Blood smear examination was done but found negative. Blood sample was collected for CBC, LFT, KFT and Oxidative indices. PCR was performed using standard protocol for *B. canis* having a 450 bp product. SNAP4Dx test is multivalent (enzyme linked immunosorbent assay) based test uses synthetic peptide reagents for in vitro diagnosis of *Dirofilaria immitis* antigen, *A.*

platys/ phagocytophilum antibodies, *B. burgdorferi* antibodies, *E. canis/ ewingii* antibodies.



Treatment and Discussion

Diagnosis of *B. canis* and *Anaplasma platys/phagocytophilum* was made based on the clinical signs and the demonstration of organism in agarose gel (1%) electrophoresis of PCR amplicon (450bp) specific for *B. canis*. SNAP4Dx plus test kit shows positive result as blue dot specific for *Anaplasma platys/phagocytophilum*. Hb, PCV and TEC were low in mixed *B. canis* and *Anaplasma* infection among dogs. Erythrocytic indices

showed an increase in MCV, MCH and MCHC value among affected dogs. Platelets showed decreased level among affected dogs. Monocytosis and Lymphopenia along with an increase in total leukocyte count among affected dogs (Table1).

Table 1: Haematological profile of mixed *B. canis* and *Anaplasma* infection among dogs.

S. No.	Parameter	Healthy (N=6)	Mixed <i>B. canis</i> and <i>Anaplasma</i> Infection among dogs (N= 1)
1	Hb (gm%)	14.52 ± 0.84	5
2	PCV (%)	44.95 ± 3.17	14
3	MCH (fl)	19.43 ± 0.31	23
4	MCV (pg)	60.30 ± 0.37	71
5	MCHC (%)	32.33 ± 0.54	32.4
6	Platelet count (103 µl)	218.33 ± 8.32	109
7	Agranulocytes (%)	61.33 ± 1.64	87
8	Lymphocytes (%)	34.66 ± 3.68	10
9	Monocytes (%)	0.66 ± 0.21	3
10	TLC (103 µl)	10.27 ± 0.44	11.5
11	TEC (106 µl)	7.5 ± 0.52	2.33

Table 2: Biochemical profile of mixed *B. canis* and *Anaplasma* infection among dogs.

S. No.	Parameter	Healthy (N=6)	Mixed <i>B. canis</i> and <i>Anaplasma</i> Infection among dogs (N= 1)
1	AST (IU/L)	36.15 ± 4.99	76
2	ALT (IU/L)	39.83 ± 3.55	67
3	ALP (IU/L)	61.58 ± 5.40	145
4	Bilirubin (mg/dl)	0.17 ± 0.01	0.23
5	Total protein (g/dl)	6.94 ± 0.58	5.51
6	Albumin (g/dl)	3.27 ± 0.07	2.6
7	Globulin (g/dl)	3.66 ± 0.57	2.91
8	BUN (mg/dl)	27.70 ± 4.19	34.5
9	Creatinine (mg/dl)	0.83 ± 0.10	0.95

There was decrease in the value of protein, albumin and globulin in mixed *B. canis* and *Anaplasma* infection among dogs. BUN and Creatinine showed a non-significant difference. AST, ALT, ALP and Bilirubin showed an increase among affected dogs (Table2). Values of CAT, SOD and GSH showed a decrease in mixed *B. canis* and *Anaplasma* infection among dogs. An increase in value of LPO was observed among affected dogs (Table 3). Animal was treated with fluids, Analgin (Injection Vetalgin @ 0.5 – 1mg /kg b.wt I/M) and Oxytetracycline (Injection terramycin @ 20mg/kg b.wt I/V in NSS). No further therapeutic intervention could be done as the animal collapsed within 24 hrs.

Canine babesiosis is a tick-borne disease caused by intraerythrocytic piroplasms of the genus *Babesia*, named after Dr. Victor Babes, who in 1887 established the etiology of the cattle disease in Romania. Canine babesiosis has been attributed to infection with either *Babesia canis*, large babesia species [6] or *Babesia gibsoni*, *Babesia conradae*, the small Babesia species

[7]. The large babesia of dogs have a wide distribution which includes South Africa [6] while the small babesiosis of dogs occur in South-East Asia, North East Africa, Spain, Australia and the USA [7]. *Babesia gibsoni* have been reported in India, Korea, Malaysia, Ceylon and the USA [8]. The situation regarding canine babesiosis in India is far from clear. Babesiosis can range from a very mild to a hyper acutely fatal disease. Parasites mainly affect

erythrocytes, and typically cause haemolytic anaemia, but can also result in multiple organ dysfunctions [2]. It is characterized by erythrocyte destruction, anaemia, hypoxic tissue injury leading to disseminated pigmenturia, thrombocytopenia, γ - hyperglobulinemia, and waxing and waning of fever, pallor, jaundice, extravascular haemolysis, hypoxic injury, splenomegaly and systemic inflammation.

Table 3: Oxidative profile of mixed *B. canis* and *Anaplasma* infection among dogs.

S. No.	Parameter	Healthy (N=6)	Mixed <i>B. canis</i> and <i>Anaplasma</i> Infection among dogs (N= 1)
1	SOD (U/mg of Hb)	2.86 ± 0.02	0.47
2	LPO (nmol MDA/mg Hb)	1.22 ± 0.08	3.10
3	Catalase ($\mu\text{molH}_2\text{O}_2$ utilized/min/mg of Hb)	2.10 ± 0.01	0.62
4	GSH (nmol/ml)	2.14 ± 0.02	0.37

Anaplasma phagocytophilum is a gram-negative, obligate intracellular bacteria that parasitize neutrophils in dogs, people, cats, cattle, sheep, and goats worldwide. *Anaplasma phagocytophilum* is an obligate, intracytoplasmic coccus that belongs to the family *Anaplasmataceae*. The agent affects mostly neutrophils and rarely eosinophils; it is present within intracytoplasmic vacuoles (morulae) [4]. Canine cyclic thrombocytopenia is caused by *Anaplasma platys*, a gram-negative, obligate intracellular bacterium that infects platelets [5]. Clinical signs of anaplasmosis vary but the disease most commonly presents as an acute febrile syndrome with elevated body temperature, lethargy, anorexia and reluctance to move [9]. More localized signs may be present, especially in the chronic phase of the disease, affecting the musculoskeletal system (lameness), the gastrointestinal system (diarrhoea), or the central nervous system [9,10]. Hematological changes in dogs infected with *Anaplasma* species are anaemia, thrombocytopenia, and leucopenia. The most consistent laboratory abnormality is thrombocytopenia which occurs in approximately 90% of dogs [11]. The platelet count in thrombocytopenic dogs has been reported to range from 5,000 to 164,000 platelets/ml [11,12]. Both the parasites are sensitive to oxytetracyclines but further therapeutic intervention could be done as the animal collapsed within 24 hrs.

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