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The concomitant infection of *Anaplasma phagocytophilum* and *Anaplasma platys* in a Labrador dog

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Abstract

Anaplasmosis is a vector borne disease that affects animals and human worldwide. These parasites parasitize erythrocytes, monocytes, granulocytes, and platelets. Members of the family Anaplasmataceae are obligate intra cellular gram-negative bacteria. A Labrador male dog aged about six years and admitted in a private veterinary clinic in Jaipur with the clinical signs of anorexia for a period of two months, lethargy, lameness while walking in both hind legs, anemia intermittent temperature. The dog was infested with *Ixodes* ticks. The blood smears examination of the dog revealed *A. phagocytophilum* in neutrophils and *A. platys* in the platelets, which indicates the co-infection state of both the organisms in one infected dog. The hematological parameters revealed Hb-11.7 g/dl, TLC- 5.6 thou/cu.mm, lymphocytes- 33%, RBC- 5.34 mil;l/mm³, PCV- 34.9% & platelet- 80 thou/mm³. The biochemical values revealed changes in the SGPT level of 103.60 u/l which is a high value indicated the liver enzyme dysfunction. An elevated alkaline phosphatase level i.e. 370.30 u/l was observed in this case. The other biochemical parameters were within the normal range. Overall hematology and biochemical values revealed the anemia, leucopenia, lymphocytopenia, thrombocytopenia, elevated SGPT (ALT) & Alkaline phosphate levels before treatment. It is confirmed that as with *A. phagocytophilum* infection, animals with clinical disease resulting from *A. platys* infection respond rapidly to treatment with tetracycline. The haemogram studies revealed normal values after the treatment with oxytetracycline with scanty prevalence of the *A. phagocytophilum* and *Anaplasma platys* in blood smear examination. This study confirms the concomitant infection of *A. phagocytophilum* and *Anaplasma platys* in the Labrador dog.

Keywords: *Anaplasma phagocytophilum*, *Anaplasma platys*, Labrador dog

Introduction

Anaplasmosis is a vector borne disease that affects animals and human worldwide (Atif, F.A *et al.* 2016) ^[1]. These parasites parasitize erythrocytes, monocytes, granulocytes, and platelets. (Beri Said, M *et al.* 2018) ^[2]. Members of the family Anaplasmataceae are obligate intra cellular gram-negative bacteria. The family consists of 7 genera, *Anaplasma*, *Ehrlichia*, *Neorickettsia*, *Aegyptianella*, *Wolbachia*, *Candidatus Neo ehrlichia* and *Candidatus X anohaliotis* (Rikishista *et al* 2011) ^[20]. Animal pathogens were attributed to the genus *Anaplasma* such as *A. marginale*, *A. centrale*, *A. platys*, *A. ovis*, *A. bovis*, *A. phagocytophilum*. *A. phagocytophilum* causes granulocytic anaplasmosis and infectious canine cyclic thrombocytopenia by *A. platys* in dogs (Atif, F.A *et al.* 2016; Said, M *et al.* 2018) ^[1, 2]. Granick J I *et al* (2009) ^[11] studied the prevalence of *A. phagocytophilum* in dogs. The main vectors of the *Anaplasma* are *Ixodes*, *Dermacentor*, *Rhipicephalus* and *Amblyomma*. *R. sanguineus* or the brown dog tick appears universally. (Stuen, S *et al* (2013) ^[27]. Tick acts as natural vectors for Anaplasmosis species and play a key role in the biological multiplication of these bacteria in salivary glands and guts (Stiller, D *et al* (1999) ^[26]. Concomitant infection with *A. platys* or *A. phagocytophilum* in dogs, as well as with *E. canis*, *B. vogeli*, *B. burgdoferi*, *H. canis* and or *Leishmania infantum*, has been reported (Sainz A. *et al* 2015; Dantas- Torres *et al* 2017) ^[22, 7].

Case report

A Labrador, male dog, aged six years was admitted in a private veterinary clinic in Jaipur with the clinical history of anemia, fever, in-appetence for more than two months, lethargy, popliteal lymph node swelling, congested mucus membrane of the eyes and inability to walk. Examination of the dog revealed *Ixodes* tick infestation. Blood smear studied for parasitic

infection and blood samples studied for hemato-biochemical parameters before and after the treatment of the dog.

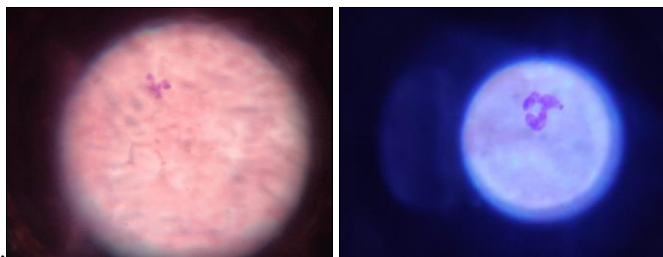


Fig 1, 2: *A. phagocytophilum* in the neutrophils

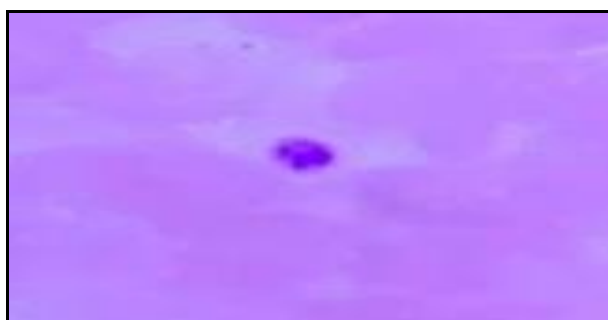


Fig 3: *A. Platys* in platelet

Results and Discussion

Clinical examination of the dog

Six years old Labrador, male dog, was examined in a private veterinary clinic in Jaipur. The dog revealed the clinical history of anemia, fever, in-appetence for more than two months, lethargy, popliteal lymph-node swelling, congested mucus membrane of the eyes and inability to walk. Kohn B. *et al* (2008) [13] reported such clinical signs in *A. phagocytophilum* infected dogs. Sainz *et al* (1999) [21] reported the same types of clinical signs in the *A. platys* infected dogs. The dog was infested with *Ixodes* ticks, which are considered as one of the major vector sources of infection for canine anaplasmosis as reported by Stuen, S *et al* (2013) [27].

Laboratory findings of the blood smears

The blood smear examination in this study revealed the presence of morulae in the neutrophils of the infected dog. (Fig.1&2). The findings of morulae within neutrophils in peripheral blood smear from endemic areas for *A. phagocytophilum* infection was reported by Beall M J. *et al* (2007) [3]. Further in the same blood smear the *Anaplasma platys* were diagnosed by blood smear examination which is unique- in the platelets as intracellular infectious agent with two large, dark blue staining *Anaplasma platys* morulae in the infected dogs (Fig.3) which was reported by Rick Alleman A. and Heather L. Wamsley (2008) [19]. Farhan Ahmad Atif *et al* (2021) [10] reported that the diagnosis is based on the identification of clinical signs, the recognition of intracellular inclusions observed by stained smear and by other methods like detecting antibodies or DNA sequencing for strain variation studies. In China, the triple detection of *A. phagocytophilum*, *A. bovis* & *A. ovis* in dogs was reported by Cui Y. *et al* (2017) [6]. Concomitant infection with *A. platys* or *A. phagocytophilum* dogs, as well as with *E. canis*, *B. vogeli*, *B. burgdoferi*, *H. canis* and or *Leishmania infantum*, have been reported by Sainz A. *et al* (2015) [22] and Dantas- Torres *et al* (2017) [7]. These findings are in concurrence with the outcome of the present one.

Hemogram

The hemogram revealed anemia, leucopenia, lymphocytopenia and thrombocytopenia. Ravnik U *et al* (2011) [18] reported lower mean values of haematocrit, haemoglobin concentration, and red cell counts in their experimental studies. In this study also hemoglobin concentration was 11.70 g/dl (as against 12.0- 18.0), and red cell counts were 5.34 mill/ mm³ (as against the normal values of 5.5- 8.5) which was below their normal range values. Labrets M. D *et al* 2011 [14]; Lilliehook I *et al*, 1998 [15]; & Grieg B *et al* (1996) [12] reported that the canine granulocytic anemia was associated with mild to moderate, non-regenerative normocytic normochromic anemia, resembling anemia of inflammation. In this case the level of hemoglobin was 11.70 g/dl (as against the normal values of 12.0- 18.0) which is in concurrence with the findings of this study. Grieg B *et al* 1996 [12]; Chierk A, *et al* (2018) [5] observed that lymphopenia is the most frequently reported WBC count abnormality in canine granulocytic anemia. In this case study also, the lymphocytes showed a level of 33% as against the normal range values of 12.0- 30.0%.

Egenval A *et al* (1998) [9] reported moderate leucopenia by experimental study. In this study also the leucopenia values were 5.60 thou/cu mm³ as against the normal values of 6.0 to 17.0 thou/cu mm³. Liberts M D *et al* 2011 [14]; Ravnik U *et al* 2011 [18]; Grieg B *et al* 1996 [12]; Chierk A, *et al* 2018 [5]; Lilliehook I *et al*, 1998 [15]; Scorpio D G *et al* (2006) [24] reported that the WBC values were non-specific and variable i.e. decreased or increased under natural conditions. In this study the WBC count was maintained in the normal range only. Beall M J. *et al* 2007 [3]; Kohn B *et al* 2008 [13]; Grieg B *et al* 1996 [12]; Chierk A, *et al* (2018) [5] reported that the most consistent abnormality is thrombocytopenia to a level of 16.5- 95% in natural cases. The severity of thrombocytopenia varies from mild to severe. Beall M J. *et al* 2007 [3]; Kohn B *et al* 2008 [13]; Grieg B *et al* 1996 [12] Chierk A, *et al*. 2018 [5] reported thrombocytopenia as most relevant abnormality in CGA after morulae detection. Mezepa A.M *et al* (2010) [16] reported that the platelet count reported to range from 5000-1, 64,000 cells/ul. Borjesson D. L *et al* (2001) [4] reported increased platelet consumption play an important role and it supports the platelet consumption hypothesis (Labrets M.D *et al* 2011) [14]. In this case study also, the platelet count was 80.0 thou/mm³ as against the normal values of 200-900 thou.mm³, which confirms the findings of the above said authors.

Biochemical studies

Ravnik U *et al* 2011 [18]; Grieg B *et al* 1996 [12]; Egenval A E, *et al* 1997 [8]; Chierk A, *et al* 2018 [5]; Silaghi C, *et al* (2011) [25] reported serum biochemistry profile modifications documented in CGA which included increased liver enzyme hyperbilirubinemia, hypophosphatemia, hyperproteinemia, hyperglobulinemia, and hypoalbuminemia. In this case study also the alanine aminotransferase (SGPT-ALT) was 103.60 u/l as against the normal values of 8-57u/l. Granick J I, *et al* (2009) [11] reported that 30% of the dogs displayed a slightly increased alanine amino transferase (ALT), activity in the absence of concurrent diseases studies and a moderate increase in alkaline phosphatase (ALP) level in 7-100% of CGA cases. In this study the level of ALP was 370.30 u/l as against the normal values of 10-100 u/l which was in concurrence with the present observation. Lilliehook I *et al* 1998; Scorpio D G *et al* (2006) [15, 24] reported that the hematological modifications associated with

A. phagocytophilum infection is similar to those induced by other members of *Ehrlichia* or *Anaplasma* genera, although they infect different blood cells, suggesting that the major mechanisms of cytological injuries are related to an immunological response or to substances secreted from the bacteria. Poitout F M *et al* (2005)^[17] suggested a successful treatment of a dog with tetracycline for full resolution of clinical signs. Rick Alleman A. and Heather L. Wamsley, (2008)^[19] confirmed *A. phagocytophilum* infection dogs with clinical disease resulting from *A. platys* infection respond rapidly to treatment with doxycycline. In this case oxytetracycline was used for a period of 6 days and the study confirmed the co-infection state of *A. phagocytophilum* with *Anaplasma platys*.

Conclusion

A Labradormale dog aged about six years examined in a private veterinary clinic in Jaipur with the clinical signs of anorexia for a period of two months, lethargy, lameness while walking in both hind legs, anemia intermittent temperature. The dog was infested with *Ixodes* ticks and was diagnosed for the concurrent presence of *A. phagocytophilum* with *Anaplasma platys*. Overall view revealed the anemia, leucopenia, lymphocytopenia and thrombocytopenia. Further studies needed on the harboring status of ticks for various blood parasites in different climatic zones.

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