

*Clinical communication***EMERGENCE OF A THROMBOCYTOPENIC DISORDER IN A GROUP OF WORKING DOGS OF SRI LANKA AIR FORCE**

**PS Jayatilaka¹, PPH Pinnagoda¹, PE Gunawardena¹, KMH Ginarathne¹,
ID Silva², N Wijeyawardena², MRCK Mallawa², TMW Karunaratne³,
A Dangolla² and RS Rajakaruna⁴**

¹ Sri Lanka Air Force, Katunayake, ² Veterinary Teaching Hospital, University of Peradeniya,
³ Government Veterinary Surgeon, Anuradhapura, ⁴ Faculty of Science, University of Peradeniya

SUMMARY: The complicated clinical manifestations of canine piroplasmosis with severe thrombocytopenia have been occasionally observed in dogs. This clinical communication describes an acute clinical condition characterized by severe bleeding tendencies with high fatality rates in five groups of working dogs of the Sri Lanka Air Force. It is a multifactorial condition in which known hemoparasites in dogs in Sri Lanka and *Anaplasma platys* possibly play roles, requiring further investigation. The clinical condition was successfully managed with a combination of oral antibiotics and prednisolone.

INTRODUCTION

In Sri Lanka, tick bone diseases (TBD) are the most common canine diseases, with Babesiosis and Ehrlichiosis diagnosed as the two most important TBDs (Silva, 2016). Hepatozoonosis has become common and symptoms similar to Ehrlichiosis caused by *Anaplasma* spp have been suspected.

Despite a favorable climate for parasites and vectors, and large populations of stray dogs, information on epidemiology, diagnosis and management of Canine Ehrlichiosis and Anaplasmosis is limited and there is a high probability of introducing new tick borne parasites to Sri Lanka through the importation of dogs.

The TBDs in dogs are reported to induce immune-mediated thrombocytopenia caused by lysis of platelets through antiplatelet antibodies which have been identified in Ehrlichiosis, Babesiosis, Leishmaniasis, and Dirofilariosis (Lewis *et al.*, 1996; Terrazzano *et al.*, 1996; Breitschwerdt *et al.*, 1988). The pathogenesis of thrombocytopenia with several infectious agents is multifactorial, involving decreased production by the bone marrow and splenic sequestration, in addition to immune-mediated destruction (Mackin, 1995)

This communication discusses the clinical manifestations and prognosis of a condition characterized by immune mediated thrombocytopenia resulting in severe bleeding tendencies and high mortality in five groups of working dogs of the Sri Lanka Air Force. The condition was successfully managed with oral medication for causative hemoparasites, oral hydration and immune suppression.

CASE PRESENTATION

The first group of 9 explosive detecting dogs (8 Labradors, 1 German shepherd of 1.5-2 years of age) at Bandaranayake International Airport at Katunayake developed fever, epistaxis and petechial hemorrhages on the ventral abdomen in July 2016 with severe thrombocytopenia (0.5-1/Field) and granular monocytes were seen in leishman stained blood smears. Three out of 9 dogs were concurrently positive for *Babesia gibsoni*. Seven dogs with slight paleness and high amplitude pulse were administered subcutaneous Imizole (1ml/ 20kg) and oral Doxycycline (5mg/ Kg 12 hourly) while the remaining 2 dogs were administered Diminazine azeturate ('Berenil') intramuscularly and oral Doxycycline (5mg/ Kg 12 hourly). Despite the treatment, all 9 dogs showed gradual to severe epistaxis and petechial hemorrhages in ventral abdomen. After 4-8 days of treatment, all dogs were given oral prednisolone (1mg/ kg twice daily). At this time, since 4 dogs still showed *B. gibsoni*, oral Clindamycin (15mg/ kg 12h) and intravenous Metronidazole (20 mg/ Kg 12 h) were also given. Weekly blood testing of the 9 dogs indicated severe thrombocytopenia and anisocytosis, which improved within a month in 3 dogs, and prednisolone was gradually tapered down. At the end of the outbreak, only 3 out of 9 dogs that were treated with the combination of three drugs (Doxycycline, Clindamycin, Metronidazole) with prednisolone, survived. The clinical picture of those given intravenous supportive therapy worsened and they succumbed to death. Necropsy of 6 dogs revealed severe hemorrhages in all internal organs and fatty



livers.

Later in the month, similar clinical signs appeared in 3 other dogs at the Katunayake Airport, and all three were Labradors of 1.5-2 years of age. Two were treated 12 hourly with oral Doxycycline (5mg/ Kg), Clindamycin (15mg/ kg 12h) and Metronidazole (20 mg/ Kg) combination with Prednisolone (1mg/ kg), while the other dog was treated only with Chloramphenicol (25mg/ Kg) and Methylprednisolone (2mg/ Kg) 12 hourly. No parenteral fluid was given and all dogs recovered.

During this time a dog handler also suffered from fever with petechiation on palms and hands. He was suspected of a zoonotic disease though he was negative for dengue antigen PCR test and for Rickettsia.

In early August 2016, four pups (2 German shepherds and 2 Labradors, 5 months of age) developed a similar condition with high fever and petechial hemorrhages on the ventral abdomen, immediately after being transferred from Diyathalawa breeding kennel to Anuradhapura Air Force camp. Blood pictures revealed severe thrombocytopenia ranging from $0.35-1.35 \times 10^3 / \mu\text{l}$. Two pups also were positive for *B. gibsoni* in peripheral blood smears and all were treated with Berenil (3mg/ kg) and Oral Doxycycline (5mg/ Kg bid). All 4 dogs died within one week and postmortems revealed severe hemorrhages in the internal organs with fatty livers.

In mid-August 2016, the same condition with fever and petechiations were reported in 10 dogs (5 Labrador, 3 Doberman Pinscher, 1 German shepherd and 1 Rotweiller, of 1.5 to 2 years) at Katunayake Air Force Kennels. Their blood smears stained with Diff quick stain revealed severe thrombocytopenia ranging from $0.35-1.35 \times 10^3 / \mu\text{l}$, low PCV (23%-25%) and granular monocytes. Five of them were treated 12 hourly with oral Doxycycline (5mg/ Kg), Clindamycin (15mg/ Kg) and Metronidazole (20mg/ Kg) while two were given Doxycycline (5 mg/ Kg) and Metronidazole (20mg / Kg). Three dogs that showed mild symptoms were given Doxycycline (5 mg/ Kg 12 hourly) only, and one died a week later. The remaining 9 dogs recovered within 10 days of treatment, and none of them were administered with intravenous fluid. Persistent high fever for longer than 12 hours was managed with a single dose Paracetamol 500 mg.

In late August 2016, 20 dogs were transferred from Diyathalawa breeding station to Anuradhapura Kennels, and 4 of them (3 Labrador and 1 German shepherd) showed similar clinical signs namely, fever and petechial hemorrhages on ventral abdomen. Hematological parameters indicated thrombocytopenia ranging from $44-176 \times 10^3 / \mu\text{l}$, leukopenia $1.61 - 2.11 \times 10^3 / \mu\text{l}$ with lymphocytosis in the range of 39.3%-66.1%. *Babesia gibsoni* was detected in peripheral blood smears stained with Diff Quick in all 4 and also in 2 out of 4 clinically normal dogs. One sick and one healthy dog showed intracellular bodies in their platelets resembling *Anaplasma platys*.

All sick pups were treated 12 hourly with oral Doxycycline (5mg/ Kg), Clindamycin (15mg/ Kg) and Metronidazol (20mg / Kg) without intravenous fluid, and

all recovered in 6 days. The treatment was continued for one month and the pups were transferred back to Diyathalawa thereafter.

DISCUSSION AND CLINICAL SIGNIFICANCE

Majority of dogs involved in this report were Labradors, German Shepherds and Dobermans within the age range of 5 months to 2 years. This multi factorial condition in dogs in which known hemoparasites and *A. platys*, possibly play roles needs further investigation. The Immune-mediated thrombocytopenia may occur in association with other autoimmune diseases in dogs such as systemic lupus erythematosus and rheumatoid arthritis, however such possibilities in these dogs were ruled out considering the infectious nature of the condition. Furthermore, blood product transfusion, vaccine or any other drug administrations had not been given prior to the incidence.

Anaplasma platys can cause infectious cyclic thrombocytopenia in infected dogs (Salinas-melendez *et al.*, 2014). Staining of buffy coat using Diff quick may increase the detection of the parasite. The Diff Quick stain when compared with conventional Leishman has been shown to detect hemoparasites with a higher predictive value. Further research in confirmation, distribution and treatment of *A. platys* is warranted.

The clinical response to the condition appeared to be better when the 3 drugs were combined with prednisolone, and when intravenous fluid in not administered. Intravenous fluids can be fatal in such situations due to plasma leakage (Silva, 2016).

A study of 72 dogs in Colombo showed the presence of *Ehrlichia phagocytophilia* in neutrophils in 4 dogs which were later found to be infected with *E. platys* (Bennet *et al.*, 2005). *Ehrlichia phagocytophilia* has been reclassified as *A. phagocytophilia* (Dumler *et al.*, 2001) which is transmitted through Ixodes spp of ticks. However, a less common species *A. platys* in platelets is transmitted via *Rhiphicephalus* and *Dermacenter* spp of ticks which have been reported in Sri Lanka.

ACKNOWLEDGMENT

Authors would like to acknowledge the Commander of the Air Force for his cooperation during the study

REFERENCES

- Bennett SR, Quinn RL, Teleford S, Rich DS, Kulasekera V, Obeysekera N, Collure J, Siriwardana D, Kirihena CD, Fernando G (2005). The prevalence and significance of canine erlichiosis in Colombo, Sri Lanka. *Sri Lanka Veterinary Journal*, **52 (1 & 2A)**: 1-8.
- Breitschwerdt EB. (1988). Infectious thrombocytopenia in dogs. *Compendium of Continuing Education for Practicing Veterinarians*, **10**:1177-1191
- Dumler JS, Barbet AF, Bekker CP, Dasch GA, Palmer GH, Ray SC, Rikihisa Y, Rurangirwa FR. (2001). Reorganization of genera in the families'

- Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. *International Journal of Systematic and Evolutionary Microbiology*, **51**:2145-2165
- Lewis DC, Meyers KM. (1996). Canine idiopathic thrombocytopenic purpura. *Journal of Veterinary Internal Medicine*, **10**:207-218.
- Mackin A. (1995) Canine immune-mediated thrombocytopenia: Part II. *Compendium of Continuing Education for Practicing Veterinarians*, **17**:515-534.
- Salinas-Meléndez JA, Villavicencio-Pedraza R, Tamez-Hernández BV, Hernández Escareño JJ, Avalos-Ramírez R, Zarate-Ramos JJ, Picón-Rubio FJ., Riojas-Valdés VM.(2014). Prevalence of anti *Anaplasma phagocytophilum* antibodies among dogs from Monterrey, Mexico. *African Journal of Microbiology Research*, **8**(8), 825-829.
- Silva ID (2016). Complex clinical presentation of Tick-borne diseases in dogs in Sri Lanka. *Sri Lanka Veterinary Journal*, **63**:2 (A):1-9
- Terrazzano G, Cortese L, Piantadosi D, et al (2006). Presence of anti-platelet IgM and IgG antibodies in dogs naturally infected by *Leishmania infantum*. *Veterinary Immunology and Immunopathology*, **110**:331-337.