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Clinico-pathological Evaluation of Critically Ailing Dogs with Special Reference to Thrombocytopenia

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ABSTRACT

Background: Thrombocytopenia is the decrease in the number of platelets in blood. In dogs, thrombocytopenia occurs due to a multitude of aetiologies such as haemoprotozoal infections, bacterial infections, viral infections, non-infectious etiologies such as immune dysregulation, neoplasia and even due to miscellaneous conditions. Diagnosis of thrombocytopenia is done mostly by estimation of platelet count in automated haematology analysers and by evaluation of platelet count in blood smears. As clinical research focusing exclusively on canine thrombocytopenia is very limited in India, the present study was carried out to study the aetiology of canine thrombocytopenia and to obtain extensive information regarding qualitative and quantitative changes in platelets in various emergency conditions of dogs.

Methods: 450 dogs were diagnosed with thrombocytopenia based on platelet count and a detailed investigation was carried out on the thrombocytopenic dogs to identify the aetiologic agents and to record the quantitative and qualitative changes in platelets.

Result: Population incidence of thrombocytopenia in critically ill dog population was recorded as 34.09 per cent. Haemoparasitic infection, renal disorders, hyperthermia and sepsis were recorded as the major causes of thrombocytopenia. Haematobiochemical investigation revealed a significant reduction ($p<0.01$) of hemoglobin, RBC and PCV values and elevation in blood urea nitrogen (BUN), creatinine, alkaline phosphatase (ALP), total bilirubin, direct bilirubin and phosphorus. A mortality of 10.67% (48/450) was recorded in the thrombocytopenic dogs.

Key words: Blood parasites, Canine, Dogs, Platelet indices, Thrombocytopenia. 10.18805/IJAR.B-5250

INTRODUCTION

Dogs are the most popular pet in India chosen for their unconditional love and companionship. They are affected by a wide spectrum of infectious agents such as bacterial, viral, parasitic, rickettsial, fungal *etc.* Further, especially in urban areas, pet dogs are treated on par with children, often being fed much more than their metabolic requirements, in the form of treats and nutritional supplements which has also led to many metabolic ailments including kidney disorders, obesity and lipoma (Subapriya *et al.* 2020). Irrespective of the aetiology and ailment, thrombocytopenia is the haematological abnormality of worrying concern observed in most of the critically ill dogs presented to veterinary hospitals in demand of immediate therapeutic intervention. Baranidharan *et al.* (2017) stated that thrombocytopenia is a common clinical syndrome seen in emergency veterinary patients and it is the most common acquired hemostatic defect of dogs. Khan *et al.* (2021) also stated that the major cause of bleeding disorders in veterinary emergency patients is thrombocytopenia and ascribed decreased production, increased destruction, abnormal loss and sequestration of platelets as the major causes of thrombocytopenia in dogs. However, clinical research dedicated exclusively on thrombocytopenia in dogs is severely lacking in India. Hence, the present research work was carried out to identify the thrombocytopenic dogs presented to the critical care unit, to identify the aetiological agent(s) of thrombocytopenia and to judge the prognosis by correlating the findings of haematobiochemical investigations in critically ailing thrombocytopenic dogs.

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MATERIALS AND METHODS

Design of study

The study was conducted on all the sick dogs(n=1320), presented to the Critical Care Unit, Madras Veterinary College Teaching Hospital (MVCTH), Chennai during the study period (February 2022 to September 2022). Particulars of animals like breed, sex and age were recorded. Specific pathological data such as history, clinical signs and presence of tick infestation were also recorded. Blood samples were collected from the ailing dogs and haematological parameters such as Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), White Blood Cell count (WBC), platelet count, Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Plateletcrit (PCT) were analyzed using auto haematology analyzer(Mindray- BC-2800 Vet, China). Thrombocytopenic dogs were identified based on the platelet count(less than 150000 platelets/cmm). Thrombocytopenia was further graded as, severe thrombocytopenia(less than 50000 platelets/cmm), moderate thrombocytopenia (50000 to 100000 platelets/cmm) and mild thrombocytopenia (100,000 to 150000 platelets/cmm).

In dogs diagnosed with thrombocytopenia, peripheral blood smears were prepared from the capillary blood of ears onto clean glass microscopic slides. The smears were stained with Leishman-Giemsa (LG) stain and screened under the microscope to identify the blood parasites and to study the morphological changes in RBCs, WBCs and platelets.

Manual count of platelets was also done in LG stained blood smears (Harvey, 2012) and by using Rees Ecker fluid in haemocytometer in all cases suspected of thrombocytopenia based on platelet count in automated haematology analyser to rule out pseudothrombocytopenia due to platelet aggregation and microplatelets wherein the platelets fail to get counted in automated haematology analysers. Similar observations have also been reported by Sakurai *et al.* (1997) who reported that an EDTA-dependent pseudothrombocytopenia can occur due to EDTA-induced exposure of platelet antigens, which causes clumping in patients with antibodies to these antigens and the result is a low platelet count that is artifactual. Carvajal-Vega *et al.* (2016) have also stated that pseudothrombocytopenia due to Ethylene diamine tetra acetic acid is a recognized phenomenon that occurs frequently and must be differentiated from true thrombocytopenia to avoid the patient to receive unnecessary platelet transfusions. In our study, seven samples identified as thrombocytopenic by automated analysers had adequate platelet counts in evaluation of blood smears. Hence, such cases were identified as pseudothrombocytopenia and were excluded from the study.

In cases with less than 40,000 platelets/cmm, Prothrombin time (PT) and Activated Partial Thromboplastin (APTT) and D-dimers were further estimated to rule out bleeding due to coagulation disorders and to identify immune mediated thrombocytopenia. For biochemical analysis, blood samples were centrifuged at 1500 rpm for 20 minutes and the extracted serum samples were analysed in automated biochemical analyzer (A-15 Biosystem Random Access Analyzer, Biosystem, Barcelona, Spain) to study the changes in serum biochemical parameters such as creatinine, BUN, total calcium (tCa), ionized calcium (iCa), phosphorus, Alanine Amino Transferase (ALT), Alkaline phosphatase (ALP), total bilirubin, direct bilirubin, total protein, glucose and lactate values in thrombocytopenic dogs. Impairment of renal function was diagnosed based on elevation in creatinine, BUN, phosphorus values, hepatic involvement was diagnosed based on elevation in Alanine Amino Transferase (ALT), Alkaline phosphatase (ALP), total bilirubin, direct bilirubin values and decrease in total protein and sepsis was diagnosed based on elevation of lactate values along with the clinical presentation.

Body fluids such as urine and ascitic fluids were collected and processed in befitting cases for cytological examination. Fine Needle Aspiration Cytology (FNAC) samples were collected and processed in thrombocytopenic dogs with neoplasms. Leptospirosis was diagnosed in thrombocytopenic dogs by dark field microscopy. Further, blood samples were collected and processed for molecular diagnosis of haemoprotozoan diseases and canine parvo viral enteritis.

In thrombocytopenic non-survivor dogs, tissue samples of organs showing pathological changes were collected during postmortem examination and processed for histopathological examination.

Statistical analysis

The data generated from different parameters of the experimental study were subjected to Chi-Square test and one-way analysis of variance (ANOVA). Analysis was performed by IBM SPSS software version 15.0 for Windows.

RESULTS AND DISCUSSION

Incidence of thrombocytopenia

Out of 1320 dogs screened during the study period, thrombocytopenia was identified in 450 dogs. This accounted to 34.09 per cent incidence of thrombocytopenia in critically ill dog population in our study period. The incidence recorded in our study was higher than the earlier reports on canine thrombocytopenia wherein Grindem *et al.* (1991) have documented 5.2% incidence of thrombocytopenia and Botsch *et al.* (2009) have reported 6.7 per cent incidence of thrombocytopenia in their study on thrombocytopenia in dogs.

Thrombocytopenia was recorded in twenty-four different breeds in our study. The highest incidence was recorded in Labrador 135(30%) followed by Non-descript109 (24.22%), Spitz 50 (11.11%), German Shepherd 36 (8%), Pug 22 (4.89%), Golden Retriever 19 (4.22%),

Rottweiler 18 (4%), Doberman 16 (3.56%), Cocker Spaniel 8 (1.78%), Siberian Husky 7 (1.56%), Beagle 6 (1.33%) and 3 (0.67%) each in Pomeranian, Chippiparai, Dachshund, Pitbull and Lhasa Apso breeds. Two cases (0.44%) were recorded in Dalmatian and one each (0.22%) was recorded in Great Dane, Kanni, Bull Mastiff, Miniature Pinscher, BulliKutta, Boxer and Shih Tzu. Similar to our findings, Khan *et al.* (2021) recorded the highest prevalence of thrombocytopenia in Labrador retriever (41.6%). An increase in the occurrence of thrombocytopenia in Labrador breed in our study could be attributed to the higher proportion of Labrador dogs reared in and around Chennai due to preference of Labrador breed by the clients.

In sexwise analysis, thrombocytopenia was recorded predominantly in males 248 (55.11%) than in females 202 (44.89%). In contrast to our findings, Schneider and Mischke (2016) reported a higher occurrence of thrombocytopenia in females (320) than in males (272) in their study. The gender bias in our findings favouring thrombocytopenia in males could be attributed to the pet owners' favouritism for having male dogs over female dogs in Chennai and its outskirts.

In age wise incidence, thrombocytopenia was recorded in critically ailing dogs of all age groups from the lowest age of 37 days in a Beagle to the highest age of 15 years in a German Shepherd dog. The incidence was highest in dogs belonging to the age group 1-5 years (219, 48.67%) and adult dogs of age group 5-10 years (129, 28.67%). The incidence was relatively less in young dogs of <1 year (61, 13.55%) and in older dogs of age group >10 years (41, 9.11%). However, published reports on agewise incidence of canine thrombocytopenia are not available for the comparison of agewise incidence recorded in our study.

Grades of thrombocytopenia

Of the 450 dogs diagnosed with thrombocytopenia, severe thrombocytopenia(less than 50000 platelets/cmm) was observed in 190 (42.22%) dogs, moderate thrombocytopenia (50000 to 100000 platelets/cmm) was observed in 166(36.89%) dogs and mild thrombocytopenia (100,000 to 150000 platelets/cmm) was observed in 94 (20.89%) dogs. Comparison of mean±SE values of platelet count in thrombocytopenic groups with the control group revealed a highly significant decrease ($p<0.01$) in mean± SE values of platelet count in thrombocytopenic dogs compared to the control group and a highly significant difference was also observed between the three groups of thrombocytopenia. Haemoparasitic diseases (35.26%, 67/190) followed by miscellaneous etiology(16.84%, 32/190), renal failure (12.10%, 23/190) and sepsis (8.95%, 17/190) were identified as the major causes that caused severe thrombocytopenia in our study.

Clinical signs in thrombocytopenic dogs

The predominant clinical signs observed in thrombocytopenic dogs were anorexia (401/450, 89.11%),

Table 1: Clinical signs recorded in various grades of thrombocytopenia.

Clinical signs	Grade 1 (n=190)	Grade 2 (n=166)	Grade 3 (n=94)	Total
Anorexia	177	146	78	401
Pyrexia	102	29	77	208
Epistaxis	98	68	32	198
Petechiae/ Ecchymoses	72	28	51	151
Vomiting	35	43	57	135
Melena	18	31	67	116
Dyspnoea	52	24	37	113
Lymphadenopathy	59	24	27	110
Pale mm	18	12	39	69
Reddish urine	26	24	15	65
Hypersalivation	3	17	20	40
Hematemesis	26	13	Nil	39
Lateral recumbency	14	3	19	36
Seizures	10	9	11	30
Icteric mm	12	8	6	26
Congested mucous membrane	12	6	8	26
Diarrhoea	5	13	8	26
Vaginal bleeding	8	3	Nil	11
Subnormal body temperature	6	2	1	9
Scrotal edema	Nil	4	3	7
Ascites	Nil	2	4	6
Gingival bleeding	4	2	Nil	6
Corneal opacity	1	3	Nil	4
Scleral haemorrhage	1	2	Nil	3

pyrexia(208/450, 46.22%), epistaxis (198/450, 44%) and petechiation and ecchymoses on the skin of ventral abdomen (151/450, 33.55%) (Table 1 and Fig 1). Similar to our findings, Kohn *et al.* (2000) also reported the classic signs of thrombocytopenia in dogs *i.e.* petechiation, ecchymosis, epistaxis and gastrointestinal blood loss. Among the three grades of thrombocytopenic dogs, clinical presentations such as epistaxis, petechiation and ecchymoses, haematemesis, reddish discolouration of urine and vaginal bleeding were manifested more in dogs with severe thrombocytopenia, compared to the dogs with moderate and mild thrombocytopenia.

Aetiology of thrombocytopenia

The causes of canine thrombocytopenia recorded in our study were haemoparasitic diseases 25.11% (113/450), renal disorders 17.55% (79/450), hyperthermia 10.44% (47/450), sepsis 8.44% (38/450), neoplasia 8.44% (38/450), hepatic disorders 6.89% (31/450), viral diseases 3.55% (16/450), immune mediated thrombocytopenia 3.11% (14/450), leptospirosis 2.44% (11/450), pneumonia 1.55% (7/450), dilated cardiomyopathy 0.55% (1/450) and ivermectin toxicity 0.55% (1/450). Fifty-four (12%) cases recorded were grouped under miscellaneous category as the cause of thrombocytopenia could not be ascertained.

Our research identified haemoparasites as the major cause of thrombocytopenia (25.11%) in critically ailing dogs. This could be attributed to the tropical climate of our geographical region which is conducive to the propagation of tick population which serve as vectors for the transmission of haemoparasitic diseases. In close concordance with our findings, Khan *et al.* (2021) also reported haemoprotozoans, followed by renal failure and neoplasia as the major etiologic groups of canine thrombocytopenia.

Haematology

Haematological parameters

Comparison of mean ± SE values of hemoglobin, PCV and RBC in thrombocytopenic groups revealed a highly significant decrease ($p<0.01$) in the mean ± SE values of hemoglobin, PCV and RBC in critically ill dogs with thrombocytopenia compared to the control group. No significant difference was observed in WBC count between thrombocytopenic groups and reference range in our study. Changes in WBC count as reported in perusal of literature such as elevation of WBC count was recorded in individual cases of thrombocytopenia with various etiologies such as sepsis, haemoparasitic infection etc and a decrease in WBC count was recorded in individual cases of ehrlichiosis, canine parvo viral enteritis *etc.* Monocytosis and neutrophila were recorded in dogs with *Ehrlichia canis* and *Hepatozoon canis* infection respectively which was in agreement with Thongsahuan *et al.* (2020).

Platelet indices

Regarding the platelet indices, no significant difference was recorded on comparison of mean ± SE values of MPV in thrombocytopenic dogs with control group as well as between the three grades of thrombocytopenic dogs.

a. Canine- Labrador- Male–7 years- Tick infestation on ears

b.Canine- Labrador- Female – 5 years - Grade 3 thrombocytopenia - Ecchymosis on ventral abdomen

c.Canine- Golden Retriever- Male – 3 years- Epistaxis

d.Canine- Non-descript- Male – 3 years- Hematemesis

Fig 1: Clinical signs recorded in thrombocytopenic dogs.

However, a highly significant increase ($p<0.01$) in the mean ± SE values of PDW was recorded in thrombocytopenic dogs which was in agreement with Souza *et al.* (2016) who also observed significant increase in the mean values of PDW in dogs with thrombocytopenia when compared to dogs with normal platelet counts. An increase in PDW can be considered as a positive indicator in thrombocytopenic dogs as increase in PDW is attributed to the release of different sized platelets resultant to increased thrombopoiesis and platelet activation to combat the platelet demand in the body. Highly significant reduction ($p<0.01$) in the mean ± SE values of PCT was recorded in thrombocytopenic dogs and between the three thrombocytopenic groups which agreed with the findings of Schwartz *et al.* (2014).

Blood picture findings

Microscopic examination of LG stained blood smears revealed, hypochromasia, neutrophilia, monocytosis and severe to moderate thrombocytopenia in *Ehrlichia canis* positive blood smears. *Babesia gibsoni* and *Babesia vogeli* positive blood smears revealed severe thrombocytopenia, presence of leptocytes, nucleated RBCs , leukopenia, polychromasia and hypochromasia. In case of *Trypanosoma evansi* positive blood smear, hypochromasia and poikilocytosis were noticed and neutrophilia was present in *Hepatozoon canis* positive smears.

Microscopic examination of blood smears revealed changes in erythrocytes such as hypochromasia, leptocytes, poikilocytosis, echinocytes and codocytes. Signs of regenerative anaemia such as nucleated RBCs, Howell Jolly bodies and polychromasia were more evident in blood smears positive for babesiosis.

The abnormalities in WBCs recorded in our study were leukocytosis in 125 cases, of which, neutrophilia was recorded in 65 cases, neutrophilia with shift to left in 57 cases and monocytosis in 60 cases. Leukopenia was observed in 54 thrombocytopenic dogs.

In LG stained blood smears, platelets appeared as pink round, ovoid, rod, discoid structures and as pleomorphic cellular fragments. The size of the platelets varied from 2μ to 4μ approximating to one-fourth to one fifth of the RBC in most of the cases. Occasionally, platelets appeared bigger, approximating the size of RBCs and were even found as giant or macroplatelets (more than 5μ) appearing bigger than the RBCs. Souza *et al.* (2016) reported on macroplatelets in thrombocytopenic animals as seen in our study and stated that large platelets can be observed in the peripheral blood when bone marrow is intact and when it is overstimulated to produce platelets. In contrary to this, platelets also appeared very small as microplatelets in smears screened for detection of thrombocytopenia.

Regarding the distribution, platelets were found as randomly scattered individual element in different fields and as few to many platelets without adherence in linear or random fashion in different fields. The platelets were also seen as small aggregates in a field as well as small to big clumps or clusters. The blood picture findings are presented in Fig 2. The platelet clumps were either seen as an exclusive platelet cluster or as neutrophils aggregated with platelet

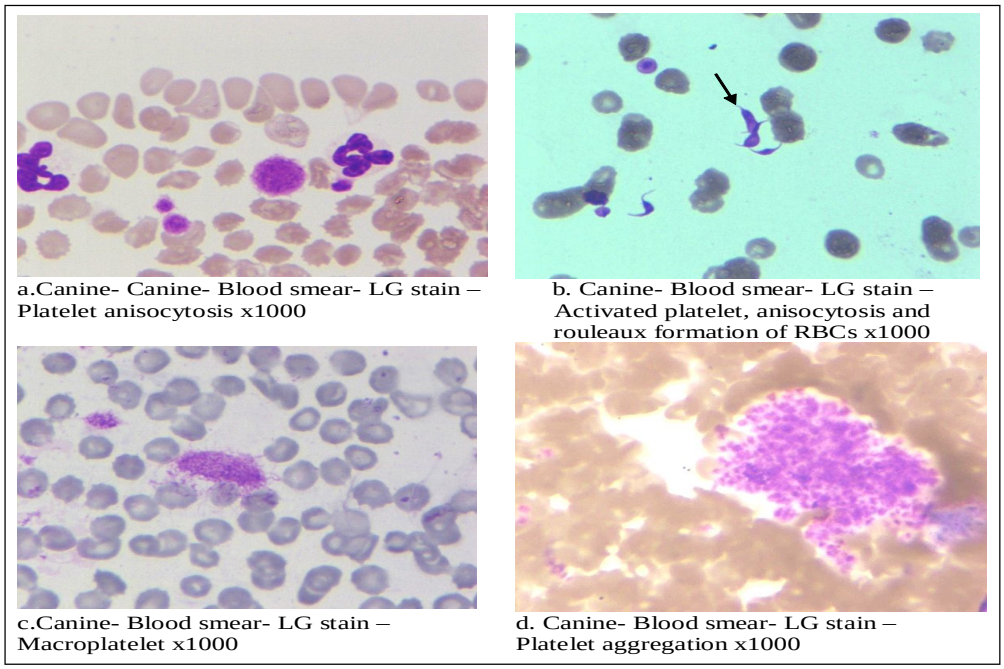


Fig 2: Platelet morphology in thrombocytopenic dogs.

clusters. These findings were in close agreement with Jones (2009) and Villiers and Ristic (2016).

Blood smear examination for the detection of blood parasites

Ehrlichia canis 54.87% (62/113), *Babesia gibsoni* 17.70% (20/113), *Babesia vogeli* 11.5% (13/113), *Hepatozoon canis* 6.19% (7/113), *Trypanosoma evansi* 4.42% (5/113) and Microfilaria 0.88% (1/113) were the haemoparasites (113/450) identified in the present study based on blood smear examination (Fig 3). Severe thrombocytopenia was recorded in 51 out of 62 dogs diagnosed with ehrlichiosis, 14 out of 20 dogs diagnosed with *Babesia gibsoni,* 12 out of 13 dogs diagnosed with *Babesia vogeli* and two out of seven dogs diagnosed with *Hepatozoon canis*. The thrombocytopenia was of mild grade in a dog diagnosed with microfilaria in blood smear. In agreement with our finding of a single case of microfilaria in thrombocytopenic dogs, Souza *et al.* (2016) have recorded microfilaria in blood smears of two thrombocytopenic dogs.

Biochemical findings

Comparison of mean±SE values of serum biochemical parameters of thrombocytopenic groups with the control group revealed a highly significant increase in the mean±SE values of blood urea nitrogen (BUN), creatinine, ALP, total bilirubin, direct bilirubin and phosphorus. Lactate was significantly higher ($p<0.01$) in non-survivor thrombocytopenic dogs. A highly significant decrease ($p<0.01$) in ionized calcium was observed in critically ill thrombocytopenic dogs and among the thrombocytopenic groups, dogs with sepsis etiology had higher incidence of ionized hypocalcemia. of the 48 thrombocytopenic dogs that died, 14 dogs had ionized calcium concentration <1 mmol/L prior to death in our study. Hence, ionized calcium concentration of <1 mmol/L can be used in future as an indicator of grave prognosis in critically ailing thrombocytopenic dogs. Similar to our observation, Sharp *et al.* (2009) and Holowaychuk and Monteith (2011) also favoured the evaluation of iCa concentrations rather than tCa in critically ill dogs. Goggs *et al.* (2017) also reported that the case fatality rate was higher in low concentration of ionized calcium and attributed that alteration in the electrolyte concentrations was associated with mortality in critically ill dogs.

Polymerase chain reaction for the detection of blood parasites and canine parvo viral enteritis

Polymerase chain reaction performed to identify haemoparasitic infections in 60 dogs with severe thrombocytopenia revealed positive infections in 47 cases (78.33%). Happi *et al.* (2018) also detected 89/116 (76.7%) positive samples of blood borne pathogens by PCR analysis. *Ehrlichia canis* (33/60, 55%), *Babesia gibsoni* (12/60, 20%) and *Babesia vogeli* (2/60, 3.33%) were the haemoparasites identified. Earlier, Macieira *et al.* (2005) in their study on the prevalence of *Ehrlichia canis* infection in thrombocytopenic dogs from Rio de Janeiro, Brazil reported that 26.8% of thrombocytopenic dogs were positive for *E.canis* in polymerase chain reaction.

Anaplasma platys is the exclusive parasite of platelets which cause thrombocytopenia. Atif *et al.* (2021) stated that *Anaplasma platys* chiefly infects the canine platelets and

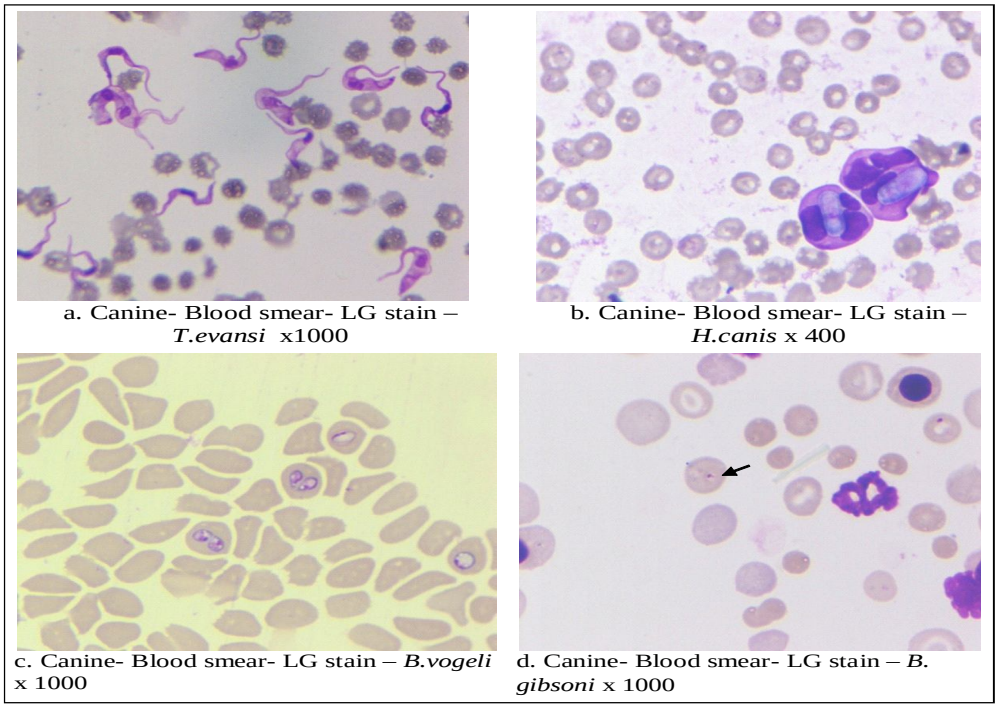
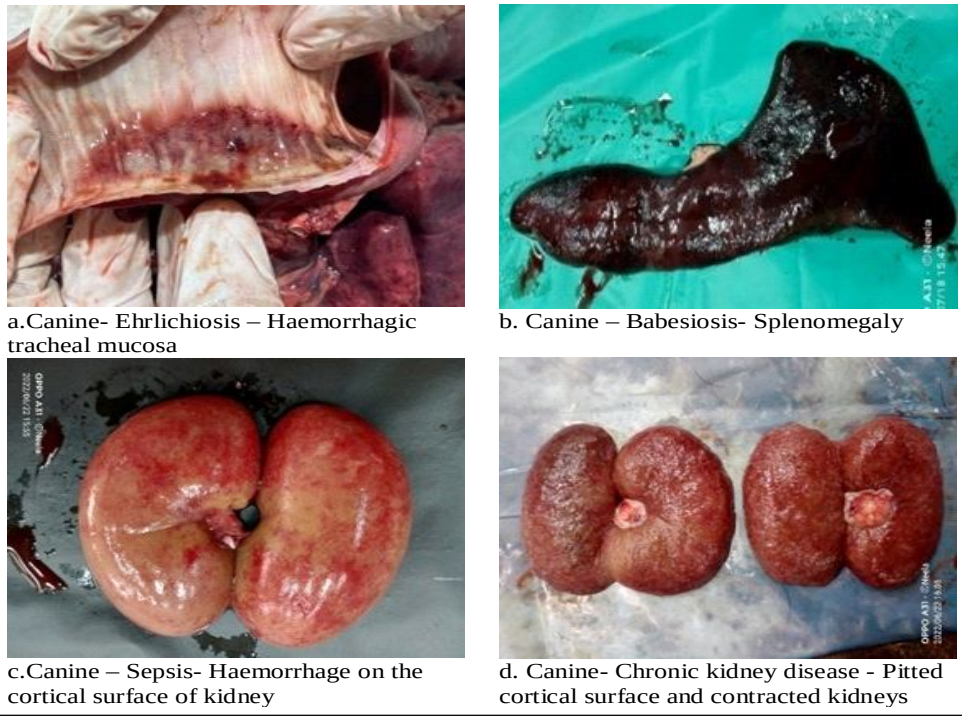


Fig 3: Haemoparasites identified in thrombocytopenic dogs.



a.Canine- Ehrlichiosis – Haemorrhagic tracheal mucosa

b. Canine – Babesiosis- Splenomegaly

c.Canine – Sepsis- Haemorrhage on the cortical surface of kidney

d. Canine- Chronic kidney disease - Pitted cortical surface and contracted kidneys

Fig 4: Pathological changes observed in thrombocytopenic dogs.

leads to severe thrombocytopenia by causing direct damage to platelets and by immune-mediated mechanisms. But, *A.platys* organisms were not detected in blood smear examination as well as by molecular study in the thrombocytopenic dogs in our research.

Canine parvo viral enteritis was diagnosed in 16 thrombocytopenic cases by Polymerase chain reaction. Earlier, Castro *et al.* (2013) also observed that thrombocytopenia were significantly more frequent among dogs infected with canine parvo viral enteritis than the control group in their study on clinical, hematological and biochemical findings in canine parvovirus enteritis.

Mortality in thrombocytopenic dogs

Of the 450 thrombocytopenic dogs recorded in our study, 134 dogs recovered completely with restitution of adequate platelet counts when evaluated concurrently by automated blood analyser and blood smear examination. 268 dogs were not reported for follow up. Unfortunately, 48 thrombocytopenic dogs (10.67%) succumbed to death. Majority of the non-survivors belonged to the severe thrombocytopenia group (37/48).

Post mortem examination was conducted on six out of the 48 dogs to study the pathological changes of non-survivor thrombocytopenic dogs. Post mortem examination could not be conducted on rest of the dogs (n=42) due to unwillingness of the pet owners. Post mortem examination revealed varying degrees of congestion and haemorrhage of the visceral organs in all the cases and based on the gross and histopathological changes, ehrlichiosis (n=2), babesiosis (n=2), chronic kidney disease (n=1) and sepsis (n=1) were confirmed (Fig 4).

CONCLUSION

In summary, in dogs suspected for thrombocytopenia based on clinical presentation and based on reduced platelet counts in automated analysers, especially in critically ailing dogs, further reaffirmation of thrombocytopenia by having a manual count of platelets in haemocytometer and in LG stained smears shall have a say in differentiating thrombocytopenia from pseudothrombocytopenia which is not clinically significant. In confirmed cases of canine thrombocytopenia, employing conventional and molecular diagnostic tools will help to identify the actual cause of thrombocytopenia and concomitant evaluation of haematobiochemical values will help to assess the prognosis of critically ailing thrombocytopenic dogs.

Conflict of interest

All authors declare that they have no conflicts of interest.

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