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Evaluation of thrombocytopenic dogs upon transfusion of platelet rich plasma

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Abstract
Thrombocytopenia is the most common haemostatic condition in dogs, and it can be fatal in dogs. Platelet transfusions can provide short-term haemostasis and fresh platelet rich plasma remains the product of choice. The present study was conducted on 12 dogs presented at Small Animal Clinics of Teaching Veterinary Hospital, Guru Angad Dev Veterinary Animal Sciences University (GADVASU), Ludhiana, India, with indications of thrombocytopenia. Melena (75.0%) was found to be most significant symptom of severe thrombocytopenia followed by epistaxis (58.30%) and ecchymotic haemorrhages/Purpura (50.0%). Physical examination, blood biochemistry and haematology were performed in these thrombocytopenic dogs to establish the etiology and PCR was performed to diagnose haemoprotozoan disease. The highest prevalence of thrombocytopenia was recorded in Labrador retriever (41.6%) followed by mixed breeds (25.0%) and German shepherd (16.6%). The highest number of cases were recorded in haemoprotozoan group (50.0%) followed by renal failure (41.6%) and neoplasia (9.30%). The blood smear examination showed no haemoprotozoa on examination however, 6 patients were positive for haemoprotozoa on PCR including *B. gibsoni* (n=1) and *E.canis* (n=5). Donor selection, blood collection, separation of packed RBCs and platelet rich plasma (PRP), and administration of PRP were done as per standard protocols. Transfusion of fresh/ stored PRP was done in severely thrombocytopenic dogs. Within one hour of transfusion, platelet count showed an increasing trend.

Keywords: Platelet rich plasma, thrombocytopenia, ecchymosis, *E. canis*

Introduction
Canine thrombocytopenia is the major cause of bleeding/haemostatic disorder and is seen as an emergency situation in veterinary patients. The major causes of thrombocytopenia in canine patients is usually attributed to decreased or lack of production, increased destruction or consumptive processes, abnormal loss, and sequestration of platelets (Neel *et al.* 2014) [3]. The leading cause of thrombocytopenia in canines is immune- mediated thrombocytopenia in dogs. The secondary causes of thrombocytopenia in dogs can be due to infectious (*Ehrlichia* spp) or inflammatory, incompatible transfusions but enhanced platelet consumption may also be observed with neoplasia, vasculitis and disseminated intravascular coagulation (DIC).
The primary indications for a platelet transfusion is the management of uncontrolled or life threatening bleeding from severe thrombocytopenia or thrombocytopathy (Callan *et al.* 2009) [10]. Platelet transfusions are only used in the case of severe uncontrolled bleeding into the nervous system or cardiopulmonary system. Platelets can only be transfused from freshly obtained blood that has been held at room temperature for at least 12 hours, though frozen or even artificial platelets are available. In clinical practice, thrombocytopenic patients who are severely bleeding can benefit the most from platelet concentrate or platelet rich plasma to replenish platelets. The studies on platelet transfusion in canines aren't available and usually human reference levels are used as for the platelet transfusions and dosage.

Materials and Methods
Study population
The present study was conducted on 12 dogs of both genders, aged 6 months to 10 years and body weight ranged from 10 kg to 20 kg. The dogs were presented at Small Animal Clinics of Teaching Veterinary Hospital, Guru Angad Dev Veterinary Animal Sciences University (GADVASU), Ludhiana, India, with signs of epistaxis, melena, hematochezia hematemesis and or ecchymotic haemorrhages or pupura.

Signalment and anamnesis
Data collected when the animals were examined included breed, age, sex, and time (days) from the onset of clinical signs. History of feed intake, water intake, fecal color, symptoms of pain, and any prior treatment given were noted in every case.

Clinical examination
Each animal was subjected to a detailed clinical examination. Each animal was thoroughly evaluated for its general condition, inspection of mucous membranes, hydration status, signs of pain, and abdominal distension.

Hematology
Blood samples (2 ml) were collected aseptically from the cephalic vein in ethylene-diamine-tetraacetic acid coated vials). Immediately after collection, the blood was used for determination of haemoglobin (Hb), packed cell volume (PCV), total leukocyte (TLC) count, total erythrocyte count (TEC), platelet count, and differential leukocyte count by and automated hematology analyzer (ADVIVA 2120 Hematology System, Siemens). Blood samples were stained with Leishman stain and subjected to microscopic examination to detect the different haemoprotozoan and rickettsial infections viz., *Babesia gibsoni*, *Babesia canis vogeli*, *Hepatozoon canis* and *Ehrlichia canis* etc.

Clinical biochemistry
For serum biochemical analysis, blood samples were collected in serum vials. After clotting, serum was separated by centrifugation and transferred to a dry clean vial for further evaluation. VITROS DT60 II chemistry system (Ortho-Clinical Diagnostics, Johnson and Johnson Company, New Brunswick, NJ, USA) was used to determine the serum activities of alanine aminotransferase (ALT), total bilirubin, total proteins, albumin, blood urea nitrogen (BUN) and creatinine.

Molecular diagnosis
The Ec-PCR was standardized by using primers targeting a partial Vir B9 gene sequence of *Ehrlichia canis* as described by (Kledmanee *et al.* 2009) [20]. The sequences of primers employed in PCR assay are as follows:
Forward primer (Ehr1401F): 5' CCA TAA GCA TAG CTG ATA ACC CTG TTA CAA 3'
Reverse primer (Ehr1780R): 5' TGG ATA ATA AAA CCG TAC TAT GTA TGC TAG 3'
The Bg-PCR was standardized by using primers targeting a partial 18S rRNA in the gene sequence of *B. gibsoni* (Singh *et al.* 2014) [25]. The sequences of primers employed in PCR assay are as follows:
Forward primer (GIB599): 5' CTC GGC TAC TTG CCT TGT C 3'
Reverse primer (GIB1270): 5' GCC GAA ACT GAA ATA ACG GC 3'
Briefly, the PCR was set up in 25 µL reaction consisting of 10X Dream Taq buffer (Thermo scientific), 200 µM of 10 mM dNTP mix (Thermo scientific), 1.0 mM of 25 mM MgCl2 (Biolabs), 1.0 U of Taq DNA polymerase (recombinant) (Thermo scientific), 15 pmol each of the respective primers and 3.0 µl of template DNA source. The final volume was made up to 25 µL by adding requisite amount of nuclease free water (Thermo scientific). PCR products were analysed using conventional agarose gel electrophoresis in 1.5% w/v agarose stained with Good view nucleic acid stain (Helix Biosciences) at 100 V for 30-45 min.

Donor selection
Donors selected for the Platelet rich plasma (PRP) donation were 1 to 8 years old, with minimum body weight of 20 kg, clinically healthy with normal feed and water intake. The donors were screened against common diseases endemic pathogens before using them for blood collection. Minimum Hb and PCV level for considering dog as donor were 13g/dL, 40.0% and minimum platelet count of 250×10^3 respectively. Thin blood smears were used for the diagnosis of babesiosis, ehrlichiosis and hepatozoonosis while, Rose Bengal plate agglutination test was used for screening of brucellosis.

Preparation of PRP
One unit (350 ml) of blood was collected by venipuncture into a double blood bag. Whole blood (WB) was centrifuged for 5 minutes at 1800x g to prepare platelet-rich plasma (PRP). The PRP was expressed into a satellite bag by plasma extractor.

Administration of PRP
Before transfusing plasma into the recipient dog, a risk note for undergoing the process got signed by the owner. The cross-matching for the plasma and platelet can be optional However; the major cross match was performed in every patient. PRP was given IV through cephalic or saphenous vein via a blood administration and to give a calculated dose. No other drug or solution containing calcium or glucose was given through the same IV line except 0.9% normal saline solution.

Result and Discussion
Observation on severely thrombocytopenic dogs
Clinical signs
Melena was found to be most significant signs of severe thrombocytopenia (75.00%) followed by epistaxis (58.33%) and ecchymotic haemorrhages/Purpura (50.00%) (Table 1). Kohn *et al.* (2000) [21] concluded that classic signs of thrombocytopenia includes petechiation, ecchymosis, epistaxis, and gastrointestinal blood loss and the most severe thrombocytopenias, are due to Immune Mediated Thrombocytopenia (IMT) often cause only mild haemorrhage. Kohn *et al.* (2000) [21] recorded that clinical signs of haemorrhage were observed in 12 of 15 dogs with pITP and in 6 of 9 dogs with Evans' syndrome. Thrombocytopenia is the most commonly acquired haemostatic disorder in dogs and can become potentially life-threatening (Grindem *et al.* 1991; Bommer *et al.* 2008) [16, 5]. The highest prevalence of thrombocytopenia was recorded in was recorded for Labrador retriever (41.67%) followed by mixed breeds (25.00%) and German shepherd (16.67%). This finding can be attributed to over representation of Labrador retriever dogs at the hospital. However, Botsch *et al.* (2009) [6] recorded the highest prevalence of thrombocytopenia in German shepherd followed by Bernese mountain dog and Golden retriever. However, according to Grindem *et al.* (1991) [16] Doberman Pinschers were most presented breed. The difference in these findings can be attributed to the presentation of different breeds in a particular geographic area.

Haematological and biochemical alterations in thrombocytopenic patients
A total of 12 thrombocytopenic cases were included in the

study which were divided on the basis of etiology into three groups; the highest number of cases were recorded in haemoprotozoan group (50.00%) followed by renal failure (41.67%) and a single case of neoplasia (Table 2). Severe thrombocytopenia in dogs is mostly caused by immune-mediated thrombocytopenia; however, low platelet counts are also commonly associated with inflammatory, infectious or neoplastic diseases (Bommer *et al*., 2008; Botsch *et al*., 2009) [5, 6].

The blood smear examination revealed no haemoprotozoa however, six patients were positive for haemoprotozoa on PCR including one case of *B. gibsoni* and five were positive for *E.canis*(Fig1). The acute thrombocytopenia could be due to increased platelet consumption due to inflammatory changes in blood vessel endothelium, increased splenic sequestration of platelets, and immunologic destruction or damage resulting in a substantially reduced platelet life (Pierce *et al*., 1977 and Kakoma *et al*., 1978) [24, 19]. All the thrombocytopenic groups showed moderate anaemia with low PCV and TEC. Similar findings were recorded by De Gopegui *et al*. (2007) [12] reported that all dogs with babesiosis had thrombocytopenia and 20 percent had disseminated intravascular coagulation syndrome. Furlanello *et al*. (2005) [15] also found that dogs presented with babesiosis had thrombocytopenia along with hyperfibrinogenemia and anaemia of variable severity. Birkenheuer *et al*. (1999) [4] recorded the clinical signs of *Babesia* range from severe haemolytic anaemia and thrombocytopenia to subclinical infections. Bulla *et al*. (2004) [8] reported thrombocytopenia in 146 samples (less than 200 000/mL) infected with *E.canis.* Similar to our study, Waner *et al*. (1995) [31] concluded that thrombocytopenia is considered to be the most common and consistent haematological abnormality of dogs naturally or experimentally infected with *E. canis*. Smith *et al*. (1975) [27] reported that platelet survival time decreased from a mean of 9 days to 4 days, 2 to 4 days after infection with *E. canis*. Thongsahuan *et al*. (2020) [30] noted the similar findings as in our study as haematological alterations caused by Ehrlichia infections include anaemia, thrombocytopenia, monocytosis, and eosinophilia. Jacobson and Clark (1994) [18] recorded haemolytic anaemia and thrombocytopenia as the predominant feature of canine babesiosis. Mechanisms of RBCs destruction include increased osmotic fragility, shortened RBC life span, and erythrophagocytosis.

In the current study, thrombocytopenia was recorded in patients with chronic kidney diseases. Supriya (2019) [9] also observed a lower than normal level of platelet count in 24 dogs out of 90 dogs with renal failure. Dorgalaleh *et al*. (2013) [14] also observed the presence of anaemia and thrombocytopenia in patients with renal failure. However, Mann, (2013) [22] found the mean total platelet count to be 145.12±20×10^3/cu mm in dogs suffering from renal failure and reported thrombocytopenia in 10 out of 46 dogs. Devipriya *et al*. (2018) [13] found that 77 dogs of the 150 with renal insufficiency had significantly lower PCV, Hb, TEC and higher TLC and high levels of BUN and creatinine. Mann, (2013) [22] recorded the mean BUN to be 134.65±14.27 mg/ dL in renal failure dogs. Singh, (2017) [26] also found high levels of BUN (58.44 mg/ dL) in renal failure dogs, and Srinivasan *et al*. (1993) [28] also reported high mean BUN (78.18 mg/d dL) in renal failure dogs. BUN elevation is associated with increased protein catabolism, oliguria, vomiting, or gastrointestinal bleeding (Bartges and Polzin, 2011) [3].

In our study, the ALT and BUN in haemoprotozoan patients were unaffected. However, in contrast to our study, Boozer and Macintire (2003) [7] noted the increased serum ALT, creatinine and BUN levels. Our results were in contrast to Burghen *et al*. (1971) [9] & Harrus *et al*. (1999) [17]. They recorded hypoalbuminemia, hyperglobulinemia, and hypergammaglobulinemia are the predominant biochemical abnormalities found in dogs infected with *E. canis*. Our findings could be due to the reason that all the cases in our study had acute onset and presented immediately.

Evaluation of platelet transfusion in severely thrombocytopenic dogs

Platelet transfusion was carried out in 12 cases and follow up on these dogs either was carried out for about week after initial presentation to evaluate change in platelet counts at different intervals and to ascertain their survival. The platelet count showed an increasing trend within 1 hour of transfusion (Table 3 and Fig 2). The platelet counts were significantly increased at 1 hour 3rd day and 7th day in haemoprotozoan group ($p< 0.05$) while as non-significant increase was recorded in other two groups. The highest trend in increase was noted in haemoprotozoan group Callan *et al*. (2009) [10] found that canine platelet apheresis allows collection of a concentrate with an elevated platelet yield, typically 3–4.5 ×10^{11} vs <1× 10^{11} for whole blood-derived platelets, improving the ability to provide sufficient platelets.

Table 1: Clinical symptoms of thrombocytopenic patients before transfusion (n=12)

Mucous membrane	Severely pale 1(8.33)
	Pale 2(16.66)
	Salmon pink 9(75.00)
General state	Recumbent 1(8.33)
	Able to stand but lethargic 3(25.00)
	Active 8(66.66)
Pulse quality	Weak 1(8.33)
	Bounding 4(33.33)
	Normal(58.33)
Hematochezia	2(16.66)
Melena	9(75.00)
Epistaxis	7(58.33)
Ecchymotic haemorrhages/Purpura	6(50.00)
Ticks	3(25.00)

Table 2: Haematological and Biochemical alterations in severely thrombocytopenic cases based on etiology

	Hb (g/dL)	PCV (%)	TEC(×10^6/ µl)	Platelet(×10^3/ µl)	ALT (IU/L)	Total protein (g/dl)	Albumin (g/dl)	BUN(mg/dl)	Creatinine (mg/dl)
CKD (n=5, 41.67%)	6.36±0.56 (4.2-7.80)	19.64±1.81 (12.8-25.00)	3.91±0.38 (2.33-4.80)	18.80±6.86 (6.00-48.00)	69.6±8.6 (50.00-104.00)	6.40±0.29 (5.50-7.50)	2.40±0.16 (1.80-2.90)	152.2±22.10 (96.00-245.00)	10.94±1.44 (5.70-15.00)
Haemoprotozoan (n=6, 50.00%)	6.20±0.64 (3.20-	19.07±1.78 (12.20-	3.52±0.45 (1.20-4.40)	13.00±3.89 (3.00-	42.67±3.96 (30.00-	6.55±0.17 (6.00-7.00)	2.43±0.08 (2.20-	23.83±1.98 (18.00-30.00)	0.97±0.83 (0.60-1.20)

	7.90)	24.50)		27.00)	56.00)		2.70)		
Neoplasia (n=1, 8.33%)	6.10±0.00	18.90±0.00	3.54±0.00	2.00±0.00	50.00±0.00	6.50±0.00	2.50±0.00	15.00±0.00	0.80±0.00

Table 3: Evaluation of thrombocytopenic dogs upon transfusion of platelet rich plasma

Disease	Pre Transfusion count	1 hour	3 day	5 day
CKD	18.80±6.86 (6-48)	29.25±9.09 (14-60)	34.00±2.55[a] (28-40)	50.00±1.25[a] (30-80)
Haemoprotozoan	13.00±3.89 [a] (3-27)	29.00±5.18 [a] (15-45)	132.50±15.15 [a] (80-150)	237.50±27.20[a] (150-300)
Neoplasia	2.00±0.00	18.00±0.00	30.00±0.00	60.00±0.00
Total	14.50±3.87	28.00±4.83	77.33±18.89	145.00±38.21

*Mean platelet count of donor dogs was 403.75±13.92 (Range 300-480× $10^{3/}$ µl) Values in columns with same superscript differ significantly ($p< 0.05$)

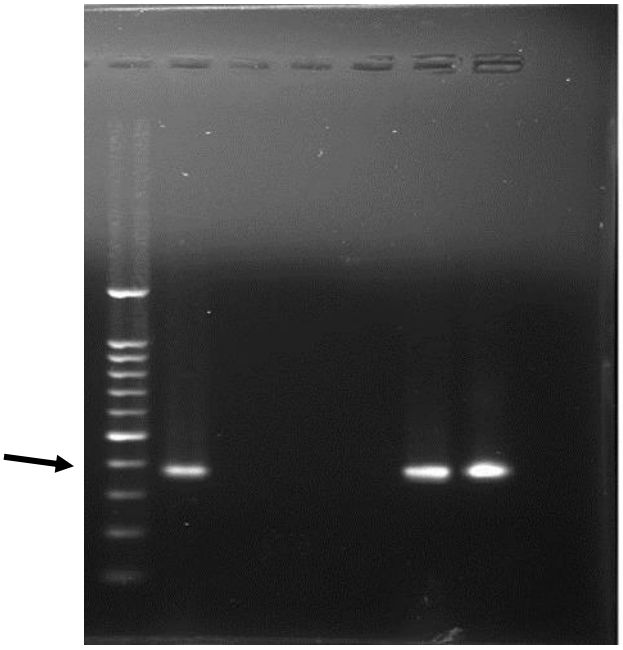

Fig 1: *E. Canis* at (380 bp-arrow) on PCR lane 1 positive control, lane 4, and 5 sample positive for *E.canis* 100 bp DNA ladder

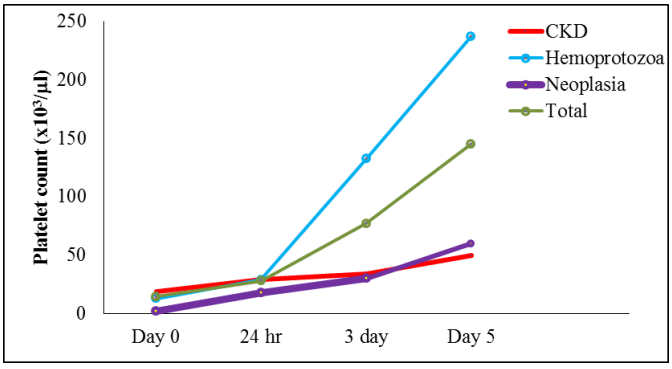

Fig 2: Line diagram showing increase in platelet count after transfusion of PRP

Conclusions

Platelet transfusions are very less done in veterinary practice due to un-availbity of blood banks at veterinary hospitals Platelet transfusions can be beneficial in upcoming time and can be very helpful to improve the platelet counts of dogs with thrombocytopenia.

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