J Bangladesh Agril Univ 21(1): 38–45, 2023 https://doi.org/10.5455/JBAU.128250

ISSN 1810-3030 (Print) 2408-8684 (Online)

Journal of Bangladesh Agricultural University

Journal home page: http://baures.bau.edu.bd/jbau

Research Article

Effects of Plasma Transfusion on Various Hematological and Biochemical Parameters in Goats

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ARTICLE INFO

Article history
Received: 09 Nov 2022
Accepted: 09 Mar 2023
Published: 31 Mar 2023

Keywords
Anemia,
Goat,
Plasma transfusion,
Hematinic

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ABSTRACT

This study investigated the clinical and hematological responses of plasma transfusion and hematinic administration in severely anemic goats. The blood profile of the recipient goats was assessed for hematological parameters: Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Hemoglobin (Hb), Packed Cell Volume (PCV), Neutrophil, Eosinophil, Lymphocyte, Monocyte, Electrolytes and Biochemical parameters: Alanine Transaminase (ALT), Aspartate Transaminase (AST), Creatinine and Total Protein (TP) before and after (1st, 7th, 14th, 21th, 28th days) treatment with plasma transfusion or hematinic administration. In plasma transfusion group (Group A), TEC, PCV, Hb, ALT, AST and TP were increased significantly ($p < 0.01$) at 1st, 7th, 14th, 21th, 28th days of post-transfusion in comparison to pre-transfusion values. In the hematinic group (Group B), two patients died treated with hematinic drugs due to severe anemia. However, no significant changes ($p > 0.05$) in clinical parameters, electrolytes (K^+, Na^+ and Cl^-) levels and creatinine values were observed in recipient goats after plasma transfusion and hematinic administration. After 28 days of treatment, all patients of plasma transfusion group (100%) and three in hematinic treatment group (60%) were recovered successfully. The plasma transfusion was found superior to hematinic administration to upgrade the hematological and biochemical parameters in anemic goats. It did not produce any adverse reaction in the recipient goats. Plasma transfusion could be an effective tool for the clinical management of anemic goats.

Introduction

Transfusion therapy is a life-saving procedure for critically ill animals and animals undergoing surgery (Howard et al.,1992). The use of plasma has emerged as a potential solution to the problems of typing and storage of blood and there is less risk of transfusion reactions. The importance of plasma transfusion for the management of acute hemorrhagic anemia by replenishing blood volume is important and has been found highly effective with 80% recovery rate and is free from incompatible reactions associated with whole blood transfusion (Srivastava and Pandey, 1992). The technique does not require expensive equipment and hence may be practiced in the field conditions (Alam and Hossain, 2005). In Bangladesh, a large amount of blood is wasted every year at the abattoir. Sources of blood, therefore, are adequate. However, collection of blood from the donor, separation of components and its storage needs to be developed. It will help to institute transfusions therapy at the time of emergency.

The goat provides meat, milk, skin and organic manure and considered as the most commercially important species of small ruminants to the rural people of Bangladesh. It is popularly reared by the rural people as it needs less space, less capital and no commercial diet (Basak et al., 1993). Among 567.34 lakh ruminants of Bangladesh, the goat population is 267.74 (DLS, 2021-2022) which plays an important economic role for rural people of this country. In Bangladesh anemia is a common and considered as challenging clinical condition to treat in goats. While anemia is a common sequelae of gastrointestinal parasite infections (Smith et al., 2021). A recent report of the Directorate of Livestock Services depicts an alarming mortality rate of 15 - 20% in goats and the increased death rate is thought to be associated with malnutrition, parasitism, anemia and declined body immunity (Shuvo et al., 2021). It may be due to colostrum deprivation, which hinders body immunity and growth. Thus, young goats become weak, emaciated, anemic and anorectic. The

Cite This Article
Saha, M., Rahman, M.A. and Alam, M.R. 2023. Effects of Plasma Transfusion on Various Hematological and Biochemical Parameters in Goats. *Journal of Bangladesh Agricultural University,* 21(1): 38–45. https://doi.org/10.5455/JBAU.128250

hematopoietic system and electrolyte levels of these animals are seriously depressed; goats become anemic and all blood parameters are remarkably diminished. The animal thus gets to a stage when conventional treatment frequently fails and as a result, death ensues. Instead of being an important part of the livestock sector, severely anemic and unhealthy animals that do not respond with the general treatment, are the burden for the poor farmer of our country and economy. The anemic animals are treated in two ways. These are treated either with nutritional supplements or with transfusion; in a few cases, treatment with combined protocols is also common. The choice of treatment is mainly determined by the severity of anemia and the hemato-biochemical parameter of the anemic patient (Naigamwalla et al., 2012). Generally, only hematinic drugs are the choice of treatment for any type of anemia in goats in the field practices in Bangladesh that cannot save lives in many cases. Blood with a PCV of 10 % will have lost two-thirds of its circulating red cells. In the mild type of anemia when PCV remains within 20-22%, hematinic drugs are the main choice of treatment for any species. In case of moderate anemia (PCV less than 20%) and severe anemia (PCV less than 15%), goats lost two-thirds of their circulating red cells, causing death. In this condition, transfusion is the immediate treatment option to save the life of these animals. However, considering the above facts, this study was performed to investigate the effects of plasma transfusion on various hematological, biochemical and clinical parameters and to observe adverse effects (if any) associated with plasma transfusion in goats.

Materials and Methods

Experimental animals

Ten Black Bengal goats weighing 10 to 15 kg and 6 to 10 months of age were used as recipients. All the goats were weak, emaciated, anorectic and had pale mucous membrane. The goats were clinically anemic. They were housed in separate building in the animal shed of the Department of Surgery and Obstetrics, Bangladesh Agricultural University. They had an access to feed and water *ad libitum*. They were allowed to graze in the pasture 7-8 h a day. The recipient goats were immunized against PPR (P.P.R vaccine, LRI, Bangladesh) and tetanus (TT vax, Popular Pharmaceuticals, Bangladesh, Ltd) and dewormed with albendazole (Bol. Alben, Sk +F Pharmaceuticals, Bangladesh, Ltd). The goats were randomly divided into two groups; Group-A: consisted of 5 anemic goats which were treated with plasma transfusion, and Group-B: consisted of 5 anemic goats which were administered hematinic. Five indigenous non-pregnant healthy goats were used as blood donors. They were free from infectious diseases and blood parasites and protozoa and had not received any vaccines recently. The blood profile of each donor was assessed before blood collection. Cross-matching of the recipient blood to that with the donors was performed before transfusion.

Collection of blood and separation of plasma

Blood was collected from the donors by jugular venipuncture, in the sterile plastic containers containing 3.8% sodium citrate (0.1 mL mL-1 blood). The containers were gently agitated during collection of blood to ensure proper admixture of the blood with preservative. Plasma was separated by centrifuging the blood at 2000 rpm for 15 min immediately after collection.

Transfusion of plasma

The recipient goats were controlled in lateral recumbency. The left jugular furrow area was aseptically prepared for transfusion of plasma. Fresh plasma was administered to the jugular vein using an intravenous cannula and a blood donor set with a built-in filter at a rate of 1 ml/kg/h for the first 15 min, then, the rate was doubled to 2 ml/kg/h for the next 15 min. At 30 min, the rate was doubled again to 4 ml/kg/h, and then was doubled one last time at 60 min to a rate of approximately 8–10 ml/kg/h.

Post-transfusion blood sample analysis

Post-transfusion blood samples collected at Days 1, 3, 7, 14, 21 and 28 and were analyzed for the determination of TEC, TLC, Hb, PCV, DLC, TP, Creatinine, ALT, AST, K^+, Na^+, Cl^-. The pre-transfusion values of these parameters were considered as control and were compared to those obtained on different post-transfusion periods. Hematological, biochemical and clinical parameters were recorded prior to plasma transfusion and hematinics treatment period. The values recorded prior to plasma transfusion and hematinics treatment were taken as control. The control values were compared with experimental values obtained at different post transfusion days and post hematinic administration period. Hematological parameters TEC, TLC, Hb, PCV and DLC were determined from the blood samples of both donors and recipients according to previously published methods (Sastry,1989). The biochemical parameters: TP, Creatinine, ALT, AST, K, Na, Cl were determined by using Microlab Bio-chemistry Analyzer (Germany) by kinetic method and serum electrolytes (K+, Na+, Cl-) level were determined by EasyLyte PLUS Na/ K/Cl ANALYZER.

Clinical parameters

Respiration rate, heart rate and rectal temperature of the recipient goats were recorded before transfusion, during transfusion and immediately after transfusion.

Statistical analysis

The collected data on various parameters were statistically analyzed using STATA 14 statistical software. The statistical analysis on heart rate, respiratory rate, rectal temperature was performed by means of One-way repeated measurement analysis of variance (ANOVA), followed by Bonferroni's mean comparison test. The different experimental groups were compared at the different times and the variables at different times were compared with the control values.

Results

Effects of plasma transfusion and hematinics administration on various hematological and biochemical parameters in recipient goats

Changes in Total Erythrocyte Count (TEC), Hemoglobin (Hb), Packed Cell Volume (PCV)

In plasma transfusion group, the pre-transfusion control values of TEC, Hb, PCV started increasing progressively from day-1 and the significant increases of TEC and PCV were recorded from day-7 ($p<0.01$). The significant increases of Hb were recorded from day-14. The elevated values of TEC, Hb and PCV sustained throughout the experimental period (Table 1 and Table 2). In hematinics group, the mean pre-transfusion control values of TEC, Hb, PCV started increasing from day-1 and sustained throughout the experimental period but the values were not significant when compared to the pre transfusion values. When compared between two groups, the value of TEC, Hemoglobin (Hb) and Packed Cell Volume (PCV) was significantly ($p < 0.01$) higher in the animals of Group A in comparison to those of the animals in Group B (Figure. 1A, 2B, 2C).

Changes in TLC and DLC

The TLC values recorded on different post-transfusion periods did not show any significant change in the animals of both the groups (Table 1, Table 2 and Figure. 1B). In both the groups, a mild change in DLC were noticed during the experimental period. The number of lymphocytes increased progressively throughout the experimental period but this change was not statistically significant (Table 1 and Table 2).

Table 1. Blood parameter resulted from before and after treatment with plasma transfusion (n = 5)

Parameters	Pre transfusion values + (Control)	Post transfusion values (Mean ± SE)					Level of significance	Normal range
		Day-1	Day-7	Day-14	Day-21	Day-28		
TEC (million/cmm)	11.37±0.45	12.10±0.66	13.62±0.57**	13.84±0.73**	14.02±0.68**	14.37±1.04**	**	8.02-17.05
TLC (thousand/ cmm)	9.77±0.49	10.14±0.62	9.76±0.64	9.56±0.91	9.53±0.50	9.45±0.56	ns	2.5-14.2
Hb (g%)	4.24±0.73	4.76±0.65	5.82±0.80	5.98±0.56*	6.24±0.58**	6.36±0.66**	**	3.6-6.6
PCV (%)	10.20±0.58	11.40±0.40	14.20±0.37**	17.00±0.71**	19.60±0.81**	22.40±0.68**	**	18-27.2
Neutrophil (%)	30.20±0.73	31.00±0.89	31.40±0.68	31.60±0.75	32.40±0.51	32.80±0.20	ns	20-40
Lymphocyte (%)	18.60±2.60	19.40±2.04	20.60±2.11	21.20±2.35	22.40±2.16	24.60±2.93	ns	1.6-38
Monocyte (%)	3.08±0.29	2.64±0.39	2.44±0.19	2.44±0.39	2.40±0.11	2.20±0.35	ns	0.4-10.6
Eosinophil (%)	2.80±0.20	2.60±0.24	2.40±0.24	2.40±0.24	2.28±0.31	2.20±0.20	ns	5-7

± = Standard Error, ns = Not significant, **= P<1% of Significance, *= P<5% of Significance

Table 2. Blood parameter resulted from before and after treatment with hematinic drugs (n= 5)

Parameters	Pre transfusion values + (Control)	Post transfusion values (Mean ± SE)					Level of significance	Normal range
		Day-1	Day-7	Day-14	Day-21	Day-28		
TEC (million/cmm)	10.90±0.36	11.33±0.58	11.52±0.40	11.70±0.40	11.88±0.56	12.02±0.61	ns	8.02-17.05
TLC (thousand/ cmm)	8.84±0.71	8.93±1.09	9.07±1.18	9.23±1.24	9.30±1.21	9.43±1.23	ns	2.5-14.2
Hb (g%)	3.27±0.06	3.35±0.03	3.40±0.03	3.45±0.03	3.50±0.03	3.63±0.20	ns	3.6-6.6
PCV (%)	9.92±0.38	10.10±0.55	10.25±0.56	10.35±0.61	10.43±0.61	10.53±0.64	ns	18-27.2
Neutrophil (%)	30.94±0.34	31.50±0.76	31.83±0.67	32.00±1.04	32.67±0.44	33.00±0.29	ns	20-40
Lymphocyte (%)	19.40±1.47	22.00±2.31	23.47±2.53	24.00±2.65	24.67±2.40	26.07±2.99	ns	1.6-38
Monocyte (%)	3.56±0.20	2.90±0.38	2.83±0.38	2.57±0.23	2.50±0.29	2.30±0.30	ns	0.4-10.6
Eosinophil (%)	3.10±0.18	3.00±0.50	2.83±0.60	2.67±0.33	2.50±0.29	2.37±0.30	ns	5-7

± = Standard Error, ns = Not significant

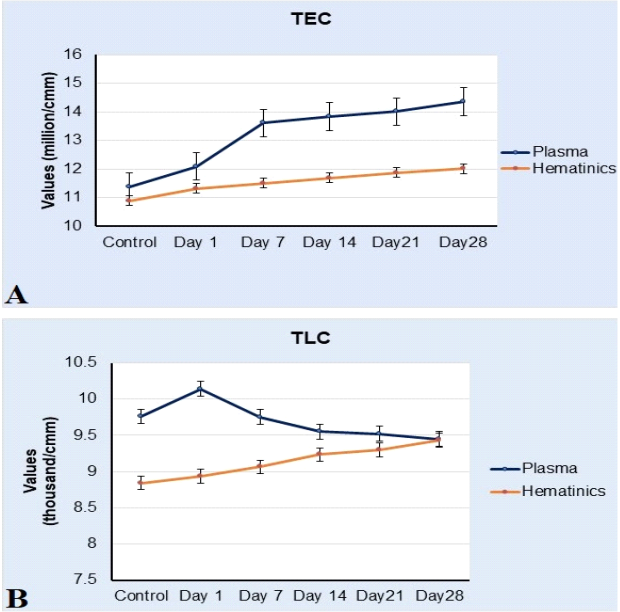

Figure 1. Effects of plasma transfusion and hematinics administration on various hematological parameters in recipient goats. (A) Effects on total erythrocyte count (TEC), and (B) Effects on total leucocyte count (TLC).

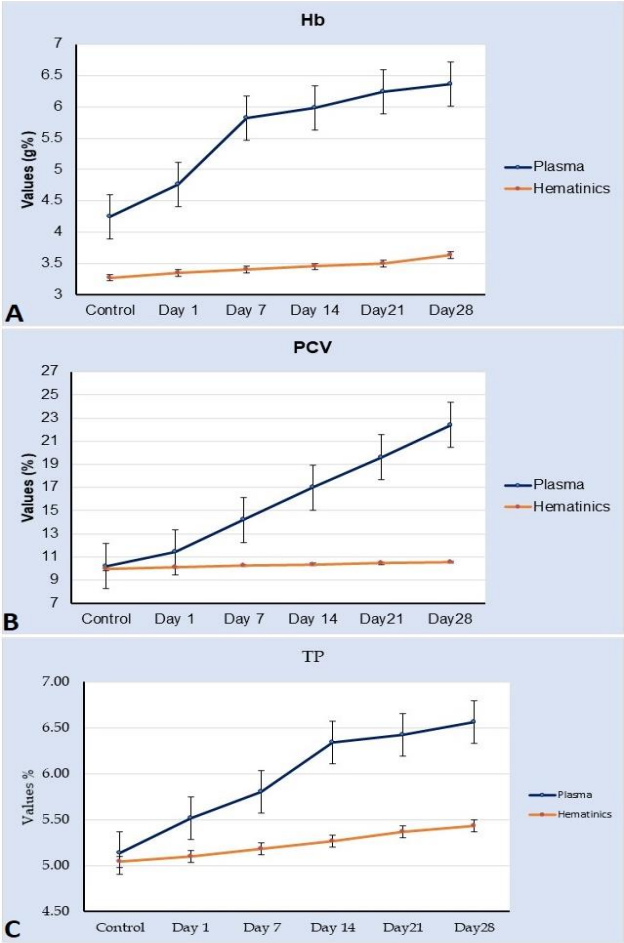

Figure 2. Effects of plasma transfusion and hematinics administration on various hematological & biochemical parameters in recipient goats. (A) Effects on hemoglobin (Hb) content, (B) Effects on packed cell volume (PCV) and (C) Effects on total serum protein.

Changes in Na, K, Cl

In both the groups, the control values of Na, K, Cl exhibited mild change but not significant throughout the experimental period (Table 4 and Table 5).

Changes in TP concentration

The effects of plasma transfusion and hematinic administration on total protein (TP) estimation is shown in Figure. 2C. The mean pre-transfusion values of TP in the animals of Group A and Group B were 5.14 ± 0.07(gm/dl) and 5.04± 0.15(gm/dl), respectively (Tables 4 and 5). However, these values started to increase from Day-1 to Day-28. The maximum increase in the Group A was recorded to be 6.56 ± 0.21(gm/dl) and in Group B was 5.43 ± 0.12(gm/dl) at Day-28. The changes in TP observed at different days of experiment in Group A was significant while in Group B, the changes were statistically not significant (Tables 4 and 5).

Changes in AST, ALT, Creatinine values

In plasma transfused group, the control values of ALT and AST increased during post transfusion period which was not statistically significant (P >0.05). In hematinic group, there were insignificant increase in the control values of the ALT and AST after treatment. In plasma group, the changes of creatinine values during different experimental periods were not significant (P>0.05). The changes in creatinine values were also not significant (p<0.05) in hematinic group. When compared between two groups, plasma transfusion showed better result than that of hematinic group (Table 4 and 5, and Figure. 3A, 3B, 3C).

Changes in clinical parameters

Respiration rate and heart rate, showed a mild increase 5 min after onset of the transfusion, thereafter gradually returned to normal. The rectal temperature during transfusion showed mild change (Table 3).

Table 3. Effects of plasma transfusion on clinical parameters in recipient goats (n=5)

Parameters	Pre-transfusion values(control) (Mean±SE)	Values during transfusion				1 hour after transfusion (Mean±SE)	Level of significance
		5 min (Mean±SE)	10 min (Mean±SE)	15 min (Mean±SE)	20 min (Mean±SE)		
Heart rate	78±2.75	82.4 ±3.18	80.2 ±3.23	79.2 ±2.22	78.4 ±2.31	77.6± 3.18	ns
Respiratory rate	13.2 ± 1.01	18.8± 1.85	18±1.89	16.2± 1.01	15.4 ±1.6	13.6 ±1.46	ns
Rectal temperature	102.4±.65	104 ±.77	103.6 ±.50	102.9± .43	102.9± .43	102.2 ±.68	ns

± SE= Standard Error, ns = Not significant

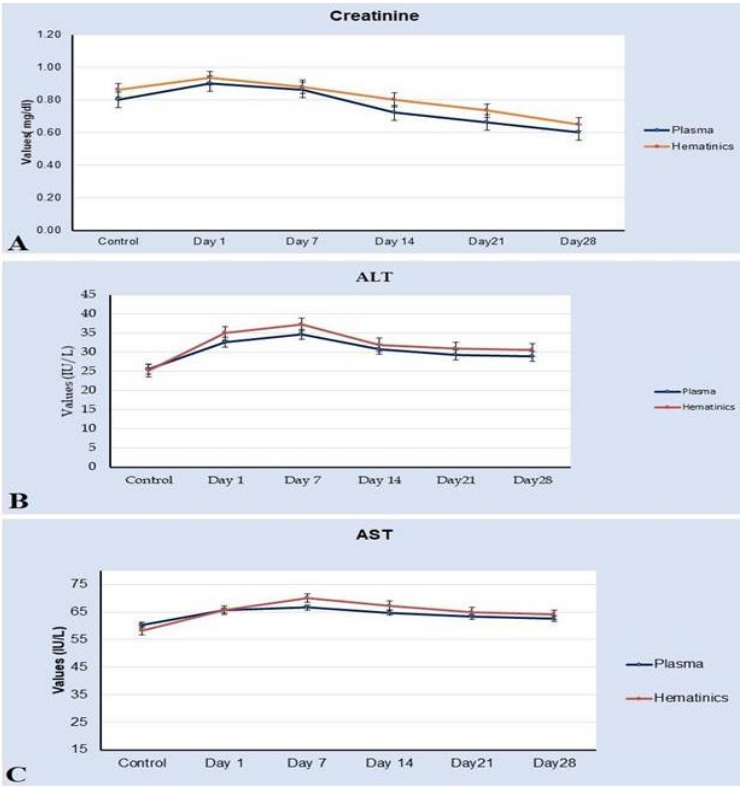

Figure 3. Effects of plasma transfusion and hematinics administration on various biochemical parameters in recipient goats. (A) Effects on creatinine content, (B) Effects on alanine aminotransferase (ALT), and (C) Effects on aspartate aminotransferase (AST) content.

Table 4. Biochemical parameter resulted from before and after treatment with plasma transfusion (n= 5)

Parameters	Pre transfusion values + (Control)	Post transfusion values (Mean ± SE)					Level of significance	Normal Range
		Day-1	Day-7	Day-14	Day-21	Day-28		
ALT (U/L)	25.64±1.81	32.68±2.54	34.64±2.48	18.7-48.2	29.30±1.73	28.96±1.86	ns	18.7-48.2
AST (U/L)	60.28±1.92	65.55±2.23	66.60±2.40	55.5-72.2	63.33±1.45	62.56±1.25	ns	55.5-72.2
K^+ (mmol/L)	4.87±0.08	4.90±0.11	4.98±0.15	3.90-5.80	5.11±0.08	5.12±0.08	ns	3.90-5.80
$Na^{+(}$mmol/L)	137.28±2.33	138.96±1.84	141.32±2.32	132-152	142.76±2.29	145.80±2.20	ns	132-152
Cl^- (mmol/L)	100.28±2.19	103.22±1.79	104.12±2.05	97-114	105.68±2.23	106.14±2.22	ns	97-114
Creatinine (mg/dl)	0.86±0.07	0.93±0.03	0.88±0.06	0.3-2	0.73±0.09	0.65±0.05	ns	0.3-2
Total protein (gm/dl)	5.14±0.07	5.52±0.17	5.80±0.11	6.2-10	6.42±0.15	6.56±0.21	**	6.2-10

± = Standard Error, ns = Not significant, ** = P<1% of Significance, * = P<5% of Significance

Table 5. Biochemical parameter resulted from before and after treatment with hematinic drugs (n=5)

Parameters	Pre transfusion values + (Control)	Post transfusion values (Mean ± SE)					Level of significance	Normal Range
		Day-1	Day-7	Day-14	Day-21	Day-28		
ALT (U/L)	25.20±2.06	35.00±3.61	37.33±4.81	25.20±2.06	31.00±3.79	30.67±3.71	ns	18.7-48.2
AST (U/L)	58.20±3.53	65.67±3.48	70.00±2.89	58.20±3.53	65.00±4.04	64.00±4.00	ns	55.5-72.2
K+ (mmol/L)	4.83±0.27	4.97±0.22	5.04±0.13	4.83±0.27	5.24±0.14	5.29±0.17	ns	3.90-5.80
Na+(mmol/L)	139.20±1.02	140.60±2.60	141.50±2.75	139.20±1.02	145.93±4.99	146.40±4.21	ns	132-152
Cl^{-1} (mmol/L)	101.70±1.50	103.33±2.91	104.47±3.47	101.70±1.50	106.40±2.27	107.20±2.80	ns	97-114
Creatinine (mg/dl)	0.86±0.07	0.93±0.03	0.88±0.06	0.80±0.07	0.73±0.09	0.65±0.05	ns	0.3-2
Total protein (gm/dl)	5.04±0.15	5.10±0.32	5.18±0.38	5.27±0.29	5.37±0.23	5.43±0.12	ns	6.2-10

± = Standard Error, ns = Not significant

Discussion

Transfusion of blood and blood components as a specialized modality of patient management can provide the life-saving element for critically ill animals (Tocci, 2010). Total blood volume of animals is about 8% of the body weight and that of the plasma is about 5%. Acute loss of 20 to 25% of the blood volume results in marked clinical signs of anemia, including tachycardia and maniacal behaviour. In the present study, plasma transfusion was started at a rate of 1 ml/kg/h for the first 15 min. Then, the rate was doubled to 2 ml/kg/h for the next 15 min. At 30 min, the rate was doubled again to 4 ml/kg/h, and then was doubled one last time at 60 min to a rate of approximately 8–10 ml/kg/h which is in agreement with the previous reports (Smith et al., 2021). Temperature, pulse, and respiratory rates were collected at 5, 10, 15, 20 and 60 min post initiation of the transfusion. During the transfusion procedure, the animals were closely monitored for signs of transfusion reactions. In anemic condition sudden infusion of plasma in the circulation of gastrointestinal tract, signals the organ to function normally resulting defecation. If the blood volume is increased too rapidly, the heart may be unable to cope and as a result pulmonary congestion may develop. The expected signs of circulatory overload include a dry cough, vomiting, dyspnea, urticaria and eventual possibility of developing pulmonary edema (Hunt and Wood,1999). In animal body kidneys are the main organs. This organ suffer much due to vasoconstriction and hypoxia as a result of severe anemia. Since blood is directed towards vital organs like brain, heart and lungs (Bitterman et al., 1996) and this is the cause behind the micturition and defecation of some animals. There were several reviews in the veterinary literature on blood transfusions in cats, dogs, and horses about the incidence and type of transfusion reactions ranging from 3 – 13% (Hurcombe et al., 2007). The mean control TEC values progressively significant increased during the experimental period in goats of Group A and B. Alam and Hossain (2005) reported that plasma transfusion increased erythrocyte count significantly in calves. Increased erythrocytes in case of plasma transfusion may be due to increased supply of circulating blood constituents and this might be due to erythropoietin present in the donor's plasma (Tamanna et al., 2022). In the anemic goats the depleted erythropoietin level might be inadequate to trigger erythropoiesis. Following plasma transfusion, the erythropoietin level increased to stimulate erythropoiesis (Alam and Hossain, 2005). In Group B, the increase of TEC value following administration of hematinic may be associated with erythropoiesis which

results increased erythrocytes in the circulation. Several earlier studies reported an increase of TEC value in lambs due to additional iron intake which increased the number of red blood cells and hemoglobin concentrations (Asadi et al., 2022). The increased levels of PCV and Hb in case of plasma transfusion is thought to be due to TEC values (Srivastova and Pandey, 1992; Alam and Hossain, 2005; Luethy et al., 2017). In hematinic treatment group, this may result from the administration of hematinic and Iron, copper, cobalt present in the hematinic may be utilized for hemoglobin synthesis (Rahman et al., 2005). In this present study, the animals showed PCV 9-10% before treatment which indicates severe anemia and a study in feline patients also reported severe anaemia to be the leading indication for transfusion (Weingart et al., 2004). Variations in creatinine values were minimal and remained within the reference values for goats (0.5-2.0) mg/dl) in both groups (Kanika et al., 2021). This finding indicates that the transfusion of plasma or administration of hematinic do not bring about any alteration in the renal functions. The increased total leukocytes might be due to epinephrine released at stress condition inducing granulocytosis and the presence of antibodies against the blood cells antigens, which triggered immune responses and consequent increase in the WBC (Nielsen et al.,1997; Tamanna et al., 2022). Variations in AST, ALT activities were minimal and remained within the reference values for goats in the experiment (Kaneko et al., 2008). Therefore, it can be postulated that no hepatocellular alteration occurs after plasma transfusion in the goat and it was evident from this finding that the transfusion of plasma or administration of hematinic do not bring about any alteration in the renal functions. Increased potassium value may be due to increased concentration of donor plasma which is similar with previous studies found in calves (Srivastova and Pandey, 1992; Alam and Hossain, 2005). For maintaining equilibrium and homeostasis, kidney excreted Na+ and Cl and adjustment occurs which increases Na+ and Cl.

Conclusions

Plasma transfusion and hematinic administration in goats were found to upgrade the hematological profile (TEC, Hb, PCV) and this elevated trend was maintained throughout the experimental period. However, the increase in the hematological parameters were significant in case of plasma transfusion in comparison to those of the hematinic administered animals. The plasma transfusion was found to be superior in upgrading hematological (TEC, Hb, PCV) and biochemical (TP) parameters in goats in comparison to that of the hematinic administration. Plasma transfusion and hematinic administration do not adversely affect kidney functions and hepatic injury. There were also no remarkable changes in the vital signs (heart rate, respiratory rate and rectal temperature). Plasma transfusion in goats appeared to improve hematological parameters and biochemical parameters and thereby might play a significant role in clinical management of anemia.

List of abbreviations

TEC : Total Erythrocyte Count
TLC: Total Leukocyte Count
Hb: Hemoglobin
PCV: Packed Cell Volume
DLC: Differential Leukocyte Count
TP: Total Protein
mg: Miligram
g: Gram
L: Litre

Acknowledgements

The authors would like to thank Bangladesh Agricultural University Research System (BAURES) for funding the research work.

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