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Efficacy of Whole Blood Transfusion as an Adjunct Therapy in Severe Cases of Haemolytic Anaemia in Cattle

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ABSTRACT

Background: In view of common occurrence of haemoprotozoan infections leading to severe haemolytic anaemia and increased case fatality rate in cattle in study area the present investigation was planned to evaluate efficacy of whole blood transfusion as an adjunct therapy in severe cases of hemolytic anaemia in cattle.

Methods: Sixteen cattle suffering from one or other haemoprotozoan infections induced haemolytic anaemia and having haemoglobin values less than 5 gm/dl were randomly divided in to two treatment groups. Group A (n=8) cattle were treated with standard treatment of haemoprotozoan infection without blood transfusion while group B (n=8) cattle were treated with whole blood transfusion in addition to standard treatment for haemoprotozoan infections.

Result: Clinical and haematological parameters revealed rapid improvement in group B cattle as compared to group A cattle. The survival rate was significantly higher in group B cattle (100%) as compared to group A cattle (62.5%). The haemoglobin and haematocrit values were also found significantly higher in group B as compared to group A at 12 hours as well as on 21st day post treatment. Thus, it is concluded that, whole blood transfusion could be used as an adjunct therapy to hasten clinical recovery and to increase the survivability in cattle suffering from severe haemolytic anaemia due to haemoprotozoan infections.

Key words: Blood transfusion, Cattle, Haemolytic anaemia, Haemoprotozoan infections, Treatment.

INTRODUCTION

Under field conditions, haemolytic anaemia is most commonly encountered in the haemoprotozoan infections in cattle and buffaloes (Singh *et al.,* 2014; Bhosale *et al.,* 2020). The tick-borne haemopro-tozoan diseases of cattle are causing heavy economic losses to farmers due to high morbidity, mortality and reduced production in recovered animals (Kachhawa *et al*., 2016). The projected annual economic loss due to tropical theileriosis and babesiosis in India are INR 8092 and 580.16 crore, respectively (Narladkar, 2018). In India, introduction of exotic and superior germ plasm in dairy industry has increased the occurrence of haemoprotozoan diseases by many-fold. These haemoprotozoan diseases are characterized by acute blood loss due to haemolytic crisis resulting in high case fatality rate especially in crossbred cattle in spite of treatment with specific drugs and supportive treatment. The blood transfusion is advocated in severe cases of anaemia for increasing survival rate.

Blood transfusion is the process of transfer of homogenous blood from one individual to another of same species. It is a very useful in patients with lower oxygenation capacity of blood due to decrease in its volume or cellular contents (Choudhary *et al*., 2017). It is being practiced for centuries for saving life of human beings and animals. Richard Lower in 1665 transfused the blood in a dog for the first time in the history (Kumar, 2017). It is vital part of veterinary emergency and critical care medicine (Mamak and Aytekin, 2012). Nowadays blood transfusion is commonly performed in pet animal practice apart from human medicine (Lanevschi and Wardrop, 2001). However, whole blood transfusion is rarely practised in large animal medicine (Sousa *et al.,* 2012). So, keeping this in view, the present study was designed to evaluate efficacy of whole blood transfusion as an adjunct therapy in cattle suffering from severe haemolytic anaemia due to haemoprotozoan infections.

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MATERIALS AND METHODS

Study animals

The present study was carried out at Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Udgir, Dist. Latur, Maharashtra, India. The study was approved by the Institutional Animal Ethical Committee (IAEC) of College of Veterinary and Animal Sciences, Udgir (Approval no: VCU/IAEC/CPCSEA/20/2019 dated 09.01. 2019). The cattle presented to clinics with history of tick infestation, high fever, lymphadenopathy, pale mucous membranes and no response to usual antibiotic treatment

were subjected to haematology and blood smear examination. Blood smears prepared from cattle were screened for haemoprotozoan infections and positive samples were selected based on presence of intra-erythrocytic organisms of *Theileria spp., Babesia spp.* and *Anaplasma spp.* and Koch blue bodies of *Theileria spp*. The cattle positive for haemoprotozoan infection(s) with Hb < 5 gm/dl and HCT< 15 gm/dl were included in the study. Sixteen animals comprising of 14 crossbred cows and two Deoni male cattle were included in the study.

Transfusion trial

Sixteen cattle suffering from one or other haemoprotozoan infections and having Hb < 5 gm/dl and HCT< 15 gm/dl were randomly divided in to two groups. In group A eight cattle suffering from severe haemolytic anaemia received standard treatment for haemoprotozoan infections without blood transfusion, whereas eight cattle from group B were treated with whole blood transfusion @ 3 ml/kg BW in addition to standard treatment for haemoprotozoan infections. Standard treatment comprised of specific drug therapy for haemoprotozoan infections and supportive treatment for correction of anaemia. The clinical cases of babesiosis were treated with imidocarb dipropionate @ 1.2 mg/kg SC once, theileriosis with buparvaquone @ 2.5 mg/kg IM once and anaplasmosis with oxytetracycline @ 10 m/kg IV once daily for 5 days. The supportive treatment in both groups comprised of vitamin B-complex @ 10 ml IM daily, Dextrose 20 per cent 1000 ml IV daily for 5 days and oral haematinic bolus (Ferrous fumarate) @ 1 boli PO BID daily for 21 days. The efficacy of whole blood transfusion as an adjunct therapy was judged on the basis of survival rate and improvement in clinical signs and haemato-biochemical parameters in treated cattle.

Clinical observations

In all treated cattle, the daily observations pertaining to feed intake, water intake, rumination, body temperature, respiration rate, heart rate, rumen motility, colour of mucous membrane, colour of urine, act of defaecation, consistency of faeces, behaviour, gait and body condition were recorded daily during the entire course of treatment.

Hematology

The blood samples collected from jugular venipuncture in EDTA vials before treatment and 12 hours, 7th day and 21st day post-treatment in both groups were subjected to complete blood count on automated hematology analyzer (Abacus Junior Vet, Diatron GMBH, Austria).

Biochemical analysis

Plasma samples collected before and on 21st day of treatment were analyzed for total, direct and indirect bilirubin, total protein, globulin, BUN and creatinine on UV-Visible Double-beam Spectrophotometer (SPECORD® 200 PLUS -Analytikjena, Endress and Hauser Company, China) using standard diagnostic kits manufactured by Agappe Diagnostics Ltd., Kerala, India.

Donor selection

Apparently healthy, adult, non-pregnant, tick free cattle from College Cattle Breeding Farm (7 Deoni cattle) or private farm (1 Red Kandhari cattle) were tentatively selected. Then the blood collected from these animals were subjected to blood smear examination as well as major and minor cross match to rule out any haemoprotozoan infection and incompatibility, respectively. The cattle negative for haemoprotozoan infections having compatible blood with recipient blood and having Hb > 9 gm/dl and HCT> 27 per cent were selected for transfusion therapy.

Collection of blood from donor

Blood was collected from jugular vein of donor cattle in ready to use blood collection bag of 350 ml (Containing 49 ml of anticoagulant Citrate Phosphate Dextrose Adenine) capacity. Approximately 3 ml of blood/kg body weight was collected from donor cattle.

Calculation of dose for blood transfusion

The dose was calculated based on the percentage of haemoglobin present in the recipient and the percentage to which it should be raised *i.e.* upto 75 per cent of the normal level. The amount of blood to be transfused for the animal was calculated according to the formula described by Prathaban (1986) and 1050 ml of blood was transfused in all adult animals.

$$\text{Blood (in ml) required to raise Hb level by 1\% } = \frac{40 \times \text{Body weight in pounds (lbs)}}{100}$$

(Since an animal contains about 40 ml of blood per pound of body weight) [*e.g.* For cattle weighing 400 kg (881.85 pounds) to increase Hb by 1 per cent = 40×881.85/ 100 =352.8 ml of blood is required. Thus blood required for cattle weighing 400 kg to increase Hb by 3 per cent=352.8× 3=1058.2 ml].

Blood transfusion therapy

Prior to the blood transfusion, specific and supportive treatment as mentioned above was given and 15 min before the transfusion antihistaminic was administered to prevent the adverse reaction. The whole blood was administered @ 3 ml/kg to the cattle in group B while group A cattle were treated with standard and supportive therapy.

Statistical analysis

The paired 't' test for equal number of observations was used for comparison of values before and after treatment in affected animals using SPSS 20 version software to estimate the level of significance at 99 per cent and 95 per cent.

RESULTS AND DISCUSSION

In present therapeutic trial on blood transfusion, 16 cattle positive for haemoprotozoan infection and having Hb<5 gm/ dl and HCT<15 per cent were included. Divers (2005) opined that serious haemorrhagic or haemolytic disorder and

euvolemic cattle with Hb<5 gm/dl (approximately 15 per cent HCT) should be considered at risk for having inadequate oxygen carrying capacity and tissue hypoxia. Balcomb and Foster (2014) recommended whole blood transfusion when the HCT decreases to <15 per cent- 20 per cent in cases of acute blood loss or <10 per cent to 15 per cent in cases of chronic anaemia. A haematocrit value of 12% or less following an acute episode of anaemia is considered as "critical". Myocardial oxygenation is compromised at Hb < 5 g/dl (HCT 15 per cent), increasing the risk of tissue hypoxia. Briggs (2014) reported that blood transfusion in cattle is indicated on priority when there is severe anaemia with HCT< 10 per cent to save the life as well as in moderate anaemia with HCT< 15 per cent to hasten the recovery. Earlier Hunt and Wood (1999) suggested that cattle having less than 12 per cent HCT with clinical signs of a significant anaemia should be considered at risk for having inadequate oxygen carrying capacity and tissue hypoxia and requires immediate blood transfusion. Several clinicians attempted to identify a reliable, consistent threshold for initiation of a blood transfusion termed as the transfusion trigger. Haematocrit value (HCT) and haemoglobin (Hb) concentration are important transfusion indicators in haemolytic disorders but should not be considered as the sole transfusion trigger measurement. Euvolemic humans with Hb concentrations < 7 gm/dl are generally considered in need of transfusion. Because normal HCT concentrations are higher in humans than cattle, this trigger value may be lower in cattle (Herbert *et al.,* 2005). A far more important indicator for transfusion is the presence of tachycardia or an ever-accelerating heart rate >110 beats/minute in bovine.

The comparative efficacy of standard treatment without blood transfusion (group A) and with blood transfusion therapy (group B) was evaluated in 16 clinical cases of severe haemolytic anaemia (Hb <5 gm/dl) due to haemoprotozoan diseases in cattle based on recovery rate, recovery period and improvement in haemato-biochemical parameters. The clinical signs such as appetite, water intake, haematuria, mucous membranes (Fig 1, 2) and body condition (Fig 3, 4) were improved rapidly in group B as compared to group A. In transfused animals (group B), appetite and water intake improved immediately after transfusion. Recumbent animals stood up during or immediately after whole blood transfusion (Fig 5, 6). Similar observations were recorded by Pradhan *et al.* (1993) who performed whole blood transfusion in cattle (Hb<6 gm/dl) suffering from haemoprotozoan infections. Saritha *et al.* (2016) also performed whole blood transfusion in a calf having anaemia (3 gm/dl) due to iron deficiency and parasitism and uneventful recovery was recorded. Kachhawa *et al.* (2016) also observed good improvement in theileriosis affected calves treated with standard treatment and single blood transfusion.

The physiological values of body temperature, pulse rate, respiratory rate and ruminal motility (Table 1) showed highly significant (P<0.01) changes and restored progressively to normal after standard treatment along with whole blood transfusion (group B) as compared to group A cattle (Table 1). Similarly, Kachhawa *et al.* (2016) observed significant decrease in body temperature, heart rate and respiratory rate after treatment with whole blood transfusion. The clinical improvement noticed may be due to restoration

Fig 1: Pale mucous membrane of 7 year old crossbred female affected with severe theileriosis before transfusion (group B).

Fig 2: Regaining of normal pink colouration of mucous membrane in same cow after blood transfusion (group B).

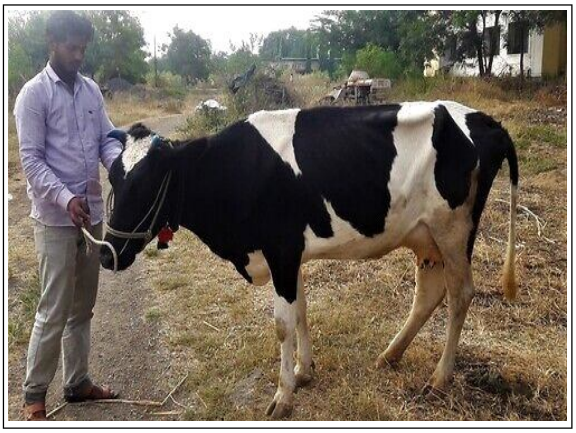

Fig 3: Six year old crossbred female affected with theileriosis showing emaciation before transfusion (group B).

of oxygen carrying capacity owing to replenishment of cellular components (Prathaban, 1986; Divers, 2005; Kachhawa *et al.*, 2016; Chand *et al.*, 2017).

The haematological values improved in both groups (Table 1 and 2) but the comparative analysis of values revealed highly significant increase in Hb and HCT values in group B as compared to group A during the course of treatment. At 12 hours of treatment in group A, the Hb and HCT values reduced by 0.24 gm/dl and 0.18 per cent, whereas in group B the same values increased by 0.45 gm/dl and 1.39 per cent, respectively indicating beneficial effects of whole blood transfusion therapy. This suggested that administration of 1050 ml of blood to adult cow resulted in net increase in HCT by 1.39 per cent. Similarly, Soldan (1999) mentioned that the administration of 1000 ml blood to adult anaemic cow raised HCT by approximately 0.75 per cent. Hunt and wood (1999) reported that in small animals 2.2 ml/kg of blood elevated HCT by 1 per cent having donor HCT> 40 per cent. Whereas 3 ml blood per kg body weight of recipient is required to get comparable increase in HCT as bovine donors have lower HCT than 40 per cent. On 21st day of treatment in Group A, Hb and HCT values were increased by 2.44 gm/dl and 6.42 per cent, whereas in-group B the same values increased by 4.10 gm/dl and 10.36 per cent, respectively suggesting significant restoration in Group B cows as compared to Group A cows. Kachhawa *et al*. (2016) recorded significant improvement in Hb, HCT, total erythrocyte count and total leukocyte count after treatment with single blood transfusion in *Theileria* affected calves having anaemia (<6 gm/dl). In both groups, the improvement in Hb, HCT and TEC values may be attributed to cessation of intravascular haemolysis and oral supplementation of haematinics. However, the marked improvement in Hb, HCT and TEC values in group B could be attributed to replenishment of RBC's through whole blood transfusion.

Fig 4: Improved body condition of same cow one month after whole blood transfusion (group B).

Fig 5: Sternal recumbency in 5 year old Red Kandhari bullock affected with babesiosis before transfusion (group B).

Fig 6: Immediate recovery after blood transfusion in 5 year old Red kandhari bullock affected with babesiosis (group B).

Highly significant (P<0.01) increase in total plasma proteins, albumin, globulin and highly significant (p<0.01) decrease plasma total bilirubin, direct bilirubin, indirect bilirubin, urea nitrogen, creatinine showed rapid improvement in transfused (group B) cows as compared to group A. The present observations corroborate the findings of Kachhawa *et al*. (2016) who observed significant (P<0.01) improvement in total protein, albumin and globulin values after treatment with single blood transfusion in Theileria affected calves. These findings indicated restoration of liver and kidney function due to cessation of intravascular haemolysis.

The survival rate was significantly higher in group B cattle received standard treatment along with whole blood transfusion (100 per cent) as compared to group A cattle (62.5 per cent). This could be attributed to improvement in oxygen carrying capacity of blood due to replenishment of RBC's owing to blood transfusion. It can also be concluded from present study that even transfusion of small quantity of blood in critically anaemic patient is lifesaving. Further, it was surprising to note that 62.5 per cent critically anaemic cattle survived without blood transfusion. These findings support the opinion of Divers (2005) who reported that ruminants that have very low hematocrits but appear relatively comfortable and have adjusted to their anaemic condition may survive without transfusion.

In current study no adverse reaction to blood transfusion was noticed in any of the transfused animals, although out of 8 recipients, 7 were crossbred cows and one was Deoni male whereas among 8 donors 7 were Deoni cattle and one Red Kandhari cattle including 2 male and 6 females. These observations suggests that first transfusion is safe in cattle irrespective of breed and sex. However, Soldan (1999) mentioned that donor and recipient from the same breed reduces the risk of a transfusion reaction.

Major and minor cross-matching of blood from all 8 donors and recipients revealed no signs of incompatibility. Before transfusion cross-matching of recipient and donor blood should be carried out to minimize the risk of transfusion reaction (Choudhary *et al.,* 2017). Cross- matching is similar to blood typing, except that specific antisera are not used and consists of a major and minor part. The major cross-matching involves the cross-matching of donor RBCs with recipient serum, whereas, the minor matching is the cross-matching of recipient RBCs with donor serum. The major cross match detects antibodies previously present in recipient serum and *vice versa* in minor cross match. However, Smith (2002) opined that generally first transfusion

Table 1: Vital, haematological and biochemical parameters in study cows before treatment and after recovery.

Parameter	Group A			Group B		
	Before treatment (n=8)	After treatment (n=5)	't' value	Before treatment (n=8)	After treatment (n=8)	't' value
Vital parameters						
Body temperature (°F)	101.95±1.04	101.46±0.11	0.054^{NS}	102.22±0.45	101.02±0.23	2.286*
Pulse rate (per minute)	87.50±5.97	67.20±6.12	2.074^{NS}	94.50±3.02	71±1.96	6.387**
Respiration (per minute)	28.88±3.46	24.80±2.50	0.466^{NS}	37.25±3.00	25±1.20	3.648**
Ruminal motility (/5 min)	2.94±0.49	4.4±1.38	-2.147^{NS}	1.75±0.52	5.06±0.43	-5.16**
Hematology						
Hb (gm/dl)	3.73±0.33	6.68±0.21	-7.520**	2.75±0.25	6.85±0.37	-8.10**
HCT (%)	10.66±1.16	19.07±1.27	-4.587**	8.37±0.70	18.73±1.02	-8.19**
TEC (× 10^6/µl)	2.35±0.31	4.27±0.24	-4.658**	1.62±0.18	4.00±0.43	-5.33**
MCV (fl)	47±3.55	51.2±2.71	-0.991^{NS}	53±2.53	49±3.60	1.938^{NS}
MCH (pg)	17.87±0.77	16.38±0.37	1.734^{NS}	17.53±0.86	17.94±1.44	0.463^{NS}
MCHC (g/dl)	36.4±2.32	33.6±0.90	1.209^{NS}	33.10±0.66	35.95±0.67	-3.01**
TLC (× 10^3/µl)	7.30±1.83	9.85±1.86	-2.392*	6.00±1.09	9.63±1.67	-2.77*
Lymphocyte (%)	54.04±6.77	55.64±7.83	0.212^{NS}	44.99±6.48	69.05±2.74	-3.55**
Monocyte (%)	1.76±0.75	3.44±1.43	-1.049^{NS}	1.32±0.31	2.16±1.06	-0.82^{NS}
Neutrophils (%)	44.2±6.66	40.94±6.42	-0.062^{NS}	53.71±6.45	35.21±6.01	2.101^{NS}
PLT (× 10^3/µl)	102.6±31.90	261±69.56	-1.960^{NS}	85.38±18.34	246.6±82.42	-1.40^{NS}
Biochemistry						
Total protein (gm/dl)	6.21±0.22	6.84±0.33	-1.697^{NS}	5.46±0.35	7.59±0.26	-4.944**
Albumin (gm/dl)	2.52±0.19	3.50±0.34	-2.648*	2.46±0.24	3.68±0.17	-4.281**
Globulin (gm/dl)	3.69±0.13	3.32±0.44	0.708^{NS}	3±0.20	3.91±0.19	-3.455**
Total bilirubin (mg/dl)	1.94±0.12	0.95±0.07	6.951**	2.21±0.16	0.56±0.12	8.022**
Direct bilirubin (mg/dl)	0.88±0.04	0.59±0.05	3.461**	0.9±0.08	0.28±0.10	4.887**
Indirect bilirubin (mg/dl)	1.06±0.08	0.36±0.09	5.447**	1.31±0.09	0.29±0.05	10.344**
Blood urea nitrogen (mg/dl)	23.5±1.44	16.5±0.73	4.304**	25.25±2.60	10.38±0.68	5.537**
Creatinine (mg/dl)	1.6±0.13	0.76±0.05	5.798**	1.81±0.14	0.98±0.10	4.514**

NS-Non significant; *-Significant (p<0.05); **-Highly significant (p<0.01).

Table 2: Variation in Hb and HCT values in cattle treated for haemoprotozoan infections with and without blood transfusion.

Group	Day	Hb (g/dl)		HCT (%)	
		Mean	Average variation	Mean	Average variation
Group A (n=5)	0 hrs	4.24±0.30	-	12.65±0.96	-
	12 hrs	4.00±0.41	-0.24	11.84±1.55	-0.81
	21st day	6.68±0.21	+2.44	19.07±1.26	+6.42
Group B (n=8)	0 hrs	2.75±0.25	-	8.37±0.70	-
	12 hrs	3.20±0.28	+0.45	9.76±0.89	+1.39
	21st day	6.85±0.37	+4.10	18.73±1.01	+10.36

- Decrease, + Increase.

is safe in cattle even if done without cross-matching due to absence of naturally occurring alloantibodies. Divers (2005) also mentioned that cross-matching is not required for first transfusion in cattle because of the likelihood of a reaction during first transfusion is low in cattle. It is possible that J positive donor cells transfused into J negative recipients might cause reaction on first transfusion because cows with J-negative antigen on the RBCs may have J antibodies. Stormant (1982) stated as anideal donor cow should have J-negative RBCs, but cattle have minimal agglutinating antibody and cross-matching is of little benefit in predicting a transfusion reaction in cattle. Blood typing and examination of plasma for alloantibodies of both, donor and recipient are ideally required before transfusion but this is not feasible in cattle. Cross-matching is performed to safeguard against any transfusion reaction but it is not 100 per cent safe. The cross-matching detects only antibodies to red blood cells and not to white blood cells and platelets, therefore immunogenic transfusion reactions can occur even after cross-matching (Chand *et al*., 2017). Hunt and Wood (1999) reported that bovine blood has 13 major types of blood group systems. Of these, anti-J isoantibodies are the most important. In ruminants, these isoantibodies are present in very low quantities in serum. Thus, initial blood transfusions can be done without matching blood types and without serious threat of fatal reaction. Compatibility tests should only be necessary if the animal is known to have been previously transfused.

CONCLUSION

The findings of the present study concludes that whole blood transfusion even in small amount has beneficial effect on increasing oxygen carrying capacity, speed of recovery and restoration of vital, haematological and biochemical parameters towards normal physiological range, reduced convalescence period and hence should be routinely employed as adjunct therapy in severe cases of haemolytic anaemia arising from haemoprotozoan infections.

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