Effect of intravenous plasma transfusion on granulocyte and monocyte oxidative and phagocytic activity in dairy calves with failure of passive immunity

Victoria C. Yang^a, Maire C. Rayburn^a, Munashe Chigerwe^{a*}

Abstract

Plasma administration has been recommended in calves older than 48 hours with failure of passive immunity (FPI) to provide immunity consistent with adequate colostral ingestion. However, the protective serum immunoglobulin G (IgG) concentrations (≥ 1000 mg/dL) of plasma derived IgG only lasts up to 12 hours. In addition to IgG, maternally derived colostral cells also confer immunity. The objective of the study was to determine the effect of intravenous plasma transfusion on granulocyte and monocyte oxidative and phagocytic activity in calves with FPI. Twenty- seven, one day-old, Jersey calves were assigned into 3 groups. The colostral (CL, N = 9) group received 3 L of colostrum once by oroesophageal tubing. Two other groups of calves received 1 L of colostrum once by oroesophageal tubing and were assigned based on their health status (sick or non-sick) at 4 days of age, as the sick-group (SG, N = 7) or the non-sick (NG, N = 11) groups. At 4 days of age, the SG and NG groups were administered plasma intravenously at 30 mL/kg. Granulocyte and monocyte oxidative and phagocytic activity was determined by flow cytometry. There was no significant difference in the granulocyte and monocyte oxidative or phagocytic activity among the 3 groups ($P > 0.05$). Plasma administration had no significant effect on the oxidative or phagocytic activity of granulocytes or monocytes. In clinical practice, plasma administration for enhancing oxidative or phagocytic activity of granulocytes or monocytes, alone, might not be justified in calves with FPI.

Keywords: calf, granulocyte, immunity, monocyte, plasma, bovine

1. Introduction

Failure of passive immunity (FPI) is the most common cause of immunodeficiency in newborn calves (Weaver et al., 2000). In calves, colostral component absorption occurs efficiently during the first 12 hours and ceases after 24 – 36 hours of life (Matt et al., 1982). Serum immunoglobulin G (IgG) concentrations of ≥ 1000 mg/dL after colostral ingestion are indicative of adequate transfer of immunity in calves (Besser et al., 1991). Calves with FPI have decreased weight gain and increased risk for morbidity and mortality (Furman-Fratczak et al., 2011; Windeyer et al., 2014).

Sick calves > 48 hours of age with FPI are managed in part with intravenous administration of bovine plasma assuming that administered plasma IgG achieves immunoprotection consistent with adequate transfer of immunity. However, recent studies indicated that the protective serum IgG concentrations (≥ 1000 mg/dL) of plasma derived IgG only lasts up to 12 hours (Pipkin et al., 2015). Thus, plasma administration for the purpose of supplementing IgG alone in calves with FPI may not be justified due to the short duration of the protective levels. In addition to the passive immunity conferred by IgG, colostral derived immune cells are known to stimulate maturation of calf's immune system when exposed to environmental pathogens (Reber et al., 2008). In septic foals, plasma administration was beneficial through increased neutrophil function (McTaggart et al., 2005). Studies on the effect of intravenous plasma administration on immune cells in cattle are lacking. We hypothesized that intravenous bovine plasma transfusion at an optimum recommended dose rate of 30 mL/kg (Murphy et al., 2014) improves phagocytic and oxidative burst activity of granulocytes and monocytes in dairy calves with FPI. Assuming that diarrhea is the most common disease condition in pre-

weaned calves, with leukocytes being the major cell types recruited to sites of inflammation, the objective of this study was to determine the effect of intravenous plasma transfusion on granulocyte and monocyte activity of calves with FPI.

2. Materials and Methods

2.1. Calves

The study was approved by the University of California Davis Institutional Animal Care and Use Committee (Protocol #18722). Sample size was determined assuming that calves with FPI and transfused plasma are 5 times more likely to experience mortality (Pipkin et al., 2015), power = 80 % and alpha = 0.05. The required sample size was at least 6 calves in each group. To account for a 20 % dropout before 7 days of age, at least eight calves were enrolled in each group. A total of 29 Jersey bull calves was enrolled.

Calves delivered from observed births were immediately separated from their dams prior to nursing colostrum, weighed, identified using ear tags and housed in individual calf hutches. A stratified (different levels of passive immune status), non-probability (non-randomized) sampling technique was used to assign the calves into 3 groups. The first 9 calves were assigned into the control group (CL) and fed 3 L of fresh colostrum, once by oroesophageal tubing within 2 hours after birth to ensure adequate passive immunity. The next 20 calves were separated into two treatment groups; sick group (SG) and the non-sick group (NG) based on their health status at 4 days of age. Assessment of calf health status prior to assignment into the NG and SG groups included daily assessment of rectal temperature, appetite, and evidence of diarrhea or coughing. Both NG and SG calves received 1 L of fresh colostrum, once by oroesophageal tubing

119 within 2 hours after birth to insure FPI. A total of 150 – 200g total IgG is required for
120 adequate transfer of colostral immunity (Chigerwe et al., 2008). Thus, based on the
121 average colostral concentration of IgG of 60g/L on the farm of study, feeding 1 L of
122 colostrum insured FPI. After colostrum administration, all calves were fed 2 L of milk
123 replacer twice daily, and 0.5 kg of commercial calf concentrate twice daily, with *ad*
124 *libitum* access to water.

125 At 4 days of age, plasma administration was performed in the SG and NG groups.
126 Bovine plasma purchased from the UC Davis Veterinary Medical Teaching Hospital
127 Blood Transfusion service was used. The plasma was evaluated for sterility and
128 considered free of transmissible blood-borne pathogens. A 5 mL aliquot of the plasma
129 was collected prior to administration for subsequent IgG concentration determination.
130 Plasma was administered through the external jugular vein via an intravenous catheter
131 (Angiocath, Becton Dickinson, Franklin Lakes, NJ, USA) at 30 mL/kg. Infusion of
132 plasma was performed at 10 mL/kg/h while monitoring for transfusion reactions.
133 Monitoring for transfusion reactions included monitoring heart rate, respiratory rate,
134 mucous membranes color, and abnormal behavior. In the absence of an immediate
135 transfusion reaction, the remainder of the plasma was transfused over 20-30 minutes. In
136 case of a plasma transfusion reaction, transfusion was discontinued for 10 minutes and
137 resumed at 5 mL/kg/h.

138 The timeline for blood collection in lithium heparin for oxidative and phagocytic
139 activity of granulocyte and monocytes, and in Ethylenediaminetetraacetic acid (EDTA)
140 for complete blood count (CBC) or no anti-coagulant for serum IgG determination is
141 summarized in Table 1. Serum was harvested from the blood samples and stored at -20°C

until immunoglobulin G (IgG) determination. Daily calf monitoring procedures included assessment of rectal temperature, appetite and evidence of diarrhea or coughing. Sick calves were medically treated by a study blinded UC Davis campus veterinarian. Calves that died during the study period were submitted for necropsy at the California Animal Health and Food Safety Laboratory in Davis, CA. Enrollment of all calves was completed within 2 weeks, in June 2015.

2.2. Serum IgG concentration and Complete Blood Count (CBC) analysis

A CBC was performed on EDTA **treated** blood samples using an automated hematology analyzer (Advia 120 Hematology System, Siemens, Malvern, PA, USA). Immunoglobulin G determination was performed on serum samples by radial immunodiffusion (RID) using a commercial bovine IgG test kit with a serum IgG determination range of 196 -2748 mg/dL (Bovine IgG kit, Triple J Farms, Bellingham, WA, USA) as recommended by the manufacturer, and described as in previous studies (Pipkin et al., 2015).

2.3. Granulocyte and monocyte oxidative burst activity determination

Oxidative burst activity of granulocytes and monocytes was evaluated using a commercial test kit (Phagoburst, Glycotope Biotechnology, Heidelberg, Germany) according to the manufacturer's recommendations. The Phagoburst test determined the percentage of leukocytes that oxidized the fluorogenic substrate dihydrorhodamine 123 to rhodamine 123 and their enzymatic activity (amount of rhodamine 123 per cell). Oxidative activity of the granulocytes and monocytes was stimulated with opsonized, non-labeled *Escherichia coli* (*E. coli*), phorbol 12-myristate 13-acetate (PMA), and peptide N-formyl-Met-Leu-Phe (fMLP). The *E. coli*, PMA and fMLP were the particulate

stimulus, strong stimuli, and weak stimuli, respectively. The cell suspension was evaluated within 30 minutes after preparation using a flow cytometer (FACScan, Beckton Dickinson, Franklin Lakes, NJ, USA).

2.4. Granulocyte and monocyte phagocytic activity determination

Phagocytic activity of granulocyte and monocytes was determined using a commercial test kit (Phagotest, Glycotope Biotechnology, Heidelberg, Germany) according to the manufacturer's recommendations. The Phagotest test allowed quantitative determination of phagocytosis by granulocytes and monocytes by assessing the percentage of cells that ingested bacteria and their activity (number of opsonized fluorescein-labeled *E. Coli* per cell). Cells were analyzed within one hour after preparation using a flow cytometer.

2.5. Oxidative burst and phagocytic activity of granulocytes and monocytes analysis using flow cytometry

Oxidative and phagocytic activity of cells data was acquired from the flow cytometer using a commercial software (CellQuest, Beckton Dickinson, Franklin Lakes, NJ, USA) and then analyzed using a commercial software (FlowJo, FlowJo LLC, Ashland, OR, USA). Forward and side scatter, fluorescence-1 and fluorescence-2 were used. A live gate was set in the fluorescence-1 histogram to include all granulocytes and monocytes stimulated. A forward and side scatter dot plot was created with gates set for granulocytes and monocytes to determine cells that underwent phagocytosis or oxidative activity. Median fluorescence intensity detecting the number of cells ingested or oxidative species produced was calculated based on the fluorescence-1 height on the histogram.

2.6. Statistical analysis

Descriptive statistics was used to summarize the data. Data was checked for normality using the Shapiro-Wilk Test. Mean $\pm$ standard deviation (SD) and median (range) were reported for normally and non-normally distributed data, respectively. Predicted serum IgG concentrations following plasma transfusion in the SG and NG groups were estimated as previously reported (Murphy et al., 2014). White cell blood counts from the CBC before and after plasma transfusion in the SG and NG groups were compared using the Wilcoxon rank sum test. Repeated measures of oxidative and phagocytic activity of the granulocytes or monocytes among the three groups was compared using the Friedman test. Post-hoc analysis of differences in the activity of phagocytes and monocytes among the 3 groups was determined using the Dunnett test. Half-life of serum IgG in each group was determined using non-linear regression. Differences in serum IgG half-life among the three groups were evaluated using an *F*-test. Survival of the calves over the 7-day period was determined by survival analysis using the Cox proportional hazard analysis. Calves with serum IgG concentrations < 1000 mg/dL were considered to have FPI. Data analysis was performed using two commercial statistical software (SAS, SAS Institute, Cary, NC, USA; GraphPad, LaJolla, CA, USA). In all analyses $P < 0.05$ was considered significant.

3. Results

3.1. Serum IgG concentrations and IgG half-lives

Of the 29 calves enrolled, 9 in the CL group, 7 in the SG group and 11 in the NG group had complete sample collections. Two calves were excluded, with one dying before 3 days old and the second calf had serum IgG >1000 mg/dL, despite ingesting

only 1 L of colostrum. Thus 27 calves were considered for analysis. Mean $\pm$ SD for calf birth weights were 31.5 ± 4.7 , 33.3 ± 4.0 and 32.3 ± 4.1 kg for CL, NG and SG groups, respectively. There was no statistical difference in the birth weights among the three groups ($P = 0.613$). Serum IgG concentrations in all enrolled calves was < 196 mg/dL prior to ingestion of colostrum indicating that calves had not ingested colostrum prior to enrollment. All calves in the CL group achieved serum IgG > 1000 mg/dL at 2 days of age and maintained the serum IgG concentrations above 1000 mg/dL throughout the 7-day study period. Mean $\pm$ SD of IgG concentrations in the plasma transfused in the SG and NG group calves was 2674.6 ± 302.3 mg/dL. Median (range) serum IgG concentrations prior to plasma transfusion at 4 days of age in the SG and NG groups were 423.4 mg/dL (308.2 – 636.4) and 528.1 (195 – 708.1), respectively. Median (range) serum IgG concentrations following plasma transfusion in the SG group were 1014.9 (656.1 – 2937.1) mg/dL at 2 hours' post plasma transfusion but were < 1000 mg/dL by 12 hours' post plasma transfusion (885.0 mg/dL, range, 759.8 – 1454.0). Median (range) serum IgG concentration in the SG group at 24 hours' post transfusion was 897.0 mg/dL (557.9 – 1245.8). Median (range) serum IgG concentrations following plasma transfusion in the NG group were 1398.8 (451.1 – 2751.0), and 1136.8 (758.2 – 2843.6), at 2 and 12 hours, respectively. Median (range) serum IgG concentrations in the NG were < 1000 mg/dL by 24 hours' post transfusion (950.1 mg/dL, range, 360.1 – 2637.1). Serum IgG concentrations at 2 days of age, 4 days of age, pre-transfusion, post-transfusion and 7 days of age for all calves are depicted in Figure 1.

Serum IgG half-life was 3.8, 6.9 and 19.4 days for the NG, SG and CL groups, respectively. The serum IgG half-life for the CL group was significantly longer compared

to the SG ($P < 0.0001$) or NG groups ($P < 0.0001$). The IgG half-life was not different between the SG and NG groups ($P = 0.134$).

Complete blood counts

There was no statistical difference in the total leukocyte counts in either the SG ($P = 0.156$) or NG ($P = 0.465$) before and 24 hours after plasma transfusion.

Oxidative and phagocytic activity of granulocytes and monocytes

Oxidative activity (median florescence intensity) of granulocytes and monocytes are depicted in Figure 2 and Figure 3, respectively. Oxidative activity for granulocyte and monocytes did not differ by treatment group (granulocytes, $P = 0.569$; monocytes, $P = 0.704$), time (granulocytes, $P = 0.325$; monocytes, $P = 0.222$) or treatment group by time interaction (granulocytes, $P = 0.188$; monocytes, $P = 0.804$). Phagocytic activity of granulocytes and monocytes are depicted in Figure 4 and 5, respectively. Although differences in phagocytic activity of granulocytes and monocytes did not differ by treatment group (granulocytes, $P = 0.382$; monocytes, $P = 0.330$) or treatment group by time interaction (granulocytes, $P = 0.078$; monocytes, $P = 0.057$), activity was influenced by time (granulocytes, $P < 0.0001$; monocytes, $P = 0.021$) in the SG and NG groups. The phagocytic activity of granulocytes ($P < 0.0001$) and monocytes ($P = 0.041$) decreased between 2 hours' post plasma transfusion and 7 days of age. The phagocytic activity of granulocytes ($P < 0.0001$) and monocytes ($P = 0.0036$) decreased between 12 hours' post plasma transfusion and 7 days of age.

Morbidity, mortality and survival analysis

All calves were treated for at least one morbidity (diarrhea) event. One calf from the CL and 2 calves from the SG groups were treated prior to 4 days of age. None of the

calves in the NG group were treated prior to 4 days of age. Sulfadimethoxine (Albon, Pfizer Animal Health, New York, NY, USA) and electrolytes (Hyr-Lyte, Agri Laboratories, St. Joseph, MO, USA) administered orally were used for treatment of sick calves. Three calves died during the 7-day study period (one calf from each group). The calves from the CL, NG and SG groups died on day 2, 6 and 7 of life respectively. One calf died from septicemia secondary to diarrhea due to *Salmonella spp.* Two calves died from necrotizing enteritis secondary to enterotoxigenic *E. coli*, rotavirus and *Cryptosporidium* infection. Differences were not detected ($P = 0.682$) in the likelihood of mortality among the 3 groups.

Discussion

The major finding in this study indicated that intravenous plasma administration did not alter oxidative or phagocytic activity of granulocytes or monocytes in sick or non-sick calves with FPI. In contrast, plasma administration was beneficial in septic foals because of enhanced neutrophil function (McTaggart et al., 2005). Thus, administration of bovine plasma to enhance granulocyte and monocyte oxidative or phagocytic activity, alone, might not be justified in clinical practice. Serum IgG concentrations were consistent with adequate transfer of passive immunity (≥ 1000 mg/dL) following plasma transfusion in both the SG and NG groups. However, the serum IgG concentrations of ≥ 1000 mg/dL in the SG and NG groups lasted less than 12 and 24 hours, respectively. In contrast, the CL group maintained serum IgG concentrations ≥ 1000 mg/dL throughout the 7-day study period. The half-life of IgG was significantly shorter in the SG (6.9 days) and NG (3.8 days) compared to the CL group (19.4 days). This suggests that plasma administration is an inadequate source of long-lasting IgG in calves with FPI compared to

colostral derived IgG. The mechanism resulting in increased catabolism of plasma derived IgG may be similar to the mechanism suggested in human studies, which indicated that IgG aggregates formed during plasma preparation, might activate complement following transfusion resulting in increased catabolism of IgG (Lundblad and Londeree, 1988).

Time was the only significant variable resulting in a decrease in phagocytic activity of granulocytes and monocytes in the SG and NG groups while no changes were observed in the CL group. Specifically, the oxidative and phagocyte activity decreased between 2 hours or 12 hours after plasma transfusion and 7 days of age. This observation may be explained in part by a possible reduction in immune function due to the stress from the plasma transfusion procedure. Previous studies indicated that decreased immune function following abrupt weaning in beef calves decreased the percentage of phagocytic neutrophils (Lynch et al., 2010). Additionally, other studies in calves demonstrated that the oxidative burst activity of phagocytes decreased with age in colostrum fed calves (Menge et al., 1998). Other nutritional factors or disease conditions reported to alter oxidative or phagocytic activity of leukocytes in cattle include vitamin E deficiency (Hogan et al., 1990).

There was no difference in the risk for mortality rates among the 3 groups. However, it should be noted that the study period was only 7 days. Previous studies indicated that colostrum deprived calves administered plasma were 5 times more likely to experience mortality compared to calves with adequate transfer of immunity (Pipkin et al., 2015). We chose to collect samples up to 7 days only because antibodies against bacterial, protozoal and viral antigens do not appear in blood prior to 14 -30 days of age

(Kerr, 1956; Smith and Ingram, 1965). Thus, we chose a time prior to appearance of the endogenously synthesized immunoglobulins to minimize the effects on the serum IgG half-life determination and mortality. The half-life for colostrum IgG (19.4 days) reported in this study is consistent with previous studies, which reported half-lives of 17.9 days (Besser et al., 1991) and 17.1 days (Pipkin et al., 2015). The half-life of plasma derived IgG for the SG (6.9 days) and NG (3.8 days) groups are consistent with previous studies, which reported a half-life of 4.4 days in colostrum-deprived calves (Pipkin et al., 2015).

In clinical practice, plasma administration is recommended in sick calves with FPI. At the sufficient dose rate (30 mL/kg) reported in this study, the cost of bovine plasma might be prohibitive. While there was no effect by plasma transfusion on granulocyte and monocyte activity in this study, practitioners may recommend plasma transfusion to maintain serum IgG concentrations consistent with adequate transfer of immunity (≥ 1000 mg/dL). Due to the rapid decline in serum IgG plasma following plasma transfusion, repeated transfusions may be warranted within 12 hours in sick calves with failure of passive immunity to maintain serum IgG concentrations consistent with adequate transfer of immunity. However, repeated plasma transfusions are more likely to predispose to transfusion reactions.

The limitations of this study should be noted. Only oxidative and phagocytic functions of granulocytes and monocytes were determined. Other essential cell functions such as chemotaxis, degranulation, or cytokine expression were not determined. The half-life of IgG was calculated based on the cumulative IgG concentrations from both colostrum and plasma in the SG and NG groups. The rate of decay of colostrum derived IgG and plasma derived IgG is different thereby affecting IgG half-life determination of

IgG in the SG and NG groups. A placebo control group consisting of calves with FPI administered crystalloid therapy also was not included in this study. Thus, the placebo effect on the oxidative and phagocytic activity of monocytes and granulocyte could not be determined.

Conclusions

In this study, plasma administration did not alter oxidative and phagocytic activity of monocytes or phagocytes in calves with FPI. The half-life of IgG in sick or non-sick calves with FPI transfused with plasma was significantly shorter than colostrum derived IgG. In clinical practice, the administration of plasma in sick calves with FPI might be difficult to justify due to the shorter half-life of plasma derived IgG and absence of enhancement of oxidative or phagocytic activity monocytes and granulocytes.

Acknowledgements

The research was supported in part by the by the USDA National Institute of Food and Agriculture (Formula Funds) and the UC Davis Student Training in Advanced Research (STAR) grants. The authors would like to thank James Moller, Cynthia Mata, Jenny Orzechowski, Jujhar Dhillon, and Kendall Swanson for their assistance.

References

1. Besser, T.E., Gay, C.C., Pritchett, L., 1991. Comparison of three methods of feeding colostrum to dairy calves. *J. Am. Vet. Med. Assoc.* 198, 419-422.
2. Chigerwe, M., Tyler, J.W., Middleton, J.R., Spain, J.N., Dill, J.S., Steevens, B.J., 2008. Effect of colostrum administration by use of oroesophageal intubation on serum IgG concentrations in Holstein bull calves. *A. J. Vet. Res.* 69, 1158-1163.
3. Furman-Fratczak, K., Rzasa, A., Stefaniak, T., 2011. The influence of colostral immunoglobulin concentration in heifer calves' serum on their health and growth. *J. Dairy. Sci.* 94, 1536-1539.
4. Hogan, J.A, Smith, K.L., Weiss, W.P., Todhunter, D.A., Schockey, W.L., 1990. Relationship among vitamin, selenium, and bovine blood neutrophils. *J. Dairy. Sci.* 73, 2372-2378.
5. Kerr, W.R., 1956. Active immunity experiments in very young calves. *Vet. Rec.* 68, 476-477.
6. Lundblad, J.L., Londeree, N., 1988. The effect of processing methods on intravenous immune globulin preparations. *J. Hosp. Infect.* 12, 3-15.
7. Lynch, E.M., Early, B., McGee, M., Doyle, S., 2010. Effect of abrupt weaning at housing on leukocyte distribution, functional activity of neutrophils, and acute phase protein response of beef calves. *BMC. Vet. Res.* 6, 39.
8. Matt, J.J., Girard, C.L., Seoane, J.R., 1982. Absorption of colostral immunoglobulin G in the newborn dairy calf. *J. Dairy. Sci.* 65, 1765-1770.
9. McTaggart, C., Penhale, J., Raidal, S.L., 2005. Effect of plasma transfusion on neutrophil function in healthy and septic foals. *Aust. Vet. J.* 88, 499-505.

10. Menge, Ch., Neufeld, B., Hirt, W., Schmeer, N., Bauerfeind, R., Baljer, G., Wieler, L.H., 1998. Compensation of preliminary blood phagocyte immaturity in the newborn calf. *Vet. Immunol. Immunopathol.* 64, 309-321.
11. Murphy, J.M., Hagey, J.V., Chigerwe, M., 2014. Comparison of serum immunoglobulin G half-life in dairy calves fed colostrum, colostrum replacer or administered with intravenous bovine plasma. *Vet. Immunol. Immunopathol.* 158, 233-237.
12. Pipkin, K.M., Hagey, J.V., Rayburn, M.C., Chigerwe, M., 2015. A randomized clinical trial evaluating metabolism of colostrum and plasma derived immunoglobulin G in Jersey bull calves. *J. Vet. Intern Med.* 29, 961-966.
13. Reber, A.J., Donovan, D.C., Gabbard, J., Galland, K., Aceves-Avila, M., Holbert, K.A., Marshall, L., Hurley, D.J., 2008. Transfer of maternal colostrum leukocytes promotes development of the neonatal immune system Part II. Effects on neonatal lymphocytes. *Vet. Immunol. Immunopathol.* 123, 305-313.
14. Smith, A.N., Ingram, D.G., 1965. Immunological responses of young animals. II. Antibody production in calves. *Can. Vet. J.* 6, 226-232.
15. Weaver, D.M., Tyler, J.W., VanMetre, D.C., Hostetler, D.E., Barrington, G.M., 2000. Passive transfer of colostrum immunoglobulins in calves. *J. Vet. Intern Med.* 14, 569-577.
16. Windeyer, M.C., Leslie, K.E., Gooden, S.M., Hodgins, D.C., Lissemore, K.D., LeBlanc, S.J., 2014. Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. *Prev. Vet. Med.* 113, 231-240.

Table 1: Procedure schedule for blood collection and plasma transfusion in 29 Jersey dairy calves in CL (colostrum), SG (sick) and NG (non-sick) groups.

Group	D = 0	D = 2	D = 4				D = 5	D = 7
CL	IgG	IgG	Act CBC IgG				IgG Act CBC	IgG Act
			T = -1hr	PT	T = 2hr	T = 12hr		
NG and SG	IgG	IgG	IgG Act CBC		Act	Act	IgG Act CBC	IgG Act

IgG: serum IgG determination; Act: phagocytic and oxidative burst activity; CBC: complete blood count; PT = plasma transfusion; D = 0, 2, 4, 5 and 7 represent age of calves in days at the time of blood sample collection with D = 0 representing time prior to ingestion of colostrum. T = -1, 0, 2 and 12 represent 1-hour prior, 2 hours after and 12 hours after plasma transfusion blood collection time points in the SG and NG groups only.

Figure 1: Median serum IgG concentrations in the CL (n = 9), SG (n = 7) and NG (n = 11) groups at different time points. Time represent age of calves with Day 0 representing period prior to ingestion of colostrum. 4d +2 and 4d +12 represent 2 hours and 12 hours post plasma transfusion in the SG and NG group, respectively. The horizontal line at 1000 mg/dL represents serum IgG consistent with adequate transfer of passive immunity. CL = control group; SG = sick group; NG = non-sick group.

Figure 2: Granulocyte oxidative activity (median fluorescent intensity, MFI) at different time points for the CL (n = 9), SG (n = 7) and NG (n = 11) calf groups. CL = control group; SG = sick group; NG = non-sick group. 4d +2 and 4d +12 represent 2 hours and 12 hours' post plasma transfusion, respectively, in the SG and NG groups. No blood samples were collected at 4d +2 or 4d +12 hours in the CL group.

Figure 3: Monocyte oxidative activity (median fluorescent intensity, MFI) at different time points for the CL (n = 9), SG (n = 7) and NG (n = 11) calf groups. CL = control group; SG = sick group; NG = non-sick group. 4d +2 and 4d +12 represent 2 hours and 12 hours' post plasma transfusion, respectively, in the SG and NG group. No blood samples were collected at 4d +2 or 4d +12 hours in the CL group.

Figure 4: Granulocyte phagocytic activity (median fluorescent intensity, MFI) at different time points for the CL (n = 9), SG (n = 7) and NG (n = 11) calf groups. CL = control group; SG = sick group; NG = non-sick group. 4d +2 and 4d +12 represent 2 hours and 12 hours' post plasma transfusion, respectively, in the SG and NG group. No blood samples were collected at 4d +2 or 4d +12 hours in the CL group.

Figure 5: Monocyte phagocytic activity (median fluorescent intensity, MFI) at different time points for the CL (n = 9), SG (n = 7) and NG (n = 11) calf groups. CL = control group; SG = sick group; NG = non-sick group. 4d +2 and 4d +12 represent 2 hours and 12 hours' post plasma transfusion, respectively, in the SG and NG group. No blood samples were collected at 4d +2 or 4d +12 hours in the CL group.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5