Influence of Injectable Immunoglobulin on Serum Immunoglobulin Concentrations in Dairy Calves

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ABSTRACT

The influence of a purified Ig preparation on morbidity, mortality, and performance of purchased calves was evaluated. Calves (n = 187) were 3 to 8 d of age and were infused i.v. (blocks 1 and 2) or s.c. (blocks 3 and 4) with a purified Ig preparation derived from bovine blood that was obtained from an abattoir (n = 88) or were infused with 0.9% NaCl saline (n = 99). The Ig solution was purified by column chromatography and $(NH_4)_2SO_4$ precipitation (blocks 1 and 2) or by column chromatography only (blocks 3 and 4). Calves were infused with 504 g of Ig solution containing a mean of 47 g of IgG. Serum IgG concentrations at 24 h postinfusion increased 2.9 g/L when calves in blocks 1 and 2 were infused with Ig, but concentrations declined when calves in blocks 3 and 4 were infused with Ig or when calves were infused with NaCl saline. Total protein in serum increased with infusion of Ig in blocks 1 and 2 also. Serum urea N increased when calves in blocks 3 and 4 were infused with Ig. Mean BW at 28 d after arrival, BW gain, DMI, mortality, and scour scores were unaffected by treatment. Infusion of Ig increased the concentration of serum IgG but was dependent on the method of processing. Increased serum Ig did not markedly influence incidence or severity of disease in young dairy calves.
(**Key words**: calves, immunoglobulin, antibodies)

INTRODUCTION

Calves are born hypogammaglobulinemic, and consumption of colostrum is required to provide passive immunity until the immune system of the calf matures (5). For approximately 24 h after parturition, intestinal epithelial cells absorb macromolecules such as Ig (18, 19), which are transported through the cell to the lymphatic system and then to the general circulation (5, 18, 19). If calves do not ingest a sufficient mass of Ig prior to cessation of macromolecular transport, morbidity and mortality may increase (1, 11, 16, 18). Klaus et al. (12) reported that 30% of calves that consumed colostrum remained hypogammaglobulinemic. A recent survey by the USDA (6) reported that 41% of 2177 calves sampled between 24 and 48 h of age failed to attain serum IgG concentrations ≥10 g/L. Furthermore, the estimated risk of mortality (53.6%) among calves with serum IgG concentrations ≤10 g/L was associated with inadequate Ig transfer and colostral intake. Techniques are needed to provide Ig to hypogammaglobulinemic calves after cessation of macromolecular transport by the intestinal epithelia. Serum transfusions are available but are difficult, time consuming, and costly. Purified Ig preparations (2, 4, 7, 8) have the potential to provide Ig via injection at a lower cost. Recently, Crawford et al. (7) reported an increase in serum IgG concentrations in hypogammaglobulinemic calves receiving s.c. injections of purified IgG. In this study, our objective was to investigate the effect of a purified Ig source on serum IgG concentration, health, and growth of purchased calves.

MATERIALS AND METHODS

One hundred eighty-seven calves were purchased in four blocks from dairy farms or sale barns and were transported approximately 325 km to the experimental facility. Calves were approximately 1 wk of age, although ages were not recorded. Six calves were female. Nine calves were crossbred Holstein × Angus or Holstein × Jersey, and the remaining calves were Holstein. Serum IgG concentrations of calves were not determined prior to purchase. Upon arrival at the facility, calves were unloaded, weighed, and placed in individual fiberglass hutches for the 28-d experiment. Hutches were bedded with straw and cleaned as needed.

Calves were assigned randomly to receive i.v. (blocks 1 and 2) or s.c. (blocks 3 and 4) infusions of 0.9% NaCl saline or approximately 500 ml of a purified solution of Ig. The Ig solution (American Protein Corp., Ames, IA) was derived from bovine blood that was obtained from an abattoir, separated and purified by column chromatography (8), precipitated with

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$(NH_4)_2SO_4$, and suspended in 0.9% NaCl saline. Precipitation with $(NH_4)_2SO_4$ was deleted in blocks 3 and 4. The solution was filtered (0.22 μm) and refrigerated (block 1) or frozen (blocks 2 to 4) prior to use. Each bottle of Ig solution was weighed to the nearest 0.01 g prior to and following infusion to determine the amount of solution administered.

Solutions were infused by shaving and disinfecting the area and inserting an 18-gauge needle into the jugular vein (i.v.) or in the shoulder of each calf (s.c.). Solutions were infused through an i.v. set (Abbot Laboratories, North Chicago, IL), and the needle was held in place with a small amount of glue. The s.c. infusions required 10 to 20 min; i.v. infusions required 10 to >60 min.

Within 24 h of arrival, calves were given a routine physical examination to determine signs of preexisting disease. Calves in blocks 2 to 4 were injected with vitamins A, D, and E within 24 h of arrival. A 10-ml sample of jugular blood was collected into evacuated tubes approximately 16 h after arrival. Serum was separated after coagulation and centrifugation (3000 $\times$ *g*) and then stored (−20°C) until analyzed for IgG by radial immunodiffusion (VMRD, Inc., Pullman, WA), total protein (Sigma total protein kit 690; Sigma Chemical Co., St. Louis, MO), and urea N (Sigma urea kit 640; Sigma Chemical Co.). Approximately 24 h after infusion, a second blood sample was collected from the jugular vein for analysis of IgG, total protein, and urea N.

Commercial calf starter (Tennessee Farmers Cooperative, LaVergne, TN) was offered for ad libitum consumption from d 1 after arrival. Amounts offered and remaining were recorded daily. Commercial milk replacer (Tennessee Farmers Cooperative) was reconstituted to 12.5% DM, and 2 L were offered at approximately 0800 and 1600 h. Refused milk replacer was reported daily. Water was available at all times. Fecal consistency was scored (four-point scale where 1 = normal feces to 4 = severe scours) at the a.m. feeding to estimate the incidence and severity of scours (14). Electrolyte solution was administered when calves scoured (fecal score ≥2) or were visibly dehydrated. Antibiotic and electrolyte therapy was initiated when the scours score was ≥3. Samples of milk replacer and calf starter were taken monthly, composited, and analyzed for DM, CP, ash, and NDF (calf starter).

Data were analyzed as a split-plot experimental design with method of infusion (i.v. vs. s.c.), treatment (Ig vs. saline) block within method, and appropriate interaction terms using a mixed model algorithm. Block within method of infusion was used as the error term to test differences caused by treatment and method of infusion. However, block within method was not significantly different from zero in any analysis; therefore, data were reanalyzed as a randomized complete block experimental design by analysis of covariance. Covariates for serum IgG, total protein, and urea N concentrations 24 h after infusion were preinfusion IgG, total protein, and urea N concentrations, respectively. Body weight, BW gain, intake of milk replacer and calf starter, and feed efficiency were analyzed using initial BW as the covariate. Days scouring, severity of scours, and use of antibiotic and electrolyte therapy were analyzed by ANOVA. Significance of $P < 0.05$ was used unless otherwise noted.

RESULTS

Mean serum IgG concentration of calves upon arrival was 17.4 g/L (SE = 0.7). Concentration of IgG in serum of 51 calves (27%) was <10 g/L and of another 24 calves (12.8%) was ≥10 and ≤15 g/L, indicating that many calves had marked or moderate failure of passive transfer of immunity.

Mean infusate of Ig solution was 503.6 g (SE = 2.0). Mean IgG concentration of the Ig preparation was 94 g of IgG/L (SE = 5.0); therefore, calves received a mean of 47 g of IgG with infusion.

Concentration of IgG in serum of calves at 24 h postinfusion was increased by IgG infusion (Table 1), and serum IgG concentration declined when NaCl saline was infused. An interaction of method of infusion and treatment was significant ($P < 0.0016$) for serum IgG concentration at 24 h postinfusion (Table 1). Serum IgG concentration increased when calves were injected with Ig during blocks 1 and 2 (i.v. injection), but no measurable increase in serum IgG occurred when calves were injected with Ig solution in blocks 3 and 4 (s.c. injection). The similar interaction of method of infusion and treatment ($P < 0.0005$) indicated that the concentration of total protein in serum was higher 24 h after IgG was infused i.v. Changes in total protein concentration did not differ from zero when calves were infused with NaCl saline or with Ig by s.c. injection.

Serum urea N declined when calves in blocks 1 and 2 were injected with Ig (i.v. injection) or with NaCl saline, but increased ($P < 0.002$) when calves in blocks 3 and 4 were injected with Ig (Table 1), suggesting significant catabolism of protein when Ig was injected into calves in these blocks. The lack of increase in serum Ig or protein and the increase in serum urea N concentration with infusion of IgG in calves in blocks 3 and 4 suggested that most or all of the protein injected was catabolized by calves by 24 h after infusion.

Body weight, BW gain, starter intake, and feed efficiency were unaffected by treatment (Table 1). Intake of milk replacer was greater when calves were injected i.v. (blocks 1 and 2) and might have been related to the fewer number of days scouring for all calves in these blocks. Mean BW gain for the 28-d study was 312 g/d (SE = 13), which was typical for calves during the 1st mo of life. Mean BW at 28 d was 45.5 kg (SE = 0.4).

Mortality was unaffected by treatment and averaged 11.7% (SE = 2.3). Eleven calves on each treatment died during the study. Mean age at death was 10.9 d (SE = 1.4). Mortality of calves in blocks 3 and 4 was markedly increased ($P < 0.001$); incidence of death was 4 calves in block 1, 3 calves in block 2, 8 calves in block 3, and 7 calves in block 4. Necropsy indicated that the primary cause of mortality was enteritis (19 of 22 calves), although a causative agent was not determined in most cases. Serum IgG concentration was ≤10 g/L in 9 calves (41%) that died.

Calves scoured (fecal score ≥2) for a mean of 9.4 d (Table 1); mean duration of treatment with antibiotics, electrolytes, and intestinal gelling compounds was 9.1 d.

DISCUSSION

To be effective, infusion of Ig derived from blood must increase serum IgG concentrations and reduce

TABLE 1. Performance of calves infused i.v. (blocks 1 and 2) or s.c. (blocks 3 and 4) with 0.9% NaCl saline or purified IgG solution.

	i.v.		s.c.			*P*		
Item	IgG	Saline	IgG	Saline	SE	Method[1]	Trt	Method × Trt
Calves started, no.	40	50	48	49	...	...	...	...
Calves ended, no.	38	45	39	43	...	...	...	...
Mortality, %	5.0	10.0	18.7	12.0	4.9	0.11	NS[2]	NS
Serum IgG, g/L								
Preinfusion	19.4	15.4	17.0	18.0	1.4	NS	NS	0.09
Postinfusion[3]	20.3	16.2	17.4	17.0	0.6	0.07	0.0016	0.0016
Change[3]	2.9	−1.2	−0.1	−0.4	0.6	0.07	0.0016	0.0016
AEA,[3,4] %	32	...	−1	...	7	0.01	...	...
Serum protein, g/L								
Preinfusion	62.7	59.2	61.0	60.7	2.0	NS	NS	NS
Postinfusion[5]	67.7	60.0	57.5	62.5	1.8	0.03	NS	0.0005
Change[5]	7.0	−0.8	−3.2	1.8	1.8	0.03	NS	0.0005
Serum urea N, mg/dl								
Preinfusion	13.4	12.0	12.4	10.9	0.7	NS	0.03	NS
Postinfusion[6]	11.1	10.6	13.7	11.8	0.7	0.007	0.08	NS
Change[6]	−1.0	−1.5	1.6	−0.3	0.7	0.007	0.08	NS
BW, kg								
Initial	36.6	35.7	36.1	37.5	0.7	NS	NS	0.10
Final[7,8]	46.2	44.9	45.6	45.9	0.7	NS	NS	NS
Gain, g/d[7,8]	336	291	315	323	26	NS	NS	NS
DMI, g/d[7,8]								
Total	622	592	597	591	20	NS	NS	NS
Milk replacer	422	422	408	402	4	0.001	NS	NS
Calf starter	200	170	188	189	17	NS	NS	NS
BW Gain:DMI, g/kg[8]	525	467	505	526	39	NS	NS	NS
Days scouring[9]	6.2	7.4	12.5	11.3	0.9	0.001	NS	NS
Fecal score[9]	2.44	2.38	2.46	2.45	0.05	NS	NS	NS
Calves scouring, %[9]	87.2	80.0	87.0	88.8	5.5	NS	NS	NS
Calves treated, %[10]	67.9	70.9	100.0	100.0	5.0	0.001	NS	NS

[1]Method of infusion: i.v. or s.c. Trt = Treatment.

[2]$P > 0.10$.

[3]Adjusted by covariance for initial oncentration of serum IgG.

[4]Apparent efficiency of absorption, calculated as grams of Ig in serum + grams of Ig consumed.

[5]Adjusted by covariance for initial concentration of serum protein.

[6]Adjusted by covariance for initial concentration of serum urea N.

[7]Adjusted by covariance for initial BW.

[8]Excludes calves that died.

[9]Days with fecal score ≥2 (four-point scale, where 1 = normal feces to 4 = severe scours).

[10]Treatments included electrolytes, antibiotics, and intestinal gelling compounds.

morbidity and mortality prior to weaning without affecting later production. Infusions of Ig should be simple and rapid and should place minimal stress on the calves to be incorporated into a farm management scheme. In this study, i.v. infusion (blocks 1 and 2) was more difficult than the s.c. infusion (blocks 3 and 4). Although the Ig solution was purified and contained no fibrin, erythrocytes, or other foreign material, signs similar to those indicating transfusion reactions were commonly observed. Such signs were probably due to complement activation caused by IgG aggregates (15). When signs of discomfort occurred, infusion was slowed or stopped until the calf recovered sufficiently to allow continued infusion. Intravenous administration of Ig solution was stressful to the calves; therefore, the s.c. infusion was used for calves in blocks 3 and 4. Increased serum IgG concentrations in calves (7, 8), pups (4), and humans (9, 10) has been reported with s.c. injection of Ig preparations. No adverse reactions were noted in the current study when calves were injected s.c. In addition, the method of IgG preparation was altered after treatment of calves in blocks 1 and 2 by eliminating the $(NH_4)_2SO_4$ precipitation to reduce the amount of NH_3 in the Ig solution. Consequently, route of administration and method of preparation were confounded. Deletion of the precipitation step increased the concentration of total protein and non-Ig proteins in the solution. Mean total protein concentration of the Ig solutions (measured by biuret) were 139 and 151 g/L for calves in blocks 3 and 4, respectively, compared with 69 and 104 g/L for calves in blocks 1 and 2, respectively.

Infusion of Ig into calves by i.v. (blocks 1 and 2) and s.c. (blocks 3 and 4) routes increased serum IgG by 1.3 g/L, or approximately 7%, over preinfusion concentrations. Serum IgG declined 0.8 g/L when calves were infused with the saline. However, an interaction ($P < 0.0016$) of method of infusion and treatment indicated that, when calves in blocks 1 and 2 were injected with Ig, serum IgG concentration increased 2.9 g/L (14.9%) over preinjection concentrations. No effect was observed when calves in blocks 3 and 4 were injected with Ig. Decline in serum IgG concentration in calves injected with NaCl saline was probably due to an expansion of serum volume as a result of milk replacer feeding and NaCl saline infusion. If the expansion of serum volume was similar between calves injected with Ig or saline, the net increase in serum IgG concentration when calves in blocks 1 and 2 were injected with Ig was 4.1 g/L (2.9 + 1.2), a 21% increase over preinjection concentrations.

The lower purity and higher protein concentration of the Ig solution might have impaired absorption of Ig from the injection site when calves in blocks 3 and 4 were injected so that measurement at 24 h did not correctly quantify absorption of Ig. However, Crowley et al. (8) measured total Ig in calves that had been injected s.c. with Ig solution (12.7 g of Ig/L) and found no difference between serum Ig concentrations at 24 or 48 h after injection. Crawford et al. (7) injected calves with Ig solutions (15, 30, or 60 g of IgG/L) and measured serum IgG and IgM concentrations at 48 h postinfusion. The IgG concentration of the solution had no effect on serum IgG or IgM at 48 h. Serum IgG and IgM concentrations were increased with s.c. injection at all Ig concentrations. The Ig solution used by Crawford et al. (7) was prepared with the two-step purification process similar to that for blocks 1 and 2. Others (4, 9, 10) have successfully used s.c. injection for administration of Ig solutions containing large amounts of protein; therefore, delayed absorption was probably not responsible for a lack of increase in serum IgG at 24 h postinfusion in the current study.

The Ig in the solution administered to calves in blocks 3 and 4 might have been damaged during preparation or administration and thus subsequently might have been catabolized by the calves. Also, Ig might have formed aggregates of molecules that could not be utilized by the calf. Increased urea N in serum of calves in blocks 3 and 4 (Table 1) supported this hypothesis. Although Ig might have been damaged or aggregated, quantification of Ig by radial immunodiffusion would have been possible, because this method of analysis does not depend on intact Ig molecules. Methods of preparation and administration appear to be critical for successful utilization of isolated Ig by calves.

Approximately 32% of IgG infused in calves in blocks 1 and 2 was measured in the serum at 24 h after infusion, based on serum volume of 10% of BW. The metabolic fate of the remaining IgG was unknown. Kruse (13) reported that approximately 50% of the IgG that was ingested by calves <24 h of age was found in extravascular space. A significant portion of the injected Ig probably moved into extravascular pools, thereby reducing the amount measured in the serum.

Slopes of the regression of preinfusion and postinfusion IgG concentrations were similar and were 0.82 and 0.85 for Ig and saline treatments, respectively (Figure 1), suggesting expansion of plasma volume or other reduction in concentration of serum IgG in the 24 h after infusion. The similarity of slopes indicated that the increase in concentration of serum IgG with IgG infusion of calves in blocks 1 and 2 was independent of preinfusion IgG concentrations.

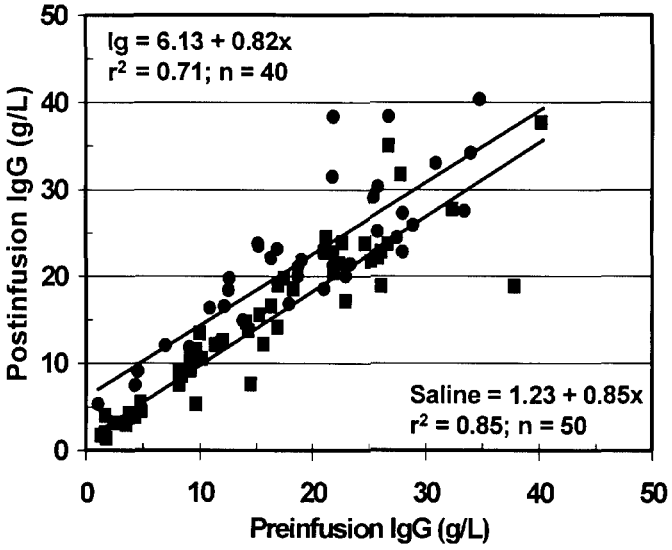

Figure 1. Regression of preinfusion versus postinfusion concentrations of IgG in calves in blocks 1 and 2 injected with NaCl saline (■) or with purified Ig solution (●).

Calves scoured (fecal score ≥2) for a mean of 9.4 d; calves that scoured were treated with antibiotics, electrolytes, and intestinal gelling compounds. More than 80% of calves on the study developed scours during the study, indicating considerable exposure to pathogens. Shipment of calves from dairy farms and sale barns probably contributed to the amount of morbidity and mortality in this study. Most calves (90 of 165) scoured for ≤8 d; the median number of days scouring was 8. Provision of Ig into peripheral circulation might not markedly affect the incidence of enteric disease. Others (1, 17) have suggested that passive antibodies in the intestinal lumen are primarily responsible for prevention of enteric disease in young calves; therefore, injection of Ig into the circulation might not completely inhibit growth of enteric pathogens. However, Besser et al. (2) reported increased resistance to oral rotavirus challenge when calves were injected s.c. with whey from hyperimmunized cows, but calves injected with whey from nonimmunized cows were not resistant to challenge. Thus, circulating IgG might serve as a reservoir of antibody to prevent enteric infection. Movement of IgG into the intestine (3) also suggested that circulating IgG are important in resistance to enteric disease. Specificity of IgG infused in this study was not determined and might have been incompatible with pathogens in the environment of the calves. Also, the pathogen load might have been too great to allow differences in immune status to be observed.

CONCLUSIONS

Exogenous Ig increased concentrations of serum IgG in calves 24 h after infusion, but only for calves in blocks 1 and 2. Method of preparation was probably responsible for the lack of significant effect for calves in blocks 3 and 4. Infusion of Ig had no effect on morbidity or mortality. The large and diverse population of donors used to collect the Ig solution requires considerable preparation and expertise to eliminate contamination and to maintain sterility. As determined in this study, method of preparation and handling is also critical to ensure the stability of the Ig and its efficacy in the calf. Furthermore, Ig sources other than colostrum may provide Ig that are not specific for antigens present in the environment or might be insufficient when calves have preexisting diseases or are exposed to large loads of pathogens. Therefore, these Ig sources should not be considered as a potential replacement for maternal colostrum or for proper management and hygiene. However, this study and previous studies (7, 8) indicate that production of a concentrated source of Ig can provide a source of Ig for neonatal dairy calves after cessation of macromolecular transport. Further research is needed to determine the optimal methods of Ig isolation and storage from abattoir blood to maximize the efficacy of purified Ig.

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