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Intravenous immunoglobulin transfusion in colostrum-deprived dairy calves

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ABSTRACT

Immunoglobulin transfusion is employed in the management of the failure of passive transfer (FPT). The aim of this study was to investigate the dose of immunoglobulin G (IgG) needed to reach a protective concentration (>10 g/L) in colostrum-deprived dairy calves. Twenty-eight Holstein Friesian newborn male calves were randomly assigned to either a control group (CG) or a treatment group (PG). Calves in the CG received 4 L of high quality colostrum within 12 h of birth. Calves in the PG received 62.7 ± 3.1 g of IgG IV in 2.6 ± 0.3 L of plasma within 6 h after birth. Serum immunoglobulin G (sIgG) and serum total protein (sTP) concentrations were assayed before and after (24 h, 72 h and 1 week after birth) plasma transfusion or colostrum ingestion.

Serum (s) IgG and sTP concentrations increased in both groups throughout the period of observation. Mean sIgG and sTP concentrations after colostrum ingestion or plasma transfusion were higher in the CG than in the PG ($P < 0.01$). Nine treated calves developed diarrhoea during the study and four were humanely euthanased due to progressive clinical deterioration. None of the calves in the CG showed signs of disease or died during the study. The dose of IgG used in this trial effectively provided an adequate sIgG concentration in colostrum-deprived calves (>10 g/L). Calves in the CG had significantly lower morbidity and mortality rates compared to those in the PG, suggesting that plasma transfusion alone is ineffective in providing complete protection against neonatal disease.

Introduction

Failure of passive transfer (FTP) is generally defined as serum (s) IgG < 10 g/L (Tyler et al., 1996). Low sIgG concentrations affect the health and survival of calves beyond the neonatal period (Trotz-Williams et al., 2008; Beam et al., 2009; Vogels et al., 2013).

Plasma transfusion has been empirically recommended as a therapeutic intervention in calves with FPT (Weaver et al., 2000; Barrington and Parish, 2009) and has been used successfully in primary or supportive therapy of many equine diseases (Feige et al., 2003). In cattle, data regarding the efficacy and safety of plasma transfusion are limited. The published literature does not clearly specify the clinical effectiveness of transfused IgG in reaching adequate serum concentrations, mainly because of: (1) lack of information on sIgG concentrations pre- and post-plasma transfusion (Smith and Little, 1922); (2) lack of information on the concentration of IgG in transfused products (Anderson et al., 1987; Selim et al., 1995); (3) the administration of inadequate doses of IgG (Chigerwe and Tyler, 2010); (4) transfusion in calves with adequate passive transfer (Quigley and Welborn, 1996); or (5) immunoglobulin aggregates formed during plasma preparation can increase IgG catabolism after 6 h (Pipkin et al., 2015), 24 h (Quigley and Welborn, 1996) or 48 h after transfusion (Murphy et al., 2014).

This study compared sIgG and sTP in a control group (CG) of calves fed high quality colostrum with a treated group of colostrum-deprived calves administered IV IgG by plasma transfusion (PG). Our aim was to evaluate the efficacy of a centrifuged plasma preparation in reaching protective sIgG concentrations (>10 g/L) in colostrum-deprived dairy calves. In addition, differences in morbidity and mortality between the two groups were determined.

Materials and methods

Animals

A randomised, parallel-group, clinical controlled trial was performed at the Clinic for Ruminants, Swine and Management (CRSM), at the University of Milan. All procedures were approved by the ethical committee of the University of Milan (CE 19/12/12 number 40/12). From September to December 2013, a total of 28 Holstein

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Friesian bull calves from a single farm in Lodi, Italy were enrolled. Healthy calves from eutocic and observed parturitions were separated from their dams within 10 min after birth. The calves were then weighed, transported to CRSM (15 km) and housed in individual calf brick pens (1.8 m × 1.2 m) bedded with straw and cleaned every day, in indoor 30-place stalls with a controlled temperature of 18 °C. After enrolment, calves were randomly assigned (1:1) to the CG or PG using a random sequence generator [1]. The first group consisted of 14 calves that received high quality bovine colostrum, and the second group consisted of 14 colostrum-deprived calves that received fresh frozen plasma transfusion IV within 4 h after birth. After the calves had been discharged, the pens were cleaned with warm water and a 1% NH_4Cl solution before introducing new calves.

Clinical procedures

High quality colostrum from individual dairy cows was bottle fed to calves in the CG in two separate feedings: 2 L within 3 h after birth, and another 2 L within 12 h after birth. Colostrum was collected 1 month earlier from the first milking of cows from the same herd. Four litres (Brix score ≥22%; Bielmann et al., 2010) were stored at –20 °C until administration. Calves in the PG were transfused with non-pooled fresh frozen plasma from individual donor cows. Plasma transfusion was performed in calves in the PG through the jugular vein with a 14 G IV catheter, using an IV line with filter (Transfusion set 601-TS; Ferrari). Infusion of plasma was performed at 10 mL/kg/h for the first 20 min. In the absence of an immediate transfusion reaction, the remaining plasma was transfused at 50 mL/kg/h. Blood samples were collected into tubes without additives from the jugular vein: at birth before receiving colostrum or plasma transfusion (T_0), at 24 h (T_1), 72 h (T_3), and at 1 week (T_7) of age. Samples were allowed to clot and then centrifuged at 20 °C for 10 min at 900 *g*. Serum was stored at –20 °C until analysis was performed to determine sTP and sIgG concentrations. After colostrum or plasma transfusion, calves were fed 2 L milk replacer (Solvor MG + Instant; Bonilait) three times daily. Both groups were monitored by daily clinical examination for 3 weeks by the same experienced veterinarian who was not involved in the study and was masked to treatment allocation.

Prediction of IgG dose required

The total IgG amount required to reach a post-transfusion sIgG of 10 g/L (Tyler et al., 1996) in calves in the PG was estimated using the following formula modified by Chigerwe and Tyler (2010):

$$\text{Total IgG in transfused plasma (g)} = (\text{desired post-transfusion sIgG concentration (g/L)} \times \text{bodyweight (BW, kg)} \times \text{estimated plasma volume (\% BW)}) / \text{estimated pre-transfusion sIgG (g/L)}.$$

The plasma volume in Holstein Friesian calves was estimated to be 8.9% of calf BW (Quigley et al., 1998). Estimated pre-transfusion sIgG (g/L) in Holstein Friesian calves was 0.639 g/L (Chigerwe et al., 2008). For each calf, after the IgG dose calculation, the volume of plasma transfused was calculated with the known IgG concentration in each plasma bag.

Plasma collection and storage

Non-pooled bovine plasma was obtained from 37 dairy cows during the dry-off period. Ten millilitre blood samples were collected by jugular venepuncture into tubes without additives and used to assay sIgG concentration with an ELISA catching system. A total of 20 cows with the highest sIgG concentrations were enrolled in the study. From each cow, 5 L of whole blood was collected 7–10 days after blood sampling according to standard techniques (Soldan, 1999) using a 450 mL double-bag closed-collection system (11 bags from each cow; CPD-A 450; Grifols). Bags were immediately refrigerated at 4 °C and centrifuged at 3500 *g* for 15 min at 4 °C (Rotixa 500 Rs; Hettich Zentrifugen). A plasma extractor (Plasma Extractor; Fenwal) was used to generate one bag of plasma from each bag of whole blood. To assess IgG concentrations in plasma bags, an aliquot was collected from each bag during extraction and IgG concentration was assayed with an ELISA catching system [2]. Bags were then immediately frozen at –20 °C.

Determination of sTP and sIgG concentrations

The concentration of sTP was determined with a colorimetric assay (Total Proteins Quantitative Colorimetric Assay; Biochemical Enterprise), following the manufacturer's protocol. The results of the colorimetric assay were determined using a clinical chemistry analyser (Roche Cobas Mira Classic; Hoffmann-La Roche).

Immunoglobulins in both calf serum and single donor cow plasma bags were analysed using an ELISA catching system (Bovine IgG ELISA Quantitation Set; Bethyl Lab) according to the manufacturer's instructions. Intra- and inter-assay coefficients of variation were <10% for all samples.

[1] See: www.random.org (accessed 17 November 2015).

[2] See: Quigley, J.D. III, 2008. Calf Note #135 – On methods of IgG analysis. http://www.calfnotes.com/pdffiles/CN135.pdf (accessed 17 November 2015).

Table 1
Mean ± standard deviation serum gamma immunoglobulin (sIgG) and serum total protein (sTP, g/L) for calves in the plasma group (PG) and control group (CG) groups at different sampling times.

Group	T_0[a]	T_1[b]	T_3[c]	T_7[d]
CG	2.62 ± 1.97	32.06 ± 6.63	30.32 ± 6.45	28.17 ± 8.14
	41.14 ± 3.79	60.78 ± 7.39	61.00 ± 5.44	57.85 ± 7.17
PG	1.52 ± 2.40	18.98 ± 5.89	19.98 ± 6.26	18.17 ± 6.50
	36.64 ± 2.87	49.92 ± 5.58	50.78 ± 5.04	46.46 ± 7.12

[a] Serum samples collected at birth before receiving colostrum or plasma transfusion.
[b] Serum samples collected at 24 h after treatment (colostrum/plasma transfusion).
[c] Serum samples collected at 72 h after treatment (colostrum/plasma transfusion).
[d] Serum samples collected 1 week after treatment (colostrum/plasma transfusion).

Statistical analysis

Changes in sIgG and sTP concentrations from T_0 to T_1 were calculated for both CG and PG. A two-tailed *t*-test was then used to assess statistical differences between the CG and PG. A linear mixed model was also used to compare sIgG and sTP concentrations in the CG and PG at 24 h (T_1), 72 h (T_3) and 1 week (T_7) after colostrum/plasma administration. Response variables were tested for normality using the Shapiro–Wilk test. The fitted model included the fixed effects of treatment (colostrum/plasma administration), time-point, their interaction, and a random calf effect. The model r^2 was obtained following Nakagawa and Schielzeth (2013). Least square means for fixed effects and their interaction were obtained. The proportions of calves that developed diarrhoea during the experiment were compared using a χ^2 test. All statistical analyses were performed using the R programming environment. The R packages lme4 and lmerTest were used to fit the model and to estimate least square means.

Results

Calves, plasma transfusion and serum analysis

Mean bodyweight ± standard deviation (SD) for the CG and PG was 43.6 ± 3.2 kg and 45.1 ± 2.5 kg, respectively. All calves in the CG ingested 4 L of colostrum within 12 h after birth. The total volume of collected plasma was 60.2 L distributed in 220 bags. Serum IgG concentrations in the 37 potential donor cows ranged from 9.84 to 41.52 g/L. In the 20 enrolled donor cows, the serum IgG concentrations ranged from 22.08 to 41.52 g/L with an average value of 26.86 ± 4.39 g/L. Immunoglobulin transfused to the calves in the PG ranged from 58 to 69 g with an average value of 62.7 ± 3.1 g, corresponding to a mean transfused plasma volume of 2.6 ± 0.3 L (57.6 ± 6.6 mL/kg of BW). Because of the predetermined content of IgG within the plasma bags, the effective administered dosage was characterised by a maximum error of ± 5%. No calves in the PG showed clinical signs of transfusion reactions, and the transfusion lasted for maximum 3 h.

The results of sIgG and sTP concentrations are summarised in Table 1. All calves in the PG had serum IgG concentrations >10 g/L at all time-points. At T_0, 11 calves in the CG and six calves in the PG had detectable sIgG concentrations with means of 2.62 ± 1.97 and 1.52 ± 2.40 g/L, respectively. At T_0, sTP concentrations in calves in the CG and PG ranged from 36 to 46 g/L, and from 33 to 41 g/L, respectively. At T_1, sIgG and sTP concentrations increased in both CG and P.

The mean increases in sIgG and sTP concentrations, respectively, at different time points are reported in Figs. 1 and 2. The average sIgG increase from T_0 to T_1 was 29.44 ± 7.06 g/L and 17.46 ± 7.06 g/L in CG and PG, respectively. These average increases were compared between treatment groups (CG vs. PG), and were statistically significant (*t* test; $P < 0.01$). The average sTP increase from T_0 to T_1