



## Short communication: Effect of feeding pooled and nonpooled high-quality colostrum on passive transfer of immunity, morbidity, and mortality in dairy calves

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### ABSTRACT

Pooling colostrum is commonly practiced on Irish dairy farms. Pooling can result in dilution when colostrums with high and low IgG concentrations are mixed, thereby predisposing calves to failure of passive immunity. The objectives of this study were to compare IgG concentrations in colostrum from individual cows with colostrum pooled from several cows, and assess serum IgG concentrations, morbidity, and mortality among calves fed colostrum from their own dam, from a different cow, or pooled from several cows. We hypothesized that pooling colostrum reduces IgG concentration due to dilution compared with colostrum from individual cows, and that calves fed pooled colostrum achieve lower serum IgG concentrations than calves fed colostrum from individual cows. Calves were randomly assigned to 1 of 3 groups: (1) fed colostrum from their own dam ( $n = 20$ ); (2) fed colostrum from a different dam ( $n = 20$ ); or (3) fed pooled colostrum ( $n = 18$ ). A sample of colostrum fed to each calf was collected. Serum samples were collected from calves at birth (0 h) and at 24 h after colostrum feeding. Colostrum and serum IgG concentrations were measured by radial immunodiffusion. Calves were weighed at birth and at weaning, and the health status of each calf was assessed twice daily. Health assessment was based on general demeanor, rectal temperature, fecal consistency, respiratory rate, and the presence of cough, nasal, or ocular discharge. Colostrum and serum IgG concentrations, and weaning weights were compared using ANOVA. Associations between group and morbidity or mortality rates were compared using  $\chi^2$  or Fisher's exact tests. Median and 95% confidence intervals (95% CI) of IgG concentrations of colostrum were 99.4 (81.8–111.5),

95.2 (84.1–107.2), and 100.7 (90.5–104.4) g/L for own dam, different dam, and pooled groups, respectively. We did not find any differences in colostrum IgG concentrations among the colostrum sources. Median (95% CI) serum IgG concentrations at 24 h were 52.0 (45.6–65.9), 55.7 (51.2–65.9), and 53.1 (46.2–63.7) g/L for calves that received colostrum from own dam, different dam, and pooled, respectively. All calves achieved adequate passive immunity. Serum IgG concentrations at 24 h, weaning weights, and proportions of morbidity and mortality were not different among the 3 groups. Our results suggest that on dairy farms where median colostrum IgG concentrations are high and colostrum management is optimal, pooling has a minimal effect on passive immunity and subsequent calf health.

**Key words:** colostrum, calf, pooling, immunoglobulin

### Short Communication

Colostrum is the first secretion from the mammary gland following parturition and contains IgG, which is essential for providing the neonate with passive immunity (Morrill et al., 2012). Adequate passive transfer (APT) of immunity is achieved when calves are fed a sufficient volume of high-quality colostrum shortly after birth (Lorenz et al., 2011). High-quality colostrum contains  $>50$  g/L IgG (McGuirk and Collins, 2004). Below this IgG threshold, there is a potential risk of failure of passive transfer (FPT), which is associated with increased rates of morbidity and mortality (Todd et al., 2018). Pooling colostrum has associated risks, such as disease transmission and an IgG dilution effect (Williams et al., 2014); however, pooling continues to be practiced on over 40% of Irish dairy farms (Barry et al., 2019). Despite the associated risk, pooling colostrum can reduce labor requirements associated with feeding newborn calves and has been reported to provide APT of immunity in North American dairy herds (Williams et al., 2014). Furthermore, pooling of colostrum may be

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beneficial, particularly in scenarios where new animals join the herd and might not have been exposed to all diseases prevalent in that herd. Consequently, pooling colostrum allows all calves to ingest colostral immunoglobulins relevant to antigens to which cows were exposed to during the dry period on that farm.

Although studies investigating the effect of pooled colostrum feeding on passive immunity have been conducted in North America, (Beam et al., 2009), no such studies have been performed in Ireland, where the calving season is condensed into a 12-wk period during spring (February–April). The objectives of this study, therefore, were to compare IgG concentrations in colostrum from individual cows and pooled colostrum, as well as serum IgG concentrations, morbidity, and mortality in calves fed their own dam's colostrum, colostrum from a different cow, or pooled colostrum. We hypothesize that pooling bovine colostrum reduces the overall colostrum IgG concentration due to dilution, and consequently, calves fed pooled colostrum achieve lower serum IgG concentrations than calves fed colostrum from individual cows.

A prospective randomized cohort study was conducted at Teagasc, Moorepark Dairy Research Farm (Fermoy, Co. Cork, Ireland). Sixty Holstein Friesian (**HF**) and HF × Jersey cows and 60 calves were enrolled in the study. Calves were stratified based on breed and BW at birth and randomly assigned, within stratum, to their experimental treatment group. The number of calves required per group was calculated by considering multiple factors. Based on a previously reported mean IgG concentration of 112 g/L in dairy calves in Ireland (Conneely et al., 2013), a mean difference in IgG concentration of at least 10 g/L among the 3 groups, a standard deviation of 10 g/L, power of 80%, and  $\alpha$  of 0.05, the minimum sample size required per group was 18 calves. To allow for a 10% dropout rate due to missed samples or incomplete data, 2 calves were added to each group. The final sample size required was 20 calves per group. Commercial software (JMP Pro version 14, SAS Institute Inc., Cary, NC) was used to calculate the sample size. Randomization of calves into each group was achieved by generation of random numbers using Microsoft Excel (Microsoft Corp., Redmond, WA). All calves were enrolled over a period of 6 wk from February to March 2018.

The 3 treatment groups were (1) calves fed unpasteurized colostrum from their own dam (**OD**,  $n = 20$ ); (2) calves fed unpasteurized colostrum from a different dam (**DD**,  $n = 20$ ); and (3) calves fed unpasteurized pooled colostrum (**PC**,  $n = 18$ ) from a group of cows of variable IgG concentrations. The DD group was included because it represents another colostrum

management technique practiced on Irish dairies and is another type of nonpooled colostrum feeding group. In cases where the dam of the calf cannot be milked in time or the dam does not produce colostrum, producers often feed colostrum from a different dam. Effective transfer of maternal leukocytes has been demonstrated in calves fed colostrum from its own dam (Donovan et al., 2007) or from pooled colostrum (Parreño et al., 2004) with no known negative effects of absorption.

All cows were blood sampled before calving, and cows that tested positive for paratuberculosis (*Mycobacterium avium* ssp. *paratuberculosis*) or *Mycoplasma* spp. were not included in the study. Calving was supervised by trained and experienced personnel. Immediately after calving, calves were separated from their dams and 10 mL of blood (pre-colostral) was collected from the jugular vein into blood tubes without anticoagulant (red top Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Cows were milked with a single portable milking unit (DeLaval Mobile Milking Unit, Carlow, Ireland) within 2 h of calving. A 250-mL composite sample of colostrum was collected from each cow or pooled colostrum. Calves were fed colostrum at 8.5% of their birth weight (Conneely et al., 2014), and the required colostrum volume was calculated for each individual calf. The required total colostrum volume was divided over 2 feedings, one within 2 h of birth and the other at 12 h of age. Colostrum was fed by esophageal tubing. Only fresh colostrum was fed, and the source was based on which cows had calved that same day. The pooled colostrum samples comprised samples from 2 to 4 cows, depending on availability. Each calf in the pooled group received their own unique pooled sample. The individual IgG content of each contributor to the pool was not measured.

At 24 h of age, 10 mL of blood was collected into tubes without anticoagulant from all calves. Serum was harvested from blood samples after centrifugation at  $2,880 \times g$  for 5 min at 4°C. Both colostrum and serum samples were then stored at  $-80^{\circ}\text{C}$  until used for IgG determination by single radial immunodiffusion (**SRID**). Following colostrum feeding, all calves were fed milk replacer thereafter. Calves were initially housed in individual pens for 5 d and then moved into group pens of 10 to 15 calves. Calves were weaned after reaching a target weight of 95 kg for HF calves, and 80 kg for HF × Jersey crosses.

Colostrum and serum samples were analyzed for IgG concentrations by collaborators blinded to the group assignments using a commercial SRID kit (Radial Immunodiffusion Test for Quantitation of Bovine IgG in Serum or Plasma, Triple J Farms, Bellingham, WA) according to the manufacturer's recommendations.

The SRID plates were stored at 4°C, and contained specific anti-bovine IgG, agarose gel, 0.1 M phosphate buffer (pH 7.0), 0.1% sodium azide as a bacteriostatic agent, and 1 µg/mL amphotericin B as an antifungal agent. The IgG concentration determination range of the SRID kit was 0.09 to 1 g/L for serum samples collected from calves at 0 h, which allowed detection of IgG; low concentrations of IgG have been reported in serum of calves before colostrum ingestion (Chigerwe et al., 2008). The IgG concentration range of the kit was 1.96 to 28.03 g/L for colostrum and serum samples collected at 24 h. Colostrum and serum samples were thawed at room temperature (approximately 20°C) for 24 h. Colostrum samples were diluted at 1:4 or 1:6 and serum samples were diluted 1:2 or 1:4 depending on the IgG concentration; PBS was used as the diluent. Test kits were incubated at room temperature for 30 min before inoculation with 5 µL of colostrum or serum per well. Once all samples had been added, plates were incubated at room temperature (20–24°C) for 24 h. Zones of precipitation in the plate were then measured using a SRID plate reader (Digital RID Plate Reader, The Binding Site, San Diego, CA). The concentration of IgG in the samples was determined by comparing the diameter of the precipitated ring to the standard curve produced by the reference sera provided with the kit. Coefficient of determination ( $R^2$ ) values for the standard curves were between 0.96 and 0.99 for the regression equations produced, indicating accurate prediction of the inoculum IgG concentrations. Calves with serum IgG concentrations  $\geq 20$  g/L at 24 h were considered to have adequate passive immunity (Chigerwe et al., 2015).

All calves were examined twice daily until weaning by trained personnel blinded to the group assignments. Assessment of morbidity was made based on general demeanor, rectal temperature, presence of diarrhea using fecal consistency scores (McGuirk, 2008), and presence of respiratory disease using respiratory rate and presence of cough, nasal, and ocular discharge (Love et al., 2014). Presence of omphalitis was assessed by

digital palpation of the umbilicus, and presence of joint disease was evaluated by digital palpation of joints. The decision to treat sick calves was based on the farm's standard operating procedures. All mortality events and all causes of morbidity were recorded.

Analyses were performed using a commercial software (JMP Pro version 14, SAS Institute Inc., Cary, NC). Normality of the data was determined using the Shapiro-Wilk test. Descriptive statistics were calculated for lactation number, birth weight, colostrum IgG and serum IgG concentrations, and weaning weights. Means  $\pm$  standard error of mean (SEM) were reported when data were normally distributed, whereas median and 95% confidence interval (95% CI) were reported when data were not normally distributed. Differences in distribution of lactation number and colostrum IgG concentrations among colostrum samples from the OD, DD, and PC groups were compared using ANOVA or Kruskal-Wallis test when data were not normally distributed. Differences in birth weights, serum IgG concentrations at 0 and 24 h in calves fed colostrum from OD, DD, and PC, and weaning weights were compared using ANOVA or Kruskal-Wallis test when data were not normally distributed. Differences in proportions of calves experiencing morbidity and mortality among the 3 groups during the preweaning period were compared using a  $\chi^2$  test or Fisher's exact test when a cell had  $< 5$  counts in a  $2 \times 2$  frequency table. The results were considered significant when  $P < 0.05$ .

Median (95% CI) lactation numbers for OD, DD, and PC group cows were 3 (1, 4), 2 (1, 3), and 1 (1, 2), respectively. Median lactation number was higher ( $P = 0.01$ ) in the OD than in the PC group. Lactation numbers were not different between OD and DD ( $P = 0.99$ ) or DD and PC ( $P = 0.18$ ) group cows. Median (95% CI) colostrum IgG concentrations were 99.4 (81.8, 111.5), 95.2 (84.1, 107.2), and 100.7 (90.5, 104.4) g/L for OD, DD, and PC group cows, respectively. Colostrum IgG concentrations, summarized in Table 1, were not different ( $P = 0.93$ ) among the OD, DD, and PC group cows.

**Table 1.** Summary of median (95% CI) colostrum IgG concentrations (g/L), serum IgG concentrations (g/L), and birth and weaning weights of calves fed colostrum from different sources

Variable	Colostrum source		
	Different dam	Own dam	Pooled colostrum
0-h serum IgG (g/L)	0.09 (0.09, 0.4) <sup>a</sup>	0.09 (0.09, 0.09) <sup>a</sup>	0.09 (0.09, 0.09) <sup>a</sup>
Colostrum IgG (g/L)	95.2 (84.1, 107.2) <sup>a</sup>	99.4 (81.8, 111.5) <sup>a</sup>	100.7 (90.5, 104.4) <sup>a</sup>
24-h serum IgG (g/L)	55.7 (51.2, 65.9) <sup>a</sup>	52.0 (45.6, 65.9) <sup>a</sup>	53.1 (46.2, 63.7) <sup>a</sup>
Birth weight (kg)	34.3 (30.6, 35.0) <sup>a</sup>	33.3 (28.0, 36.5) <sup>a</sup>	34.0 (31.0, 36.0) <sup>a</sup>
Weaning weight (kg)	91.7 (88.5, 94.5) <sup>a</sup>	93.7 (90.8, 95.5) <sup>a</sup>	94.0 (86.0, 95.5) <sup>a</sup>

Median (95% CI) birth weights, weaning weights, and serum IgG concentrations at 0 and 24 h from calves fed colostrum from OD, DD, and PC cows are summarized in Table 1. Serum IgG levels at 0 h, before colostrum ingestion, was 0.09 g/L across all calf groups, indicating that the calves had not nursed before IgG levels were measured. We found no differences in median birth weights ( $P = 0.97$ ), serum IgG concentrations at 0 h ( $P = 0.19$ ) or 24 h ( $P = 0.62$ ), or weaning weights ( $P = 0.59$ ) among the calves. All calves achieved adequate transfer of immunity.

Twenty-five morbidity events were recorded: 8, 10, and 7 across the OD, DD, and PC treatment groups, respectively. The proportion of calves experiencing morbidity did not differ ( $P = 0.60$ ) among the groups, with diagnosed events consisting of diarrhea or respiratory disease. Twelve of the morbidity events required medical treatment with antimicrobials or oral electrolytes, and a combination of both in some cases; 2, 7, and 3 calves from the OD, DD, and PC groups were treated, respectively. The proportion of calves that experienced morbidity and were treated did not differ ( $P = 0.13$ ) among the groups. All calves survived to weaning.

Our study results indicated no differences in colostrum IgG concentrations from individual cows compared with that of pooled high-quality colostrum. We also did not find differences in serum IgG concentrations at 24 h or in morbidity or mortality among calves fed colostrum from their own dam, a different dam, or pooled colostrum. In contrast to our hypothesis, pooling bovine colostrum did not affect colostrum IgG concentration compared with feeding colostrum from individual cows, and calves fed pooled colostrum achieved similar serum IgG concentrations to calves fed colostrum from individual cows. Our results differ from previous reviews (Weaver et al., 2000) and observational studies in North American dairies (Beam et al., 2009), which reported increased odds of FPT of immunity among calves fed pooled colostrum. However, a recent study (Williams et al., 2014) reported APT of immunity in calves fed pooled colostrum on a Californian dairy farm. A possible reason for these different results may be the high median colostrum IgG in both our study and that of Williams et al. (2014). Thus, when the colostrum IgG concentration is higher, pooling of colostrum has an insignificant effect on the quantity of IgG delivered to the calf. Many studies assessing colostrum and FPT also investigated other variables affecting transfer of colostrum immunity, such as timing of colostrum ingestion, method of ingestion, colostrum pasteurization, and the presence or absence of dystocia. In contrast, our study focused on the effect of pooling colostrum alone with other variables similar among calves. A previous study

by Kehoe et al. (2011) reported that IgG in colostrum increased with parity, with cows in first, second, third, and fourth or greater lactations producing colostrum with IgG concentrations of 83.5, 92.9, 107.4, and 113.3 g/L, respectively. In our study, median lactation number was higher in OD than in PC group cows. Despite this difference in lactation stage, all calves fed pooled colostrum achieved APT of immunity.

Pooling colostrum is a common practice on Irish dairy farms. Kennedy et al. (2014) reported that 73.5% of 312 randomly selected Irish dairy herds pooled colostrum. The mean (and 95% prediction interval) total costs per calf with FPT were estimated to be €60 (€10–€109) and €80 (€20–€139) for dairy and beef, respectively (Raboison et al., 2016). Thus, economic losses due to FPT are significant to Irish farmers, and achieving APT in calves is an area of colostrum management that requires improvement. The national prevalence of FPT in dairy herds in the United States is reported to be 19.2% (Beam et al., 2009). Irish surveillance data reported FPT prevalences of 50, 46, 38, and 38% in 2013, 2014, 2015, and 2016, respectively, based on the zinc sulfate turbidity test on samples submitted voluntarily by veterinary practitioners (AFBI/DAFM, 2016). Although the zinc sulfate turbidity test is not considered the reference for assessing FPT in calves, and submissions were likely biased samples submitted by veterinarians due to suspicion of FPT, the data (AFBI/DAFM, 2016) reflect potential deficiencies in colostrum management practices on Irish dairy farms. Recent studies indicated that 49% of Irish calves were classified as having FPT when optimum globulin cut-off points of 29 to 34 g/L were used (Todd et al., 2018). Based on the high prevalence of pooling colostrum and FPT on Irish dairy farms, as well as the financial consequences of FPT, it is of practical interest to farmers to ensure that pooling colostrum does not contribute to FPT.

Although results from the present study indicated that pooling colostrum did not affect achievement of APT, these results should be interpreted with caution because the herd used in this study had relatively high median colostrum IgG concentrations, and all calves were fed colostrum within 2 h after birth. Consequently, pooling colostrum might be undesirable in herds with relatively lower colostrum IgG concentrations or when other factors that maximize acquisition of APT of immunity (such as time of feeding colostrum and volume fed) are not optimized. Less optimal feeding scenarios that affect effectiveness of pooling colostrum include feeding colostrum later in the 24-h optimal window of absorption (Mech et al., 2011), feeding colostrum contaminated with bacteria (Short et al., 2016), and



pooling colostrum with a very high IgG concentration with colostrum with a very low IgG (<25 g/L) concentration (Weaver et al., 2000). Pooling colostrum is not without risk because it has been demonstrated that pathogens such as *Mycobacterium avium* ssp. *paratuberculosis* (Stabel et al., 2014) and *Mycoplasma* (Sasaoka et al., 2015) can be transmitted in colostrum. Pasteurization of the colostrum was not performed in this study because it is not routine practice on Irish dairy farms. However, the cows were screened for *Mycobacterium avium* ssp. *paratuberculosis* and *Mycoplasma* before their colostrum was included in the study.

The main limitation of our study is that a single farm with high colostrum IgG concentrations and optimum colostrum feeding management was enrolled, whereas other factors that affect passive immunity of colostrum were similar among calves. Thus, the external validity of our results might be limited. Replicating the study across multiple, nonresearch commercial dairies to ensure applicability to the typical Irish dairy farm is recommended. However, it should be noted that to examine the influence of pooling alone on the achievement of APT, other variables that effect achievement of APT were controlled. A study to determine the national prevalence of FPT in Irish dairy herds is warranted. We also recommend determination of colostrum IgG concentration cut-off points for when to pool and not to pool colostrum.

In conclusion, pooling had no effect on colostrum IgG concentration or serum IgG concentration of calves, likely because of the high median colostrum IgG concentration. In herds where colostrum IgG concentrations are high, the influence of a dilution effect lowering IgG concentration when pooled is minimized. On dairies with an overall high median colostrum IgG and optimum colostrum management practices, pooling of colostrum is a feasible strategy to reduce the labor associated with calf rearing on farms.

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