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Effect of Freezing Colostrum on Resistance of Neonatal Lambs to Experimental Infection with *Escherichia coli*

K. R. MARTIN AND J. D. QUIGLEY III

Agricultural Experiment Station, University of Tennessee, P.O. Box 1071, Knoxville, TN 37901–1071, USA

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Effects of freezing and thawing colostrum on resistance of neonatal lambs to experimental infection with Escherichia coli *were evaluated using 16 newborn lambs. Eight sets of twins were fed colostrum from the ewe at 3.3 and 15.4 h of age. Colostrum was obtained from the ewe and divided into two equal portions. One portion was frozen in liquid nitrogen and then thawed in a water bath prior to feeding. The second portion was held at approximately 39°C in a water bath. Four sets of twins were orally inoculated with* 3×10^8 *to* 10^{11} *cfu of enterotoxigenic* E. coli *at 24 h of age. Blood was sampled at 0 and 24 h for IgG and differential leukocyte counts. Freezing and thawing reduced cell viability in colostrum from 43.1 to 10.1%. Neither freezing and thawing colostrum nor* E. coli *inoculation affected plasma IgG or total or differential leukocyte counts, fecal scores, respiration rates, rectal temperatures, fecal coliform excretion or intake. Shedding of K99+* E. coli *was increased and body weight gain from 7 to 14 d was decreased when lambs were inoculated with* E. coli. *Results of this study suggest that freezing and thawing colostrum does not destroy components that provide resistance to* E. coli *challenge in newborn lambs.*

Keywords: *Lambs, colostrum, leukocytes, immunity*

INTRODUCTION

Leukocytes in colostrum consist of lymphocytes, neutrophils, monocytes, eosinophils, and basophils (Lee & Outteridge, 1981; McDonald & Anderson, 1981a, b). These cells are immunologically active (Smith & Schultz, 1977; Norcross, 1982) and functionally intact (Riedel-Caspari & Schmidt, 1991a). Absorption may take place through intracellular spaces of the epithelial layer (Ogra *et al.*, 1977; Seelig & Billingham, 1981; Tuboly *et al.*, 1995) or through endocytosis (Sheldrake & Husband, 1985). Blood leukocyte concentrations and

Correspondence to: J. D. Quigley III. Present address: American Protein Corporation, 1 VisionAire Place, Suite 2, Ames, IA 50010, USA. Tel: +1 515 268 2573; Fax: +1 515 268 2660; E-mail:jim.quigley@amerprotcorp.com

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functions in newborn ruminants may be influenced by colostral leukocyte absorption (Sheldrake & Husband, 1985) or by cytokines produced by leukocytes which influence proliferation of neonatal leukocytes (Riedel-Caspari & Schmidt, 1991a). Leukocytes also synthesize lysozyme, complement factors (Pitt, 1979), and Ig (Gershwin *et al.*, 1985). Riedel-Caspari and Schmidt (1991b) found that when colostral cells were added to milk replacer of colostrum-deprived calves, these calves showed a neutrophil increase in response to natural infection, while animals that received only milk replacer did not. Colostral leukocytes may play a role in protecting the neonate from pathogens. Pitt *et al.*, (1977) challenged neonatal rats with *Klebsiella pneumoniae* and found that frozen and thawed milk was not successful in protecting the neonate from enterocolitis. However, when viable leukocytes were added to frozen milk or artificial formula, protection was restored. Morgan *et al.* (1984) determined that human colostral leukocytes provided neonatal protection against *Shigella sonnei*. Colostral leukocytes were able to destroy *Shigella sonnei* both intracellularly and extracellularly by ADCC and natural killer cytotoxicity. Riedel-Caspari (1993) inoculated calves with *E. coli* and fed them colostrum with leukocytes and leukocyte-depleted colostrum. Calves fed the cell depleted colostrum shed larger amounts of bacteria for longer periods of time than those fed colostrum with leukocytes. Serum of calves that received cell depleted colostrum had lower concentrations of IgM and IgA against *E. coli*.

The importance of humoral components of colostrum is well understood, however, less is known about the role of leukocytes in the protection of the neonate from pathogens. Therefore, the objective of this study was to investigate effects of viable colostral leukocytes on resistance of neonatal lambs to experimental infection with *E. coli*.

MATERIALS AND METHODS

Animal Assignments and Experimental Design

Eight sets of twin lambs which were offspring of polypay ewes and the same Suffolk ram were blocked by dam and assigned randomly to one of four treatments. Treatments consisted of fresh colostrum (FrE–), fresh colostrum with *E. coli* challenge (FrE+), frozen and thawed colostrum (ThE–), and frozen and thawed colostrum with *E. coli* challenge (ThE+). Fresh (Fr) or frozen/thawed (Th) colostrum was fed at 3.3 h and 15.4 h. At 24 h of age, four sets of twin lambs received K99+ *E. coli*.

Animal Management

Pregnant ewes were subjected to transrectal ultrasonography to determine the presence of twins between day 25 and 36 of gestation (Schrick & Inskeep, 1993). Ewes were brought to the holding facility one week prior to expected lambing. After birth, lambs remained with the ewe for a limited time to be cleaned and stimulated. Once standing, lambs were removed from the ewe before nursing. Lambs were weighed, identified, and navels were dipped with 7% iodine solution as soon as possible. Each set of twins was placed in individual pens divided with plywood boards to prevent contact between animals. Pens were cleaned and disinfected prior to use with a 50/50 solution of chlorine bleach and water. Tails were docked and males ($n = 6$) were castrated by 5 days of age. Males were equally divided among Fr and Th treatments. Feeding equipment was cleaned and sterilized after each feeding.

Feeding Management

Each ewe was milked by hand after birth. Colostrum was divided into two equal portions. One portion was kept warm, while the other portion was frozen in liquid nitrogen in 50 ml aliquots for 5 min. After removal from liquid nitrogen, colostrum was thawed in a 50°C waterbath. Cell viabilities of fresh and frozen/thawed colostrum were then determined by

hemacytometer count using trypan blue exclusion. The process was repeated 12 h later for the second colostrum feeding. Lambs were fed equal amounts of colostrum within block, simultaneously when possible. At 24 h of age and for the duration of the study, lambs were offered 300 g of commercial milk replacer (Ultra Fresh Lamb Milk Replacer, Land O'Lakes, Fort Dodge, IA, USA; 24.1% CP) twice daily. Commercial lamb feed (Co-op Lamb Grower/Finisher Coarse, Tennessee Farmers Cooperative, LaVergne, TN, USA) was offered for *ad libitum* consumption once daily from 3 days of age. Amount of milk replacer consumed was measured at each feeding and amount of lamb feed consumed was measured once daily. Water was available at all times.

E. coli Challenge

At 24 h of age, lambs receiving FrE + and ThE + treatments were challenged orally with 3 ml of *E. coli* suspension containing 10^8 to 10^{11} colony forming units (cfu) ml^{-1} of K99 + *E. coli* (09:K30:K99) obtained originally from the *E. coli* Reference Center (University Park, PA, USA). The challenge strain of *E. coli* was tested by the *E. coli* Reference Center (University Park, PA, USA) for STa and K99 genes by polymerase chain reaction. Presence of the K99 pili was verified using an *E. coli* antigen test kit (K99 Pilitest, VMRD, Pullman, WA, USA).

Sampling and Analysis

Samples of fresh colostrum were diluted 1:5 with bovine serum and slides were made for differential leukocyte counting. Slides were stained using Diff-Quik Stain Set (Baxter Healthcare Corporation, Miami, FL, USA), which is a modification of the Wright stain technique.

Colostrum was diluted 1:5 with phosphate-buffered saline (PBS) and samples were transported to the Tennessee DHIA lab to obtain total cell count. Samples of fresh and frozen/thawed colostrum were collected and cell viabilities were determined by hemacytometer count using trypan blue exclusion.

Blood was collected by jugular venipuncture into vacutainers containing 1.5% EDTA as soon as possible after birth (0 h) and 24 h after first sampling. Plasma was separated by centrifugation (3000 × *g*) and stored (–20°C) prior to analysis of IgG by radial immunodiffusion. An additional sample was collected and analyzed for total and differential leukocyte counts using a Cell-Dyn 3500 (Abbott Laboratories, Stone Mountain, GA, USA).

Feces were collected twice daily for the first 7 days of the study at the a.m. and p.m. feedings. Samples were composited daily for each lamb and analyzed for fecal dry matter (DM), total coliforms, and the presence of K99 + *E. coli*. Fecal DM was determined by drying approximately 1 g of feces in a 60°C drying oven for 48 h. Feces were cooled to room temperature and weighed again.

Total coliforms were determined by making a 1:10 dilution of feces in PBS (pH 7.2). The suspension was diluted serially. Dilutions (0.1 ml) were plated on MacConkey agar and plates were incubated overnight at 37°C to determine concentration of the challenge organism. Presence of K99 + *E. coli* was determined by making a 1:10 dilution of feces in nutrient broth and incubating overnight at 37°C. MacConkey plates were streaked for isolated colonies and incubated at 37°C overnight. Eight colonies from each MacConkey plate were streaked on E medium which was then incubated overnight at 37°C. Colonies were removed from the E medium and suspended in sterile saline. This suspension was tested for the presence of K99 pili using the K99 Pilitest (VMRD, Pullman, WA).

Fecal consistency was scored once daily at the a.m. feeding, following the method of Larson *et al.* (1977) by three different individuals during the study. Rectal temperatures and respiration rates were measured daily at the a.m. feeding. Body weights were determined at birth and on days 7 and 14.

Statistical Analysis

Plasma IgG at 24 h of age, body weight (BW), and BW gain were analyzed as a randomized complete block experimental design using the Proc GLM procedure of SAS (1994). Daily intake of starter and replacer, fecal scores, rectal temperatures, and respiration rates from days 1–14, fecal coliforms, K99+ shedding, and fecal DM from days 1–7 were analyzed as a completely randomized experimental design with repeated measures analysis of covariance using Proc Mixed procedure of SAS. Initial values of parameters or initial BW were used as covariables. Significance at $P < 0.05$ was used to determine treatment effects unless otherwise indicated.

RESULTS

E. coli Testing

Results from the *E. coli* Reference Center (University Park, PA, USA) indicated the presence STa enterotoxin and K99 genes. The K99 Pilitest confirmed the production of K99 pili by the challenge organism when cultured *in vitro*.

Morbidity and Mortality

All lambs were healthy throughout the study and no mortality was recorded. Lambs consumed 256 ml of colostrum by 15.4 h of age. Fresh colostrum provided 1.9×10^6 leukocytes ml^{-1} with 43.1% viability, while frozen/thawed colostrum provided 1.9×10^6 leukocytes ml^{-18} with 10.1% viability. Freezing and thawing colostrum reduced cell viability ($P < 0.005$), although a small number of leukocytes survived freezing. Colostral leukocytes consisted of 75.4% neutrophils, 8.3% lymphocytes and 16.3% macrophages (Table 1).

Fecal scores, rectal temperatures, and respiration rates were similar for all treatments (Table 2). No severe scouring occurred during the experiment for all treatments and mean daily fecal scores were consistently ≤ 2. Mean rectal temperatures ranged from 39.3 to 39.5°C, which is considered normal for sheep (Parker, 1983). Rectal temperatures gradually increased from day 6 to 14. Respiration rates decreased with age of lamb ($P < 0.001$) but were unaffected by treatment.

Mean fecal DM ranged from 36.1 to 38.9% (Table 2) and was unaffected by treatment. Fecal DM decreased for lambs on ThE+ treatment at day 2, possibly due to *E. coli* challenge (Figure 1). However, fecal DM increased or did not change for lambs on other treatments on day 2. Fecal DM then increased on day 3 for lambs on all treatments except ThE-. Fecal DM decreased for other treatments from day 3 to 6.

TABLE 1. Amount and quality of colostrum fed to lambs

Block	ml fed	Cells ml^{-1}	Cell viability (%)		Differential count (%)			IgG ($g\mu l^{-1}$)
			Fresh	Frozen	Lymph.	Neuro.	Macro.	
1	250	1.3×10^6	20	6	10.0	60.0	30.0	302.0
2	180	1.1×10^5	43	12	8.5	81.0	10.5	70.9
3	220	8.5×10^5	48	11	13.7	79.0	7.3	120.8
4	250	3.9×10^5	52	16	5.0	82.0	13.0	123.9
5	195	1.2×10^6	63	4	6.5	82.5	11.0	82.1
6	280	4.3×10^5	43	7	10.5	63.5	26.0	83.8
7	170	4.3×10^5	31	7	6.3	69.9	23.8	149.1
8	305	2.5×10^7	60	6	6.0	85.5	8.5	164.3

TABLE 2. Least squares means of fecal DM, total coliforms, K99 shedding, fecal scores, rectal temperatures, and respiration rates in experimental lambs

Item[c]	Treatment[a]				SE	Contrasts[b]		
	FrE–	FrE+	ThE–	ThE+		1	2	3
Fecal DM (%)	37.3	36.1	38.9	36.2	2.6	NS[d]	NS	NS
Total coliforms ($\log_{10}$ cfu g^{-1})	9.24	9.08	8.87	9.37	0.75	NS	NS	NS
Fecal K99+ (% of lambs shedding)	27.5	71.5	28.5	56.8	9.8	NS	0.007	NS
Fecal score[e]	1.43	1.50	1.61	1.32	0.20	NS	NS	NS
Rectal temp. (°C)	39.2	39.5	39.3	39.4	0.2	NS	NS	NS
Respiration per min.	42.0	41.2	41.6	42.1	2.5	NS	NS	NS

[a] FrE– = lambs that received fresh colostrum; FrE+ = lambs that received fresh colostrum and *E. coli*; ThE– = lambs that received frozen/thawed colostrum; ThE+ = lambs that received frozen/thawed colostrum and *E. coli*.
[b] Contrasts: 1 = fresh vs frozen/thawed colostrum; 2 = *E. coli* vs no *E. coli*; 3 = interaction.
[d] $P > 0.10$.
[e] Feces were scored on a scale of 1 = normal, 2 = mild scours, 3 = moderate scours, and 4 = severe scours.

Total coliforms in feces were unaffected by treatment but increased from day 1. Total coliforms in feces of lambs on ThE-treatment increased from 7.6 to 10.2 $\log_{10}$ cfu g^{-1} from day 2 to 3 and then decreased to 8.5 $\log_{10}$ cfu g^{-1} on day 5. Coliform excretion in feces of lambs on treatment ThE+ increased from 8.2 to 10.5 $\log_{10}$ cfu g^{-1} from day 1 to 2 and decreased to 8.8 $\log_{10}$ cfu g^{-1} on day 6. Coliform excretion in feces from lambs on the FrE-treatment group increased from 8.4 to 11 $\log_{10}$ cfu g^{-1} from day 1 to 3, then decreased to 8.4 $\log_{10}$ cfu g^{-1} on day 5. Total coliforms increased from 8.2 to 10.2 $\log_{10}$ cfu g^{-1} for lambs on the FrE+ treatment from day 1 to 4. Total coliforms for all lambs were similar from day 5 to 7.

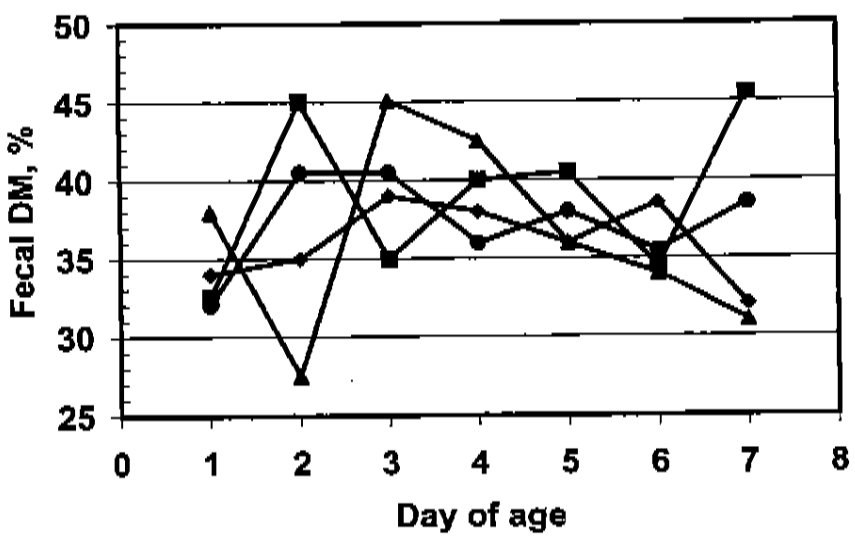

FIG. 1. Least squares means of fecal DM in lambs fed fresh colostrum and not challenged with *E. coli* (●), lambs fed fresh colostrum and challenged with *E. coli* (◆), lambs fed frozen and thawed colostrum and not challenged with *E. coli* (■), and lambs fed frozen and thawed colostrum and challenged with *E. coli* (▲).

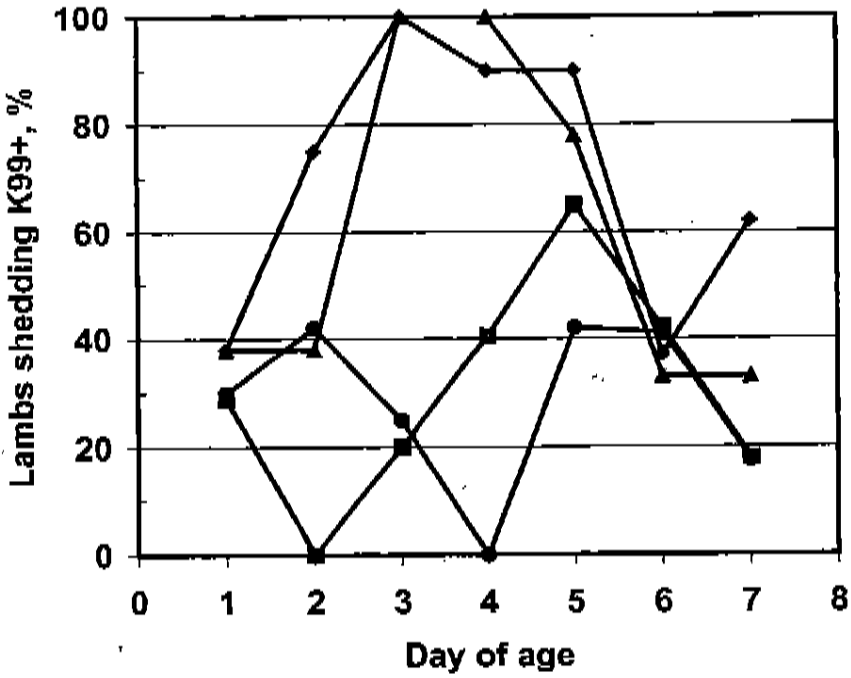

FIG. 2. Least squares means of proportion of lambs shedding K99+ *E. coli* in feces when fed fresh colostrum and not challenged with *E. coli* (●), lambs fed fresh colostrum and challenged with *E. coli* (◆), lambs fed frozen and thawed colostrum and not challenged with *E. coli* (■), and lambs fed frozen thawed and thawed colostrum and challenged with *E. coli* (▲).

The proportion of lambs shedding K99+ *E. coli* was greater when lambs were challenged with *E. coli* ($P < 0.007$; Figure 2). By 3 days of age all challenged lambs shed K99+ *E. coli*. At least one lamb on all treatments shed K99+ *E. coli* on day 1, suggesting that K99+ *E. coli* may have been present in the flock and acquired by lambs shortly after birth. Lambs were born naturally and allowed contact with the dam for a limited amount of time.

Plasma IgG and Leukocyte Counts

Plasma IgG increased from undetectable at birth to 21.0 gl^{-1} at 24 h of age (Table 3). There was no effect of freezing on plasma IgG concentration at 24 h. Total number of leukocytes also increased with colostrum intake from 2971 cells ml^{-1} at 0 h to 3874 cells ml^{-1} at 24 h of age. Freezing colostrum had no effect on the absorption of leukocytes or on the differential counts at 24 h of age (Table 3).

Body Weight, Intake and Feed Efficiency

Lambs challenged with *E. coli* had lower initial BW (Table 4); therefore, performance and intake data were adjusted by analysis of covariance for initial BW. Body weights at 7 and 14 days were unaffected by treatment; however, BW gain from 7 to 14 days was reduced by 24% when lambs were challenged with *E. coli*. Reduced BW gain occurred irrespective of leukocyte viability. Intake of DM and milk replacer were unaffected by treatment; mean intakes of total DM and milk replacer were 164 and 157 g day^{-1}, respectively. Both total DM and milk replacer intakes increased with increasing age ($P < 0.01$). Lambs challenged with *E. coli* had higher starter intakes than lambs not challenged; however, starter intake was low

TABLE 3. Plasma IgG, total leukocyte and differential counts in lambs fed fresh or frozen colostrum

Item	Fresh	Frozen/Thawed	SE	P[a]
Day 0				
Plasma IgG (gl^{-1})	0.0	0.0	0.0	NS
Total leukocytes (cells ml^{-1})	2391.0	2550.0	171.0	NS
Lymphocytes (%)	39.7	38.9	8.2	NS
Neutrophils (%)	57.3	57.6	6.7	NS
Monocytes (%)	1.5	3.3	0.8	NS
Day 1				
Plasma IgG (gl^{-1})	20.9	21.1	1.5	NS
Total leukocytes (cells ml^{-1})	3861.0	3726.0	513.0	NS
Lymphocytes (%)	22.3	23.8	4.1	NS
Neutrophils (%)	75.9	74.2	1.1	NS
Monocytes (%)	1.5	1.4	0.8	NS

[a] Probability of a significant treatment effect; NS = $P > 0.10$.

throughout the study (< 20 g day^{-1}) and many lambs spilled starter, resulting in numerous missing observations. Efficiency of utilization of DM intake for BW gain was numerically reduced in lambs challenged with *E. coli*; however, differences were not statistically significant.

TABLE 4. Least squares means of BW, average daily BW gain, intake, and feed efficiency in experimental lambs

	Treatment[a]					Contrasts[b]		
Item[c]	FrE–	FrE+	ThE–	ThE+	SE	1	2	3
BW (kg)								
Birth	3.7	2.9	3.7	3.3	0.3	NS[d]	0.006	NS
7 d	4.0	4.1	4.0	4.1	0.1	NS	NS	NS
14 d	4.8	4.7	4.8	4.8	0.1	NS	NS	NS
BW gain (g day^{-1})								
0 to 7 d	82	97	90	106	13	NS	NS	NS
7 to 14 d	113	85	114	88	8	NS	0.009	NS
0 to 14 d	98	91	102	97	9	NS	NS	NS
DM intake (g day^{-1})								
Total	164	164	161	167	4	NS	NS	NS
Milk replacer	158	154	158	159	3	NS	NS	NS
Lamb starter	5	10	3	7	2	NS	0.03	NS
BW gain:DM intake (g kg^{-1})	598	558	634	585	52	NS	NS	NS

[a] FrE– = lambs that received fresh colostrum; FrE+ = lambs that received fresh colostrum and *E. coli*; ThE– = lambs that received frozen/thawed colostrum; ThE+ = lambs that received frozen/thawed colostrum and *E. coli*.
[b] Contrasts: 1 = fresh vs frozen/thawed colostrum; 2 = *E. coli* vs no *E. coli*; 3 = interaction.
[c] Means were covariately adjusted for BW at birth, except birth BW.
[d] $P > 0.10$.

DISCUSSION

Lack of clinical signs (fever, scours, increased respiration rate) in lambs challenged with *E. coli* may be attributed to adequate transfer of passive immunity by colostrum consumption irrespective of leukocyte viability. However, shedding of *E. coli* and BW gain were depressed when lambs were challenged, suggesting a deleterious effect of the challenge organism on the lambs. Mean plasma IgG concentrations in lambs at day 1 was 21.0 gl^{-1}, indicating adequate transfer of passive immunity. Others (Morgan *et al.*, 1978; Sojka *et al.*, 1978; Gregory *et al.*, 1983; Valente *et al.*, 1988) have reported that neonatal piglets, lambs, and calves challenged with *E. coli* did not exhibit clinical signs when fed colostrum, particularly when colostrum was obtained from dams vaccinated against K99+ *E. coli*. Wray *et al.* (1984) orally challenged colostrum fed lambs with B44 (O9:K–:K99) and S13 (O8:K85:K99) *E. coli* that tested positive for heat-stable enterotoxin production. Two of four lambs challenged with B44 remained healthy while two of three lambs challenged with S13 remained healthy. Wray *et al.* (1989) challenged calves with 10^{10} verocytotoxic *E. coli*. Three of nine calves (two colostrum-fed, one colostrum-deprived) passed normal feces, although the challenge organism was recovered in the feces of all animals. Duchet-Suchaux *et al.* (1982) orally challenged colostrum deprived Berrichons and Prealpes lambs with B41 (O101: K99:H–) *E. coli*. Heat-stable toxin production was confirmed by infant mouse assay and presence of K99 pili was confirmed by slide agglutination with a monovalent antiserum. A nonpathogenic strain (C12) was given to controls. Three of 20 lambs challenged with 1.7×10^8 to 3.1×10^8 B41 did not develop diarrhea. With these data and other data from the study, they determined that differences in susceptibility may depend upon strain of *E. coli* and breed of animal. Logan *et al.* (1974) challenged neonatal calves with a preparation of *E. coli* at 24 h of age. Calves fed colostrum remained healthy, whereas calves fed colostral preparations containing individual Ig survived but developed severe diarrhea and dehydration. All colostrum-deprived calves challenged with *E. coli* died. Gregory *et al.* (1983) reported that vaccination of pregnant ewes with multiple strain enterotoxigenic *E. coli* protected 193 of 216 lambs against *E. coli* exposure when lambs were allowed access to the ewe to consume colostrum. Only 4 of 216 lambs (1.9%) died after colostrum consumption. Data from the present study suggest that protection provided by colostrum intake is not degraded by freezing and thawing. Protection to oral challenge was probably mediated primarily by IgG; reduction in the number of viable leukocytes consumed appeared to have little effect.

Absorption of colostral IgG (as indicated by serum IgG concentration) was unaffected by freezing (Table 3). Besser *et al.* (1991) reported that calves fed frozen colostrum were at higher risk of failure of passive transfer than calves fed fresh colostrum. However, the authors were unable to determine if freezing colostrum was associated with loss of IgG because all samples in the study were frozen prior to analysis.

Total leukocyte counts at 0 h and 24 h were unaffected by treatment (Table 3). Cell counts increased in all animals after colostrum intake. Lombardo *et al.* (1979) and Clover and Zarkhower (1980) reported that calves fed frozen colostrum had elevated numbers of total leukocytes at 12 and 24 h compared to colostrum deprived calves. The proportion of neutrophils increased with colostrum ingestion, while the proportion of lymphocytes changed only slightly. Riedel-Caspari and Schmidt (1991b) demonstrated increased numbers of PMN at 2 days of age in calves fed cell-free colostrum.

Freezing colostrum reduced viability from 43.1 to 10.1%. We found it impossible to completely kill leukocytes without negatively affecting nutrient quality or delay feeding for an extended period. First feeding of colostrum occurred at 3.4 h of age; our goal was to feed all lambs by 4 h of age. Remaining viable leukocytes may have played a role in supporting the immune response; however, in most cases, the contribution of viable leukocytes was small. Also, it is possible that effects due to freezing and thawing may have been masked by microbial contamination of fresh colostrum during incubation and prior to feeding. Colostrum was not collected aseptically; therefore, some microbial contamination was likely.

Colostral leukocytes may have lost immunologic function due to contact with microorganisms or cellular debris.

CONCLUSIONS

Lambs challenged with *E. coli* shed more K99 *E. coli* than lambs that did not and BW gain was transiently reduced after challenge. However, other indicators of health showed no effect of treatment. This could be due to lambs receiving colostrum prior to the oral challenge. Frozen and thawed colostrum with reduced cell viabilities provided neonatal lambs with sufficient immunity to withstand an experimental *E. coli* challenge. Colostrum consumed contained normal IgG concentrations, therefore, colostrum provided lambs with sufficient passive immunity. Further study is required to determine effects of prolonged freezing of colostrum and acquisition of passive immunity and effects of colostral leukocytes on the development of the neonatal immune system.

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REFERENCES

Besser, T. E., Gay, C. C. & Pritchett, L. (1991) Comparison of three methods of feeding colostrum to dairy calves, *Journal of the American Veterinary Medical Association*, **198**, 419–422.

Clover, C. K. & Zarkhower, A. (1980) Immunologic responses in colostrum-fed and colostrum-deprived calves, *American Journal of Veterinary Research*, **41**, 1002–1007.

Duchet-Suchaux, M., Bertin, A., De Rycke, J. & Lechopier, P. (1982) Experimental *Escherichia coli* diarrhoea in colostrum deprived lambs, *Annals of Veterinary Research*, **13**, 259–266.

Gershwin, M. E., Beach, R. S. & Hurley, L. S. (1985) Immunological considerations of breast milk, in *Nutrition and Immunity*. Academic Press. Orlando, p. 285.

Gregory, D. W., Cardella, M. A. & Myers, L. L. (1983) Lamb model in the study of immunity to enteropathogenic *Escherichia coli* infections, *American Journal of Veterinary Research*, **44**, 2073–2077.

Larson, L. L., Owen, F. G., Albright, J. L., Appleman, R. D., Lamb, R. C. & Muller, L. D. (1977) Guidelines toward more uniformity in measuring and reporting calf experimental data, *Journal of Dairy Science*, **60**, 989–991.

Lee, C. S. & Outteridge, P. M. (1981) Leukocytes of sheep colostrum. milk, and involution secretion, with particular reference to ultrastructure and lymphocyte subpopulations, *Journal of Dairy Research*, **48**, 225–237.

Logan, E. F., Stenhouse, A., Ormrod, D. J. & Penhale, W. J. (1974) The role of colostral immunoglobulins in intestinal immunity to enteric colibacillosis in the calf, *Research in Veterinary Science*, **17**, 280–301.

Lombardo, P. S., Todhunter, D. A., Scholz, R. W. & Eberhart, R. A. (1979) Effect of colostral ingestion on indices of neutrophil phagocytosis and metabolism in newborn calves, *American Journal of Veterinary Research*, **30**, 362–368.

McDonald, J. S. & Anderson, A. J. (1981a) Total and differential somatic cell counts in secretions from noninfected bovine mammary glands: The early nonlactating period, *American Journal of Veterinary Research*, **42**, 1360–1365.

McDonald, J. S. & Anderson, A. J. (1981b) Total and differential somatic cell counts in secretions from noninfected bovine mammary glands: The periparum period, *American Journal of Veterinary Research*, **42**, 1366–1368.

Morgan, D. L., DuPont, H. L., Gonik, B. & Kohl, S. (1984) Cytotoxcity of human peripheral blood and colostral leukocytes against *Shigella* species, *Infection and Immunity*, **46**, 25–33.

Morgan, R. L., Isaacson, R. E., Moon, H. W., Brinton, C. C. & To, C. C. (1978) Immunization of suckling pigs against enterotoxigenic *Escherichia coli* induced diarrheal disease by vaccinating dams with purified 987 or K99 pili; protection with pilus homology of vaccine and challenge, *Infection and Immunity*, **22**, 771–777.

Norcross, N. L. (1982) Secretion and composition of colostrum and milk, *Journal of the American Veterinary Association*, **18**, 1057–1060.

OGRA, S. S., WEINTRAUB, D. & OGRA, P. L. (1977) Immunologic aspects of human colostrum milk. III. Fate and absorption of cellular and soluble components in the gastrointestinal tract of newborn, *Journal of Immunology*, **119**, 245–248.

PARKER, R. (1983) *The Sheep Book: A Handbook for the Modern Shepherd*. Scribner, NY.

PITT, J. (1979) The milk mononuclear phagocyte, *Pediatrics*, 64 (Suppl), 745–749.

PITT, J., BARLOW, B. & HEIRD, W. C. (1977) Protection against experimental necrotizing enterocolitis by maternal milk. I. Role of milk leukocytes, *Pediatric Research*, **11**, 906–909.

RIEDEL-CASPARI, G. (1993) The influence of colostral leukocytes on the course of an experimental *Escherichia coli* infection and serum antibodies in neonatal calves, *Veterinary Immunology and Immunopathology*, **35**, 275–288.

RIEDEL-CASPARI, G. & SCHMIDT, F. W. (1991a) The influence of colostral leukocytes on the immune system of the neonatal calf. I. Effects on lymphocyte responses, *Deutsche Tierarztliche Wochenschrift*, **98**, 102–107.

RIEDEL-CASPARI, G. & SCHMIDT, F. W. (1991b) The influence of colostral leukocytes on the immune system of the neonatal calf. III. Effects of phagocytosis, *Deutsche Tierarztliche Wochenschrift*, **98**, 330–334.

SAS (1994) *SAS User's Guide*. SAS Inst., Cary, NC.

SCHRICK, F. N. & INSKEEP, E. K. (1993) Determination of early pregnancy in ewes utilizing transrectal ultrasonography, *Theriogenology*, **40**, 295–299.

SEELIG, L. L. & BILLINGHAM, R. E. (1981) Capacity of 'transplanted' lymphocytes to traverse the intestinal epithelium of adult rats, *Transplantation*, **32**, 308–314.

SHELDRAKE, R. F. & HUSBAND, A. J. (1985) Intestinal uptake of intact maternal lymphocytes by neonatal rats and lambs, *Research in Veterinary Science*, **39**, 10–15.

SMITH, J. W. & SCHULTZ, T. S. (1977) Mitogen and antigen-responsive milk lymphocytes, *Cellular Immunology*, **29**, 165–173.

SOJKA, W. J., WRAY, C. & MORRIS, J. A. (1978) Passive protection of lambs against experimental enteric colibacillosis by colostrum transfer of antibodies from K99 vaccinated ewes, *Journal of Medical Microbiology*, **11**, 498–499.

TUBOLY, S., BERNATH, S., GLAVITS, R., KOVACS, A. & MEGYERI, Z. (1995) Intestinal absorption of colostral lymphocytes in newborn lambs and their role in the development of immune status, *Acta Veterinaria Hungarica*, **43**, 105–115.

VALENTE, C., FRUGANTI, G., TESSI, B., CIORBA, A., CARDARAS, P., FLORIS, A. & BORDONI, E. (1988) Vaccination of pregnant cows with K99 antigen of enterotoxigenic *Escherichia coli* and protection by colostrum in newborn calves, *Comparative Immunology, Microbiology and Infectious Diseases*, **11**, 189–198.

WRAY, C., DAWSON, M., APSHAR, A. & SOJKA, W. J. (1984) Experimental diarrhoea in lambs, *Journal of Veterinary Medicine, Series B*, **31**, 381–390.

WRAY, C., MCLAREN, I. & PEARSON, G. R. (1989) Occurrence of 'attaching and effacing' lesions in the small intestine of calves experimentally infected with bovine verocytotoxic *E. coli*, *Veterinary Record*, **125**, 365–368.