Effects of Housing and Colostrum Feeding on the Prevalence of Selected Infectious Organisms in Feces of Jersey Calves[1]

J. D. QUIGLEY, III,[1,2] and K. R. MARTIN
Institute of Agriculture
Department of Animal Science
University of Tennessee
Knoxville 37901-1071

D. A. BEMIS, L.N.D. POTGIETER, C. R. REINEMEYER, and B. W. ROHRBACH
Department of Comparative Medicine
College of Veterinary Medicine
University of Tennessee
Knoxville 37901-1071

H. H. DOWLEN and K. C. LAMAR
Dairy Experiment Station
USDA-ARS
Lewisburg, TN 37091

ABSTRACT

Neonatal Jersey calves (n = 96) were used to evaluate effects of housing (individual hutches or wooden pens in a barn) and colostrum feeding (calves were separated from the dam and fed 2 L of colostrum in nipple-bottles or allowed to nurse the dam for 3 d) on the prevalence of selected organisms in feces. Prevalence of *Cryptosporidium* and *Eimeria* were reduced, and prevalence of rotavirus tended to be reduced, when calves were housed in hutches. Prevalence of coronavirus was unaffected by treatment. Weekly prevalence of *Giardia* was increased when calves were left to nurse the dam for 3 d. Mean prevalence of *Cryptosporidia* (wk 1 to 4), *Eimeria* (wk 4 to 5), *Giardia*, rotavirus, and coronavirus (wk 1 to 5) were 34.7, 20.6, 27.1, 15.8, and 4.9%, respectively. *Escherichia coli* (K99 positive) were observed in 3 of 174 samples cultured. Methods of housing and colostrum feeding affected acquisition of enteropathogens in this study.

(**Key words**: colostrum, housing, Jersey)

INTRODUCTION

Dairy calf morbidity and mortality are significant losses associated with the dairy enterprise. A recent US dairy heifer management study (3) reported that mean mortality of preweaned calves was 8.4% (SE = .4); over 52% of fatalities were associated with scours. Two factors that are related to mortality and morbidity are colostrum feeding and housing.

The importance of consumption of an adequate amount of colostrum on morbidity and mortality is widely recognized (6). However, less clear are effects of the method of feeding colostrum (allowing the calf to suckle the dam or bottle feeding a known amount of previously collected colostrum) on the temporal acquisition of enteropathogenic organisms. Calves left with the dam for 72 h may be exposed to greater numbers of infectious organisms associated with the dam and environment, thereby increasing the risk of disease.

Housing may affect exposure of calves to infectious organisms and subsequent morbidity and mortality (5, 9, 20, 21). Waltner-Toews et al. (20) indicated that calves raised in hutches were 25 times less likely to be treated for pneumonia and 8 times less likely to be treated for scours than were calves raised in individual pens in a calf barn. Conversely, Martin et al. (12) and James et al. (8) reported that housing did not predispose calves to increased morbidity or mortality, but the person caring for

Received December 2, 1993.
Accepted April 15, 1994.
[1]Supported in part by the American Jersey Cattle Club, 6486 East Main Street, Reynoldsburg, OH 43068-2362.
[2]Reprint requests.

the calf and the overall level of calf management were more important factors influencing morbidity and mortality.

The objectives of this study were to determine the effects of housing and method of colostrum feeding on the prevalence of selected infectious organisms in neonatal Jersey calves.

MATERIALS AND METHODS

Experimental Design

Ninety-six Jersey heifer (n = 48) and bull calves were blocked by sex and date of birth and assigned to one of four treatments in a randomized complete block design. Treatments were a 2 × 2 factorial arrangement of colostrum feeding (calves were allowed to nurse the dam for 3 d or were separated from the dam and fed 1 L of colostrum in nipple-bottles at 0 and 12 h after birth) and housing (individual fiberglass hutches or individual pens in an unheated calf barn).

Cows were housed in a drylot until approximately 2 d prior to parturition, when they were moved to an individual calving pen bedded with straw. Cows were not immunized with viral or bacterial vaccines during the dry period. Calves fed colostrum from nipple-bottles were moved to the hutch or barn before nursing. The unheated calf barn contained 40 individual wooden pens (1.2 × 2.4 m) and housed calves prior to initiation of the study. Mechanical ventilation was provided in the barn throughout the study. Calf hutches were located in an area not previously exposed to calves. Pens and hutches were thoroughly washed, disinfected, and allowed to dry prior to each use. Hutches were moved to a new location after each use. Calf pens and hutches were bedded with sawdust and straw.

Calf Management

Calves fed by nipple-bottle were fed colostrum from the first milking, obtained from the dam shortly after parturition. Each cow was milked by hand, and a 50-ml sample of colostrum was obtained and stored (–20°C). Colostral composition has been reported (15). Calves were fed 1 L of colostrum as soon as possible after birth and again 12 h later. After the second feeding, calves were fed twice daily to 3 d of age using second and third milking colostrum from the dam.

Calves that nursed the dam were left with the dam until 3 d of age. Calves were assisted if nursing did not commence by 4 h. No attempt was made to measure quantitatively the colostrum consumed by nursed calves.

Commercial calf starter (Tennessee Farmers Cooperative, LaVergne, TN) was offered for ad libitum consumption from d 3. Calf starter was formulated to contain a coccidiostat (Deccox®; Rhône-Poulenc, Atlanta, GA) at 25 mg/kg. Commercial milk replacer (Land O'Lakes, Inc., Ft. Dodge, IA) was reconstituted to 12% DM and fed twice daily (.95 L per feeding) from 3 to 35 d of age. Milk replacer was formulated to contain oxytetracycline (138 mg/kg) and neomycin base (250 mg/kg). Refused milk replacer and calf starter were weighed and reported daily. Water was available at all times. Second-cutting alfalfa hay was offered for ad libitum consumption after 14 d. Calves were weighed at birth and every 7 d thereafter to 35 d.

Sampling and Analyses

Fecal grab samples were collected from all calves every Tuesday and Friday during the study. Samples were divided into approximately equal portions, placed into two plastic sample cups, and stored at 3 or –20°C. Occasionally, no feces could be collected from calves. When insufficient quantities of feces were collected for two samples, samples were refrigerated only. Refrigerated samples were shipped to the laboratory to determine the presence of *Cryptosporidium*, *Giardia*, and *Eimeria*. Qualitative fecal examinations were performed with a modified flotation technique by the method of Cox and Todd (4), except that 1 g of feces was used. Samples were examined microscopically at 400×. Frozen samples were shipped to the laboratory, thawed, pooled within week of age, and refrozen prior to analysis for rotavirus and coronavirus by direct electron microscopy. A

10% suspension of feces was made in distilled water and clarified by centrifugation (27,000 × *g* for 40 min). The supernatant was decanted, and the pellet was resuspended in 2 to 3 ml of distilled water. Approximately 100 μl of the suspension was added to 1 ml of distilled water. One hundred microliters of 3% phosphotungstic acid were added to the sample for negative staining. Stained samples were sprayed onto copper grids coated with Formvar™ (Monsanto Co., St. Louis, MO) and carbon and scanned with an electron microscope (Philips 201 transmission electron microscope; Philips Electronic Co., Mahwah, NJ) at a plate magnification of 30,000 × with an 80-kV beam. Morphological identification of virus particles was made by examination at 300,000×.

Prevalence of enterotoxigenic *Escherichia coli* was determined using a commercial latex agglutination test for K99+ pili (K99 pilitest; VMRD, Inc., Pullman, WA) on eight colonies isolated from weekly samples after preenrichment in nutrient broth for 24 h.

Approximately 10 ml of jugular blood were taken at 24 h after birth and allowed to clot. Serum was separated by centrifugation (3000 × *g*) and frozen (–20°C) until analysis for IgG and IgM by radial immunodiffusion (VMRD, Inc.).

Statistical Analyses

Weekly prevalence of infectious organisms was analyzed as a randomized complete block design in a repeated measures analysis of covariance using a general linear mixed models algorithm (2). Body weight at birth was used as a covariable. Terms in the model were block, treatment, block × treatment, week of age, week × treatment, and error. Block × treatment was used as the error term to test effects of treatment, and error was used to test week and week × treatment. Significance was determined at $P < .05$ unless otherwise noted.

RESULTS AND DISCUSSION

Seven calves died, and 4 calves were stillborn during the experiment (Table 1). Stillborn calves and 1 calf that died at 3 d of age were replaced; therefore, a total of 90 calves completed the study. Calculated mortality of all calves during the experiment was 10.9% and, of calves born alive, 7.2%. Mortality of 7.2% is less than mortality reported in a recent survey of calf mortality in herds across the US (3). Of the 7 calves that died after assignment to treatment, 5 calves were fed colostrum by nipple-bottle. Serum IgG and IgM concentrations at 24 h were generally low in calves that

TABLE 1. Mortality of Jersey calves.

Calf	Sex	Treatment and housing	Birth	Death	Age	Reason	Serum IgG[1]	Serum IgM[1]
					(d)		(g/L)	
1	F	Nursed + hutch	9/06/92	10/06/92	30	Scours	65.1	4.8
8[2]	F	. . .	9/12/92	9/12/92	0	Stillborn	. . .	. . .
14	F	Fed + hutch	9/20/92	10/08/92	18	Scours	12.9	3.3
20	F	Fed + hutch	10/07/92	10/28/92	21	Scours	22.1	2.1
700[2,3]	M	. . .	9/12/92	9/12/92	0	Stillborn	. . .	. . .
701[2,3]	M	. . .	9/12/92	9/12/92	0	Stillborn	. . .	. . .
712[2]	M	. . .	8/04/92	8/04/92	0	Stillborn	. . .	. . .
715[2]	M	Nursed + barn	8/07/92	8/10/92	3	Septicemia	3.3	1.8
751	M	Fed + barn	9/28/92	10/08/92	10	Scours[4]	7.0	1.8
761	M	Fed + hutch	10/12/92	10/30/92	18	Pneumonia[5]	15.0	1.1
996	F	Fed + barn	8/31/92	9/14/92	14	Scours[6]	36.5	2.9

[1]At 24 h after birth.

[2]Calf was replaced.

[3]Twins.

[4]Fecal sample was positive for *Cryptosporidium* 2 d prior to death.

[5]Fecal sample was positive for *Cryptosporidium* and *Giardia* 1 d prior to death.

[6]Fecal sample was positive for *Cryptosporidium* 1 d prior to death and rotavirus 3 d prior to death.

died, although serum IgG exceeded 30 g/L in 2 calves (Table 1).

Cryptosporidium was shed by 86 calves during the study and was found in 28% of all samples collected. This prevalence (96% of calves) is higher than other reports of prevalence in young calves (10, 13) but similar to a recent survey of dairy herds throughout the US (3). Samples were positive for *Cryptosporidium* during wk 1 to 4 only. When pooled across wk 1 to 4, prevalence of *Cryptosporidium* oocysts in feces of calves housed in the barn was greater (Table 2), and a significant interaction of housing × colostrum feeding indicated a greater prevalence in feces of calves housed in the barn and fed colostrum in nipple-bottles. Most shedding occurred during the first 3 wk of life. An interaction of period × treatment was significant ($P < .01$) for the prevalence of *Cryptosporidium* (Figure 1a). At 1 wk of age, calves that nursed the dam had a higher prevalence of *Cryptosporidium* infection than calves removed from the dam and fed colostrum by nipple-bottle. This effect was probably due to earlier exposure of calves to *Cryptosporidium* from the dam and surrounding environment. The lack of difference between calves housed in the barn or in individual hutches at 1 wk of age suggests that the major source of inoculation was the dam in the 1st wk of life. At 2 wk of age, most calves (n = 68, 76%) shed oocysts; however, shedding was greatest from calves fed colostrum by nipple-bottle and housed in the barn. Shedding of oocysts during wk 3 was greatest when calves were housed in the barn, suggesting that infections later in life were associated with exposure in the barn.

Lopez et al. (10) reported that most fecal shedding of *Cryptosporidium* occurred during the first 24 d of life and was highly related to serum IgG concentrations in calves at 24 h of age. Stepwise multiple regression indicated that age was the primary factor affecting prevalence of *Cryptosporidium* in our study. Other significant independent variables included housing, mean daily BW gain, and the prevalence of *Giardia*. However, neither serum IgG nor IgM was significant in predicting prevalence of *Cryptosporidium*, suggesting that level of transfer of passive immunity did not affect level of infection in this study, in agreement with other reports (7, 19) but in contrast with data of Lopez et al. (10). Prevalence of *Giardia* was negatively associated with shed-

TABLE 2. Prevalence of selected infectious organisms in fecal samples of Jersey calves fed colostrum by nipple-bottle or allowed to nurse the dam and housed in individual hutches or pens in a calf barn.

Item	Treatment				SE	Contrasts[1]		
	Bottle + barn	Bottle + hutch	Nursed + barn	Nursed + hutch		1	2	3
	(% of samples positive)							
Cryptosporidium[2,3]	41.8	25.3	37.4	33.2	3.1	NS[4]	**	*
Giardia[5]	26.4	23.1	29.9	29.5	3.9	*	NS	NS
Eimeria[2,6]	32.4	6.6	32.3	10.3	5.9	NS	**	NS
Rotavirus	19.6	13.2	17.0	12.4	3.3	NS	†	NS
Coronavirus[5]	6.4	7.1	1.8	4.4	2.9	NS	NS	NS

[1]Contrasts: 1 = effect of colostrum feeding, 2 = effect of housing, and 3 = colostrum feeding × housing interaction.

[2]Significant age × treatment interaction ($P < .01$).

[3]Means for wk 1 to 4.

[4]$P > .10$.

[5]Significant effect of age.

[6]Means for wk 4 and 5.

†$P < .10$.

*$P < .05$.

**$P < .001$.

ding of *Cryptosporidium*, possibly because of differences in temporal acquisition of the organisms. *Cryptosporidium* is considered to be an opportunistic organism that does not cause significant morbidity unless it is associated with another agent (14). No other organism measured in this study was associated with prevalence of *Cryptosporidium*, indicating that it was a primary pathogen. Only 25.4% of samples positive for *Cryptosporidium* were positive for other organisms (primarily rotavirus and *Giardia*).

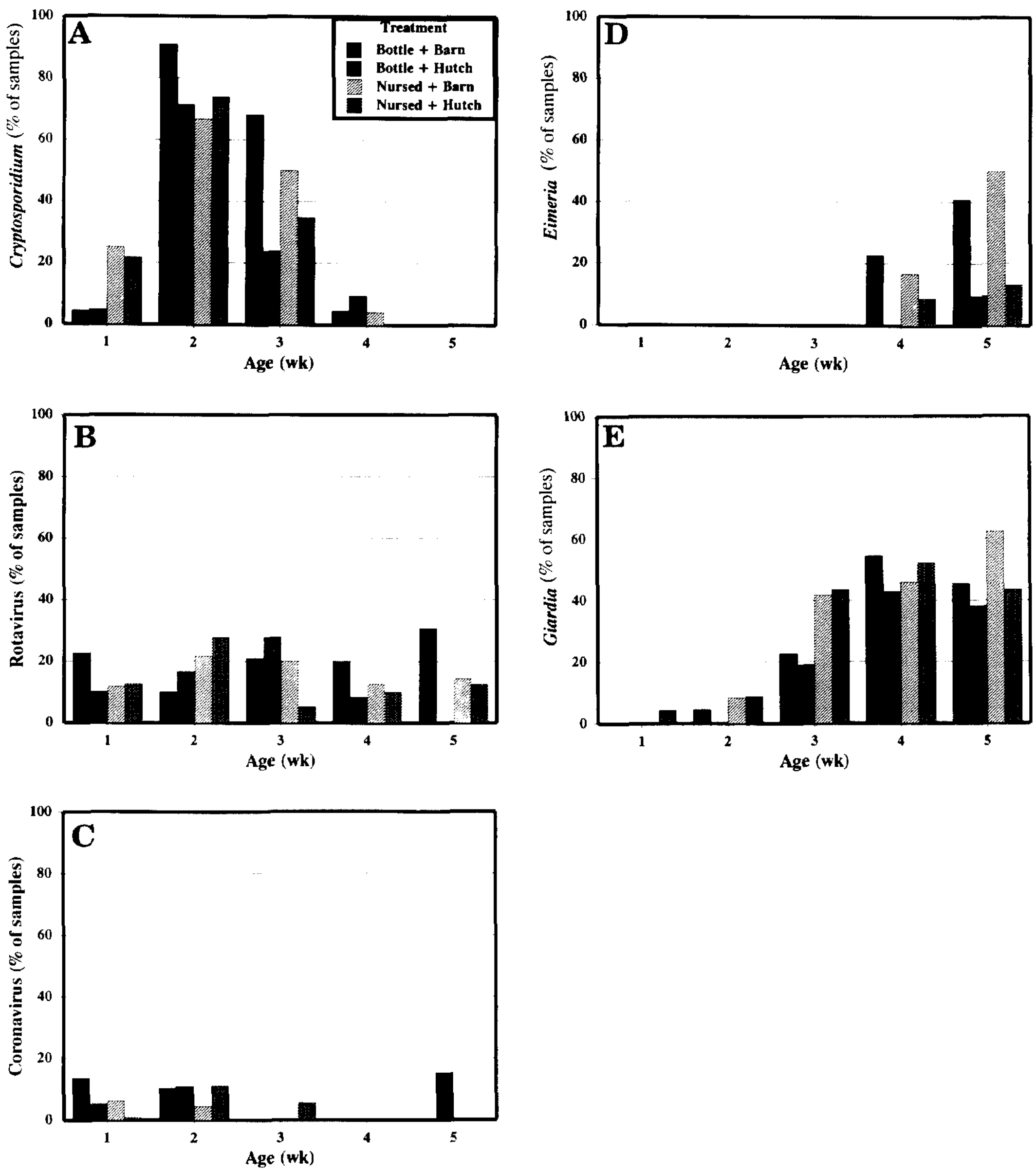

Figure 1. Prevalence of selected organisms in fecal samples of calves fed colostrum via nipple-bottle or allowed to nurse the dam and housed in calf hutches or in individual pens in a calf barn. Standard errors = 8, 9, 5, 5, and 8% for parts A to E, respectively.

Direct electron microscopy detected rotavirus and coronavirus as the primary viruses in fecal samples during the study, although parvovirus and coronavirus-like particles were occasionally observed.

Rotavirus was shed by 50 calves during the 35-d study. Mean prevalence of 15.8% (SE = 2.0) compares favorably with data of Lucchelli et al. (11), who reported prevalence of rotavirus shedding of 16.4% in calves from birth to 30 d of age. Age had no effect on the prevalence of rotavirus in fecal samples (Figure 1b), and prevalence tended ($P < .10$) to be greater in feces from calves housed in the barn. Lopez et al. (10) also reported no significant effect of age or time on the shedding of rotavirus during the first 31 d after birth. Because rotavirus is ubiquitous in the environment (16), placement of calves in individual hutches was unlikely to eliminate potential for rotavirus infection. Calves housed in hutches shed rotavirus by 1 wk of age, indicating early transmission; however, leaving the calf with the dam for 3 d did not increase prevalence of rotavirus shedding in wk 1, thereby eliminating the dam as a major source of the virus. Saif (16) suggested that strict hygiene and isolation of calves may reduce transmission of rotavirus. These factors probably contributed to the reduction in prevalence of rotavirus in calves housed in hutches.

There was no evidence that serum Ig concentrations influenced prevalence of rotavirus. Besser et al. (1) reported that transfer of rotavirus antibody from serum to the intestinal lumen may be important in providing immunity against rotavirus. Because cattle in this study were not immunized against rotavirus prior to parturition, concentrations of colostral antibody may have been insufficient to increase serum titers in calves and to affect prevalence of the virus. Saif and Smith (17) reported that lactogenic immunity (presence of colostral or milk antibodies in the gut lumen) is important for protection against enteric viruses, including rotavirus. Apparently, colostrum feeding for 3 d after birth did not provide sufficient lactogenic immunity to inhibit shedding of rotavirus, as indicated by increased fecal shedding and scours during the first 2 wk of life.

Coronavirus was observed in fecal samples of 13 calves during the study; mean weekly prevalence was 4.3% (SE = 1.1). Coronavirus was observed most frequently during the first 2 wk of age, although some samples were positive for coronavirus at 5 wk (Figure 1c). Coronavirus, like rotavirus, is widespread in most dairy herds. A lack of treatment effect (Table 2) suggested that neither housing nor method of colostrum feeding influenced the prevalence of coronavirus.

Stepwise multiple regression indicated that date of birth ($P < .01$), concentration of IgG in serum, and age affected the prevalence of coronavirus shedding. Calves born later in the study were more likely to shed coronavirus, indicating that fecal shedding was seasonal or that an outbreak occurred during the study. Increased concentration of IgG in serum reduced the prevalence of coronavirus in feces.

Fecal samples from 30 calves were positive for *Eimeria* oocysts during the 35-d study. *Eimeria* shedding occurred only after 4 wk of age (Figure 1d) and was affected by housing type ($P < .02$) and a period × treatment interaction ($P < .02$). Prevalence of *Eimeria* oocysts was greater in feces from calves housed in the barn, particularly during the last week of the study. Few calves housed in hutches shed *Eimeria* oocysts. Davis et al. (5) also reported that shedding of *Eimeria* oocysts was reduced when calves were housed in individual outdoor pens in an uncontaminated area. Multiple stepwise regression also identified date of birth as a significant factor affecting prevalence of *Eimeria* shedding, particularly when calves were housed in the barn. Calves born later in the study had a greater prevalence of *Eimeria* oocysts in their feces, apparently because of contamination of the barn environment by calves born earlier.

Giardia was observed in fecal samples of 71 calves during the study, and a total of 122 samples (27.1%; SE = 2.1) were positive for *Giardia*. Prevalence of *Giardia* pooled across all weeks was greater when calves were allowed to nurse the dam (Table 2). Because *Giardia* is transmitted by ingestion of infected feed, water, or feces (18), allowing the calf to remain in proximity to the dam for 72 h may have increased exposure to *Giardia*. Fecal excretion of *Giardia* generally occurred after 2 wk of age (Figure 1e), although feces from 1 calf contained *Giardia* oocysts during wk 1.

Stepwise multiple regression indicated that method of colostrum feeding, mean daily BW gain, intake, and date of birth were significant for the prevalence of *Giardia*. Positive regression coefficients indicated that intake and mean daily BW gain increased as prevalence of *Giardia* increased; this effect may be related to the establishment of *Giardia* later in the study when intake and rates of gain increased, but also suggests that prevalence of *Giardia* did not significantly impair intake or BW gain in this study.

Escherichia coli was isolated from only 174 of 450 samples cultured. Thawing and refreezing of samples during pooling may have contributed to the apparent loss of viability. Three of the 174 samples (1.7%) were positive for K99(F5) antigen, suggesting that enterotoxigenic *E. coli* did not contribute to the incidence or severity of scours in this study. The observed low prevalence of K99+ *E. coli* in this herd was similar to that recently reported in a case-control study of veal calves in Canada (22).

CONCLUSIONS

Management strategies can affect the prevalence of infectious organisms, incidence and severity of scours, growth, and intake in young calves. When the calf was permitted to nurse the dam for 3 d exposure to *Cryptosporidium* and *Giardia* increased, although nearly all calves were exposed to *Cryptosporidium* within 2 wk. Barn housing of calves increased fecal shedding of *Eimeria* and tended to increase shedding of rotavirus.

ACKNOWLEDGMENTS

The authors thank L. Wallis, D. Johnson, and the farm crew at the Dairy Experiment Station in Lewisburg, Tennessee, for their capable assistance in care, feeding, and sampling of calves; M. Kennedy and A. Tineo for competent laboratory assistance; the American Jersey Cattle Club for partial financial support; and A. M. Saxton for statistical advice.

REFERENCES

1 Besser, T. E., C. C. Gay, T. C. McGuire, and J. F. Everman. 1988. Passive immunity to bovine rotavirus infection associated with transfer into the intestinal lumen. J. Virol. 62:2238.

2 Blouin, D. C., and A. M. Saxton. 1990. General Linear Mixed Models (GLMM) User's Manual. Agric. Exp. Stn., Louisiana State Univ., Baton Rouge.

3 Center for Animal Health Monitoring. 1993. Dairy Herd Management Practices Focusing on Preweaned Heifers. USDA, Anim. Plant Health Inspect. Serv., Vet. Serv., Fort Collins, CO.

4 Cox, D. D., and A. F. Todd. 1962. A survey of gastrointestinal parasitisms in Wisconsin dairy cattle. J. Am. Vet. Med. Assoc. 141:706.

5 Davis, L. R., K. M. Autrey, J. Herlich, and G. E. Hawkins. 1954. Outdoor individual portable pens compared with conventional housing for raising dairy calves. J. Dairy Sci. 37:562.

6 Gay, C. C. 1983. Failure of passive transfer of colostral immunoglobulins and neonatal disease in calves: a review. Page 346 *in* Proc. 4th Int. Symp. Neonatal Dis. Vet. Infect. Dis. Org., Saskatoon, SK, Can.

7 Harp, J. A., D. B. Woodmansee, and H. W. Moon. 1989. Effects of colostral antibody on susceptibility of calves to *Cryptosporidium parvum* infection. Am. J. Vet. Res. 50:2117.

8 James, R. E., M. L. McGilliard, and D. A. Hartman. 1984. Calf mortality in Virginia dairy herd improvement herds. J. Dairy Sci. 67:908.

9 Jorgensen, L. J., N. A. Jorgensen, D. J. Schingoethe, and M. J. Owens. 1970. Indoor versus outdoor calf rearing at three weaning ages. J. Dairy Sci. 53:813.

10 Lopez, J. W., S. D. Allen, J. Mitchell, and M. Quinn. 1988. Rotavirus and *Cryptosporidium* shedding in dairy calf feces and its relationship to colostrum immune transfer. J. Dairy Sci. 71:1288.

11 Lucchelli, A., S. E. Lance, P. B. Bartlett, G. Y. Miller, and L. J. Saif. 1992. Prevalence of bovine group A rotavirus shedding among dairy calves in Ohio. Am. J. Vet. Res. 53:169.

12 Martin, S. W., C. W. Schwabe, and C. E. Franti. 1975. Dairy calf mortality rate: influence of management and housing factors on calf mortality rate in Tulare County, California. Am. J. Vet. Res. 36:1111.

13 Ongerth, J. E., and H. H. Stibbs. 1989. Prevalence of *Cryptosporidium* infection in dairy calves in western Washington. Am. J. Vet. Res. 50:1069.

14 Pohlenz, J., H. W. Moon, N. F. Cheville, and W. J. Bemrick. 1978. Cryptosporidiosis as a probable factor in neonatal diarrhea of calves. J. Am. Vet. Med. Assoc. 172:452.

15 Quigley, J. D., III, K. R. Martin, H. H. Dowlen, L. B. Wallis, and K. Lamar. 1994. Immunoglobulin concentration, specific gravity, and nitrogen fractions of colostrum from Jersey cattle. J. Dairy Sci. 77:264.

16 Saif, L. J. 1992. Rotavirus. Page 126 *in* Veterinary Diagnostic Immunology. A. E. Castro and W. P. Heuschele, ed. Mosby Year Book, St. Louis, MO.

17 Saif, L. J., and K. L. Smith. 1985. Enteric viral infections of calves and passive immunity. J. Dairy Sci. 68:206.

18 St. Jean, G., Y. Conture, P. Dubreuil, and J. L. Fréchette. 1987. Diagnosis of *Giardia* infection in 14 calves. J. Am. Vet. Med. Assoc. 191:831.

19 Tzipori, S., M. Smith, and C. Halpin. 1983. Ex-

perimental cryptosporidiosis in calves: clinical manifestations and pathological findings. Vet. Rec. 112:116.

20 Waltner-Toews, D., S. W. Martin, and A. H. Meek. 1986. Dairy calf management, morbidity and mortality in Ontario Holstein herds. III. Association of management with morbidity. Prev. Vet. Med. 4:137.

21 Waltner-Toews, D., S. W. Martin, and A. H. Meek. 1986. Dairy calf management, morbidity and mortality in Ontario Holstein herds. IV. Association of management with mortality. Prev. Vet. Med. 4:159.

22 Wilson, J. B., S. A. McEwen, R. C. Clarke, K. E. Leslie, D. Waltner-Toews, and C. L. Gyles. 1992. A case-control study of selected pathogens including verotoxigenic *Escherichia coli* in calf diarrhea on an Ontario veal farm. Can. J. Vet. Res. 56:184.