A Clinical Trial Evaluating Prophylactic and Therapeutic Antibiotic Use on Health and Performance of Preweaned Calves

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ABSTRACT

The objective of this clinical trial was to evaluate the influence of prophylactic and therapeutic antibiotics on health and performance in preweaned dairy calves on a calf ranch. One hundred twenty 1-d-old calves were enrolled into 3 management systems for antibiotic use and raised until 4 wk of age. Sixty calves were not eligible to receive prophylactic or therapeutic antibiotics. Thirty calves were eligible to receive individual antibiotic treatment for disease, but no prophylactic antibiotics in milk replacer. The remaining 30 calves received milk replacer medicated with neomycin and tetracycline HCl, and could be treated with antibiotics. Health status and treatments were monitored and recorded daily. The primary study outcomes were weight gain, morbidity, and mortality. The most important factor associated with morbidity and mortality was passive immune transfer through colostrum. In-feed antibiotics delayed onset of morbidity, decreased overall morbidity, and increased weight gain. Nonantibiotic therapies for clinical disease were associated with increased mortality and morbidity compared with antibiotic treatments. The study has shown that minimizing or eliminating the use of antibiotics in the feed requires measures to ensure adequate passive transfer of immunity, but that in the face of inadequate passive transfer of immunity, animal welfare may be endangered by replacing medicated milk replacer with nonmedicated milk replacer, and therapeutic antibiotics with nonantibiotic alternatives.

(Key words: antimicrobial, food animal production, herd health and medicine, dairy calf)

Abbreviation key: **APT** = adequate passive transfer of immunity, **FPT** = failure of passive transfer of immunity, **HR** = hazard ratio, **PH** = Cox proportional hazard model.

INTRODUCTION

Producers involved with rearing dairy-source bull or heifer calves are faced with numerous challenges, the most significant being protecting calf health in the preweaning period. Calf health is adversely affected by weather, nutrition, exposure to infectious agents and, most significantly, by the calf's passive immune status.

The importance of adequate passive transfer (**APT**) for minimizing morbidity and mortality has been demonstrated in several studies (Quigley et al., 1997; Donovan et al., 1998a; Weaver et al., 2000). Although the health benefits of APT are unequivocal, the reality of calf rearing is that a high proportion of calves on calf ranches and dairies are colostrum-deprived (Gay et al., 1983; Fallon et al., 1987; Wilson et al., 2000). Calf ranches receive 1-d-old calves with a high proportion of them having failure of passive transfer (**FPT**) from multiple sources (Moore et al., 2002; Berge et al., 2003). One management strategy for successfully rearing these calves is to add antibiotics, such as neomycin and tetracycline, to the calf milk replacer. Although there are many commercial milk replacers supplemented with antibiotics available to small and large dairy farms, some large calf ranches choose to custom mix milk replacer and supplement it with antibiotics in quantities that exceed those commonly used as feed additives.

All uses of antibiotics in animal agriculture are under increased scrutiny due to concerns that such uses promote the development of bacterial antimicrobial resistance. The European Union has banned the use of several antibiotics for growth promotion; eventually all antibiotics for growth promotion will be banned in the European Union (European Commission, 1998; Commission Press Room, 2003). The World Health Organization recently recommended the cessation of use for all growth-promoting antibiotics (Ferber, 2003). Moreover, there is growing consumer interest in organic products, which provides an additional impetus to raise calves without antibiotics.

There are few studies evaluating health and performance of calves raised without in-feed or therapeutic antibiotics under field conditions. One study evaluated the clinical efficacy of tetracycline in milk replacer dur-

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ing an outbreak of enteric and respiratory disease. The calves receiving antibiotics in the milk replacer experienced lower morbidity compared with those not receiving any antibiotics in the milk replacer (Braidwood and Henry, 1990). In a study comparing a probiotic to tetracycline and neomycin supplementation of milk replacer, no differences in weight gain, feed efficiency, or fecal consistency in 45 calves were detected. The investigators concluded that it might be possible to replace antibiotics with nonantibiotic alternatives such as fructooligosaccharides, allicin, and probiotic bacteria (Donovan et al., 2002). Although published literature on efficacy is lacking, nonantibiotic treatments such as bismuth salts, kaolin-pectin, probiotic bacteria, enzymes, and charcoal are often used to treat calf diarrhea. These few studies have not sufficiently addressed the effect of rearing calves without prophylactic and therapeutic antibiotic use on calf health and productivity.

The objective of this study was to compare calf morbidity, mortality, and weight gain in preweaned calves reared with and without antibiotics for therapy and prophylaxis. The study hypothesis was that calf weight gain, morbidity, and mortality are not affected by antibiotics in the milk replacer or given as individual therapy.

MATERIALS AND METHODS

Study Site

The clinical trial was conducted on a commercial calf ranch in the southern San Joaquin Valley in California. The ranch raised calves as heifer replacements, veal, and dairy beef, maintaining a daily inventory of approximately 2000 calves on milk. One-day-old calves from local dairies arrived daily and were placed in wooden housing units, each of which held 3 calves in individual hutches. Each unit had a plywood roof that could be folded down to provide protection from sun and rain, and slatted floors. The hutch units were placed on cement blocks that raised them approximately 30 cm off the ground. The 3 calves in each unit had the opportunity for nose-to-nose contact with their immediate neighbor. The calves destined for beef and heifer replacement were bottle-fed twice daily with 1.86 L of milk replacer composed of 23% protein and 18% crude fat (proprietary blend). Vitamins and minerals were added at each milk feeding according to a farm protocol. Starter grain in buckets was introduced to calves on d 3 and provided fresh daily. Fresh water was continually available in buckets. On the day of arrival, all calves received 1.86 L of plasma-derived colostrum supplement (Lifeline, APC, Ames, IA). Calves were vaccinated with a *Salmonella*-mutant core antigen bacterin (Endovac-bovi, Immvac, Columbia, MO). On d 14 of the trial,

calves received a vitamin E and selenium injection (Mu-Se injection, 1 mL s.c., Schering-Plough, Kenilworth, NJ), and on d 18 they were vaccinated with modified live infectious bovine rhinotracheitis, bovine viral diarrhea, bovine respiratory syncytial virus, and parainfluenza 3 vaccine (Pyramid MLV 4 Vaccine, Fort Dodge, Overland Park, KS).

Enrollment and Treatment Allocation

One hundred twenty, random-source, 1-d-old calves from multiple dairies were purchased from a commercial calf supplier, and represented the typical dairy bull calf destined for beef. All calves were enrolled over a 2-d period, followed through 28 d of age, and then sold. No information on dairy source was provided with the calves, although no more than 3 calves could have come from a single farm. The calves from multiple dairies were commingled on the trailer and were randomly off-loaded from the trailer by the calf dealer. No further randomization was attempted and calves were assigned to treatment groups and housing in the order they were removed from the calf trailer. Calves were housed in blocks according to study group allocation. This was done to ensure compliance with the feeding protocols. They were ear-tagged thereafter, weighed, had their navels dipped with 7% iodine solution, and received a veterinary visual inspection.

Calves in the trial were placed in new wooden housing units that had not previously housed calves. The veterinarian in charge of the trial and 2 technicians were responsible for all aspects of calf and trial management, including preparation of feed, feeding, and treatments. All equipment for feeding and treating study calves was separate from that used for calves belonging to the ranch. Calves were fed colostrum supplement and vaccinated according to farm protocol. Calves were bottle-fed 1.86 L of milk replacer at 0600 and 1700 h. Before the study, samples of water used to mix the milk replacer were collected and analyzed using standard microbiological techniques for the presence of *Salmonella* and *Escherichia coli*, with negative results (American Public Health Association, 1995; Farmer, 1999).

Health and Performance Monitoring

Calves were monitored for feed intake at all feedings, and received a recorded visual health appraisal twice daily by a veterinarian (blinded to treatment allocation) and by the manager of the calf facility who was responsible for treatments on the ranch. Calf health was assessed visually using objective criteria of appetite, fecal consistency, hydration status, respiratory effort, and attitude (Table 1). Subsequent to these evaluations,

Table 1. Criteria for clinical diagnosis and therapeutic decisions used in a clinical trial assessing the efficacy of antibiotic use in milk replacer and as disease therapy in 120 preweaned calves.

Health condition	Clinical signs	Score	Therapeutic choices	
			Nonantibiotic therapy	Antibiotic therapy
Diarrhea	Formed	0		
	Semiformed	1		
	Watery	2	Bismuth, kaolin-pectin	Ceftiofur ¹
	Watery with mucous	3	Kaolin-pectin,	Ceftiofur ¹
	Blood in feces	4	Kaolin-pectin, UAA ²	Ceftiofur ¹
Respiratory signs	Normal	0		
	Rhinitis	1		
	Coughing	2		Ceftiofur ¹
	Heavy thoracic breathing	3		Ceftiofur
	Abdominal breathing	4		Ceftiofur
Dehydration	Normal appearance	0		
	Sunken eyes	1	Electrolytes ¹	
	Skin tented 5 to 10 s	2	Electrolytes	
	Skin tented >10 s	3	Electrolytes	
Attitude	Alert	0		
	Depressed	1	Flunixin meglumine	
	Nonresponsive	2	Flunixin meglumine	
Appetite	Normal	0		
	Consuming < ¼ bottle	1		
Eye appearance	Normal	0		
	Swelling/redness/discharge	1		Penicillin G (subconjunctival)
Umbilicus/joints	Normal	0		
	Swelling/heat/pain/lameness	1	Iodine (topical)	Penicillin G
	Normal	0		
Otitis	Head tilt, hanging head	1		Tilmicosin

¹Treatment initiated if combined with appetite loss, depressed attitude, or multiple diagnosis.

²UAA = Universal animal antidote, Vedco Inc., St. Joseph, MO.

calves were designated as “normal” or “to be treated”. Neither appraiser was involved with feeding or treatment administration. Treatments were administered by the veterinarian in charge of the study. Calves received treatment based on the health category strategy as described in Table 1, and according to their assigned experimental group. Calves were weighed on the first and last day of the trial using a scale accurate to the nearest 0.5 kg.

Calves were removed and censored from the study when their clinical status appeared critical and they were not expected to survive. These calves were placed in hutches elsewhere on the ranch and treated with antibiotics according to the standard ranch policy. The decision to remove an animal from the study was made by the veterinarian in charge and was done to ensure compliance with Institutional Animal Care and Use Committee concerns on animal welfare.

Experimental Approach

The assumptions for determining sample size were: Type I error = 0.10, Type II error = 0.10, and a mean number of 5 morbid days for calves not exposed to antibiotics compared with 3.5 morbid days for calves exposed to antibiotics in milk and treated with antibiotics,

with a sample standard deviation of 2 d. Allowing for 15% loss to follow up, we enrolled 120 calves allocated to 4 study groups (30 calves/treatment group).

Study groups. Sixty calves received no antibiotics in the milk replacer, and clinical disease was treated using only nonantibiotic alternatives. Bismuth subsalicylate (Biocode, Vedco, St. Joseph, MO), electrolytes (Biolyte, Pharmacia Animal Health, Kalamazoo, MI), kaolin-pectin (Kaolin-Pectin Plus, Agripharm, Grapevine, TX), activated attapulgite, and activated charcoal (UAA gel, Vedco) were used according to label dose to treat diarrhea; and flunixin meglumine (Banamine, 50 mg/mL, 2.2 mg/kg, Schering-Plough) was used to treat calves with a depressed attitude (Table 1). These 60 calves were split into 2 housing groups of 30 calves. One group was housed with other study calves along with commercial calves. The other group was housed away from the study calves and separated from the commercial calves. Because there were no health differences between these groups, these 2 study groups were treated as one group for all statistical analyses.

Thirty calves received no antibiotics in the milk replacer, but antibiotics were used to treat clinical disease. Treatment protocols followed standard protocols of the farm (Table 1). The primary antibiotic treatment was ceftiofur hydrochloride (2.2 mg/kg per d, 3 to 5 d;

Excenel RTU, Pfizer Inc., NY). Penicillin G procaine (300,000 U/mL, 2.2 mL/100 kg per d, 1 to 3 d) and tilmicosin (10 mg/kg per d, 1 to 3 d; Micotil 300, Elanco Animal Health, Greenfield, IN) were available but used sparingly. In addition, nonantibiotic alternatives (described above) could be used concurrently with antibiotic treatments.

Thirty calves received medicated milk replacer containing tetracycline hydrochloride (22 mg/kg per d, TET-324, Agripharm) and neomycin sulfate (22 mg/kg per d, Neomix AG 325, Pfizer Inc.) according to protocols used on the ranch. The antibiotics were diluted in 20 mL of purified water and added directly to each bottle at the milk-mixing stage before addition of the milk replacer. These calves were treated with antibiotics and nonantibiotic alternatives as described (Table 1).

Fecal and Postmortem Samples

Using 2 sterile cotton-tipped swabs, fecal samples were taken per rectum on d 1, 14, and 28 for isolation of *Salmonella*. Samples were placed in 9 mL of tetrathionate pre-enrichment broth and incubated overnight at 37°C. The broth was plated on xylose-lysine desoxycholate agar and brilliant green agar and incubated at 37°C for 18 to 24 h. Plates were evaluated for suspect colonies of *Salmonella*. Two suspect colonies per plate was picked and transferred to triple sugar iron, lysine iron, and urea agar slants, incubated at 37°C for 24 h. *Salmonella*-positive colonies were serogrouped using the slide agglutination technique, and *Salmonella enterica* isolates were thereafter sent to the California Animal Health and Food Safety laboratory for serotyping. Antibiotic susceptibility to 12 antibiotics was tested using the disk diffusion method according to the National Committee of Clinical Laboratory Standards using *E. coli* ATCC 25922 and *Salmonella* Typhimurium (VMTRC bovine clinical isolate) as quality control standards (Bauer et al., 1966; NCCLS, 1999). The antibiotic discs included in the panel were amikacin 30 µg, amoxicillin/clavulanic acid 20/10 µg, ampicillin 10 µg, cephalotin 30 µg, ceftiofur 30 µg, chloramphenicol 30 µg, gentamicin 10 µg, nalidixic acid 30 µg, streptomycin 10 µg, sulfisoxazole 250 µg, tetracycline 30 µg, and sulfamethoxazole/trimethoprim 23.75/1.25 µg.

Field necropsies were performed on calves more than 6 d old. Gross pathology was noted and samples from lung, liver, spleen, and intestines were taken for isolation of *Salmonella*. Results from the *Salmonella* fecal cultures from the 0-, 2-, and 4-wk-old calves, and the isolates from lung, liver, spleen, and intestines obtained from postmortem samples were assessed to evaluate risk factors for morbidity and mortality.

Passive Transfer of Immunity

On the day of arrival, a blood sample was collected from each study animal to evaluate passive transfer of immunity by measuring serum IgG using a single radial immunodiffusion assay (IgG Single Radial Immunodiffusion kit, VMRD Inc., Pullman, WA). The IgG values in mg/dL were calculated by comparing sample values to the standard curve obtained using a set of 4 standards (400, 800, 1600, 3200 mg/dL) applied to each plate. Passive immune transfer status was classified as IgG status 0 for IgG = 86 mg/dL (this corresponded to the y-intercept of the standard curve, and represented no evidence of IgG), IgG status 1 for values $87 \leq \text{IgG} \leq 999$ mg/dL, and IgG status 2 for IgG ≥ 1000 mg/dL. Immunoglobulin G status 0 or 1 indicated FPT and IgG status 2 indicated APT (Tyler et al., 1996).

Measuring Serum Bacterial Inhibition Levels

Serum from calves at enrollment was tested for the presence of antimicrobial inhibitory substances using a serum inhibition bioassay (Bennett et al., 1966). The assay was a modified agar-well diffusion system containing *Bacillus subtilis* spores. The agar was poured over a 20 × 20 cm plate with 64 wells. A standard concentration of penicillin in 8 serial dilutions was added to the wells to determine a standard curve. One hundred microliters of serum was added in duplicate to the wells. The plates were incubated for 18 h at 37°C. The diameters of the inhibition zones surrounding the wells were measured and compared with the standard curve to estimate equivalent units of penicillin per milliliter. The assay is a general screen for serum bacterial inhibition and detects the presence of β -lactams, tetracycline, and aminoglycosides among other inhibitory substances.

Data Analysis

All health and treatment data were recorded and entered into a spreadsheet program (Excel, Microsoft Corp., Redmond, WA) and analyzed using a statistical software package (SAS version 8.2, Proc LIFETEST, PHREG, and GLM; SAS Institute Inc., Cary, NC). For analytical purposes, calves removed from the trial for animal welfare reasons were treated as dead. Our primary outcome measures were mortality, morbidity, and survival weight-gain. For all models, predictive variables, and interaction effects with a P value < 0.10 were retained in the final models. The predictive variables included in the models were antibiotics in the milk-replacer; the calf individual treatment categorized as antibiotic, nonantibiotic or no treatment; passive im-

Table 2. Passive immune transfer status and mortality of 120 Holstein calves enrolled in a study of antibiotic use in milk replacer and as therapy over the first 28 d of life on a calf ranch.

Antibiotic exposure category	IgG status ¹			Total
	0	1	2	
	No. of calves (no. of dead ³)			
No antibiotics allowed	7 (4)	27 (14)	26 (5)	60 (23)
No in-feed or therapeutic antibiotics allowed	11 (4)	10 (1)	9 (0)	30 (5)
In-feed and therapeutic antibiotics allowed ²	5 (3)	11 (0)	14 (0)	30 (3)
Total	23 (11)	48 (15)	49 (5)	120 (31)

¹IgG status 0 = 86 mg of IgG/dL of serum, IgG status 1 = 87 to 999 mg of IgG/dL of serum, IgG status 2 > 1000 mg of IgG/dL of serum.

²Calves were eligible for antibiotic treatments for clinical disease.

³Calves censored from study for animal welfare reasons were treated as "dead".

mune transfer status categorized as IgG status 0, 1, or 2; and arrival weight.

Mortality. Mortality was defined either as a calf dying or a calf removed from the trial because of severe disease. Mortality rates were calculated using Kaplan-Meier survival plots (days-to-death) and stratified by presence or absence of antibiotics in the milk replacer, individual calf treatments, and IgG status. Log-rank and Wilcoxon statistics were used to test equality over strata. Proportionality of the survival distributions was assessed by plotting $\ln(-\ln(\text{survival}))$ against the $\ln(\text{calf-days})$. Multivariate assessment of survival time was developed using a Cox proportional hazard (PH) model. Hazard rate ratios and 90% confidence intervals for each covariate were determined (Hosmer and Lemeshow, 1999).

Weight gain. A GLM procedure calculating least square means was used to compare differences in weight gain between the calves that survived to the end of the study in the different treatment categories. For all independent variables, least square mean estimates and 90% confidence intervals were calculated.

Morbidity. A calf was considered diseased when it received a treatment. Treatments were administered when a calf was observed with a diarrhea score ≥ 2 , respiratory disease score ≥ 2 , and/or attitude score ≥ 1 (Table 1). Calf health was evaluated using 2 outcome measures. The first outcome evaluated was the number of days to first treatment using a PH model (Hosmer and Lemeshow, 1999). The second outcome measured the number of days a calf was untreated, standardized for the number of days the calf remained in the trial. This was calculated as the difference between the number of days the calf remained in the trial and the number of days the calf remained untreated. Four calf health categories corresponding to 7-d intervals were created from the difference calculation. Calves that died early or were treated for prolonged periods of time would thereby fall into categories 1 or 2, and calves that re-

mained in the trial until d 28 with fewer than 6 treatment days would be in category 4. These categories standardized the morbidity data so that a calf leaving the trial early but untreated was not evaluated as "healthier" than a calf that survived until the end of the trial but received a treatment. Multivariate relationships between calf health categories and the predictive variables were assessed using a cumulative logistic regression model (Kleinbaum et al., 1988; Agresti, 2002). The model predicted the odds of the calf being in a lower health category (having fewer standardized untreated days) compared with all the higher health categories.

RESULTS

One hundred twenty calves were enrolled in the study over a 2-d period in February 2002. During the trial, the weather was dry except for 2 d of heavy rain. Nighttime temperatures ranged between 2 and 7°C, and daytime temperatures were between 18 and 27°C. Based on the results from the serum bacterial inhibition assays, no calves had detectable levels of antibiotic, suggesting that they had not received any antibiotic treatment before arrival on the farm.

Of the 120 calves enrolled, only 41% had APT (Table 2). Twenty-one calves died during the study, 6 within the first 5 d of the trial. Ten calves, not eligible for antibiotic treatments, were removed from the study for animal welfare reasons because their clinical status was deemed critical (Table 3). These calves were considered dead for subsequent analyses.

Postmortem examination was performed on 16 of 21 calves that died. The 6 calves that died within the first 5 d of the study were not available for examination. Gross pathology indicated pneumonia, enteritis, and signs of systemic infection as primary problems. *Salmonella enterica* was isolated from 11 of the 16 dead calves from lung, liver, spleen, and intestines. The *S. enterica*

Table 3. The total number of days that calves contributed to a trial evaluating the use of antibiotics in feed and as therapy in the different antibiotic exposure categories, the number that died or were censored and average weight gain for calves that survived the study period in a study of antibiotic use in milk-replacer and as therapy in preweaned calves on a calf ranch for 28 d.

Antibiotic exposure category	No. of calves enrolled ¹	Total calf-days	No. of calves		Mean weight ² (kg)		
			Died	Censored ³	d 1	d 28	Total gain during study
No antibiotics allowed	60	1270	13	10	43.6	48.7	5.05
No in-feed or therapeutic antibiotics allowed	30	727	5	0	44.9	47.1	2.2
In-feed and therapeutic antibiotics allowed	30	769	3	0	44.7	50.3	5.6

¹Total number enrolled in study group (d 1).

²Calves that died or were removed from trial for animal welfare reasons were not included in the calculations for mean weight or weight gain.

³Calves removed from the study for animal welfare reasons.

serovars identified included *Salmonella* Newport (n = 7), Dublin (11), Typhimurium (2), and Give (2). Multiple serovars were recovered from 5 calves. Serovars Give and Montevideo were sensitive to all 12 antibiotics tested, whereas the other serovars exhibited resistance to chloramphenicol, streptomycin, tetracycline, and sulfisoxazole. Serovars *Salmonella* Newport, Typhimurium, and Reading exhibited additional resistance to beta-lactam antibiotics, including resistance to the third-generation cephalosporin, ceftiofur.

Salmonella was prevalent in the study calves throughout the follow-up period. On screening, 3 calves were positive for *Salmonella* on the day of arrival, 17 calves on d 14, and 3 calves on d 28. The *Salmonella* isolated included *Salmonella* Dublin (8), Give (8), Montevideo (5), Newport (1), and Reading (1). *Salmonella* was isolated from calves in all study groups, and there was no apparent pattern to the serotype distribution between groups.

Mortality

The Kaplan-Meier curves depicting mortality rate conditional on 1) receiving antibiotics in the milk replacer, 2) receiving antibiotic therapy, and 3) by passive transfer status are shown in Figures 1 to 3, respectively. As univariate analyses, mortality was highest in calves not receiving antibiotics in the milk replacer (Wilcoxon rank test; $P < 0.02$), receiving nonantibiotic therapy ($P < 0.01$), and with FPT ($P < 0.01$).

Plots of $\ln(-\ln(\text{survival}))$ against the $\ln(\text{calf-days})$ for the treatment effects indicated that the mortality hazards were not proportional over time and suggested that the mortality experience was different for calves that died <7 d into the trial compared with those dying between 7 and 28 d into the trial. Consequently, the data were analyzed in 2 separate PH models. The final models included the main effect of antibiotics in the feed, calf individual treatment, and IgG status.

The PH model evaluating mortality indicated that for the first 6 d of the trial, calves with IgG status 0 were more likely to die than calves with APT [hazard ratio (HR) = 17.80, 90% CI = 2.84 to 111.67; Table 4]. No mortality HR could be calculated for calves receiving antibiotics in the milk because none of these calves died during the first 6 d. Individual antibiotic treatments did not significantly alter the risk of early mortality. Between 7 and 28 d of age, antibiotics in the milk replacer had no significant influence on mortality. Treatments with nonantibiotic alternatives increased the hazard of dying (HR = 10.07, 90% CI = 1.81 to 56.06) compared with nontreated calves, whereas treatments with antibiotics did not change the hazard of death compared with a nontreated calf. Calves with IgG status 0 or 1 were more likely to die than calves with IgG status 2 (Table 4).

Weight Gain

The average weight gain of calves that survived through the 28-d follow-up period stratified by antibiotic exposure category is shown in Table 3. The least square means from the GLM and least square means evaluating weight gain by antibiotic exposure categories indicated that calves receiving antibiotics in the milk replacer had greater 28-d weight gain compared with calves raised without in-feed antibiotics (Table 5). Calves receiving antibiotic treatments had decreased weight gains compared with nontreated calves, whereas calves receiving nonantibiotic therapies had similar weight gains to the nontreated calves. The number of days that a calf was treated, IgG status, arrival weight, and interaction were not significant effects.

Morbidity

The treatments administered to the calves are summarized in Table 6. From the multivariate analysis of days-to-first-treatment, calves with FPT had an in-

Figure 1. Kaplan-Meier plot showing survival proportion of 120 calves stratified on receiving antibiotics in the milk replacer (30 calves) or no in-feed antibiotics (90 calves) in a study of prophylactic and therapeutic use of antibiotics on a commercial calf ranch. Wilcoxon test of equality of strata, $\chi^2 = 5.04$, P value < 0.02 .

Figure 2. Kaplan-Meier plot showing survival of 120 calves stratified by treatments received (no treatments, antibiotic treatments, or treatments with nonantibiotic alternatives) in a study of prophylactic and therapeutic use of antibiotics on a commercial calf ranch. Wilcoxon test of equality of strata, $\chi^2 = 9.56$, P value < 0.00 .

Figure 3. Kaplan-Meier plot showing survival of 120 calves stratified on passive transfer of immunity status (IgG status 0 = 86 mg of IgG/dL of serum, IgG status 1 = 87 to 999 mg of IgG/dL of serum, IgG status 2 = ≥ 1000 mg of IgG/dL of serum) in a clinical trial evaluating effect of prophylactic and therapeutic antibiotic use on calf health. Wilcoxon test of equality of strata, $\chi^2 = 14.63$, P value < 0.00 .

creased HR for treatment compared with calves with APT. Calves not receiving antibiotics in feed had a much higher HR for first treatment compared with calves receiving antibiotics in the milk replacer (Table 7).

A cumulative multinomial logistic regression model was used to predict the odds of belonging to a lower health category compared with higher health categories (Table 8). Calves with FPT had increased odds of be-

Table 4. Maximum-likelihood estimates and hazard ratios (HR) from a Cox proportional hazards model evaluating the effect of antibiotics in milk and as therapy on mortality in calves 0 to 6 and 7 to 28 d of age. Data are from a clinical trial conducted on a commercial calf ranch.

Calf age	Parameter	Level	Estimate	P value (Wald's)	HR	90% CI	
						Lower	Upper
0 to 6 d	In-feed antibiotics	Yes	Nonestimable ¹				
		No	Reference				
	Treated	Antibiotics	-1.82	0.13	0.16	0.02	1.17
		Nonantibiotics	-0.11	0.88	0.90	0.28	2.85
		None	Reference				
	IgG status	0	2.18	0.01	8.85	2.15	36.46
		1	0.72	0.41	2.06	0.49	8.55
7 to 28 d	In-feed antibiotics	Yes	-0.30	0.63	0.74	0.26	2.12
		No	Reference				
	Treated	Antibiotics	1.29	0.25	3.63	0.57	23.16
		Nonantibiotics	2.31	0.03	10.07	1.81	56.06
		None	Reference				
	IgG status	0	1.87	0.01	6.51	1.93	21.99
		1	1.45	0.03	4.25	1.45	12.43
		2	Reference				

¹Nonestimable due to no deaths in calves receiving medicated milk-replacer during first 6 d of life.

Table 5. Least square means¹ (LSM) estimates for the effect of antibiotics in feed and treatments for disease on weight gain over a 28-d follow-up period in 120 preweaned calves enrolled in a clinical trial on a commercial calf ranch.

Parameter	Level	LSM estimate	P value	90% CI	
				Lower	Upper
In-feed antibiotics	No	3.80	0.02	3.14	4.46
	Yes	5.48	Reference	4.47	6.48
Treated	Antibiotics	2.72	<0.01	1.68	3.76
	Nonantibiotics	5.42	0.66	4.42	6.43
	None	5.77	Reference	4.87	6.67

¹F-value = 6.84, df = 3, P value = 0.0003, and R² = 0.19.

longing to a lower health category compared with calves with APT. Calves treated with nonantibiotic alternatives were 4.8 times more likely to be in a lower health category than untreated calves ($P = 0.01$). Calves receiving antibiotic therapy did not differ significantly ($P = 0.36$) in health category compared with untreated calves. Calves not receiving antibiotics in the feed were 3.33 times more likely to belong to a lower health category than calves that received in-feed antibiotics ($P = 0.06$).

DISCUSSION

This study demonstrates the significance of ensuring APT, the presence of in-feed antibiotics, and therapeutic use of antibiotics in maintaining calf health (morbidity and mortality) on calf ranches. As found in other studies, the most significant factor associated with neonatal calf morbidity and mortality was APT (Fecteau et al., 1997; Donovan et al., 1998a; Barnett et al., 2003). Although APT has greater implication for maintaining calf health than antibiotics in the feed or the use of antibiotics for therapeutic treatments, the use of antibiotics to treat clinical disease was associated with decreased morbidity and mortality. The use of antibiotics in milk replacer was associated with decreased morbidity and increased weight gain.

Overall morbidity and mortality was high in this trial. Weather conditions may have been stressful on calves because of large night and day temperature fluc-

tuations (Martin et al., 1975). The study ranch, as well as several other calf ranches in the area, experienced unusually high morbidity and mortality during this period. Several study calves had bloody diarrhea, possibly due to *Salmonella* infections. The S. Newport recovered from several necropsied calves was multiple-antibiotic resistant and has been associated with clinical disease in dairy cattle (Berge et al., 2004). This serovar was resistant to the third-generation cephalosporin ceftiofur, which was used as the primary antibiotic for calves with diarrhea in this study.

Passive Transfer of Immunity

Adequate passive transfer of immunity was the most important predictor of calf health. Several other studies have found APT associated with decreased morbidity and increased weight gain, but in these studies, APT had no specific influence on number of diarrhea days (Quigley et al., 1995; Rea et al., 1996; Weaver et al., 2000). Although the influence of APT on the development of diarrhea is not clear, the importance of APT for minimizing morbidity and mortality has been demonstrated in several studies (Quigley et al., 1997; Donovan et al., 1998a; Weaver et al., 2000). Failure of passive transfer of immunity has also been correlated with bacteremia in calves (Fecteau et al., 1997).

Our study confirmed published findings in that mortality and morbidity in calves was higher in calves that had FPT compared with calves with APT. We did not

Table 6. Summary of antibiotic and nonantibiotic therapy used in a clinical trial evaluating the use of antibiotics in milk replacer and as therapy involving 120 preweaned calves on a commercial calf ranch.¹

Antibiotic exposure category	Number of doses of medication/100 calf-days				
	Ceftiofur	Flunixin meglumine	Bismuth salts	Kaolin-pectin	Oral electrolytes
No antibiotics allowed	0	5.75	3.35	3	3.75
No in-feed or therapeutic antibiotics allowed	13.8	9.4	3.7	2.3	4.7
In-feed and therapeutic antibiotics allowed	3.3	1.8	1.6	1.6	2.6

¹Other medicines used (activated attapulgate, penicillin G, and tilmicosin) with a frequency of less than 1 dose/100 calf-days per group were not included.

Table 7. Maximum-likelihood estimates from a Cox proportional hazards model evaluating the effect of antibiotics in the milk replacer and the calves' passive immune transfer status on days-to-first treatment. Data are from a clinical trial conducted on a commercial calf ranch.

Parameter	Level	Estimate	P value (Wald's)	Hazard ratio	90% CI	
					Lower	Upper
In-feed antibiotics	No	-0.69	0.01	2.00	1.27	3.12
	Yes	Reference				
IgG Status	0	1.03	<0.001	2.81	1.76	4.48
	1	0.53	0.03	1.71	1.14	2.56
	2	Reference				

detect any difference in weight gain attributed to the IgG status of the calves. This may be explained in part by the loss of calves with FPT due to mortality and censoring. Donovan et al. (1998b) also showed that after adjustment for disease status, passive immune status had little influence on weight gain.

Antibiotics in the Feed

Our study indicated that morbidity was lower for calves receiving in-feed antibiotics than for calves not receiving medicated milk replacer. This suggests that antibiotic supplementation of the milk protected calves from disease events during the first month of life. Because no calves died in the antibiotic-supplemented group, we were unable to demonstrate statistically that in-feed antibiotics decreased mortality rate. As shown in other studies, antibiotics in the milk replacer were associated with increased weight gain (Morrill et al., 1977; Donovan et al., 2002). It should be noted that the dosage of neomycin and tetracycline added to the milk replacer in this study, although typical for many large calf ranches, exceeded that found in Type B and C medicated feeds (FDA, 2005). The antibiotic doses used in this trial were metaphylactic and required extended

withdrawal times. It is not known whether lower doses would result in the same outcomes as we observed. Further studies to evaluate prophylactic antibiotic concentrations according to label use should be performed.

The beneficial effects of probiotics to calves have not been consistently demonstrated. Indeed, there are studies indicating that low levels of antibiotics in the feed increase feed consumption and weight gains compared with calves receiving probiotics (Morrill et al., 1977). Further research is needed to develop and evaluate nonantibiotic alternatives for improving calf health to minimize the use of prophylactic or metaphylactic antibiotics in the feed.

Therapeutic Treatments for Clinical Disease

Calves treated with nonantibiotic alternatives had increased the risk for morbidity and mortality, whereas calves treated with antibiotics had the same mortality and morbidity experience as calves that had no evidence of disease needing therapy during the trial. Calves receiving only nonantibiotic therapy were more likely to be in a lower health category compared with antibiotic-treated calves. Nonantibiotic therapy resulted in prolonged duration of treatment, possibly due to poor clinical

Table 8. Results from a cumulative logistic regression model assessing the influence of antibiotics in the milk, individual calf treatments and IgG status on the odds ratio of belonging to a lower health category compared with higher categories.¹

Parameter	Level	Estimate	P value	Odds ratio	90% CI	
					Lower	Upper
In-feed antibiotics	No	1.20	0.06	3.33	1.28	10.10
	Yes	Reference				
Treated	Antibiotics	0.65	0.36	1.91	0.60	6.55
	Nonantibiotics	1.56	0.01	4.78	1.80	14.62
	None	Reference				
IgG Status ¹	0	2.26	<0.00	9.62	3.44	28.93
	1	1.22	0.02	3.39	1.45	8.44
	2	Reference				

¹Health categories were defined as the difference between the number of days on the trial and the number of treated days. Four categories were created: category 1 = 1 to 7 d, category 2 = 8 to 14 d, category 3 = 15 to 21 d, and category 4 = 22 to 28 d. Category 4 was considered the highest health category and category 1 the lowest health category.

cal response. When calves that were not eligible for any antibiotic treatments became critically ill, they were placed in separate hutches outside the study groups and then treated with antibiotics. Nine of 10 calves recovered after having received antibiotic treatments. This further supports the importance of therapeutic antibiotics for calf health and welfare. Our study indicated that ceftiofur hydrochloride was more effective than nonantibiotic alternatives in reducing morbidity and mortality. Given the scarcity of data in the peer-reviewed literature supporting the use of antimicrobials with labels for treating calf diarrhea, our data and others indicate that the extra-label use of antimicrobials (in this case, ceftiofur) may be justified in treating calves with diarrhea (Fecteau et al., 2003; Constable, 2004).

Calves that were treated for disease with antibiotics gained less weight, whereas calves treated with nonantibiotic alternatives gained similar weight to those calves not treated during the trial. These results are biased by the fact that the calves receiving nonantibiotic alternatives were more likely to die (as shown by our analysis of mortality) and their weights were not included in the analysis.

Flunixin meglumine in conjunction with antibiotic treatment has been shown to decrease the severity and total number of treatments in calves with bloody diarrhea and may be a valuable nonantibiotic treatment option (Barnett et al., 2003). We found the use of flunixin meglumine valuable in improving the well being of the calves, but the study design did not allow for further evaluation of this treatment.

Study Design

This trial was designed as a parallel-group clinical trial with 2 main treatment effects: exposure to antibiotics in the feed and exposure to antibiotics for clinical therapy. The hallmarks of a clinical trial are randomization of subjects to the treatment groups and objective assessment of outcomes using blinding (Institute of Medicine, 2001). The intent of randomization is to eliminate selection bias and to minimize the possibility that unmeasured covariates do not bias or confound the study. For logistical reasons, randomization for the trial was achieved by receiving calves from a single supplier who purchased calves from multiple sources and delivered them as a group. Selection bias was not an issue as study group enrollment was systematic and determined by the order of calves being removed from the trailer by the calf hauler (i.e., not influenced by us). Confounding bias was controlled in 2 ways, first through the randomization, and second through the

analyses which accounted for the principle confounders of APT and arrival weight.

Management of Calves

Heifer replacement calves raised on dairies in California are not usually administered antibiotics in the milk (A. C. B. Berge, unpublished data, 2004). These heifers are not subjected to the same stressors, such as transportation and commingling, as calves on calf ranches. They are often fed fresh or hospital milk (milk withheld from human consumption because it is abnormal or contains antibiotics following treatment) that may be supplemented with milk replacer. In general, there may be more emphasis on providing heifers with colostrum as soon as possible after birth to ensure APT.

Applying the dairy model for calves raised on dedicated calf ranches would include efforts to minimize environmental stress (e.g., by not transporting neonatal calves), and improving passive transfer of immunity. These management changes could optimize calf health and may lead to a decrease in the use of prophylactic and therapeutic antibiotics. In the absence of these changes, antibiotics will continue to be an important tool to ensure the health and well-being of these calves.

CONCLUSIONS

Although ensuring that calves have APT continues to be the most significant management factor to ensure the health of neonatal calves, in-feed and therapeutic antibiotics played an important role in controlling morbidity and mortality in our study. Without the use of antibiotics for therapy or prophylaxis, it is difficult and labor-intensive to raise preweaned calves on a calf ranch without APT. If immunocompromised neonatal calves are under environmental stress and high infection pressure, such as exposure to calf-pathogenic *Salmonella*, antibiotic therapy appears necessary. Calf ranch systems need to address problems of colostrum-deprived calves, pathogen load, and environmental stress to successfully raise calves and minimize prophylactic and therapeutic antibiotic use.

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