Comparative efficacy of enrofloxacin and tulathromycin for treatment of preweaning respiratory disease in dairy heifers

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ABSTRACT

Prewaning respiratory disease continues to have a substantial effect on the current and future productivity of dairy replacement animals. Establishing an effective treatment plan for the preweaned calf may have a significant effect on well-being and lifetime productivity by limiting any early development of chronic disease. The primary objective of this study was to examine the efficacy of treatment with tulathromycin (TUL) or enrofloxacin (ENR) on the risk of re-treatment, with a secondary objective of investigating the effect of disease and subsequent treatment choice on average daily gain (ADG). A total of 1,141 Holstein heifers from 4 farms were observed and systematically scored for evidence of respiratory disease from birth through weaning or the time of death. At the time of diagnosis, calves were randomly and blindly allocated into 2 treatment groups. The overall incidence of respiratory disease was 60.9%. In the univariable analysis, the incidence of re-treatment between 7 and 10 d of initial therapy for calves treated with ENR was greater than that in calves treated with TUL (27.6 vs. 21.2%). After adjusting for farm ID, clinical score at first treatment, and weight at first treatment, the odds of re-treatment were 1.5 times higher for calves treated with ENR than with TUL. The percentage of calves that required more than one re-treatment was higher for calves that received ENR compared with those that received TUL (9.3 vs. 4.1%). We observed no difference in ADG between calves treated with ENR or TUL, and no difference in ADG between calves that were treated for respiratory disease and those that were not treated for respiratory disease. Appropriate drug therapy for preweaning respiratory disease may have an important role in reducing the odds of re-treatment during the preweaning period.

Key words: bovine, preweaning, respiratory disease, treatment

INTRODUCTION

The effect of bovine respiratory disease (BRD) on the preweaned heifer has been poorly characterized by existing scientific literature. According to the 2007 National Animal Health Monitoring Survey (NAHMS), the incidence of respiratory disease in the preweaning period was 12.4% and represented 22.5% of the mortality losses in the same period (NAHMS, 2010). These data are consistent with previous NAHMS studies from 1991, 1996, and 2002, which demonstrated respiratory disease caused mortality in 21.3, 24.5, and 21.3% of deaths, respectively (Gorden and Plummer, 2010). In 1990, a report estimated the total cost, including prevention, of preweaned calf respiratory disease to be approximately \$14.71 per calf per year (Kaneene and Hurd, 1990). A complicated disease that may be initiated by a variety of stressors, including dystocia, environment, and housing and management factors, dairy calf pneumonia may affect the future productivity of the dairy animal (Waltner-Toews et al., 1986; Svensson et al., 2003; Lago et al., 2006; Lombard et al., 2007). Often following an initial viral infection, dairy calves may be infected with one or more bacterial pathogens, including *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma* spp. These organisms represent the primary bacterial causes of the BRD complex.

Early and accurate diagnosis of BRD is paramount to ensuring treatment success and reduced losses due to excessive morbidity and mortality. Appropriate diagnostic criteria should be objective and repeatable, utilizing several clinical parameters, including rectal temperature, nasal and eye discharge, ear position, and the presence or absence of a cough (Lago et al., 2006). The use of an objective screening program during the preweaning phase may allow identification of up to 85% of active cases and contribute to reducing the number of calves weaned with active respiratory infections (McGuirk, 2008). Several antibiotic treatments are avail-

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able for use in the replacement heifer industry but little published work exists comparing the efficacy of these products in this class of animal. Inappropriate choice and application of therapy may lead to the development of a chronic respiratory disease, further dampening the future productivity of these animals. The effects of BRD in the preweaned calf on the long-term productivity and survival of the calf have not been fully described. In Colorado beef calves, respiratory disease during the neonatal period accounted for a 16.5-kg reduction in weaning weight (Wittum et al., 1994). Additional beef feedlot studies have attributed a reduction in ADG and carcass yield characteristics to respiratory disease in the feeding period (Smith, 1998; Babcock et al., 2009; Schneider et al., 2009). Disease associated with BRD in the early feeding period may prolong the number of days on feed and increase the number of therapeutic interventions required before harvest (Babcock et al., 2009). It is assumed that preweaned dairy calves suffering from respiratory disease experience a reduction in ADG, decreased weaning weight, increased mortality risk, and decreased productivity and survival following weaning, as seen in heifers treated for clinical respiratory disease following antiinfective treatment at weaning (Stanton et al., 2012). Heifers experiencing 4 or more cases of BRD before first calving may have lower odds of completing the first lactation than those not experiencing BRD (Bach, 2011).

Commingled dairy calves may represent an efficient vehicle for the spread of respiratory pathogens within a herd. In a large study, Swedish calves housed in a large group automated-feeding system were at 2.2 times greater odds of respiratory disease in the first 90 d of life compared with those in individual housing (Svensson et al., 2003). Other studies also indicated that individually housed calves may have reduced incidence and risk of respiratory disease than those housed in group environments (Hanekamp et al., 1994; Lago et al., 2006).

Tulathromycin (**TUL**; Draxxin, Pfizer Animal Health, New York, NY) and enrofloxacin (**ENR**; Baytril 100, Bayer Animal Health, Shawnee Mission, KS) are widely used in the beef and dairy replacement industry for the treatment of respiratory disease, but little clinical research exists directly comparing the use of these products in preweaned dairy calves. A previous study examining treatment options in feedlot respiratory disease at 2 feedlots in the western United States showed a higher therapeutic success following treatment with TUL (87.9 and 80%) over ENR (70.2 and 62.5%) with significant results ($P = 0.009$ and $P = 0.031$, respectively; Robb et al., 2007). There is little published research analyzing the effect and efficacy of various treatment options in preweaned dairy heifers.

Isolates of *M. haemolytica*, *P. multocida*, and *H. somni* submitted to diagnostic labs from 2000 to 2009 indicate a gradual decrease in the susceptibility of the primary respiratory organisms to the major antibiotics available for treatment, demonstrating the necessity for current research (Portis et al., 2012).

The primary objective of this randomized and blinded field trial was to examine the efficacy of treatment of clinically identified preweaning calf pneumonia with TUL or ENR on the likelihood of re-treatment. A secondary objective was to examine the effect of treatment for respiratory disease with TUL or ENR on preweaning ADG. The null hypothesis of this study was that re-treatment rates and ADG would not differ between heifers treated with either TUL or ENR.

MATERIALS AND METHODS

This study was conducted at 4 commercial dairy facilities with on-site heifer development programs. One facility was located in Georgia, and the other 3 facilities were located in New York State. One facility (farm D) in New York State raised additional replacement females from birth through breeding for a separate dairy operation. A sample size of 285 calves per treatment group was estimated to provide a power of 80% to detect a 10% difference in treatment efficacy with $\alpha = 0.05$. Enrollment in the study occurred between December 2011 and July 2012. Bull calves were excluded from enrollment in this study. This study was approved by the University of Georgia College of Veterinary Medicine Clinical Research Committee.

Calf Management

Heifers reared on farm A in Georgia received approximately 3.8 L of pasteurized frozen colostrum by esophageal feeder within 2 h of birth. Heifers were vaccinated at 7 d of age with a modified live intranasal vaccine containing bovine respiratory syncytial virus, infectious bovine rhinotracheitis virus, and parainfluenza-3 virus (Inforce 3, Pfizer Animal Health). For the first 7 to 14 d following birth, heifers were housed in individual hutches and fed 2.8 L of 28:20 milk replacer (Cornerstone, Purina Mills, Gray Summit, MO) mixed to 13 to 14% total solids as measured by a Brix refractometer (PA202x, Misco, Cleveland, OH) twice each day. Between 7 and 14 d of age, heifers were grouped by age into a pen of 18 to 25 calves and moved into group housing in an open-sided barn where milk feeding was provided by an automated feeding system (CF1000, DeLaval, Kansas City, MO). Straw bedding was provided in each pen. Beginning in February 2012, half of the heifers were randomly allo-

cated to receive 1 mL of injectable trace mineral (Multimin 90, Multimin USA, Fort Collins, CO) containing zinc (60 mg/mL), manganese (10 mg/mL), selenium (5 mg/mL), and copper (15 mg/mL) at 1 d of age and again at 13 to 16 d of age. Antimicrobial agents used for respiratory treatment before the field trial included oxytetracycline (Bio-mycin 200, Boehringer Ingelheim Vetmedica, St. Joseph, MO), enrofloxacin, and tulathromycin.

Heifers reared on farm B in New York received approximately 3.8 L of refrigerated colostrum before milk feeding. Beginning in May 2012, farm B began administering a modified live intranasal vaccine containing bovine respiratory syncytial virus, infectious bovine rhinotracheitis virus, and parainfluenza-3 virus (Inforce 3, Pfizer Animal Health) at approximately 14 d of age. Calves were housed both in individual wire pens on wood shavings and in group pens on straw bedding of 18 to 25 animals in a closed barn. Calves were fed pasteurized nonsaleable milk from December 2011 to April 2012 and then transitioned to acidified nonsaleable milk for the remainder of the trial. From birth to 4 wk of age during the pasteurized milk feeding period, calves were allowed access to 4 L/d; after 4 wk, calves were fed 6 to 8 L/d, dependent upon appetite. After April 2012, acidified nonsaleable milk was provided *ad libitum* to the calves in the group pens. Before the onset of the field trial, antimicrobial agents used for respiratory treatments included tilmicosin (Micotil, Elanco Animal Health, Greenfield, IN), tulathromycin, florfenicol (Nuflor, Intervet Inc., Merck Animal Health, Summit, NJ), and enrofloxacin.

Heifers raised on farm C, located in New York, fed each calf approximately 3.8 L of previously frozen colostrum immediately following birth. No vaccines or supplements were provided to the calves before the onset of the study. Calves were housed individually in wire pens on straw bedding inside a barn. Calves were fed 2 to 3 L/feeding twice daily. Antimicrobial agents previously used for respiratory treatments included tilmicosin, tulathromycin, florfenicol, and enrofloxacin.

Farm D, located in New York, reared additional replacement heifers for a nearby farm. Heifers from both farms received 3.8 L of fresh colostrum after birth. All calves received a modified live intranasal vaccine containing bovine respiratory syncytial virus, infectious bovine rhinotracheitis virus, and parainfluenza-3 virus (Inforce 3, Pfizer Animal Health) at approximately 14 d of age. Heifers were housed in group pens of 22 calves per group on straw bedding. Calves were allowed to consume up to 12 L/d of acidified nonsaleable milk from an automated feeding system (CF1000, DeLaval). Antimicrobial agents previously used for respiratory treatments included tilmicosin, tulathromycin, florfeni-

col (Resflor Gold, Intervet Inc., Merck Animal Health), lincomycin (Lincocin, Pfizer Animal Health), and enrofloxacin.

Dystocia scores were recorded for all calvings on a scale from 1 to 4, where 1 = no assistance, 2 = minor assistance but hands did not extend beyond the vulva, 3 = hard pull or calf manipulation required, 4 = hard pull with calving equipment or Caesarean section. All heifers were weighed (Waypig 505, Raytek, Ephrata, PA) and received a hip height measurement (Nasco Measuring Stick C11503N, Nasco, Fort Atkinson, WI) within 24 h of birth by farm or research personnel. A serum sample was collected by research personnel from 2 to 6 d of life and evaluated for evidence of passive transfer. Heifers born in Georgia with a serum total protein score ≥ 5.2 g/dL, as measured by refractometry (PA202x, Misco), were considered to have adequate passive transfer (Tyler et al., 1996). Failure of passive transfer (**FPT**) for heifers born in New York State was evaluated using a Brix refractometer with a serum cutoff value of 8.3% (Caldwell and Wenz, 2012). The data were transformed from both research sites into a positive/negative dichotomous outcome and were not analyzed on a linear scale. At the time of serum collection, an ear notch biopsy was collected from each calf to identify the presence of any heifers persistently infected with bovine viral diarrhea virus. All ear notch samples were submitted to the Athens Veterinary Diagnostic Laboratory for individual antigen-capture ELISA testing.

Enrollment Criteria

Heifers were evaluated by trained observers for evidence of respiratory disease 3 times/wk until weaned using a variation of a clinical scoring system previously published for use in dairy calves (Table 1; Lago et al., 2006). Heifers were visually evaluated for the presence and severity of nasal discharge, ocular discharge, cough, and head tilt. Heifers did not have their rectal temperature evaluated unless the cumulative score for all other score variables was ≥ 2 . One farm in New York State utilized farm labor for 2 of the weekly observations, whereas research staff completed the remaining observations. Heifers more than 4 d of age with a cumulative clinical score > 4 were eligible for enrollment. Any calf requiring treatment was weighed and measured for hip height. At the time of enrollment, a coin flip was used to randomly allocate heifers to treatment with TUL at 2.5 mg/kg or ENR at 12.5 mg/kg administered subcutaneously. All treatments were administered by farm or research personnel not responsible for observation or scoring of the heifers. Likewise, calf observers were blinded to treatment group.

Table 1. Scoring system for calf respiratory disease (Lago et al., 2006)

Item	Points allocated			
	0	1	2	3
Rectal temperature, °F	<101	101–101.9	102–102.9	>103.0
Cough	None	Induce single	Induce repeated or occasional spontaneous cough	Repeated spontaneous coughing
Nasal discharge	Normal serous	Small amount of unilateral, cloudy discharge	Bilateral, cloudy or excessive mucous	Copious bilateral, mucopurulent nasal discharge
Eye or ear	Normal	Mild ocular discharge	Bilateral purulent ocular discharge or unilateral ear drop	Heat tilt or both ears dropped

Exclusion Criteria

Heifers that had been treated with antimicrobial agents for any reason, including respiratory disease, diarrhea, sepsis, or injury, before diagnosis by research personnel were ineligible for enrollment. Treatment with flunixin meglumine also excluded calves from enrollment into the field trial. Any calves with protocol deviations or additional treatment by farm personnel not approved by research personnel were excluded from the trial. Calves initially enrolled by research personnel and re-treated by farm personnel without observation by research staff before the 7-d treatment moratorium were excluded from analysis. Treatment was not withheld from any animal for any reason. Table 2 describes the number of calves excluded from the trial due to protocol deviation or early treatment.

Microbiological Culture and Sensitivity

Deep nasopharyngeal swabs were collected on a convenience sample of approximately 10% of calves from each farm at the time of initial enrollment for bacterial flora description. An 82.5-cm-long, double-guarded swab (J273 Jorgensen Double Guarded Culture Swab, Jorgensen Labs, Loveland, CO) was introduced into each naris, stopping at the length estimated to correspond with the nasopharyngeal region, approximately 12 to 15 cm. One sample from each calf tested was placed into culture medium (Stuart's Liquid Media, Jorgensen Labs) to identify *M. haemolytica*, *P. multocida*, *H. somni*, or any other potential bacterial pathogen. A second sample was placed into charcoal medium (Amies Agar Gel with Charcoal, Fisher Scientific, Pittsburgh, PA) for *Mycoplasma* spp. isolation. All samples were submitted to the Athens Veterinary Diagnostic Laboratory (Athens, GA) for microbiological culture and antimicrobial susceptibility testing.

Primary Outcome Assessment

The experimental unit was the individual animal. Following enrollment, all heifers continued to be evalu-

ated 3 d/wk for evidence of worsening severity or clinical cure. Heifers were ineligible for re-treatment for 7 d after enrollment unless disease severity worsened and the farm requested the calf be removed from the trial. Any calf treated by farm personnel less than 7 d after initial treatment was considered a treatment deviation and excluded from the analysis. Any calf requiring re-treatment within 10 d of the initial treatment was considered a primary treatment failure and was re-treated with the initial antibiotic. Any calf requiring a third treatment within 10 d of the re-treatment was considered a chronic case and was treated with the commercially available florfenicol (40 mg/kg) and flunixin meglumine combination. If additional treatments were necessary, the standard treatment outline established by the dairy was followed. An attempt was made to perform a necropsy on each deceased animal. If the deceased animal was under treatment observation and the cause of death was determined to be BRD, that animal was considered a treatment failure. Respiratory disease that occurred more than 10 d after a treatment was considered to be a separate episode of illness (Bach, 2011). For calves experiencing more than one episode of respiratory disease before weaning, only the first episode was considered in the statistical analysis.

Table 2. Number of calves dropped from study by treatment group

Reason for exclusion	Treatment ¹	
	TUL	ENR
First treatment <8 d of age	11	11
Second treatment <7 d by farm	6	8
Protocol deviation by farm ²	14	10
Total	31	29

¹TUL = tulathromycin (Draxxin, Pfizer Animal Health, New York, NY); ENR = enrofloxacin (Baytril 100, Bayer Animal Health, Shawnee Mission, KS).

²Protocol deviations included treatment by farm personnel or veterinary staff for navel ill, sepsis, injury, or pinkeye.

Secondary Outcome Assessment

All heifers were weighed at birth and weaning on a commercial livestock scale (Waypig 505, Raytek) and received a final clinical evaluation. A 60-d adjusted weaning weight was calculated to standardize weaning age between farms and calculated as follows: adjusted weaning weight = (actual weaning weight – birth weight)/actual age at weaning $\times$ 60 + birth weight. Average daily gain was calculated as follows: ADG = (adjusted weaning weight – birth weight)/age in days at weaning.

Statistical Analysis

The primary and secondary outcomes were the risk of re-treatment and ADG for calves treated with either TUL or ENR. Univariate associations between categorical predictors and the risk of re-treatment were evaluated using Pearson's chi-squared, and the multivariable analysis was performed using logistic regression. The complete list of explanatory variables for all outcomes evaluated included farm ID, birth weight, birth height, dystocia score, twin status, passive transfer status, drug treatment group, weight at first treatment, height at first treatment, and age at first treatment. Linear regression was used to perform both the univariable and multivariable analyses for ADG. Multivariable model selection proceeded from a maximum main-effects model that included all predictor variables that were associated with the outcome of disease ($P < 0.2$) in the univariate analysis. Variables were removed from the model in a manual backward stepwise fashion until only those with $P < 0.05$ remained. Farm and the treatment variables were retained in all multivariable models regardless of their P -value. After reaching a preliminary main-effects model, the P -values of all previously excluded variables were re-evaluated by adding each one back into the model. All possible 2-way interactions with the treatment variable were considered. The most appropriate form of continuous predictor variables was determined by comparing Akaike's information criterion (AIC) for models that incorporated linear, quadratic, or categorical versions of the variable. The form of the variable yielding the lowest AIC was preferred. Following logistic modeling, data were screened for outliers by examining plots of the predicted probabilities versus delta chi-squared, delta deviance, and delta β statistics. Goodness of fit for the final logistic regression model was evaluated using the Hosmer-Lemeshow goodness-of-fit test (Hosmer and Lemeshow, 2000). Following linear regression modeling, plots of the fitted values versus the Studentized residuals, Cook's distance, and leverage were assessed to identify any potential influential ob-

servations. All tests were performed assuming a 2-sided alternative hypothesis, and $P < 0.05$ was considered statistically significant. Analyses were performed using commercially available statistical software (Stata 12.1, StataCorp, College Station, TX).

RESULTS

In total, 1,208 heifers from 4 rearing operations were evaluated from birth to weaning or death for the presence of respiratory disease. Sixty-seven calves were excluded from the trial because of protocol deviation by farm personnel, leaving a total of 1,141 calves that were eligible for enrollment. Of these calves, 696 (61.0%) had clinical respiratory disease between birth and weaning and were included in the drug treatment trial. The percentage of calves treated for respiratory disease was lower on farm A than on farms B, C, and D ($P < 0.001$; Table 3). After adjusting for farm, only calf birth height was associated with the risk of being treated for respiratory disease ($P < 0.001$). Compared with calves in the middle 2 quartiles of birth height (77–81.9 cm), those with a birth height ≥ 82 cm [odds ratio (95% CI) = 0.55 (0.36, 0.84)] and < 77 cm [odds ratio (95% CI) = 0.46 (0.32, 0.67)] had a lower risk of treatment for respiratory disease (model not shown). Fifty-six calves died over the course of the study, although only 6 calf deaths (10.7%) were determined to be due to respiratory disease based on necropsy findings, and only 4 of the respiratory deaths occurred in calves that had previously been treated (Table 3). Randomization yielded drug treatment groups that were similar with respect to the distribution of calf characteristics at the time of treatment assignment (Table 4). The randomization procedure enrolled 344 heifers into treatment with TUL and 352 heifers into treatment with ENR. We observed no adverse events associated with the administration of any injections in any calves.

Risk of Re-Treatment Between 7 and 10 Days After Initial Therapy

The risk of re-treatment for respiratory disease between 7 and 10 d of initial treatment is summarized by farm in Table 5. In the univariable analysis, the risk of re-treatment tended toward significance for calves treated with ENR compared with TUL, although this comparison did not achieve statistical significance at the 5% level (27.6% vs. 21.2%; $P = 0.05$). Univariable comparisons of the risk of re-treatment for other calf characteristics are summarized in Table 6. Along with clinical score, the age, weight, and height of calves at the time of first treatment were all associated with re-treatment in the univariable analysis. Because weight

Table 3. Characteristics (means, SD in parentheses) of heifer calves born on 4 farms participating in a randomized, blinded trial to compare the efficacy of enrofloxacin and tulathromycin for the treatment of clinical respiratory disease

Farm	State	Born, no.	Birth weight, kg	Died, no. (%)	FPT, ¹ no. (%)	Respiratory disease, no. (%)	ADG, ² kg/d
A	Georgia	368	37.5 (3.8)	48 (13.0)	28 (7.6)	76 (20.7)	0.69 (0.16)
B	New York	488	39.3 (5.7)	4 (0.8)	199 (40.8)	386 (79.1)	0.63 (0.16)
C	New York	116	38.8 (4.1)	1 (0.9)	33 (28.5)	82 (70.7)	0.75 (0.17)
D	New York	169	40.4 (5.0)	1 (0.6)	46 (27.2)	152 (89.9)	0.85 (0.18)
Total		1,141	38.8 (5.0)	54 (4.7)	306 (26.8)	696 (61.0)	0.69 (0.18)

¹Failure of passive transfer.²ADG from birth to weaning.

at the time of first treatment was correlated with both age ($r = 0.81$) and height ($r = 0.81$) at the time of first treatment, only weight at first treatment was considered for inclusion in the multivariable analysis. Results of the multivariable logistic regression are summarized in Table 7. After adjusting for the other variables in the model, calves treated with ENR had 1.5 times higher odds of re-treatment in the 7 to 10 d following initial therapy compared with calves treated with TUL ($P = 0.04$). Compared with calves on farm A, the odds of re-treatment were approximately twice as high for calves on the other farms. Calves with an initial treatment

score >6 had 1.9 times higher odds of re-treatment compared with calves with a treatment score of 5 or 6. Compared with calves in the upper 3 quartiles of BW at the time of initial treatment (≥ 44 kg), those in the lowest quartile (<44 kg) were twice as likely to require re-treatment.

Risk of a Third Treatment Between 7 and 10 Days After Re-Treatment

The risk of receiving a third treatment between 7 and 10 d after the second treatment was higher for

Table 4. Distribution of 696 calves enrolled in a randomized blinded clinical trial by calf characteristic and treatment assignment

Variable	Category	Treatment, ¹ no. (%)		<i>P</i> -value ²
		TUL	ENR	
Farm ID	Farm A (Georgia)	37 (48.7)	39 (51.3)	0.87
	Farm B (New York)	190 (49.2)	196 (50.8)	
	Farm C (New York)	44 (53.7)	38 (46.3)	
	Farm D (New York)	73 (48.0)	79 (52.0)	
Dam parity	1	164 (49.3)	169 (50.8)	0.99
	2	91 (49.7)	92 (50.3)	
	3+	83 (49.4)	85 (50.6)	
Birth weight	<36 kg	81 (50.6)	79 (49.4)	0.42
	36–41.9 kg	150 (46.7)	171 (53.3)	
	≥ 42 kg	110 (52.4)	100 (47.6)	
Calving ease	1	300 (49.7)	304 (50.3)	0.72
	2+	38 (47.5)	42 (52.5)	
Twin	No	322 (49.0)	334 (50.9)	0.52
	Yes	16 (55.2)	13 (44.8)	
FPT ³	No	230 (49.2)	238 (50.9)	0.83
	Yes	114 (50.0)	114 (50.0)	
First treatment score	5–6	288 (49.6)	293 (50.4)	0.86
	7–10	56 (48.7)	59 (51.3)	
First treatment age	0–13 d	78 (49.7)	79 (50.3)	0.85
	14–21 d	91 (51.1)	87 (48.9)	
	22–32 d	90 (50.3)	89 (49.7)	
	>33 d	85 (46.7)	97 (53.3)	
First treatment weight	<44 kg	84 (51.9)	78 (48.2)	0.78
	44–60.9 kg	172 (48.9)	180 (51.1)	
	≥ 61 kg	88 (48.4)	94 (51.7)	

¹TUL = tulathromycin (Draxxin, Pfizer Animal Health, New York, NY); ENR = enrofloxacin (Baytril 100, Bayer Animal Health, Shawnee Mission, KS).²Chi-squared test of independence.³Failure of passive transfer.

Table 5. Number of calves re-treated

Farm ID	Treatment, ¹ no./total (%)		<i>P</i> -value ²
	TUL	ENR	
Farm A	7/37 (18.9)	5/39 (12.8)	0.47
Farm B	39/190 (20.5)	54/196 (27.6)	0.11
Farm C	11/44 (25.0)	11/38 (29.0)	0.69
Farm D	16/73 (21.9)	27/79 (34.2)	0.09
Total	73/344 (21.2)	97/352 (27.6)	0.05

¹TUL = tulathromycin (Draxxin, Pfizer Animal Health, New York, NY); ENR = enrofloxacin (Baytril 100, Bayer Animal Health, Shawnee Mission, KS).

²Chi-squared test of independence.

calves treated with ENR than with TUL (9.3 vs. 4.1%; $P = 0.007$). In addition to drug treatment, only weight at the time of first treatment was associated with the risk of receiving a third treatment in the univariable analysis (models not shown). Results of the multivariable analysis for the risk of receiving a third treatment are summarized in Table 8. After adjusting for farm and weight at first treatment, calves treated with

ENR had 2.6 times higher odds of being treated a third time within 10 d of the second treatment ($P = 0.004$). Compared with calves on farm A, the odds of receiving a third treatment were approximately 5 to 6 times higher for calves on the other farms. Weight at first treatment was negatively associated with the odds of a third treatment; for each 1-kg increase in weight at initial treatment, the odds of requiring a

Table 6. Univariate analysis of potential risk factors for re-treatment of respiratory disease in 696 dairy calves in a randomized blinded clinical trial

Variable	Category	Enrolled, no.	Re-treated, no. (%)	<i>P</i> -value ¹
Farm ID	Farm A (Georgia)	76	12 (15.8)	0.20
	Farm B (New York)	386	93 (24.1)	
	Farm C (New York)	82	22 (26.8)	
	Farm D (New York)	152	43 (28.3)	
Treatment ²	TUL	344	73 (21.2)	0.05
	ENR	352	97 (27.6)	
First treatment age	0–13 d	157	43 (27.4)	0.01
	14–21 d	178	52 (29.2)	
	22–32 d	179	47 (26.3)	
	≥33 d	182	28 (15.4)	
First treatment score	5–6	581	131 (22.6)	0.01
	7–10	115	39 (33.9)	
First treatment weight	<44 kg	162	56 (34.6)	0.001
	≥44 kg	534	114 (21.4)	
First treatment height	<82 cm	170	51 (30.0)	0.009
	82–84 cm	162	45 (27.8)	
	85–87 cm	168	40 (23.8)	
	≥88 cm	175	27 (15.4)	
Birth weight	<39 kg	318	88 (27.7)	0.07
	≥39 kg	373	81 (21.7)	
Birth height	<80 cm	289	77 (26.6)	0.14
	≥80 cm	381	83 (21.8)	
Dam parity	1	333	86 (25.8)	0.74
	2	183	42 (23.0)	
	≥3	168	40 (23.8)	
Calving ease	1	604	153 (25.3)	0.20
	≥2	80	15 (18.8)	
Twin	No	656	164 (25.0)	0.17
	Yes	29	4 (13.8)	
FPT ³	No	468	112 (23.9)	0.66
	Yes	228	58 (25.4)	

¹Chi-squared test of independence.

²TUL = tulathromycin (Draxxin, Pfizer Animal Health, New York, NY); ENR = enrofloxacin (Baytril 100, Bayer Animal Health, Shawnee Mission, KS).

³Failure of passive transfer.

Table 7. Multivariable logistic regression model predicting re-treatment for clinical respiratory disease within 7 to 10 d of initial diagnosis in 696 heifers with complete covariate information¹

Variable	Coefficient (SE)	Odds ratio (95% CI)	P-value ²
Farm ID			0.18
A	Referent	Referent	Referent
B	0.546 (0.342)	1.7 (0.88, 3.4)	0.11
C	0.795 (0.410)	2.2 (0.99, 4.9)	0.05
D	0.752 (0.368)	2.1 (1.0, 4.4)	0.04
Treatment ³			
TUL	Referent	Referent	Referent
ENR	0.380 (0.182)	1.5 (1.0, 2.1)	0.04
First treatment score			
5–6	Referent	Referent	Referent
7–10	0.629 (0.227)	1.9 (1.2, 2.9)	0.006
First treatment weight			
<44 kg	0.729 (0.200)	2.1 (1.4, 3.1)	<0.001
≥44 kg	Referent	Referent	Referent
Constant	–2.210 (0.349)	NC ⁴	<0.001

¹Hosmer-Lemeshow goodness of fit ($P = 0.95$).²Based on Wald statistics.³TUL = tulathromycin (Draxxin, Pfizer Animal Health, New York, NY); ENR = enrofloxacin (Baytril 100, Bayer Animal Health, Shawnee Mission, KS).⁴Not calculated.

third treatment for respiratory disease decreased by approximately 7%.

Average Daily Gain

We observed no difference in ADG [mean (SD)] between calves treated with ENR [0.694 (0.179) kg/d] or TUL [0.691 (0.193) kg/d; $P = 0.86$]. In the univariable analysis of treated calves, ADG was associated with farm, weight at first treatment, birth weight, birth height, calving ease category, FPT, and being a twin (models not shown). Results of the multivariable linear regression analysis are summarized in Table 9.

After adjusting for the other variables in the model, we detected no difference in the mean ADG of calves that received ENR compared with TUL ($P = 0.60$). Compared with that of calves on farm A, the mean ADG of calves on farm B was lower, and that of calves on farms C and D was higher. Average daily gain was associated with the weight of calves at the time of first treatment; for every 1-kg increase in weight at treatment, ADG increased by an average of 0.005 kg/d. In contrast, for every 1-kg increase in birth weight, mean ADG decreased by 0.005 kg/d. Twin calves had a mean ADG 0.063 kg/d lower than calves that were born as singletons.

Table 8. Multivariable logistic regression model predicting a third treatment for clinical respiratory disease within 10 d following a second treatment in 682 heifers with complete covariate information¹

Variable	Coefficient (SE)	Odds ratio (95% CI)	P-value ²
Farm ID			0.30
A	Referent	Referent	
B	1.898 (1.030)	6.7 (0.89, 50)	0.06
C	1.571 (1.115)	4.8 (0.54, 43)	0.16
D	1.780 (1.065)	5.9 (0.74, 48)	0.10
Treatment ³			
TUL	Referent	Referent	
ENR	0.970 (0.338)	2.6 (1.4, 5.1)	0.004
First treatment weight, kg	–0.072 (0.017)	0.93 (0.90, 0.96)	<0.001
Constant	–1.362 (1.288)	NC ⁴	0.29

¹Hosmer-Lemeshow goodness of fit ($P = 0.87$).²Based on Wald statistics.³TUL = tulathromycin (Draxxin, Pfizer Animal Health, New York, NY); ENR = enrofloxacin (Baytril 100, Bayer Animal Health, Shawnee Mission, KS).⁴Not calculated.

Table 9. Multivariable linear regression model predicting ADG (kg/d) from birth to weaning in 669 heifers with clinical respiratory disease that had complete covariate information

Variable	Coefficient (SE)	95% CI	P-value ¹
Farm ID			<0.001
A	Referent	Referent	
B	−0.074 (0.019)	−0.111, −0.037	<0.001
C	0.056 (0.025)	0.007, 0.104	0.02
D	0.165 (0.022)	0.123, 0.207	<0.001
Treatment drug ²			
TUL	Referent	Referent	
ENR	−0.006 (0.012)	−0.029, 0.017	0.60
First treatment weight, kg	0.005 (0.001)	0.004, 0.006	<0.001
Birth weight, kg	−0.005 (0.001)	−0.008, −0.003	<0.001
Twin			
No	Referent	Referent	
Yes	−0.063 (0.030)	−0.121, −0.004	0.04
Constant	0.651 (0.050)	0.552, 0.749	<0.001

¹Based on *F* statistics.²TUL = tulathromycin (Draxxin, Pfizer Animal Health, New York, NY); ENR = enrofloxacin (Baytril 100, Bayer Animal Health, Shawnee Mission, KS).

Microbial Isolation

Bacterial respiratory pathogens isolated from a convenience sample of approximately 10% of the enrolled calves from each farm are summarized in Table 10. *Mycoplasma* spp. were the most commonly isolated pathogens, being identified in 35.7% of samples submitted, followed by *P. multocida* (32.9%) and *M. haemolytica* (10.0%). In vitro resistance to TUL was identified in 2 *P. multocida* isolates submitted from farm A. Resistance to TUL or ENR was not observed in any other isolates.

DISCUSSION

This study evaluated the efficacy of 2 antibiotics for the treatment of preweaning respiratory disease in Holstein heifers. Previous studies have established a clear link between the risk of respiratory disease and decreased productivity in replacement dairy animals (Schmoldt et al., 1979; Virtala et al., 1996; Rerat et al., 2012; Stanton et al., 2012). A recent study examining treatment of calves at weaning with antiinfective agents

described a negative effect on growth performance for calves experiencing BRD following weaning (Stanton et al., 2010). For those calves experiencing respiratory disease, little scientific evidence exists comparing therapeutic options.

Overall incidence of respiratory disease was 61.0% (20.7 to 89.9%), which is higher than that reported in previous studies (Virtala et al., 1996; Lago et al., 2006). Commingling was the primary method of housing for 3 of the 4 dairies, possibly increasing the risk of respiratory disease in this study (Hanekamp et al., 1994; Svensson et al., 2003; Lago et al., 2006). The scoring method used had a goal of identifying up to 85% of all calves with respiratory disease; frequent use may aid in the early identification of calves at the most effective time for antimicrobial therapy (McGuirk, 2008). First treatment score was an important predictor of the odds of re-treatment, indicating the importance of correct diagnosis. This may allow for more directed therapy to those calves with more severe clinical disease, reducing the risk of relapse and further disease progression.

Age, weight, and height at first treatment were strongly correlated ($r > 0.80$), and weight was retained

Table 10. Isolation frequencies for bacterial respiratory pathogens obtained from nasopharyngeal swabs of 70 calves with clinical respiratory disease¹

Farm ID	No.	No growth	<i>Mannheimia haemolytica</i>	<i>Pasteurella multocida</i> ²	<i>Histophilus somni</i>	<i>Bibersteinia trehalosi</i>	<i>Moraxella</i> spp.	<i>Mycoplasma</i> spp.
A	10	0	0	10	3	1	1	9
B	35	8	7	5	0	0	0	11
C	9	5	0	1	0	0	4	0
D	16	0	0	7	0	0	0	5
Total	70	13	7	23	3	1	5	25

¹More than one pathogen was isolated from some swabs.²Two *P. multocida* isolates from farm A were resistant to tulathromycin.

for use within the multivariable model. Calves in the lowest quartile of BW at first treatment had twice the odds of requiring a second treatment within 7 to 10 d compared with calves with a higher BW. This is not explained by the possibility of increased days at risk for those treated at a lighter weight or age, due to the re-treat eligibility limits set at the onset of the trial. Instead, additional challenges in the form of nutritional deficiencies or other infectious diseases, limiting the ability of the calf to respond to natural disease challenge, may explain this finding.

Treatment with ENR was associated with higher odds of requiring a re-treatment in the 7 to 10 d following initial therapy and with increased odds of requiring a third treatment within 7 to 10 d of re-treatment. This corresponds with previous work in beef cattle indicating greater therapeutic success for the treatment of respiratory disease with TUL (Robb et al., 2007). Of those calves treated for an initial case of respiratory disease and not re-treated in the 7 to 10 d following initial therapy, 115 calves in each group were diagnosed with a second case of respiratory disease between 11 and 38 d following the first diagnosis. It should be noted that ENR was numerically superior to TUL on farm A, which had a lower incidence of calf BRD compared with the other farms. Additionally, *in vitro* resistance to TUL was found on farm A, indicating another possible reason for the numerical advantage. The duration of effectiveness of ENR is reportedly shorter than that of TUL, potentially biasing re-enrollment toward calves in the ENR treatment group. This concern was shared during the initial planning of the study and was approved by the sponsoring organization.

Within this study, 6 of 54 (11.1%) deceased calves were identified through field necropsy to have pneumonic lesions contributing to their death during the preweaning phase of development. The increased incidence of death loss on farm A was primarily attributed through field and diagnostic laboratory necropsy to early neonatal infection with *Cryptosporidium parvum* and *Salmonella* spp. Increasing evidence indicates that heifers experiencing respiratory disease early in life are at increased risk of death or herd removal before calving or during the first lactation (Waltner-Toews et al., 1986; Bach, 2011; Stanton et al., 2012).

Failure of passive transfer was not an important predictor of the risk of respiratory disease, mortality, or improvement in ADG in neonatal calves, conflicting with previous reports (Wittum and Perino, 1995). This may be explained by the high incidence of respiratory disease and the increased odds of treatment for all calves observed during the study. This does not preclude the potential role of FPT as a risk factor in the development of respiratory disease but rather emphasizes the

potential that other risk factors may predispose calves to increased disease. Brix refractometry, utilized by the research staff in New York State (farms B, C, and D), measures the percentage of total dissolved solids present within the serum, whereas traditional refractometry, utilized by the research staff in Georgia (farm A), measures protein dissolved in the serum in grams per deciliter, serving as an indirect measurement of dissolved IgG transmitted through colostrum feeding (Tyler et al., 1996). The difference in measurement methods was due to miscommunication not noted until research data began to accumulate. Recent work evaluating the use of Brix refractometry as an alternative to traditional refractometers has shown a high correlation [$R^2 = 0.95$; D. V. Nydam L. Stephens, and L. Stanko (all from Cornell University, Ithaca, NY), unpublished data] and Brix refractometry appears promising (Caldwell and Wenz, 2012) as an alternative to traditional serum refractometry.

The results of the current study did not indicate an effect on ADG during the preweaning period as a result of treatment, conflicting with previous work that demonstrated a reduction in weight and height gains due to disease in each of the first 3 mo of life and decreased growth performance in the postweaning period (Virtala et al., 1996; Stanton et al., 2010). However, the high incidence of treatment on 3 of the farms in this study may have obscured any potential effect on ADG because those animals not demonstrating clinical disease may have been affected with subclinical disease that negatively affected the rate of growth. Additionally, procedural drift and variation in nutrition and management between farms may have clouded our ability to accurately identify the potential decrease in productivity associated with preweaning respiratory disease. Conversely, frequent observations by trained research staff utilizing objective scoring criteria as well as the elevated plane of nutrition offered by these dairies may allow for earlier therapeutic intervention for those experiencing clinical disease, reducing the effect of respiratory disease on growth performance. Despite the increased risk of re-treatment for calves treated with ENR, we found no statistical difference in ADG between treatment groups.

CONCLUSIONS

Treatment with ENR was associated with increased odds of re-treatment compared with calves initially treated with TUL. However, in clinical practice, each farm should be considered individually when making treatment recommendations because varying levels of efficacy may exist. The high incidence of treatment of calves within this study may obscure the effect of respi-

ratory disease on ADG or weaning weight. Future work is warranted to further delineate the effect of early calfhoo morbidity on lifetime productivity, including ADG, time to first conception, survival, and lifetime milk yields. As additional antimicrobial agents become available, it is increasingly important to examine the efficacy of these products to determine best treatment practices in light of changing management practices and possible microbiological resistance.

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