Antibiotic Resistance in Gut Bacteria from Dairy Calves: A Dose Response to the Level of Antibiotics Fed in Milk

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ABSTRACT

Dairy calves are commonly fed milk from cows treated with antibiotics. The concentration of β -lactam antibiotic residues found in milk from treated cows was used to determine the range of concentrations of penicillin used in a dose-regulated experiment. Thirty-one Holstein calves were randomly assigned to milk with penicillin G added at concentrations of 0, 6.25, 12.5, 25, and 50 μ l/kg. Fecal swabs were taken from each calf twice weekly. Resistance to penicillin was tested by measuring the zone of inhibition in bacterial growth around a disk impregnated with the antibiotic. Inhibition was greatest for bacteria from calves fed milk with no penicillin (2.89 ± 0.14 mm), and declined as the penicillin dose provided in the milk increased (to a low of 0.70 ± 0.10 for the 50 μ l/kg treatment group). In conclusion, resistance of gut bacteria to antibiotics increases with increasing concentrations of penicillin in the milk fed to dairy calves.

(Key words: calf management, feeding, waste milk, health)

INTRODUCTION

In the dairy industry it is estimated that between 2 to 55% of all lactations will include a mastitis infection (Kelton et al., 1998), and the majority of these cases are treated with antibiotics via intramammary infusion. Milk must be withheld from sale when cows have mastitis, are being treated with antibiotics, or are on the compulsory withdrawal period after treatment (Kesler, 1981). Although this 'waste milk' cannot be sold, it is often used to feed dairy calves. Waste milk is an economical alternative to other feeds and therefore may be fed in larger quantities or even provided ad

libitum. Antibiotics contained in milk may also act as a growth promoter when fed to calves (Kesler, 1981). The dose of antibiotics in the milk fed to calves is variable and will depend on the number of cases, the method of treatment, the withdrawal period and the amount being fed. There is great controversy over feeding farm animals food containing antibiotics due to the possible emergence of antibiotic resistant bacteria (Sailsbury et al., 2002). Antibiotic resistance among bacterial populations is thought to emerge when antibiotics are used in such a way that, although most bacteria will be susceptible, a proportion of the more resistant bacteria survive. The genetically resistant bacteria, which are not competitive without the antibiotic challenge, prosper on the resources that would have been utilized by their drug-sensitive counterparts (Heinemann et al., 2000).

A number of earlier studies have found that milk from cows treated with antibiotics can be acceptable as food for calves in terms of measures of clinical health and growth (Chardavoyne et al., 1979; Keys et al., 1979; Kesler, 1981; Loveland et al., 1983). Two other studies specifically addressed the issue of antibiotic-resistant bacterial growth in the calf gut. One study (Wray et al., 1990) found no differences in the prevalence of antibiotic resistant *Escherichia coli* in the feces of calves fed milk from cows treated with antibiotics versus those fed a milk replacer. However, the milk used in this trial had sufficiently high bacterial counts that calves often refused to drink the milk and therefore showed low growth rates. In another study (Selim and Cullor, 1997), samples of milk used to feed calves (including milk from treated cows) were tested for viable bacteria numbers and antibiotic resistant bacteria. Both the number of bacteria and antibiotic-resistance were high in the treated milk. Concentrations of antibiotics fed to calves and levels of resistant bacteria in the gut before the introduction of antibiotics were not measured in these previous studies. In this study we provide the first controlled, multiple-dose experiment on the effects of feeding calves' milk with antibiotics.

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MATERIALS AND METHODS

Determining levels of residual antibiotics in milk. Over a 3-wk period, a 50-ml sample of milk was taken at each milking from each of 11 cows being treated with penicillin G (Special formula, Upjohn Laboratories, Ontario, Canada). Samples were also taken during the 72-h withdrawal period. All samples were stored at -20°C and thawed at 8°C for 24 h before testing. Samples were tested using the LakTek Lacstation II Beta Lactam testing kit (IdeTek Inc. Sunnyville, CA) in the calibration mode (Bell and Scanella, 1994).

Calf feeding and housing. A total of 31 Holstein calves (16 males and 15 females) were used over an experimental period in the summer of the year 2000. Each calf spent 2 to 12 h after birth with its dam. Calves were separated and moved to the calf barn, where calves were kept in individual pens (1.7×1.2 m), throughout the 37-d trial. The calves had ad libitum access to water throughout the experiment. Within the first 24 h after birth calves were given at least 4 L of their dam's colostrum. During the following 24 h, calves received a mixture of colostrum from all that was produced on the day. Therefore all calves may have received a proportion of colostrum from heifers and from second-plus parity cows. The quality of the colostrum was not measured. During subsequent days calves were provided ad libitum access to milk via a nipple feeding system (Appleby et al., 2001). Calves were given new milk two times per day, and consumption was measured. Calf weights and solid feed intakes were measured two times per week. Calves were scored as experiencing diarrhea whenever a fecal consistency of three or four (Larson et al., 1977) was observed. When diarrhea was present, calves were given a 1-L electrolyte solution four times daily and 100 ml of Kaopectate twice daily. Calves were cared for according to the standards of the Canadian Council on Animal Care and a protocol approved by the University of British Columbia's Animal Care Committee.

Treatment levels. The 75th quartile level of β -lactam from the treated milk was used as the highest concentration given to calves. This corresponded to 50 μl of 10,000 IU/ml of penicillin G solution added to each kilogram of milk. Three dilutions by half (i.e., 25, 12.5, and 6.25 $\mu\text{l/kg}$) were used as the other treatment groups. No antibiotic was fed to the control group. The penicillin was added to 5 kg of milk in the morning feeding. The amount consumed was recorded before the evening feeding. Penicillin was added for 3 consecutive days each week. Untreated milk was given for the remaining 4 d. Baseline values for gut bacteria were measured before the first 3 d of penicillin feeding started at 4 to 10 d of age. At the end of the trial (d 37), all calves were given a therapeutic dose of neosulphate (12

$\times 500$ mg over 4 d) with the aim of eliminating the penicillin-resistant strains.

Bacterial and statistical analysis. Rectal swabs (Culturette bacterial transport system, Becton Dickinson, Cockeysville, MD) were taken twice a week: on the first days calves were fed treated and untreated milk. Each swab was put into a test tube containing 2 ml of peptone broth (Difco, Becton Dickinson Microbiology Systems). The tubes were mixed for 30 s to release fecal material into the peptone broth. Samples were diluted to 10^{-2} concentration. A 200- μl aliquot of each sample was added to two plates containing a soy tryptase agar (BBL prepared media, Becton Dickinson Microbiology Systems). Each sample was evenly plated out using a sterile glass rod. A 10- μg penicillin disk (BBL Sensidisc, Becton Dickinson) was placed in the center of the plate. The plates were incubated for 18 to 24 h at 37°C . After incubation, the plates were measured for zones of inhibited growth of bacterial colonies by measuring the distance from the edge of the disc to the nearest bacterial colony. The larger the distance from the edge of the disc, the less resistant the bacteria were to penicillin G.

Values from the two plates taken for each sample were averaged, and these average values are presented in Figure 1. Sample averages were then averaged for the two phases of the experiment (i.e., before and after the treatment began). Pretreatment means were analyzed to determine whether there were any unintended differences among groups in baseline resistance, using a one-way ANOVA with the effect of treatment (4 df) tested against an error term with 26 df. Treatments means were tested with the same model, but with pretreatment values included as a covariate.

RESULTS

Antibiotic concentrations in waste milk. The median concentration of β -lactam antibiotics found on the first milking after treatment was 5.6 $\mu\text{l/kg}$ (25 to 75 percentile, ± 1.2). The concentration ranged from 1.8 to 50.4 $\mu\text{l/kg}$. Concentration on the first milking of the withdrawal period was much lower (0.8 $\mu\text{l/kg}$; 25 to 75 percentile, ± 0.05). Concentration decreased to 0.01 $\mu\text{l/kg}$ (25 to 75 percentile, ± 0.01) by the last day of the withdrawal period.

The effects of feeding waste milk to calves. Growth rates and intakes of milk, calf starter, and hay were similar for all treatment groups (Table 1, $P < 0.1$ for all variables). There was also no significant difference between treatment groups in calf BW at the beginning of the treatment period (d 7), and no difference in the incidence of diarrhea.

Figure 1. Mean inhibition values (colony nearest the disk) of bacteria from each calf. Seven calves were tested at the control level (a). Six calves were tested at each of the higher doses (6.25, 12.5, 25, and 50 $\mu\text{L/kg}$; b-e). Values are shown in relation to the time when calves started on treatment (d 0).

The zones of inhibition around the penicillin-impregnated discs showed the extent of antibiotic resistance in the fecal micro flora, with the most resistant having the smallest zone of inhibition. Differences in the amount of penicillin-resistant bacteria are illustrated in Figure 1. The control group showed considerable

variation in inhibition to the disc, during both treatment and pretreatment periods (Figure 1a). Groups receiving 6.25 and 12.5 $\mu\text{L/kg}$ also showed variation in inhibition values but with a trend of lowering central tendencies during the treatment (Figure 1b and c). Groups receiving the highest doses (25 and 50 $\mu\text{L/kg}$) showed low inhibition values throughout the trial once penicillin treatment began (Figure 1d and e).

There was a statistically significant difference in inhibition among treatment groups in the pretreatment values ($P > 0.05$), with the 25 $\mu\text{L/kg}$ group showing the largest values. During the treatment period there was a highly significant effect of antibiotic treatment ($P < 0.0001$), with mean inhibition values decreasing from 2.89 ± 0.14 mm for calves fed milk without penicillin, to 0.70 ± 0.10 for calves fed milk with 50 $\mu\text{L/kg}$ of the antibiotic.

DISCUSSION

Calves were fed ad libitum by nipple, so growth rates were higher than those commonly seen with calves fed restricted quantities of milk (typically 10% of BW per day) (Jasper and Weary, 2002). Growth promoting effects of antibiotics are more likely when animals are living in relatively poor management or housing conditions (Witte, 1998), so it is not surprising that we found no treatment effects on growth rates in this study. Although other work has shown that antibiotics caused lower milk intake by calves, we found no evidence for such an effect over the range of doses we used in this study. Antibiotics in feed may also cause diarrhea from the disturbance of the gut microflora, especially in young animals (Rollin et al., 1986; Nord, 1993), but we found no such effect in the current experiment. Interestingly, recent research (Gunn et al., 2003) suggested that antibiotic-resistant bacteria were more likely to be present on farms with a high incidence of diarrhea in dairy calves, perhaps because some farms attempt to treat scours using antibiotics.

Differences among the treatment groups in the baseline values of resistance may have resulted from previous selection pressures, including from feeding colos-

Table 1. Mean ($\pm$ SEM.) daily milk, calf starter, and hay consumption, weight gain, BW at d 7, and incidence of scouring for calves ($n = 31$) fed milk containing different doses of penicillin.

	Penicillin ($\mu\text{L/kg}$ of milk)				
	0	6.25	12.5	25	50
Milk (kg)	10.19 ± 0.52	10.33 ± 0.99	10.04 ± 1.29	10.89 ± 1.10	10.89 ± 0.94
Starter (kg)	0.11 ± 0.03	0.04 ± 0.01	0.07 ± 0.02	0.09 ± 0.04	0.09 ± 0.04
Hay (kg)	0.04 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Gain/day (kg)	0.96 ± 0.08	0.88 ± 0.13	0.92 ± 0.13	0.99 ± 0.12	0.85 ± 0.14
BW (kg)	51.01 ± 1.7	51.52 ± 3.0	50.31 ± 4.2	50.52 ± 2.2	53.13 ± 2.1
Days scouring	4.14 ± 1.2	5.66 ± 1.8	6.17 ± 2.2	3.33 ± 1.0	6.00 ± 2.4

trum from cows that have been given prophylactic dry-cow mastitis treatments (Loveland et al., 1983; Teuber, 2001). Treatment groups were balanced for birth order, but not for the number of lactations, the barn in which the calf was born, or the amount of time the calf had spent with its dam. Any of these factors may have had an effect on antibiotic resistant bacteria in the calf gut prior to baseline measurements.

Despite the variation in baseline levels, we found that once treatment began, resistance increased in relation to dosage. At the lowest dosage and control levels we found a substantial zone of inhibition around the penicillin disk but at the higher doses the bacterial cultures showed little inhibition. This resistance developed rapidly and persisted over the course of the experiment. During each week, treated calves received the penicillin-treated milk for only 3 d. We saw no evidence of a decline in resistance after 4 d on untreated milk, suggesting that the resistant bacteria are stable and persistent within the general population of fecal bacteria over this period. We do not know how long resistant strains persist once feeding of waste milk ceases, but there is some evidence that resistant bacteria can persist for years after the selection pressure is removed (Nord, 1993).

In conclusion, feeding calves waste milk containing antibiotics increases antibiotic resistant bacteria in the lower gut of calves, especially if fed at higher concentrations such as from cows starting treatment for mastitis. This increase in resistance is a concern both for the health and treatment of the calf and for humans and other animals in the same environment (Lacey, 1987; Witte, 1998).

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