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Efficacy of monensin sodium for the reduction of fecal shedding of *Mycobacterium avium* subsp. *paratuberculosis* in infected dairy cattle

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Abstract

Reducing the quantity of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) being shed by cows with Johne's disease should decrease the risk of spread of this disease to young stock. Previous work has suggested that monensin sodium decreases the pathologic lesions associated with Johne's disease, but the impact on shedding of viable MAP remains unknown. After serologic screening of 32 dairy herds in southwestern Ontario, 228 cows from 13 of these herds were enrolled into a randomized clinical trial. Fecal culture and PCR were used to identify 114 cows as potential fecal shedders, while another 114 cows were enrolled as ELISA negative, herd and parity matched controls. All cows were randomized to receive either a monensin controlled release capsule (CRC) or a placebo capsule. Serial fecal and blood samples were collected for fecal culture and serum ELISA testing over a 98-day period. On day 98 of the study, treatments were switched for all cows continuing in the trial. These remaining cows were followed for another 98 days with a similar sampling protocol. Mixed effect models were used to measure the impact of treatment on the number of colony

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forming units identified on fecal cultures over time. During the first 98 days of the study, cows treated with a monensin CRC were found to shed 3.4 cfu per tube less than placebo treated cows ($P = 0.05$). The serum ELISA S/P ratio was reduced by 1.39 units in cows given monensin ($P = 0.06$). However, treatment with monensin did not reduce the odds of testing positive on serology. Only the cows shedding MAP on day 0 were found to have a reduced odds of testing positive on fecal culture when treated with monensin ($OR = 0.27$; $P = 0.03$). Monensin sodium administered to infected animals at 335 mg/day marginally reduced fecal shedding of MAP in mature dairy cattle, but the biological significance of this reduction is unknown.

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1. Introduction

Johne's disease, or bovine paratuberculosis, has long plagued the cattle industry worldwide (Chiodini et al., 1984). Progressive weight loss and chronic diarrhea are the cardinal signs for advanced stages of the disease. There are currently no drugs approved for the prevention or treatment of Johne's disease. Several drugs have been used to treat individual animals, but none have been shown to be efficacious or economical for treatment of commercial dairy cattle (St-Jean and Jernigan, 1991). Many infected dairy cows are culled prematurely due to low milk production and unthriftiness. When the cost of culling is combined with lost milk revenue, the economic impact of Johne's disease becomes quite significant at both the herd and industry level (Ott et al., 1999). More recently, there has also been increasing human health concerns in regards to a potential link between Johne's and Crohn's disease (Selby, 2000).

Many countries have moved towards developing voluntary control programs to help producers manage Johne's disease. The recommended approach to managing this disease is prevention and control (Rossiter and Burhans, 1996). Minimizing the amount of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) being shed by infected cattle may help to reduce the environmental contamination and risk of spread to susceptible calves (Goodger et al., 1996; Wells and Wager, 2000). Culling fecal shedding cows may help reduce the environmental burden of MAP, but identifying these cattle can be difficult with the current diagnostic tests available.

It has been hypothesized that monensin sodium may be beneficial in limiting fecal shedding and thus the spread of Johne's disease (Brumbaugh et al., 2000). Recent studies have indicated that MAP infected cattle and mice responded favourably to monensin sodium treatment, as demonstrated by an improvement in histological lesions in the gastrointestinal system following therapy (Brumbaugh et al., 1992, 2000). Monensin sodium is a polyether ionophore that modifies the bacterial cell membrane permeability (Prescott et al., 2000). Its large spectrum of activity includes several Gram-positive bacteria, some *Campylobacter* spp., *Serpulina* spp., *Mycobacterium* spp. as well as coccidia and toxoplasma (Prescott et al., 2000; Liu, 1982). Its minimum inhibitory concentration (MIC) for MAP was recently reported to be 0.3 $\mu\text{m}/\text{ml}$ (Brumbaugh et al., 2004). To date, the use of monensin has not been evaluated clinically for its impact on fecal

shedding of MAP, nor has the effect of monensin on diagnostic tests for Johne's disease been assessed.

The objectives of this clinical trial were to investigate the effect of monensin sodium on the quantity of MAP colony-forming units (cfu) shed in the feces of infected animals and to evaluate the influence of monensin treatment on the detection of Johne's disease using a commercial serum ELISA and fecal culture.

2. Materials and methods

2.1. Initial herd screening prior to clinical trial

2.1.1. Herd selection and sample collection

A purposive sample of 32 Ontario dairy herds with a previous history of Johne's disease were nominated by their herd veterinarian for this portion of the study. Herds were required to be enrolled in a milk recording program through dairy herd improvement (DHI) and could neither be feeding monensin in any cow rations nor be using monensin controlled-release capsules. During the months of May through August 2002, each of the farms enrolled were visited once for fecal and blood collection from each of the milking and dry cows. Fecal samples were collected with a lubricated sterile rectal sleeve and placed in 40 ml screw top vials (Starplex Scientific Inc., Mississauga, Ont.). Blood samples were collected from the coccygeal vein using a 10 ml vacuum tube (Red Top Vacutainer, Becton Dickenson, Franklin Lakes, NJ). Serum was harvested from the blood samples and stored with the fecal samples in a -20°C freezer until processing. At the herd's next DHI test, milk samples containing bronopol were collected for routine component testing by DHI staff.

2.1.2. Diagnostic testing

All 32 herds were screened with a milk and serum ELISA. Serum samples were submitted to the Animal Health Laboratory (University of Guelph, Guelph, Ont., Canada) and tested for antibodies to MAP with the IDEXX Johne's HerdChek ELISA (IDEXX Laboratories Inc., Westbrook, MA, USA), according to the manufacturer's instructions. Serum samples with an S/P ratio of greater than or equal to 0.25 were considered positive. The reported sensitivity of the serum ELISA ranged from 15.4% in subclinical cattle to 88.1% in clinical cases, while the specificity was 98.9% (Dargatz et al., 2001). Once the milk samples were processed by the Ontario DHI laboratory, they were sent to AntelBio (Lansing, MI, USA) for an in-house milk ELISA to measure antibodies to MAP. Milk samples with a corrected optical density (OD) of greater than 0.1 were considered positive for Johne's disease. The sensitivity and specificity of the milk ELISA relative to fecal culture has been reported to be 28.9% and 99.7%, respectively (Collins et al., 2005).

Cows identified as positive on either of the ELISAs had their corresponding fecal sample submitted to AntelBio for fecal culture. Fecal samples were cultured onto Herrold's egg-yolk medium using a modified double incubation method (Shin, 1989; Whitlock et al., 1991). A 2 g sample of feces was added to 35 ml of sterile water and mixed on a rotator for 30 min. After settling for 30 min, the top 5 ml portion of the sample was transferred to

25 ml 0.9% (w/v) hexadecylpyridinium chloride (HPC) in brain-heart infusion broth (BHI, 1.85%, w/v) and incubated at 37 °C overnight. The samples were concentrated by centrifugation at $900 \times g$ for 30 min, and the supernatant was decanted. The pellet was re-suspended in 1 ml of an antibiotic solution (0.01% vancomycin, 0.01% naladixic acid, 0.005% amphotericin B in BHI solution) and incubated overnight at 37 °C. A transfer pipette was used to inoculate each of four Herrold's Egg Yolk Media with 250 μ m of the suspension. After drying at 37 °C for approximately one week; the caps were tightened and the samples were incubated another 15 weeks. Samples with colonies were confirmed by polymerase chain reaction (PCR) for the presence of the IS900 genetic element. The mean colony-forming units (cfu) were reported for positive samples. AntelBio was kept blinded as to the common identity of the cows' milk and fecal samples.

Fecal samples were also sent to the Animal Health Laboratory for radiometric culture and direct fecal PCR. Fecal samples for radiometric culture were prepared as described by Eamens et al. (2000) (culture method 5), with one minor variation. The initial decontamination procedure was as described for the conventional fecal culture above. Eamens et al. (2000) inoculated each BACTEC culture vial with 0.1 ml of fecal sediment, however, 0.2 ml of inoculant was used in this study. Vials were incubated at 37 °C for up to 12 weeks. A growth index (0–999) was determined weekly using an automatic ion chamber (BACTEC 460, Johnston Laboratories). Cultures with a positive growth index (>10) were confirmed with PCR, acid-fast staining and subculture onto blood agar to identify any contamination. Direct fecal PCR was completed using the IDEXX Johne's HerdChek PCR Kit (IDEXX Laboratories Inc., Westbrook, MA, USA), as to the manufacturers instructions, to identify the IS900 genetic element. The sensitivity of conventional fecal culture, radiometric (BACTEC) fecal culture and IDEXX direct fecal PCR were reported to be 45.1%, 54.4% and 33.5%, respectively (Sokkett et al., 1992). The specificity of these tests was reported to be 100%.

2.2. Clinical trial

2.2.1. Cow selection and sample collection

It was estimated from previous culture data (AntelBio, Lansing, MI, USA) that an average fecal shedding cow would have 30 cfu per tube (S.D. 28.3). A reduction of 50% or 15 cfu per tube was the anticipated treatment effect to be measured. In total, 55 fecal shedding cows would be necessary in each treatment group if $\alpha = 0.05$ (two-sided) and $\beta = 0.2$.

Thirteen of the original 32 herds were selected to participate in a randomized clinical trial. All herds enrolled had to have a minimum of two fecal shedding cows to be enrolled. In January 2003, 87 cows identified as fecal shedders by conventional or radiometric fecal culture or fecal PCR, were randomized to receive a monensin or placebo controlled-release capsule (CRC). A treatment table was generated by tossing a coin five times where "heads" represented monensin treatment. After each coin toss, the opposing treatment was immediately given so as to keep the table balanced for a total of 10 treatments. A total of three different treatment tables were made and systematically selected to randomize the cattle for each herd. Herd and parity matched, milk and serum ELISA negative control cows were selected and similarly randomized

to treatment or placebo. Monensin CRC (Rumensin[®] CRC) and placebo capsules were supplied by Elanco, a Division Eli Lilly Canada, Guelph, Ont., Canada. The monensin CRC is a sustained-release intraruminal device that has a medicated core containing 32 g monensin in a hexaglycerol distearate matrix (45% monensin), while the placebo CRC contains only the non-medicated matrix. Each capsule delivers its product over an average period of 95 days (Rumensin CRC, Veterinary Reference Guide, Elanco Animal Health, A Division of Eli Lilly Canada Inc.). Capsules were administered orally with a specially designed administration tool. Every device is individually identified with a unique four-digit code. A record of the capsule number administered to each cow treated was recorded along with the ear tag or chain number. Capsules regurgitated prior to payout were re-administered to the appropriate animals at the following visit.

In an effort to increase cow numbers, each of the 13 herds had an additional milk ELISA completed for all milking cows at a DHI test day during the months of January or February 2003. Twenty-seven cows that were not already enrolled on the clinical trial, were found to be milk ELISA positive. These cows were then randomized to treatment or placebo and were sampled with the same frequency as fecal shedding cows. Once again, herd and parity matched ELISA negative control cows were also selected and randomized to the two treatment groups.

Herds were visited every two weeks starting at treatment (day 0). For the cows identified as fecal shedding prior to the clinical trial or milk ELISA positive during January or February 2003, fecal samples were collected at each visit (study days 0, 14, 28, 42, 56, 70, 84, 98, 112, 126, 140, 154, 168, 182, and 196). The sampling strategy used for herd and parity matched ELISA negative controls included collection of feces on days 0, 56, 98, 154 and 196 of the study. All fecal samples were collected as described above, but were stored at -80°C prior to conventional culture at AntelBio. Blood samples were obtained from all enrolled cows on days 0, 56, 98, 154 and 196, for serum ELISA testing as previously described. Cows were body condition scored by the study technician at the time of treatment allocation, and at each subsequent visit, on a scale of 1–5 using 0.25-point increments according to Ferguson et al. (1994). In addition, a subjective fecal scoring system (1 = watery diarrhea to 4 = firm stool) described by Ireland-Perry and Stallings (1993) was used to assess fecal consistency at every farm visit during the trial.

On day 98 of the study, treatments were switched for all cows remaining in the trial. Cows that had originally received a placebo CRC were given a monensin CRC and vice versa. The collection schedule continued until day 196. Lactation number, calving and dry dates for each of the cows were collected from Ontario DHI. The days in milk (DIM) of each cow were then calculated for each sampling date.

2.3. Statistical analysis

Data collected during the study were recorded and maintained in a Microsoft Access database (Microsoft Access 2000, Microsoft Corp., Redmond, WA) and were checked for errors against the written data sheets prior to analysis. All statistical analyses were completed using SAS version 8.2 (SAS Institute, Cary, NC). Descriptive statistics were

generated using the univariate and frequency procedure in SAS (PROC UNIVARIATE, PROC FREQ, SAS version 8.2). The data collected for the entire clinical trial were separated into two datasets. The first dataset included data from day 0 to day 98, and the second dataset included only the crossover data (days 98–196). The number of cfu cultured from each fecal sample was recorded as a continuous variable. Positive cultures that had colonies too numerous to count (TNTC), were coded as 200.

A separate mixed model was generated to quantify the impact of monensin on fecal shedding for each of the above datasets (PROC MIXED, SAS version 8.2). Each dataset was restricted to include only cows shedding MAP on the initial day of study (day 0 or 98). If monensin decreased fecal shedding of MAP relative to the day of treatment (a covariate in the model), then this effect would only be seen in the cattle that were shedding to begin with. That is, monensin cannot decrease the number of cfu per tube below 0 for cattle not shedding on the day of treatment. However, these non-shedding cattle can be evaluated for their probability of testing positive at a later date. Treatment refers to the effect of monensin on cattle that are shedding, while prevention describes the effect of monensin on non-shedding cattle at the start of each study. The terms treatment and prevention have been used as descriptors of the models to evaluate the effect of monensin and do not imply a mode of suggested usage.

Cows with fecal cultures that were TNTC on the day of treatment were excluded from both study datasets because it was not possible to quantify relative changes in fecal shedding. Many of these high shedders continued to have TNTC fecal cultures in subsequent samplings. Fecal shedding cattle were identified on day 0 and 98 from “fecal positive” and ELISA negative control groups. As different sampling strategies were used in these two groups, only the data collected on days 0, 56, 98, 154 and 196 were used in the analyses. The fecal culture results were normalized using a natural logarithmic transformation. Initial fixed covariates in the ‘base mixed models’ included: body condition score (BCS), fecal consistency score (FS), days in milk (DIM), and the number of cfu being shed on day 0. The day of study from which samples were collected was the only time-varying covariate included in the models. Although BCS and FS were both ordinal variables, they were treated as continuous variables in these models. For the crossover dataset, the number of cfu being shed on day 98, replaced the number of cfu from day 0 as an added covariate to the model. Each of the models also included random effect parameters to control for herd variability and the repeated measures from individual cows within these herds. A heterogeneous compound symmetry correlation structure was found to give the best model fit as indicated by Akaike’s information criterion (AIC). All multivariable analyses were carried out using manual backwards elimination at a 95% level of significance based on the likelihood ratio test. Once the model was reduced, all two way interactions with treatment were evaluated for significance. Polynomial terms were tested as means of evaluating the linearity of continuous predictors. Plots of residuals and predicted values were performed to evaluate heteroscedasticity and normality, as well as to identify possible outliers and leverage points. The two ‘base’ mixed models generated were of the form:

$$Y(\text{fecal cfu}) = X_0 + \sum \beta_i X_i + \varepsilon_i$$

where Y is the number of cfu cultured from each fecal sample (dependent variable), X_0 the intercept, X_i the i th covariate (such as treatment, DIM, day of study, etc.), β_i the effect if the i th covariate (slope), and ε_i is the random error term.

Similar mixed models were also constructed to evaluate the influence of treatment on the serum ELISA S/P ratio within each dataset. The S/P ratio of the serum ELISA was transformed using a natural logarithm to normalize the data. Just as the fecal shedding models, analyses were split according to the fecal culture results on the first day of each trial. The S/P ratio was established for all cattle shedding TNTC, so these cattle were included in the analyses. The day of study, DIM, FS, BCS and the S/P ratio on day 0 were all included in the initial model. As with the previous models, only significant variables were included in the final reduced model, unless they were found to be a confounding variable. Final models were assessed for their overall fit of the data as described above. Coefficients were back-transformed to ease interpretation of the effects.

The odds of a monensin-treated cow testing positive on either fecal culture or serum ELISA was also investigated using a generalized linear model. Fecal cultures with colonies of MAP grown were categorized as positive, and serum ELISA S/P ratios greater than 0.25 were considered positive. Multivariable logistic models were then fit for both of these dichotomous dependent variables using the GENMOD procedure, with the logit link function, and a binomial error distribution (PROC GENMOD, SAS version 8.2). The same covariates were initially added as described for previous mixed models. Separate models were constructed for previously fecal shedding and non-fecal shedding cows to evaluate the treatment and preventive effect of monensin. Cows shedding TNTC on the day of treatment were included in these analyses. Since serial fecal culture and serum ELISA results from one cow are not independent, correlation within cow was accounted for using generalized estimation equations (Zeger and Liang, 1986; Zeger et al., 1988). The fecal culture and serum ELISA results were analyzed, acknowledging that there was clustering of samples within cows, and cows within herds. The variance components at both the herd and cow level were evaluated (PROC VARCOMP, SAS version 8.2) to determine which was larger. Since herd had a very low variance component, it was included in the model as a fixed effect and cows within herds as a clustering variable. A compound symmetry correlation structure was used to give equal correlation between fecal cultures and serum ELISA results in a cow. The models would not converge when a parameter was estimated for each individual herd. Instead, herd size and housing type variables were created. Herds were classified as free-stall or tie-stall, and greater or less than 100 cows. The choice of herd size was based on inherent differences in management strategies associated with larger herds. The 'base' linearized logistic regression models can be expressed as

$$\text{logit}(\text{positive test}) = \ln\left(\frac{Y_i}{1 - Y_i}\right) = X_0 + \sum \beta_i X_i + \varepsilon_i$$

where X_0 is the intercept, X_i the i th covariate (such as treatment, DIM, day of study, etc.), β_i the effect if the i th covariate (slope), and ε_i is the random error term.

A manual backwards-stepwise procedure was used once again to determine the final model. The effects of treatment, herd size and housing type were forced into the final model. The estimated regression parameters were converted to odds ratios.

3. Results

A summary of the cattle screened and included in the clinical trial is provided in Fig. 1. Descriptive and summary statistics for both herds and cows enrolled are provided in Tables 1 and 2, respectively.

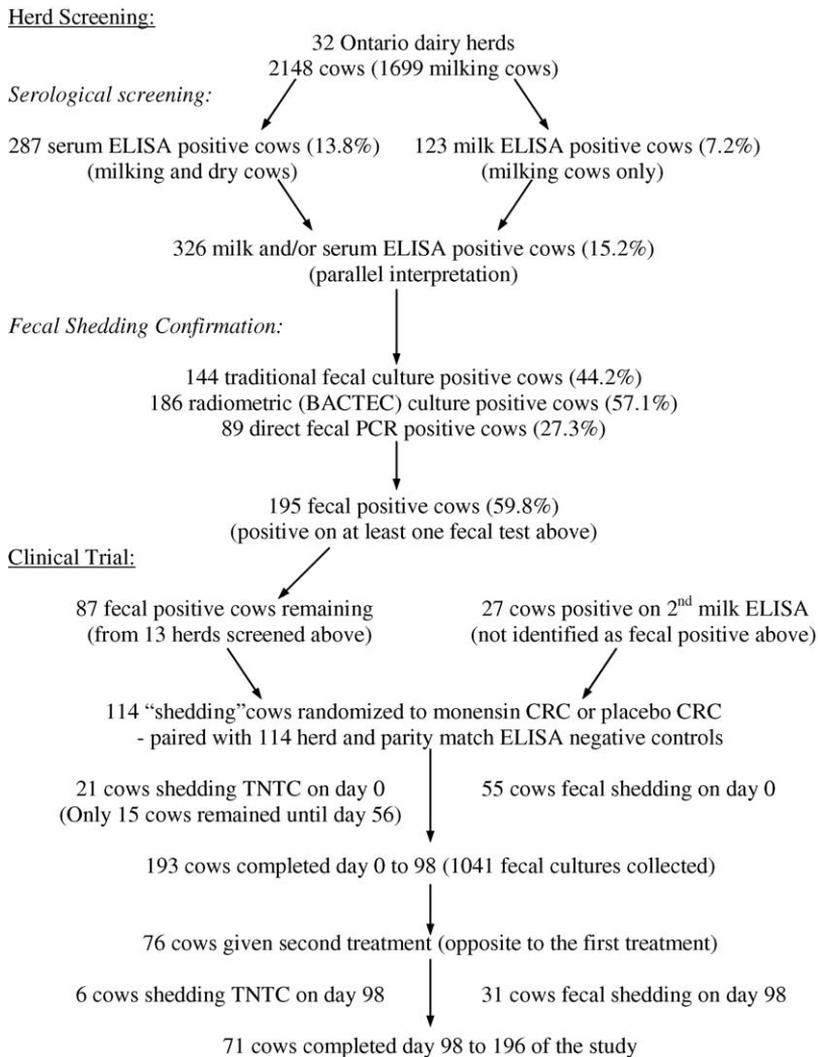


Fig. 1. A schematic to illustrate the sample collection and test results for the herd screening and subsequent randomized clinical trial to investigate the effect of monensin sodium on the quantity of MAP colony-forming units (cfu) shed in the feces of infected cattle and to evaluate the influence of monensin treatment on the detection of paratuberculosis using a commercial serum ELISA and fecal culture. Dairy herds included in this study were from Ontario, Canada.

Table 1

Summary of herd descriptive statistics for: (A) 32 herds initially screened and (B) a subset of 13 herds included in a clinical trial to investigate the effect of monensin sodium on the quantity of MAP colony-forming units (cfu) shed in the feces of infected cattle and to evaluate the influence of monensin treatment on the detection of paratuberculosis using a commercial serum ELISA and fecal culture

(A) 32 herds screened		
Number of cows	Mean = 2148/32 = 67 cows	Range = 27–171
Housing		
Free-stall	13/32 = 41%	
Tie-stall	19/32 = 59%	
Seroprevalence	287/2148 = 13%	
(B) 13 herds in clinical trial		
Number of cows	Mean = 1153/13 = 89 cows	Range = 39–171
Housing		
Free-stall	9/13 = 69%	
Tie-stall	4/13 = 31%	
Seroprevalence	191/1153 = 17%	

Table 2

Summary of cow-level statistics from: (A) 32 herds initially screened, (B) a subset of 13 herds enrolled in the clinical trial, and (C) 233 cows actually enrolled in the clinical trial to investigate the effect of monensin sodium on the quantity of MAP colony-forming units (cfu) shed in the feces of infected cattle and to evaluate the influence of monensin treatment on the detection of paratuberculosis using a commercial serum ELISA and fecal culture

	(A) 32 herds screened (N = 2148) (%)	(B) 13 herds (N = 1153) (%)	(C) Cows enrolled (N = 233) (%)
Parity			
1	32	31	22
2	26	27	33
3 and above	42	42	45
Breed			
Holstein	95	99	100
Other ^a	5	1	0

^a Other breeds include Jersey, Guernsey and Ayrshire.

To quantify the effect of monensin on fecal shedding, mixed models were generated from both datasets including only cows that were fecal culture positive on the days of treatment. The final models are shown in Table 3. During the first 98 days of the study, cows treated with monensin shed 3.4 fewer cfu than cows given a placebo CRC ($P = 0.05$). When treatments were switched on day 98, cows given a monensin CRC were found to shed 2.4 cfu less than cows given a placebo bolus but, this difference was not statistically significant ($P = 0.40$).

The S/P ratio was compared between the two treatment groups for the cattle shedding MAP on the first day of each trial (day 0 or 98), including those shedding TNTC (Table 4). The serum S/P ratio in the first 98 days was 1.39 units lower for cows treated with monensin as compared to cows given a placebo CRC ($P = 0.04$). During the second half of the trial

Table 3

The effect of monensin CRC treatment on the number of colony forming units (cfu) cultured from the feces of dairy cows shedding MAP based on mixed model analysis of a randomized crossover clinical trial

	Estimate (cfu)	Standard error	<i>P</i> -value
Model 1: days 0–98 ^a			
Intercept	3.06	1.61	0.039
Fecal cfu on day 0	1.05	1.01	<0.001
Monensin treatment	–3.42	1.84	0.047
Model 2: days 98–196 ^b			
Intercept	1.14	2.15	0.869
Fecal cfu on day 98	1.06	1.02	0.002
Monensin treatment	–2.39	2.80	0.404

Models only included cows found to be shedding on the first day of each trial (day 0 or 98).

^a *n* = 106 observations from 55 cows.

^b *n* = 48 observations from 25 cows.

Table 4

The effect of monensin CRC treatment on the serum ELISA S/P ratio of dairy cows shedding MAP based on mixed model analysis of a randomized crossover clinical trial

	Estimate (S/P ratio)	Standard error	<i>P</i> -value
Model 1: days 0–98 ^a			
Intercept	–5.51	1.21	<0.001
S/P ratio on day 0	3.11	1.11	<0.001
Monensin treatment	–1.39	1.19	0.055
Model 2: days 98–196 ^b			
Intercept	–4.29	1.32	0.001
S/P ratio on day 98	2.19	1.14	<0.001
Monensin treatment	1.10	1.33	0.744

Models included cows fecal shedding (even cows shedding too numerous to count) on the first day of each trial (day 0 or 98).

^a *n* = 130 observations from 70 cows.

^b *n* = 59 observations from 31 cows.

(days 98–196), monensin treated cows had a higher S/P ratio (1.10 units) than placebo treated cattle, although this difference was not statistically significant (*P* = 0.74).

Results of logistic regression modeling for the odds of testing positive on fecal culture or serum ELISA are presented in Tables 5 and 6, respectively. Separate analyses were completed for cows that were fecal shedding on day 0 and those that were not. These analyses also included the cows shedding TNTC on the day of treatment. For cows that were fecal shedding, the odds of testing positive on fecal culture or serum ELISA were 0.34 and 0.30 times as high for cows treated with a monensin CRC as compared to cows receiving a placebo bolus (*P* = 0.03 and 0.22), respectively. Conversely, monensin treatment numerically increased the odds of testing positive on both fecal culture and serum ELISA (*P* = 0.63 and 0.11, respectively), for cattle not fecal shedding on day 0 of the study. The herd size and cow housing type variables were retained as confounders in each model despite their non-significant *P*-values.

Table 5

The odds of a monensin CRC treated dairy cow testing positive on fecal culture as compared to a cow given a placebo CRC as estimated through generalized linear models

	OR	95% CI	P-value
Model 1: fecal shedding ^a			
Intercept	1.04	0.10–11.08	0.975
Monensin treatment	0.27	0.08–0.89	0.031
Herd size > 100 cows	0.34	0.06–2.00	0.232
Free-stall housing	1.77	0.22–14.2	0.586
Model 2: non-fecal shedding ^b			
Intercept	2.49	0.28–22.0	0.411
Monensin treatment	1.28	0.47–3.45	0.632
Herd size > 100 cows	0.28	0.03–2.26	0.232
Free-stall housing	4.34	0.47–40.1	0.196

Analyses were stratified according to fecal shedding status of MAP on the day of treatment for the randomized clinical trial (day 0). Both models used only data from day 0 to day 98 of clinical trial and model 1 also included cows shedding too numerous to count.

^a $n = 130$ observations from 70 cows.

^b $n = 261$ observations from 139 cows.

Table 6

The odds of a monensin CRC treated dairy cow testing serum ELISA positive as compared to a cow given a placebo CRC as estimated through generalized linear models

	OR	95% CI	P-value
Model 1: fecal shedding ^a			
Intercept	8.67	2.10–35.8	0.003
ELISA positive on day 0	232.0	27.0–1993	<0.001
Monensin treatment	0.30	0.04–2.07	0.222
Herd size > 100 cows	1.02	0.05–22.2	0.991
Free-stall housing	1.17	0.07–20.6	0.916
Model 2: non-fecal shedding ^b			
Intercept	4.39	1.66–11.6	0.003
ELISA positive on day 0	22.4	9.03–55.4	<0.001
Monensin treatment	2.26	0.83–6.13	0.109
Herd size > 100 cows	2.31	0.65–8.21	0.196
Free-stall housing	0.31	0.07–1.31	0.110

Analyses were stratified according to fecal shedding status of MAP on the day of treatment (day 0) for the randomized clinical trial. Both models used only data from day 0 to day 98 of clinical trial and model 1 also included cows shedding too numerous to count.

^a $n = 130$ observations from 70 cows.

^b $n = 261$ observations from 139 cows.

4. Discussion

The ability of current diagnostic tests to identify cattle that are shedding MAP in their feces is limited. If a cost-effective and safe pharmaceutical could be found to reduce the shedding of MAP in these unidentified cattle, the risk posed to susceptible stock would be significantly reduced. By no means would monensin or any other drug be a replacement for

good management practices, but rather a potential aid for the prevention and control of Johne's disease. The use of monensin as an individual cow therapy for Johne's disease is not advisable. At best, monensin may help to reduce new infections when given to the entire mature cow-herd as a preventative.

The results of this clinical trial suggest that monensin delivered as a CRC may marginally decrease the mean number of cfu shed by MAP infected cows. The mean number of cfu shed by cows that were given monensin during the first 98 days of the study was reduced compared to cows given placebo. Shedding cattle cultured an average of 4.2 cfu per tube on the initial day of treatment. Therefore, a decrease of 3.4 cfu per tube in the monensin treated cattle equates to a 80% reduction. Although this measured reduction in cfu per tube is statistically significant, the biological importance remains questionable. Without knowing the effect of monensin on high fecal shedding cattle it is difficult to conclude on the significance of these findings. The fact that cows treated with monensin were less likely to test positive on fecal culture is somewhat disconcerting in that infected animals may go undetected. However, this is probably only true for the very low fecal shedding cattle.

No difference could be found in the mean number of cfu shed by monensin treated cows as compared to those given placebo during days 98–196 of the study. Interpretation of results from the second half of the study was challenging since treatments were not randomly allocated, but were merely opposite to that given in the prior 98 days of the study, and no washout period was given. The residual effect of monensin on the fecal shedding of MAP remains unknown. The influence of culling on the study results will be discussed at more length below, but there is little doubt that the removal of cattle on day 98 resulted in more "healthy survivors" during the second trial. This may have also contributed to the lack of statistically significant findings during the second trial.

When high fecal shedding cows were removed from the datasets, the average cfu being shed was reduced. This resulted in a predominance of low shedding cows (<10 cfu) and a smaller difference between treatment groups. The greatest source of environmental contamination with MAP most likely comes from a few high shedding cattle in each herd. Evaluating the effect of monensin on such high shedding cattle is necessary to validate its use for Johne's disease prevention and control. If improved methods for the quantification of MAP could have been used on the high fecal shedding cattle, the results received from this study may have been different.

It was hypothesized that monensin may immunologically modulate the MAP infection (Brumbaugh et al., 2000). The serum ELISA S/P ratio tended to be lowered by monensin treatment during the first 98 days of the trial. However, the S/P ratio was not decreased sufficiently to alter the disease classification of these cattle. Overall, this study suggests that monensin may affect the humoral response of infected cattle, but any influence on cell-mediated immune responses remains yet to be determined.

Clinical trials to investigate the fecal shedding of MAP infected cattle are difficult to complete. Simply identifying fecal shedding cattle is complex given the biology of Johne's disease and its related test complications. Even with using a full battery of tests as in this study, recognizing the fecal shedding cattle was not easy. Sixteen percent of the ELISA negative controls were later found to be shedding MAP in their feces. An even bigger complication for these studies is the high risk of culling. A total of 162 cows were lost from

this study, and in particular at the crossover (day 98). The large loss of cattle at day 98 was a result of our encouragement to producers to maintain infected cattle until the end of the first trial. Interestingly, cows were removed equally from both treatment groups. Unfortunately the reason for culling was not recorded, but in most instances this decision was multifactorial. It was presumed that many of the culling decisions were directly or indirectly related to Johne's disease as an estimated 65% of the culled animals had developed diarrhea ($FS \leq 2$) by day 98 of the study. A washout period of one month would have improved the results of the crossover study. However, to avoid losing more cows the second treatments were given on day 98.

It should be acknowledged that the mean and standard deviation estimations used to calculate the sample size for this clinical trial were quite different than those in the actual data sets. These differences can be attributed to the natural skewed distribution of the number of cfu being shed, and the restriction of our data sets to low shedding cattle. The effect of misclassification bias is also an important consideration in any Johne's disease study. The variation in S/P ratio and cfu per tube from serial serum ELISA and fecal cultures from individual animals, respectively, have not been extensively described in the literature. It is difficult to know how this variation may have biased the results of this study. The testing strategies used in the screening phase of this study did vary for different groups of animals, but the main inclusion criterion in this trial was whether or not cattle were fecal shedding on the starting day. Ideally the randomization of the cattle would have been done based on the fecal shedding status on the initial day of the study, but this was not possible due to the delay in fecal culturing. Despite the re-grouping of cattle after randomization the parity and herd distributions remained equal for both treatment groups. In retrospect it may have been more efficient to screen herds with ELISAs and then randomize the test positive animals to treatment, rather than confirming with fecal culture first. Many of the treated cows were group housed with other infected cows in free-stall facilities. This means that the ingestion of infective feces from another cow may have resulted in some false positive culture results from fecal "pass through". Although this is phenomenon is thought to be an uncommon event, if it did occur on the initial day of the study, results would be biased towards a reduction through monensin. Overall, any Johne's disease study based on the outcome of diagnostic testing must be interpreted with some caution. Perhaps other outcomes could also be evaluated as confirmation of these diagnostic test results.

Histological lesions were evaluated as an outcome measure in previous monensin and Johne's disease studies. In the clinical trial by [Brumbaugh et al. \(2000\)](#), 13 beef and dairy cows were fed 450 mg of monensin per day for 120 days. This treatment is of a higher dose and length as compared to a monensin CRC. Even though the MIC for monensin and MAP has been established, the most appropriate dose for usage had not been determined. The fact that a small reduction in fecal shedding was found at 335 mg/day and that histological impacts in the Brumbaugh study were found at 450 mg/day, suggests that further work with monensin at higher doses may be warranted. As mentioned above, the design of this study was such that the second treatments were not randomly allocated. Therefore, the effect of extended exposure to monensin on fecal shedding of MAP could not be determined. Another difference between these two studies is that cows enrolled in the [Brumbaugh et al. \(2000\)](#) study were non-lactating and non-gestating, which may also alter the immune status and competency of the animals as compared to the field study described above. Differences

in severity or stage of clinical disease could also influence the success of treatment of cows with Johne's disease. It has been postulated from this study that perhaps monensin may be more useful in calves in preventing infections as compared to cows with established infections. More research is needed to understand the potential of monensin to control or prevent exposure to MAP.

5. Conclusions

Although previous research suggests that monensin decreases the pathological lesions associated with Johne's disease, this study does not support any definitive effect of monensin on the fecal shedding of viable MAP. Furthermore, the use of monensin does not appear to interfere with either serum ELISA or fecal culture results.

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