

Use of an ELISA to Monitor Internal Parasite Burdens in Dairy Cows

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Introduction

Internal parasites, particularly *Ostertagia ostertagi*, are considered to be economically detrimental for dairy herds. Negative effects include a reduction in heifer growth rates and decreased levels of milk production in lactating cows. A recent review by Gross *et al* reported a median increase of 0.63 kg/day in milk production following treatment with anthelmintics in a wide range of studies that were based on various study designs, treatment protocols and products.¹

Unfortunately, monitoring of parasite burdens in dairy herds currently requires the use of fecal egg counts (FEC). FEC are highly variable both within and among animals and are time consuming and expensive to carry out. An ELISA developed in the Netherlands² and evaluated under Dutch conditions³ has more recently been evaluated in dairy herds in Quebec, Canada.⁴ In this last study, ELISA optical densities (ODs) computed as an average of the 5 most recently calved heifers were found to have a reasonably high correlation with FEC taken from the same 5 animals ($r=0.49$ to 0.55).

However, comparing ELISA results to FEC assumes that FEC are a good indicator of parasite burdens in adult cows, which may not be the case. Consequently, other means of evaluating the ELISA to determine if it is an effective measure of parasite burden need to be found. One approach is to determine if factors which would be expected to influence parasite burdens have a predictable effect on ELISA optical densities.

A second approach is to evaluate relationships between ELISA test results and measures of productivity. A recently completed study in Prince Edward Island determined a strong relationship between ELISA test results from bulk tank milk samples and the amount of seasonal (summer-fall) decline in milk production that the herd experienced.⁵ A third method would be to monitor response to anthelmintic therapy using the ELISA.

This study examined the relationship between ELISA results and both herd milk production and man-

agement factors that would be expected to influence parasite burdens.

Materials and Methods

Bulk tank milk samples were obtained from all Nova Scotia dairy herds once in each of the months of July, August and September 1998 (N=415). Each sample was tested using an indirect microtitre ELISA against a crude saline extract, whole worm *Ostertagia ostertagi* antigen as previously described.² Readings from blank wells in each plate were subtracted from the sample values and the average of the results from three months was computed.

At the end of August all producers received a brief questionnaire by mail asking for basic information on factors that would potentially influence parasite burdens in their herds. Data captured included extent of exposure to pasture, mingling of cows in different age groups, use of parasite control procedures in heifers and milking cows and pasture management practices.

Twelve months of individual cow milk production data for all herds on production recording (Atlantic Dairy Livestock Improvement Corporation- ADLIC) were extracted from the Animal Productivity and Health Information Network (APHIN) database.⁶ From these data, herd average values were computed for annual milk production, summer milk production (July, August, September) and relative fall production (average of Sept., Oct., Nov. expressed as a proportion of average of May, June, July).

Descriptive statistics were computed and unconditional associations were evaluated using t-tests, ANOVA analyses and correlation coefficients. Linear regression models were used to evaluate multivariable relationships between management factors and test results (ODs) and between test results and milk production parameters. All analyses were carried out using the statistical package Stata (Stata Corp., College Stn., Texas).

Results and Discussion

Descriptive Statistics

Milk samples were tested from 415 herds and usable questionnaires were returned by 207 producers (50% of those contacted). Production data were obtained for the 280 herds on ADLIC.

Individual sample optical densities ranged from 0.15 to 1.18 with the average and standard deviation of the three month averages being 0.69 ± 0.19 . The wide range of values for the ODs suggests considerable variation between herds in levels of antibodies to internal parasites. The interquartile range for ODs was 0.58 to 0.83 (ie: 50% of herds fell in this range). Within herd correlations between monthly samples were high ($r=0.85$). The consistency within herds, across months suggests that multiple samples probably do not need to be evaluated.

Associations between Management Factors and ELISA Results

There were significant ($p < 0.1$) unconditional associations between the following management factors and the average ELISA optical density for the herd:

- increasing exposure of cows to pasture (ODs increased across the following categories: total confinement housing < access to concrete or gravel exercise yard < access to small grass covered paddock for exercise < pastured with the expectation that grazing met some of their nutritional requirements)
- increasing exposure of heifers to pasture (same categories)
 - dry cows grazing on same pastures that heifers grazed
 - milking cows grazing on same pastures that heifers grazed
 - spreading manure on pasture fields

Two linear regression models were fit to evaluate the relationships between management practices and ELISA test results. The first was based on data from all herds and included level of exposure to pasture as a predictor. Results are presented in Table 1. Increased exposure of both heifers and cows to pasture resulted in higher ELISA optical densities. Cows that spent part of their time on pasture had ODs that were, on average, 0.28 units higher than cows kept in confinement housing.

Deworming heifers in the spring of the year significantly reduced the ODs. This relationship was not significant when evaluated unconditionally, suggesting that spring deworming might be used more frequently in herds which used pasture. In unconditional analysis, the beneficial effect of the deworming was masked by the effect of greater exposure to pasture. Other parasite control practices (eg. fall treatments of heifers, sus-

Table 1. Effects of housing and management practices on test results from an Ostertagia ELISA applied to bulk tank milk samples. Data from 232 Nova Scotia dairy herds. ($R^2 = 0.43$)

Variable	Coefficient	P-value	Confidence interval	
			lower	upper
Cows - confined	baseline			
Cows - exercise yard	0.01	0.88	-0.11	0.12
Cows - paddock	0.09	0.06	-0.003	0.18
Cows - pasture	0.28	0.00	0.21	0.34
Heifers - confined	baseline			
Heifers - exercise yard	0.09	0.43	-0.13	0.30
Heifers - paddock	0.16	0.01	0.04	0.27
Heifers - pasture	0.15	0.00	0.08	0.23
Heifers - spring treatment	-0.06	0.00	-0.11	-0.02

tained release bolus use and a variety of treatments of milking cows) did not have a significant effect on ODs.

A second model, based only on herds which allowed the cows to have access to pasture, was fit to evaluate the effects of pasture management variables on ELISA test results. The results (Table 2) show that allowing dry cows and/or milking cows to graze the same pastures as heifers resulted in elevated ODs while spring treatment of heifers lowered the ODs.

All of the observed results are consistent with accepted ideas about the effects of management factors on parasite burdens. These results support the conclusion that the ELISA is effectively monitoring parasite burdens in these herds.

Table 2. Effects of management practices on test results from an Ostertagia ELISA applied to bulk tank milk samples. Data from 166 Nova Scotia dairy herds which used pasture for grazing. ($R^2 = 0.08$)

Variable	Coefficient	P-value	Confidence interval	
			lower	upper
Dry cows and heifers grazed same pastures	0.04	0.05	0.001	0.08
Manure spread on pasture	0.05	0.04	0.002	0.09
Heifers - spring treatment	-0.05	0.05	-0.09	-0.001

Associations Between ELISA Results and Production Parameters

The associations between the ELISA ODs and the following three management parameters:

- herd average annual milk production,
- herd average summer milk production, and
- seasonal decline in milk production (fall production divided by spring production) were evaluated using multiple linear regression models. In order to avoid confounding, a number of factors which are known to have strong relationships with production parameters (eg. extent of pasture use, herd average days in milk, herd average lactation number, herd average log somatic cell count), were made available to the linear regression models. Exposure to pasture was reduced from 4 categories to 2 (grazing vs confinement, yard and paddock combined) since there were relatively few herds in the three non-grazing categories and there were no differences in the levels of milk production among these three categories. The average OD was forced in to each of the regression models in order to evaluate the effect of parasite burden.

Increasing days in milk, herd age, somatic cell count and use of pasture for grazing all had negative effects on annual milk production. However, the effect of the OD was non significant ($\beta=-3.1$, $P=0.11$). There was also no significant association between OD and the seasonal decline in milk production. Exposure to pasture was the only factor which exhibited a significant effect on this parameter. These results are in contrast to those reported from PEI herds, where OD scores were highly correlated to the seasonal decline in milk production.⁵ However, it must be noted that the amount of seasonal decline demonstrated by Nova Scotia herds was considerably less than that shown by PEI herds. As a result, the power of a study to detect factors which would affect the decline would be lower.

The results from the regression model evaluating the effects of factors on summer (July, August, Sept.) production are shown in Table 3. The ELISA OD had a significant association with daily milk production during these months ($\beta=-5.0$, $P=0.02$), as did grazing, increasing days in milk and somatic cell counts. With an interquartile range of 0.58 to 0.83 and a coefficient of 5.0, a herd at the 25th percentile for OD would be expected to produce 1.25 kg ($5.0 \times (0.83 - 0.58)$) less milk per head per day than a herd at the 75th percentile. This represented the effect of the parasite burden after controlling for exposure to pasture and several other potential confounding variables. However, whether or not there were unmeasured confounding variables which may have affected this estimate is not known.

Conclusions

The results from this study indicate that factors that would be expected to affect parasite burdens in

Table 3. Effects of ELISA optical densities and other herd parameters on the herd average daily milk production during July, August and September. Data from 140 Nova Scotia dairy herds which used pasture for grazing. ($R^2 = 0.28$)

Variable	Coefficient	P-value	Confidence Interval	
ELISA optical density	-5.01	0.02	-9.29	-0.75
Pastures used for grazing	-2.64	0.02	-4.91	-0.38
Herd average days in milk	-0.06	0.004	-0.09	-0.02
Herd average log SCC	-3.15	0.000	-4.73	-1.16

dairy herds have a predictable effect on the ELISA optical densities. This, taken with other evidence of the value of the ELISA as a monitoring tool suggests that the ELISA may be a useful tool for monitoring parasite burdens in dairy herds.

There appeared to be a substantial, and statistically significant relationship between the herd OD value and the level of milk production during the summer in herds using pasture. This suggests, that herds with high OD readings may benefit from more intensive parasite control programs in the milking herd. However, this result must be interpreted with caution. Although a number of known potential confounding factors were controlled in the analysis, unmeasured confounders may have influenced the result. Confirmation of the relationship between parasite burdens as predicted by OD values and lost milk production will most likely come from trials in which response to anthelmintic treatment is compared to previous estimates of OD.

References

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