

# Gastrointestinal Nematodes in Québec Dairy Cattle: Herd Prevalence, Level of Infection Estimated by Bulk Tank Milk ELISA Testing and Related Risk Factors

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## Abstract

A cross-sectional observational study was undertaken in Québec dairy herds to evaluate regional and provincial prevalences of herds with gastrointestinal nematode (GIN) infections in lactating cows, and to determine risk factors associated with level of herd infection. The five most recently calved first-lactation cows from each of 208 randomly selected farms in seven pre-defined regions of Québec were sampled for feces collection in July-August 1995. On the same occasion, a bulk tank milk sample was collected. A questionnaire on replacement and grazing practices was filled out for each farm. Fecal samples were examined and fecal egg counts were determined for trichostrongylids, *Capillaria*, *Nematodirus*, *Trichuris*, *Strongyloides* and total GIN eggs. Weighted provincial estimates for prevalence of herds with positive coprologies to these parasites ranged from 4 to 93%. Median fecal egg count per herd was low (17 eggs per 5 grams of feces). Milk samples were submitted for an indirect ELISA test to detect antibodies against *Ostertagia* and *Cooperia*. Level of herd infection, as estimated by *Ostertagia* and *Cooperia* bulk tank milk ELISA titers (BTTs), varied ( $p < 0.05$ ) among regions. Questionnaire-derived risk factors most significantly associated with an increased bulk tank *Ostertagia* titer were: exposure of lactating cows to pasture, intensive grazing of heifers on farm, contamination history of heifers' pasture and incomplete pasture rotation for heifers. Very high stocking rates for cows and heifers and mechanical mowing during the grazing season of heifer pastures were associated with decreased ELISA

titers. The results of this study indicate that the prevalence of nematode infections in first-lactation cows is very high in Québec herds, with trichostrongylid infections being the most frequently observed. The association between bulk tank milk ELISA titers for *Ostertagia* and herd-level management practices capable of having an impact on exposure of cows to parasites seems to suggest that BTTs could be a valuable indicator of herd infection level. Proper knowledge of regional prevalences, differences among mean regional titers, and the association between titers and of herd-level risk factors can help veterinarians with their decisions regarding parasite prevention and treatment.

## Résumé

Une étude transversale corrélationnelle de troupeaux laitiers du Québec a été entreprise pour déterminer d'une part la prévalence à l'échelle régionale et provinciale des troupeaux comprenant des vaches en lactation infectées par des nématodes gastro-intestinaux et d'autre part les facteurs de risque associés à l'infection au niveau du troupeau. Dans 208 troupeaux, choisis au hasard dans sept régions pré-sélectionnées du Québec, les cinq vaches ayant vêlées le plus récemment parmi l'ensemble des vaches en première lactation ont été désignées pour la cueillette de fèces durant les mois de juillet et août 1995. Au même moment, un échantillon de lait de réservoir était recueilli. Un questionnaire sur la régie des sujets de remplacement et de l'utilisation des pâturages était complété à chaque ferme. Les échantillons fécaux furent examinés pour déterminer

le nombre d'œufs de trichostrongylidés (*Capillaria*, *Nematodirus*, *Trichuris* et *Strongyloides*) et le nombre total d'œufs produits par les nématodes gastro-intestinaux. Les estimés pondérés provinciaux pour la prévalence des troupeaux avec des coprologies positives pour ces parasites variaient de 4% à 93%. Le compte médian du nombre d'œufs par troupeau était faible (17 œufs par 5 g de fèces). Les échantillons de lait ont fait l'objet d'un test ELISA indirect pour déterminer la présence d'anticorps contre *Ostertagia* et *Cooperia*. Le niveau d'infection, tel que déterminé par les titres ELISA du lait de réservoir associés à ces deux parasites, variait significativement selon la région ( $p < 0.05$ ). Le questionnaire a permis de cerner certains facteurs de risque associés à des titres élevés contre *Ostertagia* dans le lait de réservoir. Le pâturage intensif et la contamination préalable des pâturages de taures, la rotation incomplète et l'exposition des vaches adultes au pâturage étaient associés à des titres augmentés. Le fauchage du pâturage des taures et une forte densité de population étaient associés à des titres plus bas. Les résultats de cette étude démontrent que la prévalence des infections causées par les nématodes est très forte chez les vaches en première lactation dans les troupeaux du Québec. Les infections causées par les trichostrongylidés étaient les plus fréquentes. L'association entre les titres ELISA du lait de réservoir associés à *Ostertagia* et certaines pratiques de régie au niveau du troupeau, qui ont un impact sur l'exposition des vaches aux parasites, suggère que les titres ELISA pris au niveau du lait de réservoir pourraient servir d'outil pour déterminer le niveau d'infection d'un troupeau. Une meilleure connaissance des prévalences régionales, des différences entre les titres moyens régionaux et de l'association entre les titres et les facteurs de risque au niveau du troupeau servirait à aider les vétérinaires dans la prise de décision concernant la prévention et le traitement des parasites.

### Introduction

Nematode parasitism of the gastrointestinal tract in cattle occurs under almost all climatic conditions and in all breeds of cattle managed under grazing conditions.<sup>16,18,35,46</sup> In the northern part of North America, pathogenic gastrointestinal nematodes (GIN) are widely distributed and very prevalent,<sup>16,18</sup> but are not necessarily responsible for heavy burdens in animals.<sup>18</sup> Growth impairment during first and second grazing season can occur in dairy heifers at different levels of exposure<sup>16,40,44,46,48</sup> and is thought to be caused by various pathophysiological mechanisms such as reduced dry matter intake,<sup>49</sup> mucosal hypersensitivity in response to larval and adult stages of parasites,<sup>16,18,22,23,35,49</sup> disruptions in nitrogen balance and post-absorptive utilization,<sup>18,48,49</sup> and induced metabolic hormone imbalances.<sup>18,47,49</sup> The effect

of parasites on heifer growth during the first and/or second grazing season can decrease milk production of dairy cows at their first lactation.<sup>8,36,39</sup>

While numerous research projects investigating the effect of anthelmintics on milk production have been published, the importance of these parasites in adult dairy cows remains controversial. Gross *et al* reviewed more than 80 publications based on these clinical trials, and concluded that there was a beneficial effect on milk yield to be gained from eliminating cattle nematodes using anthelmintics.<sup>19</sup> A recent Canadian clinical trial performed with 901 pastured Holstein cows treated with topical eprinomectin<sup>a</sup> at calving showed a consistent increase in daily milk production of 2.07 lb (0.94 kg) per day in the first six months of lactation.<sup>32</sup>

For practitioners, application of a strategic treatment to selected herds or cows that are most likely to experience a beneficial response is difficult because, until recently, no valuable indicator of infection levels existed for adult cattle. Although fecal egg counts can be a relatively reliable indicator of infection level in young animals,<sup>4,15,35</sup> they have not been acceptable for this purpose in adult animals because it is most likely that egg excretion is lowered when the animal's immune response has developed against gastrointestinal nematodes.<sup>11,35,46</sup> Furthermore, trials with positive milk production responses to anthelmintic treatment have failed to demonstrate a positive association of treatment effect with fecal egg counts<sup>13,30</sup> or serum pepsinogen values.<sup>34</sup>

An ELISA test for detecting antibodies against *Ostertagia* and *Cooperia* has been developed in the Netherlands.<sup>21</sup> Although considerable cross-reactivity exists between milk responses to *Ostertagia* and *Cooperia*,<sup>1,21,24</sup> the test is considered an excellent tool for use in cross-sectional surveys to measure the level of exposure of animals to gastrointestinal parasitism.<sup>1,36</sup> Individual animal ELISA titers and mean herd serum and milk titers can be correlated positively with treatment response,<sup>36</sup> and negatively with milk production in individual cows.<sup>24</sup> Further research has demonstrated that *Ostertagia* and *Cooperia* bulk tank ELISA titers (BTT) had significant, but moderate, correlations with herd serum antibody mean titers<sup>9,24</sup> and could be a reasonable indicator of parasite exposure in herds, demonstrating less variability than fecal egg counts. A recent study of 239 Nova Scotia dairy herds related questionnaire data on herd management practices to bulk tank milk *Ostertagia ostertagi* ELISA titers or optical densities. Some management factors known to be associated with an increased level of parasitism, such as use of pasture for lactating animals, were found to result in increased titers. These results serve as indirect evidence that the ELISA test for detecting antibodies against *Ostertagia* may be a useful tool for measuring parasite burdens, even at subclinical levels.<sup>20</sup>

Identification of risk factors showing strong associations with BTT could guide veterinarians in the identification of herds which would be most likely (or least likely) to benefit from anthelmintic treatment. Although research has yet failed to prove a direct significant association between treatment effect and BTT that could be used in the field,<sup>25</sup> the use of ELISA tests on bulk tank milk for estimation of the level of herd exposure to parasites seems to be a very promising avenue. Moreover, dairy practitioners often lack information, not only about the level of parasitic infection, but also about the prevalence of different parasites in their region.

In Québec, larval and adult worm identification in trials with limited numbers of animals,<sup>3,14,26,41</sup> and in two prevalence studies,<sup>12,26</sup> have indicated that *Ostertagia* and *Cooperia* were the most prevalent genera present in adult cattle. In the two prevalence studies, percentages of adult cattle infected by nematodes, as determined by identification of trichostrongylid eggs in the feces, was high on an individual and herd basis, but sampling was not designed in such a way to provide regional prevalence estimates (for each county). New pasture management techniques have gained in popularity in the last decade and endectocides have been used considerably in dairy replacement stock. Therefore, it is uncertain that prevalence estimates are currently accurate.

Although the impact of grazing techniques and management practices on gastrointestinal nematode parasitism has been much discussed and studied in other countries,<sup>28,35,46</sup> young calves were most often the focus of these studies, and it is uncertain that all conclusions would be applicable to lactating cows under Québec's climate. Moreover, the impact of these risk factors on serological parameters at the herd level have received very little attention. In comparison, the same questions have been studied much more thoroughly for *Dictyocaulus viviparus*.<sup>41</sup>

A cross-sectional epidemiological survey was conducted in Québec to evaluate coprological prevalence of GIN in lactating herds, to evaluate the degree of variation in herd exposure to *Ostertagia* (estimated by bulk tank milk ELISA titers) and to determine herd-level risk factors associated with the level of infection by *Cooperia*.

## Materials and Methods

### Study population

The province of Québec is divided into 12 regions (or counties) by the Ministry of Agriculture. For this study, the regions were grouped into seven (A to G) according to climatic and agricultural similarity. Within each of the seven regions (strata), four veterinary clinics were randomly selected.<sup>7</sup> Within each clinic's clientele, a simple random sample of eight dairy farms was chosen. Each selection was determined by random-num-

ber generation, with the next farm or veterinarian being chosen in case of refusal. The number of herds selected for each region was the same (thirty-two) in order to guarantee large enough samples to evaluate prevalence. Sample sizes were therefore not proportional to the actual number of dairy herds in the region. On each farm, the five most recently calved first-lactation cows were sampled to detect infection.

### Data collection

Fecal samples (obtained directly from the rectum) were collected by participating veterinarians between July 5 and August 16, 1995. Samples were packed in ice and sent to the Faculté de médecine vétérinaire from the Université de Montréal in Saint-Hyacinthe (Québec, Canada) where they were processed within 48 hours of sampling. Gastrointestinal nematode eggs were detected and counted (eggs per 5 grams, ep5g) using an adaptation of the Wisconsin centrifugation technique.<sup>6</sup> Eggs were classified as: *Capillaria*, *Nematodirus*, *Trichuris*, *Strongyloides* or "strongyle-type" (trichostrongylid-type including *Ostertagia* and *Cooperia*). Total GIN counts were obtained by adding all previously named genera.

A milk sample was taken from the bulk tank, which was required to contain at least two complete milkings. The tank's agitator was also required to be in motion for at least 10 minutes prior to sampling. Milk samples were centrifuged and the butterfat fraction was extracted. Whey samples were kept frozen at -70°C until further analysed as a batch. Milk samples were packed in dry ice and sent to the Atlantic Veterinary College in Charlottetown (Prince Edward Island, Canada). Duplicate milk samples were submitted to an indirect ELISA test using *Ostertagia* and *Cooperia* crude saline extracts,<sup>1,24</sup> with results reported in optical density units (referred to as titers in this paper). Samples with a coefficient of variation greater than 0.15 were re-tested. Whey samples from infected and non-infected animals were included on all ELISA plates as positive and negative controls.

A questionnaire on replacement and pasture management practices was mailed to all farms and herd owners answered each question by telephone, with a copy in hand. Questionnaire data entry was conducted using the Epi-Info software version 6.04 (Center for Disease Prevention and Control (CDC), USA, World Health Organization, Geneva, Switzerland). The data validation process was performed by researchers in order to identify any protocol deviations committed by participating veterinarians.

### Statistical analysis

All statistical analyses were performed with the SAS 6.11 for Windows software package (SAS Institute, Cary NC, USA).

**Herd prevalence of GIN.** In order to evaluate the prevalence of GIN measured by fecal egg counts (FEC), a farm was considered positive for a parasite genus if at least one fecal egg was identified in one of the five sampled heifers. Total nematode fecal egg counts (ep5g) were computed for all animals and a square root transformation was performed in order to normalize data. Herd means of raw and transformed egg counts were calculated. A weighted provincial prevalence was calculated, considering the number of herds in each region (in 1994) in proportion of the total number of dairy herds in the province.<sup>42</sup> A 95% confidence interval for the provincial weighted prevalence was calculated using a corrected provincial standard error estimate.<sup>33</sup>

**BTT and relationships with FEC.** Between-region differences for *Ostertagia* and *Cooperia* BTT were detected by ANOVA.<sup>33</sup> *A posteriori* comparisons between regional mean BTTs were made using the Tukey HSD-test, controlling for experimentwise type-I error.<sup>43</sup> Pearson correlation coefficients were calculated for association between BTTs for both antigens in all herds and for the association between ELISA titers and herd mean transformed fecal egg counts.

**Risk factors and their associations with BTT.** Frequencies were compiled for all categorical risk factors. Stocking rate (number of animals/ha) was submitted to a logarithmic transformation ( $\log(x+1)$ ) and subsequently transformed into categories (very high stocking rate or normal stocking density) since frequency distributions revealed a clearly bimodal nature. The categorization cutoff for this variable was obtained by cluster analysis (centroid method).<sup>43</sup>

In order to evaluate the association of all risk factors with *Ostertagia* BTTs, three multiple regression models were created, representing the three following situations: 1) farms where both heifers and cows were pastured (n=134); 2) all farms where heifers were pastured (n=164); and 3) all farms where lactating cows were using pasture (n=151). Categorically scaled independent variables were coded into dummy variables, with the reference category being attributed the value 0 for all dummy variables.<sup>27</sup> Ordinal variables were hierarchically coded.<sup>45</sup> Final models were built in a stepwise fashion with variables chosen at an  $\alpha = 0.15$  level. Dichotomous and ordinal variables were entered one at a time, whereas categorically scaled nominal variables were entered or removed as forced blocks of dummy variables. When a dummy variable significantly contributed to the model, all other levels of the same variable were kept in the model in the case of categorically scaled independent variables. In the case of ordinal variables, only the dummy variable coding for the significant sub-level, which corresponds to a threshold for predicted variable increase or decrease, was kept in the model. Due to insufficient number of herds, the effect of all seven regions could not be tested

in the regression models along with all questionnaire risk factors. Models were therefore built without region as an independent variable. To verify if the effect of region was sufficiently accounted for by inclusion of questionnaire risk factors, mean residuals per region were graphically plotted.

## Results

Of the 28 selected veterinarians that received sampling material and random farm lists, 26 collected and sent back samples from 208 herds (1040 animals). Questionnaires were sent to all 208 farms and were completed for 206 of them (99%). Data from the questionnaire survey made it possible to identify protocol deviations (the most often being non-first lactation cows sampled) and 20 farms were excluded from prevalence analyses. Data from the 20 excluded farms were, however, retained for the BTT analyses, since the variable of interest in that case was the estimated level of infection of the herd, notwithstanding individual animal sampling. Therefore, 188 herds were used for the prevalence estimates and 206 herds were used for the BTT and questionnaire risk factor analyses. Descriptive statistics of the questionnaire variables are presented in Tables 1 and 2.

**Herd prevalence of GIN.** The raw herd average fecal egg count was 30 ep5g, with a median of 17; the mean square root count was 3.8 ep5g, which corresponds to 14.4 ep5g in the original scale. Provincial weighted prevalence estimates and 95% confidence intervals of herds positive for strongyle-type eggs, *Capillaria*, *Nematodirus*, *Trichuris* and *Strongyloides* eggs were 92% (85-99%), 4% (0-10%), 10% (3-17%), 4% (0-8%), and 37% (17-56%), respectively, with 93% (86-99%) of herds being positive for total GINs (Figure 1).

**BTT and relationships with FEC.** The mean *Ostertagia* BTT was 0.144 and the mean *Cooperia* BTT

**Table 1.** Herd-level questionnaire variables.

Questionnaire variable	% of Herds
Number of cows in herd <sup>1</sup>	
<30	15
30 – 49	63
50 – 69	15
>70	7
Routine herd health visits <sup>1</sup>	52
Heifers grazed <sup>1</sup>	86
Cows grazed <sup>1</sup>	79
Heifers treated with anthelmintic <sup>1</sup>	68
Cows treated with anthelmintic <sup>1</sup>	32
Heifers grazed 1st summer of life <sup>2</sup>	48
Heifers are grazed with dry cows <sup>2</sup>	54

<sup>1</sup>Measured on all farms that answered questionnaire (n=206)

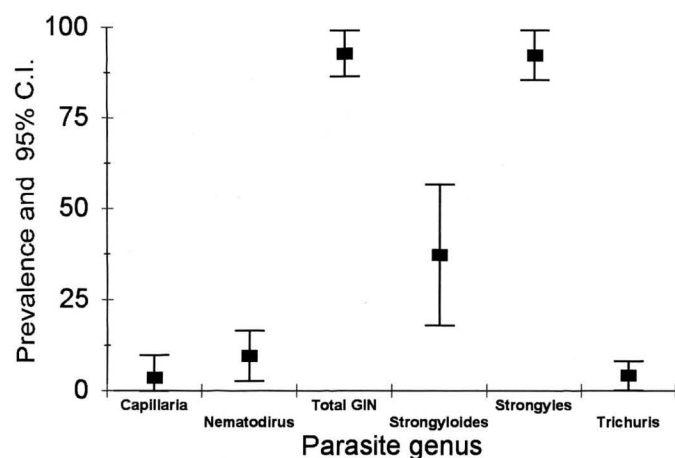
<sup>2</sup>Measured only on farms where heifers were pastured (n=176)

**Table 2.** Pasture management questionnaire variables for heifers and cows (%).

Questionnaire variable	Possibilities	% heifers <sup>1</sup>	% cows <sup>2</sup>
Pasture contamination history	first year as a pasture	3	8
	yearly rotation	17	26
	has always been a pasture	79	66
Pasture rotation type	none	61	30
	new sections opened, access to original paddock	22	23
	intensive rotation (mobile fences)	5	18
	complete paddock changes (no return)	12	29
Mechanical mowing of pasture	yes	54	68
Artificial drainage of pasture	yes	46	66
Grass proportion in heifer diet	grass only	2	
	grass = more than 50 % of diet	63	
	grass = less than 50 % of diet but significant	17.5	
	very minimal but existing grazing	17.5	

<sup>1</sup>Measured only on herds where heifers were pastured (n=176)

<sup>2</sup>Measured only on herds where cows were pastured (n=163)



**Figure 1.** Weighted provincial prevalence of herds (n=188) for various gastrointestinal nematodes with 95% confidence intervals.

was 0.143. Mean *Ostertagia* and *Cooperia* BTTs for each region of the province are represented in Figure 2. Between-regions variation for *Ostertagia* titers ( $p=0.0001$ ) and *Cooperia* titers ( $p=0.0001$ ) were detected. Correlations between *Ostertagia* and *Cooperia* BTTs were elevated and significant ( $r=0.86$ ,  $p=0.0001$ ) and correlations between titers and herd mean transformed fecal egg counts were low to moderate, but highly significant ( $r=0.28$ ,  $p=0.0001$  for *Ostertagia* and  $r=0.36$ ,  $p=0.0001$  for *Cooperia*).

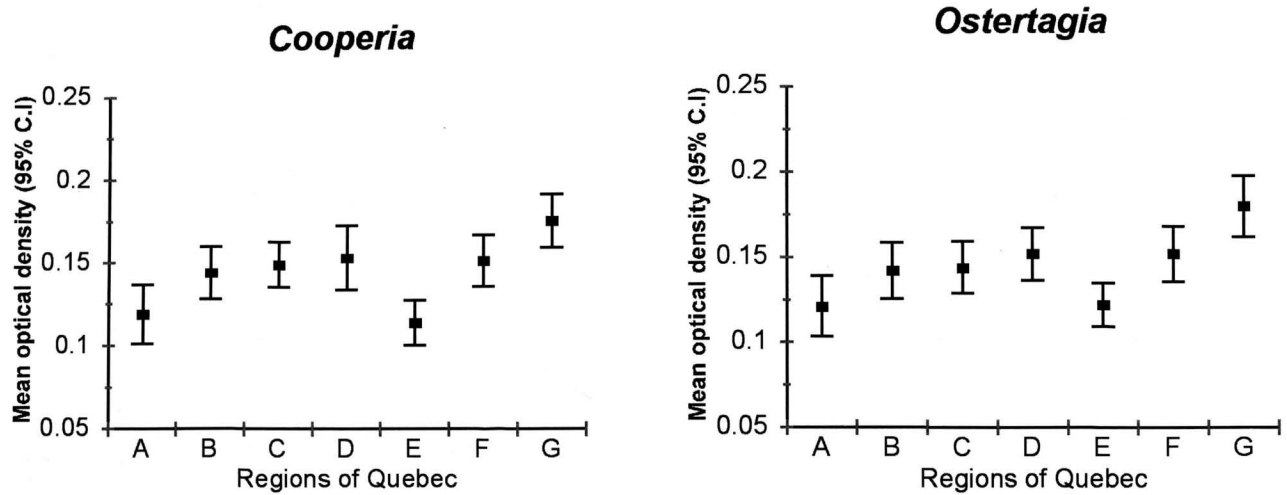
**Risk factors and their associations with BTT.** The cutoff chosen to categorize stocking rates for cows and heifers into "high" and "low" groups was 2.3 on the transformed logarithmic scale, which corresponds to 10 animals per hectare. This dividing point was very close to the first dividing point obtained by cluster analysis in case of cows (2.26) and second best in case of heifers (2.45).

Model characteristics and all independent variables included in the three multiple regression models for *Ostertagia* are presented in Table 3. The direction of the association is negative if the parameter estimate is presented with a minus sign before its value, otherwise the association is considered positive. Mean residuals per region plotted against region showed that models almost systematically underestimated titers in regions with high mean titers and systematically overestimated titers in regions with low titers. The most significant risk factors and their association with BTT in each model will be discussed in detail in the following sections.

## Discussion

**Prevalence of GIN.** The prevalence of Quebec herds with adult cows infected with gastrointestinal nematodes is as high as it was for yearlings and calves in 1981, even if we consider that our random sample included farms where cows did not graze.<sup>26</sup> Another Québec prevalence study reported lower prevalences for adult dairy cows.<sup>12</sup> This confirms that modern treatment and grazing practices have not strongly decreased the prevalence of pathogenic nematodes from Québec dairy herds in any region. Results also suggest that sampling the five most recently calved first-lactation cows in July or August is adequately sensitive for detection of gastrointestinal nematodes in the lactating herd. What may have helped to attain this degree of sensitivity is that sampling was strategically done in July and August, which have been shown to be the months where egg excretion in cattle is the highest in North America.<sup>12,17,41</sup>

The predominance of strongyle-type eggs in fecal samples confirms previous findings that reported *Ostertagia* and *Cooperia* as the most frequent genera found in herbage samples, calves, yearlings and adult cows.<sup>3,12,14,26,41</sup>



**Figure 2.** Mean *Cooperia* and *Ostertagia* bulk tank ELISA titers in 188 herds from 7 regions of Québec.

**Table 3.** Model characteristics and risk factors included in 3 different models predicting *Ostertagia* bulk-tank titers.

	Model <i>Ostertagia</i> 1 Farms where heifers and cows are pastured (n=134)		Model <i>Ostertagia</i> 2 Heifers are pastured (whether or not cows are) (n=164)		Model <i>Ostertagia</i> 3 Lactating cows pastured (whether or not heifers are) (n=151)	
Model r-square	0.29		0.28		0.18	
Model significance (P(F > f))	p < 0.0001		p < 0.0001		p < 0.0003	
Risk factors	Parameter estimate	P-value	Parameter estimate	P-value	Parameter estimate	P-value
<b>Cows:</b> exposed to pasture	—	—	0.037	<b>0.0001</b>	—	—
Heifers: intensive pasture rotation	0.054	<b>0.002</b>	0.047	<b>0.004</b>	—	—
<b>Cows:</b> high stocking rate	-0.021	<b>0.05</b>	—	—	-0.029	<b>0.005</b>
Heifers: high stocking rate	-0.020	0.07	-0.022	<b>0.030</b>	—	—
Heifers: pasture mowed	-0.014	0.056	-0.014	<b>0.042</b>	—	—
Heifers: “old” pasture	0.046	<b>0.02</b>	0.030	0.119	—	—
<b>Cows:</b> all-summer access to same paddock while opening other sections	NS <sup>1</sup>	NS	—	—	0.023	<b>0.038</b>
Heifers: all-summer access to same paddock while opening other sections	NS	NS	0.016	0.057	—	—
<b>Cows:</b> “old” pasture	NI <sup>2</sup>	NI	—	—	0.02	0.062
Heifers: minority of diet is grass	NI	NI	-0.012	0.106	—	—
Heifers: complete paddock changes during summer	NS	NS	0.016	0.119	—	—
<b>More than 30 cows in herd</b>	NI	NI	-0.012	0.10	-0.014	0.119
<b>Cows</b> treated with anthelmintic	NI	NI	NI	NI	-0.013	0.083
Herd health program in place	NI	NI	NI	NI	0.012	0.085

<sup>1</sup>NS: forced in model but not significant at  $\alpha \leq 0.15$

<sup>2</sup>NI: not included in model by the stepwise procedure ( $\alpha$  level for selection: 0.15)

The variable and relatively high prevalence of *Strongyloides* in this study is surprising and should be interpreted cautiously, considering that some smaller strongylid eggs can occasionally be mistaken for non-embryonated *Strongyloides* eggs. This can greatly influence herd prevalences when only one egg in one animal is sufficient to declare a herd positive. The other genera, however, are easy to identify, which suggests that the other prevalence estimates are accurate.

**BTT and relationships with FEC.** The very high correlations observed between *Ostertagia* and *Cooperia* BTTs are not surprising since the antigens used for the ELISA test are from crude saline extracts, which have been demonstrated to show considerable cross-reactivity.<sup>1,21,24</sup> In fact, it has been stated that these tests were not accurate to assess genus predominance in field exposure but were more reliable to quantify total nematodes, which was precisely their purpose in this study.<sup>21,24,35</sup>

Low to moderate correlations were observed between mean transformed fecal egg counts and *Ostertagia* or *Cooperia* BTTs from our study. Those findings were not reported in Dutch studies where the authors observed no significant correlations between coprological and serological measures on individual heifers and dairy cows.<sup>36-39</sup> These former findings, in conjunction with other results from a subsample of the herds from which individual serum and milk samples were taken,<sup>9</sup> seems to indicate that fecal egg counts and antibody titers demonstrate better correlation when they are used as herd means than on an individual basis. The fact that correlations between fecal egg counts and BTTs are low should not be taken as an indicator that BTTs are not good indicators of infection level since fecal egg counts themselves are not.

**Risk factors and their associations with BTT.** The multiple regression models that were built only from questionnaire-derived farm management and pasture utilization variables were explanatory at appreciable levels and significant. Many management factors known to be associated with increased levels of parasitism were found to increase optical density results. This finding is in agreement with Guitian *et al* and serves as indirect evidence that *Ostertagia* bulk tank ELISA titers (BTTs) may be a useful tool for measuring parasite burdens.<sup>20</sup> Although the models were quite explanatory, the mean residuals plotted against regions clearly showed that region still has an effect on BTTs, which is not explained by the model. This suggests that geographical region is associated with one or many factors not included in the questionnaire that have a significant effect on BTTs. It is logical to speculate that these factors are climatic.

Risk factors which showed significant associations with *Ostertagia* BTTs in this study are interesting and informative. Although only lactating cows

contribute to the bulk tank, many of the significant risk factors were related to heifer pasture management, which suggests that level of infection in lactating cows is greatly influenced by heifer management. This may be an indication that heifer pasture management has an impact on the importance of worm burdens that are acquired before calving and may survive until calving and lactation in first-lactation cows as hypobiotic larval stages. It can also indicate that the exposure of the whole lactating herd to pasture contamination due to egg excretion by first-lactation cows may be influenced by the pasture management practices these cows were exposed to as heifers. Among these practices, one that was correlated with an increased level of herd infection is intensive pasture rotation in heifers. This type of pasture rotation has not been demonstrated to lower worm burdens in the past because heifers are usually brought back to contaminated parcels when infective larvae are still present on pasture, or left unmoved long enough to permit self-contamination.<sup>10,28,31</sup> Some researchers, however, have shown that very rapid rotation could have a beneficial effect if animals are treated before being put to pasture or if unoccupied pastures are mowed, facilitating larval desiccation.<sup>46</sup>

The management practice of mowing or clipping the heifer's pasture during grazing season was related to diminished *Ostertagia* BTTs, whereas in another study on *Dictyocaulus viviparus* in Québec, pasture mowing was significantly associated with an increased risk of infection.<sup>5</sup> This apparent discrepancy may be explained by differences in the life cycles of GINs and lungworms. Lungworm infective forms are less mobile than GIN third-stage larvae and are largely dependent on the fungus *Pilolobus* for dispersion from fecal pats. Mechanical mowing of pastures probably diminishes total numbers of infective L<sub>3</sub> on pasture by removing the favorable humid and cool microclimate under long grass coverage for lungworms and GINs, but increases the risk of one animal ingesting *Dictyocaulus viviparus* L<sub>3</sub>, by considerably increasing their dispersion.<sup>31,46</sup>

Classification of heifer pastures based on contamination history (Table 2) made it possible to demonstrate that grazing of heifers on a parcel that has always been used as a pasture ("old" pasture in Table 3) leads to higher BTTs than does the use of new pastures or parcels that are alternatively used for pasture and crop production on a yearly basis. The only pasture rotation method for adult cows that was significantly associated with an increase in BTTs was the opening of new sections (eg. haycut regrowths) while keeping access to an original contaminated paddock. This may be due to the fact that the opening of new sections gives chance to grass in the original pasture to stay a little longer, creating a favorable microclimate for L<sub>3</sub> survival.

The effect of stocking rate on level of infection with GINs has never been clearly demonstrated and has been subject to much debate.<sup>31</sup> This study demonstrated that high stocking rate was very significantly associated with a decrease in BTTs for *Ostertagia* in cows and heifers. However this variable measured the importance of being in a very high stocking rate situation compared to normal population densities on pasture. It is possible that what caused the stocking rate distribution to be bimodal is that a proportion of farms sent animals outside in paddocks with a small grass area. This detail may not have been properly identified by any other questionnaire variable. In these situations, grass as a source of nutrition is of very limited importance and animals ingest very few  $L_3$ , even if in theory the high stocking rate could amount to a high number of  $L_3$  per surface unit of pasture. It is possible that stocking rate as a continuous variable measured on all farms where animals are not in paddock conditions would have had a different effect, but this hypothesis could not be tested in our study.

Although most of the significant pasture management variables were for heifers, exposure of lactating cows to pasture was the most significant of all variables related to increased *Ostertagia* BTTs in all models. The relatively high slope for this factor in the regression equation (0.037) indicates that a good part of the antibody levels in milk is due to exposure of lactating cows to parasites from pasture contamination. The use of anthelmintic treatment in cows demonstrated a trend to decrease BTTs in herds where lactating cows had access to pasture (Table 3). It is possible that statistical significance for this variable was not achieved because all possible types of products used in any kind of treatment strategies were included in the question, thereby combining the effect of adequate and sub-optimal treatment regimens.

### Conclusion

The results of this study suggest that the prevalence of herds with nematode infections in first-lactation cows is very high in Québec, with trichostrongylid type eggs being the most frequently observed category. The significant associations observed between bulk tank milk ELISA titers for *Ostertagia* and herd-level management seems to suggest that BTTs could be a valuable indicator of herd infection level. Further research is needed, however, to clearly demonstrate the value of BTTs as predictors of anthelmintic treatment response. A classification tool based on *Ostertagia* BTTs which could help identify herds that are more likely to show a positive milk production response to anthelmintic treatment would be of great interest to veterinarians.

### Acknowledgments

The authors desire to express their gratitude to Guy Beauchanp for his assistance with statistical analysis and Ms. Stéphanie Cazalais for her excellent technical assistance.

This work was supported by a grant from Merial Canada.

Dr. Caldwell was supported by The National Sciences and Engineering Research Council of Canada (NSERC), Merck Agvet Canada and the Fonds du Centenaire de la Faculté de médecine vétérinaire de l'Université de Montréal.

### Footnote

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