INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

Parasites in lactating dairy cattle:

epidemiology and response to treatment

A Thesis

Submitted to the Graduate Faculty

in Partial Fulfilment of the Requirements

for the Degree of

Master of Science

in the Department of Health Management

Atlantic Veterinary College

University of Prince Edward Island

Ane Christine Wammer Nødtvedt

Charlottetown, P.E.I.

August, 2001

SIGNATURE PAGES

iii-iv

REMOVED

Abstract

Gastrointestinal nematodes rarely cause signs of clinical disease in adult cattle. However, they have been shown to exert a negative impact on production in lactating animals, as seen by improved production following elimination of the worms using anthelmintics. The epidemiology of bovine gastrointestinal nematodes and chorioptic mange was investigated through a one-year longitudinal study in 38 Canadian dairy herds from four different provinces (Prince Edward Island, Quebec, Ontario and Saskatchewan). For each of the herds included in the study, fecal egg counts from eight randomly selected animals and whole herd mange scores were performed on a monthly or quarterly basis. Larval cultures were performed once, and all producers were interviewed regarding herd management practices using a standardized questionnaire.

The occurrence of mange was low throughout the duration of the trial, with a test day within-herd prevalence of 6.1 %. A total of 75.7 % of the herds had animals affected with tail head mange at one point in time during the study. The herds in Saskatchewan had the highest mange prevalence throughout the year.

The observed fecal egg counts were low in this study, with a range from 0 to 419 trichostrongyle type eggs per 5 grams of feces. The average count was 9.8 and the median was 1. A zero inflated negative binomial model was applied in order to assess factors that would influence the fecal egg counts. The lowest egg counts were seen in the winter and the highest were in the late spring. First lactation animals yielded higher counts than older cows. Counts were higher in herds where lactating animals were given access to pasture. Mechanical spreading of manure on pastures used by lactating animals gave higher fecal egg counts and if manure was spread on heifer pastures, the adult cows would have lower counts. In herds where pasture use was more extensive, the monitored animals had higher fecal egg counts. The difference in exposure to pasture was found to be a main contributor to an observed difference in fecal egg counts between the selected herds in the four provinces.

The 28 herds from the two eastern locations were also enrolled in a clinical trial of the effect of treatment with eprinomectin pour-on solution at calving on production. Cows in these herds were randomly allocated to treatment or placebo groups in blocks of ten based on calving date. Milk production results were obtained from the Canadian Dairy Herd Management System database, and analysed using a mixed model with herd as a random effect and tests within cow as a repeated measurement. Test day milk yields from the first six tests after treatment were included in the model, representing a time period of approximately 180 to 200 days in milk. Treated cows produced an additional 0.94 kg of milk per day when compared to the controls over this period. The production effect was independent of calving season, age of the animal and geographical location. No effect of treatment was seen on milk composition, somatic cell count or on the selected health parameters that were recorded for all included animals.

In conclusion, gastrointestinal nematodes are common in Canadian dairy cows that have had some degree of pasture exposure, although fecal egg counts are low. Eliminating the subclinical parasite burdens produces a consistent increase in milk production that yields economic benefits for the dairy producer.

Acknowledgements

First of all I would like to thank my supervisor Dr Ian Dohoo for offering me the opportunity to come to Prince Edward Island for my graduate studies. It has been two wonderful years for me, and I appreciate all the time and effort invested in turning me into an epidemiologist in heart and mind. What I have learned from Ian, both through his excellent courses and through his patient explanations during our discussions, is valuable knowledge that will have a great impact on my future career.

The many co-investigators in the study also need to be acknowledged: Dr Luc DesCôteaux in Quebec, Dr Ken Leslie in Ontario and Dr John Campbell in Saskatchewan. The farm service veterinarians at the AVC helped out with sampling and herd selection. Thank you Drs Greg Keefe, John VanLeeuwen and Jeff Wichtel. Dr Gary Conboy has been very helpful and his insight in parasitology was a necessity for the completion of my work. Technical assistance provided by Theresa Rogers and Dr Dominique Cecyre was invaluable.

Finally, I would like to thank all my friends within the graduate student community at AVC. Without you my stay in Canada wouldn't have come close to being as much fun as it was! All the time wasted around the health management carrels with Drs Emery Leger, Carol McClure and Javier Sanchez was well worth it. A special thanks also goes to Karen Bedard for her honest attempt to turn me into a triathlete.

Please forgive me if I forgot to mention anyone, and please come see me in Norway or wherever else I might happen to relocate myself to in the future!

Ane, PEI August 2001

Contents

1. Introduction: Bovine gastrointestinal nematodes and chorioptic mange

There are several reasons why gastrointestinal parasites in adult cattle are a topic that continues to catch the interest of researchers from various fields. While numerous clinical trials investigating the effect of anthelmintics on milk production have been published, the importance of these worms in adult dairy cows remains somewhat controversial. Gross et al. (1) reviewed more than 80 publications based on these trials, and concluded that there was a beneficial effect on milk yield to be gained from eliminating cattle nematodes using anthelmintics. The recent development of second generation macrolide endectocides, which require no withdrawal of milk after treatment, provide an attractive treatment option for lactating animals (2). Additionally, diagnostic tests that enable herd level monitoring of subclinical parasite burdens, such as antigen ELISA and blood pepsinogen, are currently being evaluated and show promising results (3).

Studies done in Eastern Canada indicate that subclinical parasite burdens in lactating dairy cattle from this region negatively affect milk production. Work done by Hovingh (4) in the early 1990's determined that the bulk tank ELISA optical density (OD) for gastrointestinal parasites (crude *Ostertagia ostertagi* antigen) was highly correlated with a summer/ fall drop in milk production. Guitian et al (5) collected bulk milk tank samples and management questionnaire data from Nova Scotia dairy herds, and concluded that animals in herds with high optical densities on average produced less milk than low OD herds during the summer/fall.

This general introduction includes sections on the properties of gastrointestinal nematodes in cattle, along with their diagnosis and control. A discussion of the different classes of anthelmintics is included, as well as the issue of anthelmintic resistance. Finally, the chorioptic mange that affects cattle is given a brief presentation.

1 The trichostrongyles

1.1 Systematic placement

The "hairworms" of ruminants parasitize the abomasum and small intestines, and belong to the superfamily Trichostrongyloidea of the order Strongylida, phylum Nematoda. The different species often occur in mixed infections, and mature females lay typical "strongylid" or "strongyle" type eggs that cannot be distinguished from each other microscopically. The abomasal nematode *Ostertagia ostertagi* is the most commonly found and most important species under North American conditions. *Cooperia* spp. are also common, but are believed to be of lesser economic importance to bovine producers. Other species from the superfamily Trichostrongyloidea that occur in the gastrointestinal system of pastured cattle are *Nematodirus* spp., *Haemonchus* spp., *Oesophagostomum* spp. and *Trichostrongylus* spp. (6).

1.2 Life cycle

The worms of the superfamily Trichostrongyloidea all have a direct life cycle. Eggs are shed by mature females and passed in the feces where a first stage larvae (L_1) develops within few days. After two molts the resulting infective third stage larvae (L_3), covered by a protective "sheath" which is the outer cuticle from the second stage larvae

(L_2), moves away from the fecal pat to be ingested by grazing animals. Development within the host includes larval migration into the abomasal or intestinal mucosa and two more molts. Approximately three weeks after ingestion of third stage larvae, egg-shedding mature females can be found in the lumen of the gastrointestinal tract, if hypobiosis or arrested development at the fourth larval stage (L_4) does not occur. *Ostertagia ostertagi* has a particularly strong tendency to undergo this dormant stage of low metabolic activity, that may last for months when conditions are unfavourable for survival of the free-living stages of the parasites (6).

The mechanisms behind the initiation of inhibited development are not completely understood, and are believed to depend both upon host immunity and climatic conditions. In cold temperate zones, the worms will typically undergo arrested development in the late fall and "wake up" again in the spring, and it has been shown that exposure of free living stages to low temperatures has induced hypobiosis. This pattern of inhibition is seen in temperate zones and is sometimes referred to as winter inhibition, while in other regions the onset of hypobiosis might be observed before a particularly hot or dry period (7).

Larvae from both *Ostertagia ostertagi* and *Cooperia* spp. have been shown to be able to survive the winter on pastures under cold temperate conditions in Atlantic Canada (8). These larvae will be able to infect susceptible animals shortly after spring turn-out of cattle onto pasture, but then lose infectivity or die early in the pasture season. Hypobiotic L_4 that over- winter within the gastrointestinal tract of stabled animals also contribute to the contamination of pastures in the spring, when they develop into mature worms and start shedding eggs.

1.3 Pathogenesis

The mechanisms resulting in production losses during infections with gastrointestinal nematodes in ruminants include: changes in feed intake, alterations in alimentary tract function and altered metabolism. Reduced voluntary feed intake is recognized as a major factor behind the impaired production, and studies have shown that as much as 73% of the difference in weight gain between *Ostertagia ostertagi* infected cattle and controls can be explained by decreased appetite (9).

When maturing *O. ostertagi* emerge from the gastric glands 18 to 21 days post ingestion, changes occur in both the affected and neighbouring mucosal cells. The result is a reduction in the number of functional parietal cells in the abomasum, leading to reduced acid production and an increase in abomasal pH. This increase leads to an increase in blood gastrin to send signals to the parietal cells that more acid needs to be produced. Because pepsinogen only will be converted to pepsin in an acid environment, the precursor will accumulate leading to impaired protein digestion and increased levels of pepsinogen in the blood. Consequently, both serum gastrin and serum pepsinogen levels increase during heavy infections with *Ostertagia* and this can be used in the diagnosis of the disease. Another adverse effect of the changes in the gastric environment is that the mucosal changes lead to increased permeability and protein loss into the gut lumen (10).

1.4 The effect of gastrointestinal nematodes

Young animals that are exposed to *Ostertagia* infected pastures during their first grazing season may show clinical signs of nematodiasis, including reduced growth or

weight loss, diarrhea, dull hair coat and dehydration. This disease complex is also known as type I Ostertagiosis. Type II Ostertagiosis occurs when a wave of hypobiotic larvae simultaneously emerge from the gastric glands causing widespread mucosal damage. Clinical signs of type II Ostertagiosis are reduced feed intake, diarrhea, dehydration and hypoalbumenia. The disease occurs in yearlings and sometimes even in adult cows, and will be seen during the winter housing period, but does not seem to occur very often anymore (10).

Older animals have usually developed a sufficient level of immunity to gastrointestinal nematodes to prevent clinical signs from occurring. An increasing level of immunity will manifest itself as the following sequence of events: lower egg output by female worms; decreased size of mature worms; increased numbers of hypobiotic larvae; elimination of adult worms; and finally resistance to new infections (11). For species such as *Cooperia* and *Nematodirus*, host resistance is usually well developed within one year, while for *Ostertagia* it takes longer and older animals can therefore harbour substantial worm burdens (12).

In North America, as well as in other developed parts of the world, clinical signs of gastrointestinal parasitism are rare in adult dairy cows. This is mainly due to the frequent use of highly efficient anthelmintics. By far the most important effect of the gastrointestinal nematodes under modern production systems is the production effects caused by subclinical parasite levels. These effects include reduced weight gain in young animals and decreased milk production in adult cattle (13). The beneficial effect of deworming adult dairy cattle is somewhat controversial, and a review by Reinemeyer (14) concluded that due to the lack of large scale clinical trials in North America, no firm

recommendations can be made as to how subclinical parasitism should be dealt with in this age group. Gross et al. (1) summarized the results from more than 80 clinical trials on the effect of anthelmintic treatment on milk production and concluded that the median increase in milk production across these studies was 0.63 kg per cow per day. However, the trials included in this review contained major differences in sample size, geographic location, anthelmintic substance applied, outcome measured and stage of lactation at which treatment was applied. It seems clear that more information is needed regarding trichostrongyles in adult North American dairy cows, and the potential effect of these worms on milk production.

2 Diagnosis

There are a number of methods identifying and/or quantifying gastrointestinal parasitism in cattle. Each of them has their advantages and disadvantages, which may depend on the age of the animal to be tested and the type, and level, of parasite to be detected. These methods of identification include: clinical signs, fecal egg counts, larval culture, levels of gastrin and pepsinogen and serum or milk levels of antibodies against certain species of parasites.

In young animals with clinical signs of parasitic gastroenteritis, a tentative diagnosis can be made based upon age, symptoms, and the history of pasture use and anthelmintic prophylaxis. Confirmation of the diagnosis can be done by fecal egg counts (FEC), and more than 1000 eggs per gram of feces are often recovered. If a species determination between the trichostrongyles is required, larval cultures must be performed before the individual species can be identified. However, in adult cattle, there is a poor

correlation between the level of strongyle type eggs recovered in fecal egg counts and the number of worms present in animals suffering from subclinical parasitism or even Type II Ostertagiosis (10).

The existing evidence of an increase in milk production following anthelmintic treatment of subclinical parasite burdens suggests that the effect may vary not only between animals but also to a larger degree between herds. It would therefore be useful to be able to apply a test that could predict whether or not a certain group of animals would benefit from such treatment (13). In Western Europe, an inadequate development of immunity against gastrointestinal nematodes has been seen in cattle during their second grazing season due to over-protection against, or under-exposure to, parasites following aggressive use of anthelmintics in first season grazing cattle (3). A diagnostic test capable of quantifying parasite levels could potentially detect susceptible older animals before they suffer from adverse effects of the helminth burden carried, and would enable the producer to adjust the treatment strategy accordingly.

Due to the poor correlation between fecal egg counts and infection levels in adult animals, fecal egg counts are not suitable for herd level parasite monitoring. They are however still used since thoroughly validated alternatives are not commercially available, but must be interpreted with caution and do not appropriately reflect infection levels based on samples from individual animals. If conclusions are to be drawn about the parasite status of a specific herd, a group of animals that has a similar composition as the herd in total needs to be sampled (15). Larval culturing from fecal samples will offer an estimate of the species breakdown of the worm burden present, but is not very accurate when the number of worms recovered is low, as will be expected in older animals.

Gastrin levels are elevated during abomasal nematode infections, but the test is not sensitive to infections at subclinical levels. However, pepsinogen levels show some promise as a diagnostic test of subclinical parasitism. The same is true for serology based on an ELISA test using either crude worm antigen or recombinant parasite antigen (3).

The majority of the published work on serologic testing for subclinical parasitism in cattle has been done in the Netherlands (16-20). However, Hovingh et al. (4) found that gastrointestinal parasite levels, as measured by antibody levels against *Ostertagia ostertagi*, was significantly associated with the summer/fall decrease in milk production seen in dairy herds on Prince Edward Island. A study of 239 Nova Scotia dairy herds related questionnaire data on herd management practices to bulk tank milk optical densities (crude antigen *Ostertagia ostertagi* ELISA). Some management factors known to be associated with increased levels of parasitism, such as use of pasture for lactating animals, were found to give increased optical density results. This serves as indirect evidence that the ELISA may be a useful tool for measuring parasite burdens, even at subclinical levels (5).

3 Control

The choice of a control strategy against helminth infections was discussed by Vercruysse and Claerebout (13). Three different thresholds for treatment were defined; therapeutic, production based or preventive. In modern production systems, clinical outbreaks of gastrointestinal disease were considered rare and the need of therapeutic treatment in clinically affected animals limited. Production based treatment was aimed to prevent economic losses due to subclinical parasitism. For adult dairy cows the threshold

parasite burden determining when production based treatment should be applied had not yet been defined due to the lack of diagnostic tests that accurately predict such losses. Preventive anthelmintic treatment was meant to protect future generations of animals from parasitic disease by keeping pastures "clean". Preventive treatment was suggested applied at a herd or pasture-group level, as opposed to therapeutic treatment of individual animals.

The following three sections deal with the various tools that are available for the management of gastrointestinal parasites in pastured cattle. The different classes of anthelmintics commonly applied are presented, together with control strategies based on pasture management. The issue of anthelmintic resistance among ruminant gastrointestinal nematodes is also briefly discussed.

3.1 Anthelmintics

The most frequently used class of anthelmintics used in cattle today is the macrocyclic lactones. Before presenting some of their properties, a couple of other drug classes that have been commonly used in the past, and are still available today, will be discussed. Table 1 summarizes the most important properties of the anthelmintic drugs mentioned in the text.

3.1.1 Benzimidazoles

The benzimidazoles are broad spectrum anthelmintics that inhibit parasite microtubule formation and cell division, through binding with tubulin molecules. In cattle today, albendazole (Valbazen®, Pfizer) can be used against cestodes, trematodes and

trichostrongyles including hypobiotic L_4 stages. However, treated animals can not be slaughtered for food consumption within 27 days of treatment, and the drug can not be used in breeding age heifers or cows in the first 45 days of gestation. Fenbendazole (Safe-Guard® /Panacur®, Intervet) is another broad spectrum anthelmintic in this class that is approved for use in cattle, including lactating animals (6).

3.1.2 Imidazothiazoles

The imidazothiazoles cause parasitic contraction and tonic death through the pathway of nicotinic agonists. Mitochondrial energy production is also compromised because of interference with the fumarate reduction system. The only drug in this class that is still available is levamisole (Tramisol®, Ayerst) which is active against many trichostrongyles, but not hypobiotic stages of *Ostertagia ostertagi* (6).

3.1.3 Macrolides/ macrocyclic lactones

The avermectins and milbemycins belong to the family of macrolides/ macrocyclic lactones. The avermectins (*a* without + *verm* worm + *ect* ectoparasites + *in* pharmaceutical product) in particular, have become very important broad spectrum anti-parasite drugs. The mechanism of effect is by high affinity binding to a glutamate-gated chloride channel, which in turn leads to chloride influx, hyper-polarisation and subsequent paralysis and death of the parasite. Both compounds are produced through fermentation by soil-living bacteria from the genus *Streptomyces*. Because the avermectins have effect against both endo- and ectoparasites, they are commonly termed as endectocides (21).

Ivermectin (Ivomec®, Merial) was the first commercially available macrolide, and it is a first generation avermectin together with doramectin (Dectomax®, Pfizer). They all have effect against a wide range of both nematodes and arthropods, but do not affect cestodes and trematodes. Eprinomectin (Ivomec-Eprinex®, Merial) is a second generation avermectin that has similar anti-parasite properties to the already mentioned products, but has the additional benefit of no withdrawal on milk or meat and can therefore be used safely in lactating animals (6). Moxidectin (Cydectin®, Ayerst) is also approved in lactating dairy cattle and has meat but no milk withdrawal after treatment. It is a milbemycin that has a similar range of efficacy as the first generation avermectins.

3.2 Anthelmintic resistance

A recent report by Fiel et al. (22) from Argentina documented the finding of *Cooperia* in Argentina that was resistant to ivermectin. The worms were parasitizing grazing cattle on the Humid Pampa that had been subject to frequent endectocide treatments. Although anthelmintic resistance has been reported less frequently in cattle than in sheep and goats, the authors suggested that this may have been the first stage of an emerging problem.

The small ruminant sector has struggled with anthelmintic resistance in many parts of the world, and in South Africa the commercial sheep farming industry was devastated due to parasite problems following the development of worms that were resistant to all the anthelmintic drugs available (23). So far the reports of resistant helminths in cattle have been fewer than in sheep, but there are concerns that this might be an emerging problem. Resistance can be expected to occur more commonly in areas

where frequent treatments are relied upon in order to keep parasites under control, and the chances of developing resistance are greater if the applied treatments are less than 100 percent effective.

3.3 Pasture management strategies

In order to reduce the number of anthelmintic treatments required, and thereby theoretically also reduce the selection pressure for resistance, a *preventive, evasive* or *diluting* strategy can be applied. These three different strategies for worm control by pasture management were discussed by Barger (24). A *preventive* approach seeks to keep infection level in the herd low by placing worm-free animals on clean pastures, or by treating (sometimes repeatedly) with an anthelmintic early in the grazing season until the overwintered larvae have died out or lost infectivity. *Evasive* strategies consist of moving animals away from a pasture before high levels of infective worms build up, and *diluting* means that susceptible stock is grazed with more resistant animals and hence a lower density of infective larval stages in the pasture is generated.

As of today, anthelmintic resistance does not seem to be a major concern in cold temperate zones of cattle production. A successful treatment strategy against gastrointestinal nematodes does however require an understanding of the epidemiology of the parasites. The most meaningful approach to restricting losses due to parasites, will be to use this knowledge combined with anthelmintics to apply effective, strategic parasite control. The introduction of endectocides with no milk withdrawal provides more flexibility when developing a parasite control strategy in lactating dairy cows, because treatment can be applied at any time during the year independent of lactation stage.

4 Chorioptic mange

Chorioptes bovis is an arthropod belonging to the family Psoroptidae. It is an ecto-parasite for which cattle are the principal host, even though the mites can be found on several other species such as horses, sheep and rabbits. Clinical signs of mange infestations in cattle include itchy dermatitis in the tail and udder area and is commonly known as tail head mange. Lesions typically occur in stabled animals in the late winter, to disappear when the animals are let out on pasture (6). A recent Ontario workshop for veterinarians in bovine practice identified mange as an increasing problem in many Canadian dairy herds. Application of endectocides enables elimination of the mites, and combined with knowledge on prevalence and seasonal occurrence of mange these drugs can become useful tools in herd prevention strategies.

5 Overall objective

The overall objective of the current study was to develop a more complete understanding of the epidemiology of trichostrongyle infestation in adult dairy cows under cold temperate, North American conditions. The natural history of gastrointestinal nematode infections and tail head mange was investigated through a longitudinal study that included dairy herds in four Canadian provinces. To reach the first specific objective, fecal egg counts, larval cultures and mange scores were obtained from these herds during a study period of twelve months. The results from the internal parasite monitoring is presented in Chapter 2, and the mange results are summarized in Chapter 4. The second specific objective was to investigate the impact of the subclinical worm burden on production in lactating dairy cattle. This was done by means of a clinical trial where the

endectocide eprinomectin was used to eliminate the parasites from the host, after which milk production was measured. The trial was carried out in 28 herds from Prince Edward Island and Quebec, and the production results are presented in Chapter 3.

References

(1) Gross SJ, Ryan WG, Ploeger HW. Anthelmintic treatment of dairy cows and its effect on milk production. Vet Rec 1999; 144(21):581-587.

(2) Shoop WL, Egerton JR, Eary CH, Haines HW, Michael BF, Mrozik H et al. Eprinomectin: a novel avermectin for use as a topical endectocide for cattle. Int J Parasitol 1996; 26(11):1237-1242.

(3) Eysker M, Ploeger HW. Value of present diagnostic methods for gastrointestinal nematode infections in ruminants. Parasitol 2000; 120 Suppl:S109-S119.

(4) Hovingh E. An investigation into factors affecting summer/fall milk production and profitability in PEI dairy herds. PhD thesis. University of Prince Edward Island, Canada., 1998.

(5) Guitian FJ, Dohoo IR, Markham RJ, Conboy G, Keefe GP. Relationships between bulk-tank antibodies to Ostertagia ostertagi and herd-management practices and measures of milk production in Nova Scotia dairy herds. Prev Vet Med 1999; 47(1-2):79-89.

(6) Bowman DD. Georgis' parasitology for veterinarians. 7th ed. Philadelphia: W.B. Saunders Company, 1999.

(7) Eysker M. Some aspects of inhibited development of trichostrongylids in ruminants. Vet Parasitol 1997; 72(3-4):265-272.

(8) Smith HJ. On the persistence of infective Ostertagia ostertagi, Cooperia oncophora and Nematodirus helvetianus on pastures. Can J Comp Med Vet Sci 1972; 36(4):333-338.

(9) Fox MT. Pathophysiology of infection with gastrointestinal nematodes in domestic ruminants: recent developments. Vet Parasitol 1997; 72(3-4):285-297.

(10) Radostits OM, Gay CC, Blood DC, Hinchcliff KW. Diseases caused by helminth parasites. Veterinary Medicine. London: W.B.Saunders, 2000: 1339-1347.

(11) Vercruysse J, Claerebout E. Immunity development against Ostertagia ostertagi and other gastrointestinal nematodes in cattle. Vet Parasitol 1997; 72(3-4):309-316.

(12) Armour J. The influence of host immunity on the epidemiology of trichostrongyle infections in cattle. Vet Parasitol 1989; 32(1):5-19.

(13) Vercruysse J, Claerebout E. Treatment vs non-treatment of helminth infections in cattle: defining the threshold. Vet Parasitol 2001; 98:195-214.

(14) Reinemeyer CR. Should you deworm your clients' dairy cattle? Vet Med 1995; 90:496-502.

(15) Gasbarre LC, Leighton EA, Bryant D. Reliability of a single fecal egg per gram determination as a measure of individual and herd values for trichostrongyle nematodes of cattle. Am J Vet Res 1996; 57(2):168-171.

(16) Ploeger HW, Kloosterman A, Rietveld FW, Berghen P, Hilderson H, Hollanders W. Quantitative estimation of the level of exposure to gastrointestinal nematode infection in first-year calves. Vet Parasitol 1994; 55(4):287-315.

(17) Ploeger HW, Kloosterman A, Bargeman G, von Wuijckhuise L, van den BR. Milk yield increase after anthelmintic treatment of dairy cattle related to some parameters estimating helminth infection. Vet Parasitol 1990; 35(1-2):103-116.

(18) Ploeger HW, Schoenmaker GJ, Kloosterman A, Borgsteede FH. Effect of anthelmintic treatment of dairy cattle on milk production related to some parameters estimating nematode infection. Vet Parasitol 1989; 34(3):239-253.

(19) Ploeger HW, Kloosterman A, Borgsteede FH. Effect of anthelmintic treatment of second-year cattle on growth performance during winter housing and first lactation yield. Vet Parasitol 1990; 36(3-4):311-323.

(20) Kloosterman A, Borgsteede FH, Eysker M. The effect of experimental Ostertagia ostertagi infections in stabled milking cows on egg output, serum pepsinogen levels, antibody titres and milk production. Vet Parasitol 1985; 17(4):299-308.

(21) Shoop WL, Mrozik H, Fisher MH. Structure and activity of avermectins and milbemycins in animal health. Vet Parasitol 1995; 59(2):139-156.

(22) Fiel CA, Saumell CA, Steffan PE, Rodriguez EM. Resistance of Cooperia to ivermectin treatments in grazing cattle of the Humid Pampa, Argentina. Vet Parasitol 2001; 97(3):213-219.

(23) Waller PJ. Anthelmintic resistance. Vet Parasitol 1997; 72(3-4):391-405.

(24) Barger I. Control by management. Vet Parasitol 1997; 72(3-4):493-500.

Table 1. Commonly used anthelmintics in cattle including active ingredient, trade names (manufacturers), routes of administration, spectrum of activity and presence of withdrawal restrictions for meat and milk for each drug.

Active substance	Trade names	Route	Spectrum of activity	Withdrawal
Fenbendazole	1.Safe-Guard 2.Panacur (Intervet)	oral	Cestodes Trematodes Nematodes, incl hypobiotic L_4	1.Meat: + Milk: - 2.Meat: + Milk: +
Albendazole	Valbazen (Pfizer)	oral	Cestodes Trematodes Nematodes, incl L_4	Meat: + Milk: +
Levamisole	Tramisol (Ayerst)	oral	Trichostrongyles, except L_4	Meat: + Milk: +
Ivermectin	Ivomec (Merial)	oral topical injection	Nematodes, incl L_4 Arthropods	Meat: + Milk: NA*
Doramectin	Dectomax (Pfizer)	topical injection	Nematodes, incl L_4 Arthropods	Meat: + Milk: NA*
Moxidectin	Cydectin (Ayerst)	topical injection	Nematodes, incl L_4 Arthropods	Meat: + Milk: -
Eprinomectin	Ivomec-Eprinex (Merial)	topical	Nematodes, incl L_4 Arthropods	Meat: - Milk: -

* Not applicable; drug not approved for lactating animals.

2. A longitudinal study of gastrointestinal parasite burdens in adult Canadian dairy cows

1 Introduction

It has been well established that gastrointestinal nematodes in young cattle can have detrimental effects on how well the animals grow and thrive during their first grazing season. *Ostertagia ostertagi* is the most economically important of the trichostrongyles in pastured cattle from temperate zones, but various *Cooperia* spp. are also commonly seen (1;2). Clinical manifestations of infection with these genera of nematodes include reduced growth rates, diarrhea and in severe cases death by dehydration and malnourishment. Young animals during their first grazing season will commonly display such clinical signs following exposure to contaminated pastures. This is known as type I Ostertagiosis (3). Adult cows that have previously been exposed to parasites will harbour worms but rarely show clinical signs of infection. In a recent abattoir study performed in the Netherlands 96% of 125 adult dairy cows were found to have worms present in their abomasa (6), and previous studies in North America and Western Europe have found proportions between 84 and 100% (4-6).

Both *Ostertagia* and *Cooperia* can overwinter outside on pastures under Canadian conditions (7), or they can undergo arrested development and survive as hypobiotic L_4 larvae in the host (8). Cases of clinical disease seen when such worms again become metabolically active in late winter are known as "winter" or Type II ostertagiosis, but are uncommon (1). There is, however, evidence of a subclinical effect of gastrointestinal

nematodes in adult cows, because production has been shown to increase when the worms are eliminated(9).

In order to formulate a rational plan for the control of parasitism in cattle, both the actual prevalence of the parasites and factors that influence that prevalence need to be determined. These factors include climatic conditions, herd and pasture management, types of test used, characteristics of the individual animal and the parasite species present as described below.

Climatic conditions, such as temperature and humidity, will affect the life-cycles of the parasites (10). Management factors such as choice of parasite control program, level of access to pasture and pasture management strategies can all be expected to exert an impact on the level of parasitism in the environment, and hence also within the host (11). Both in the UK and in the Netherlands, researchers have found that different strains of trichostrongyles may have different propensities to undergo arrested larval development (8;12). If genetic constitution of the worms play a role in their ability to survive the winter, this can also be expected to contribute to differences in parasite populations between regions, herds and management practices.

The techniques used to detect trichostrongyle infections in cattle is another factor that will impact the estimation of both the prevalence and intensity of infection in these studies. Fecal egg counts are the most commonly used diagnostic tool but show poor correlation with actual worm burden, except in young animals during their first season on pasture (13). Fecal egg counts have been reported to be able to detect 50 or 25 strongyle type eggs per gram using the McMaster technique (14), and 0.2 eggs per gram by Wisconsin sugar flotation (15). Both Kloosterman (14) and Ploeger et al. (16) report

better ability to detect parasites as their reason for choosing larval cultures over McMaster fecal egg counts when monitoring parasite levels in clinical trials of anthelmintics. Frécette (17) utilized both a simple sodium nitrate flotation and the McMaster technique in a study of gastrointestinal helminths in Quebec cattle, and concluded that the flotation had a better sensitivity. Borgsteede et al. (6) concluded that larval cultures have a better ability to detect parasite eggs in fecal samples than the McMaster egg count procedure. Overall, the least sensitive of the methods appears to be the McMaster technique and it should only be used in young animals that shed high numbers of parasite eggs. The Wisconsin sugar flotation technique would be expected to have a similar performance as larval culturing. However, neither egg counts nor larval cultures will detect parasites that are not shedding eggs at the time of sampling. The gold standard for detection of gastrointestinal nematodes is worm counts at necropsy, which is currently also the only way to detect hypobiotic larvae (8).

Characteristics of individual animals, such as parity and stage of lactation, can be determinants of parasite egg outputs because they affect host immune status (2;14). Both stunting of the worms and lower worm egg output has been shown in nematodes from yearlings compared to calves, and mature cows will have even lower egg counts (18).

Because adult worms of the genus *Cooperia* typically produce more eggs than those of *Ostertagia,* the species distribution of a worm burden will potentially also have an effect on the number of eggs detected at sampling (13). A recent study, performed on abattoir material in Belgium, found worms in 91% of the abomasa from 110 slaughtered adult cattle. Fecal egg counts from the same animals using the McMaster technique only identified 14% positive samples, while 64% were larval culture positive. The same study

also failed to find any significant relationship between the age of the animal and the *Ostertagia* worm burden present in the abomasum (19). A similar lack of correlation between worm burden and age was documented by Borgsteede et al. in the Netherlands (15), Guitieres et al. in Wisconsin (4) and Overend et al. in Australia (20). This suggests that zero or low fecal egg counts in older animals are likely to be due to host immunity suppressing the parasite egg production rather than to a total elimination of the worms.

Larval cultures enable the species present to be identified, but are time consuming and hence not suitable for large scale studies. Fecal egg counts are therefore used as a crude indicator of the presence of parasites in adult cattle, even though they are known to be poor indicators of the burden in individual adult cows (13). Using a Wisconsin sugar flotation technique for the fecal egg counts is expected to increase the ability to detect parasite eggs over the McMaster technique. Despite this fact, almost all animals have trichostrongyles present but only a limited proportion will test positive using standard tests. The use of fecal egg counts in adult cattle is controversial, and has little value as a diagnostic tool in individual animals due to the lack of correlation with adult worm counts observed at necropsy and with clinical signs of parasitism (3;15;21). However, a series of repeated fecal egg counts from a number of animals may offer information on population dynamics at a group or herd level (22;23).

Fecal egg count data usually do not follow a normal distribution because of their wide range and many zero counts. Various standard transformations, the log-transformation in particular, along with non-parametric tests are often used by parasitologists in the analysis of these data. Applying a negative binomial error distribution in generalized linear models has been suggested as a better way of dealing

with the lack of normality in macro parasite data (24). One important benefit of this methodology is that it also allows one to account for the dependence between observations that arises by the clustering of animals at farms, or from repeated samples taken from the same animal over time (25). The negative binomial error distribution is a modification of the Poisson distribution for count data that allows for extra-Poisson variation. Even though the Poisson model can be considered the standard way of describing count data, it rarely fits since it assumes the mean and the variance to be approximately the same (26). The negative binomial model takes over-dispersion into account, but it may not adequately deal with the high number of zero counts present in fecal egg count data from mature cows.

An alternative approach to dealing with the high number of low counts in fecal egg count data would be to categorize the data and use models appropriate for ordinal outcomes (27). Alternatively, separate analyses could be carried out to model the probability of a zero count versus any value greater than zero using logistic regression, and using ordinary least squares regression for non-zero observations. The latter assumes that there is a separate mechanism involved in generating the zero observations and that different factors might be determinants for whether the outcome is zero or not than for the magnitude of the non-zero outcome. As described, it does however assume that the positive observations follow a normal distribution (27).

Zero-inflated negative binomial (ZINB) models are negative binomial models that allow for additional over-dispersion via a splitting process that models the probability of a zero outcome by logistic regression, while the continuous outcome is modelled using a negative binomial error structure (26). It is assumed that different underlying mechanisms

can be involved in generating zero and non-zero counts. Both zero and positive counts are generated by a negative binomial process, and in addition zero counts can arise separately through a logistic process. The Vuong statistic (26;28) is applied to assess the usefulness of a zero-inflated versus a regular negative binomial model, with a high positive value favouring the zero-inflated version. Estimated coefficients in the negative binomial part of the model are interpreted as for count models, while coefficients from the logistic part are related to the probability of a zero outcome. A positive coefficient in the logistic part of the model would be interpreted as an increased probability of a zero count (i.e. not having any trichostrongyle type eggs present in a fecal sample). Applied to parasite data, zero inflated negative binomial models might offer a useful way of dealing both with the high number of zero observations and with the extra-Poisson variation present among the non-zero counts.

The main objective of this study was to compare the level of parasitism in Canadian dairy cattle as measured by fecal egg counts across different regions, seasons and age-groups. The species breakdown of the present parasite population was also investigated through larval culturing. A second objective was to determine how various factors affected parasite burdens at the herd or animal level and this required applying a sound statistical method for analysing fecal egg count data.

2 Materials and methods

2.1 Test animals

Eight Holstein cows from each of 38 dairy farms in Canada were monitored for one year from October 1999 to September 2000. The farms were located in four different

regions of Canada from Prince Edward Island (PEI) on the East coast (n=14), moving west through St Hyacinthe in Quebec (n=14) and Guelph, Ontario (n=5) to Saskatoon, Saskatchewan (n=5). Herds in PEI and Quebec were also part of a clinical trial evaluating the effect of treating cows with an anthelmintic at the time of calving. Selection of participating herds was a convenience sample, based on proximity to the four Canadian veterinary colleges and expected compliance from the producer. All herds had some exposure to pasture, and had not used broad spectrum endectocides in the milking cows in the 6 months prior to the onset of the trial.

Fecal egg counts were performed on samples collected per rectum from the eight cows randomly selected within two strata at each farm: first lactation and second lactation or older animals. The animals were chosen from herd records at the initial visit at each farm using a random numbers table. Fecal sampling was either done by the researchers or by the Farm Service Veterinarians or technicians during regular herd health visits. During the initial visit in the fall of 1999, fecal samples from fifteen additional animals on each farm were collected for larval culturing. The most recently calved primiparous (n=5) and multiparous (n=5) animals were chosen, in addition to five randomly selected nulliparous heifers. In some cases the heifers were still on pasture and unavailable at this time and these animals were sampled at a later visit.

Information on the use of pasture and other management factors was collected using a standard in-person interview questionnaire (Appendix A) at all participating farms. Data from cows that were treated with an anthelmintic as part of the above mentioned clinical trial were included only until the last sampling date before such treatment. Data from untreated cows and cows treated with placebo were kept for all

analyses.

2.2 Fecal examinations

2.2.1 Fecal egg counts (FEC)

Fecal samples were collected from the rectum, stored at 4°C and analysed within one to seven days (mean=4 days), at each of the four study sites. The number of nematode eggs per 5 grams of feces was determined for individual cows using a modified Wisconsin sugar flotation technique (21). The animals from PEI were sampled monthly through the entire duration of the study. Herds in Quebec were sampled every month for the first 6 months and then bi-monthly, while the Ontario and Saskatchewan animals were sampled quarterly through the one year period of the trial.

2.2.2 Larval cultures

Larval cultures were performed on fecal samples from the initial visit, pooled by age group at each farm. All samples were refrigerated and shipped on ice to the University of Prince Edward Island for larval culturing. Duplicate samples were cultured for each herd age group. Approximately 10 grams of feces from each of the five animals in the age groups nulliparous, primiparous and multiparous were mixed with vermiculite and left at room temperature for two weeks allowing nematode eggs to develop into L_3 larvae. The larvae were harvested using a Baerman apparatus and identified based on microscopic features (29;30). When available, 100 larvae per culture were identified, and the proportion of each species was calculated.

2.3 Data analysis

2.3.1 Descriptive statistics

The mean and median number of eggs per 5 grams of feces (ep5g) was compared between the different regions and age groups, and through the year. The log(ep5g+1) was used in the graphical presentation of the results. The proportion of different L_3 species in the larval cultures was determined and compared to the fecal egg counts. The software package Stata (version 7) was used for the analysis (31).

2.3.2 Multivariable methods

A zero inflated negative binomial (ZINB)(31) model in Stata 7 (31), was used to evaluate factors that affected fecal egg counts. The clustering of observations by cow due to repeated samples through time was accounted for by the negative binomial model which allows for extra-Poisson variation, in conjunction with the Huber/White/sandwich estimator of variance (28). A variable representing herd was included as a fixed effect in each model, to account for the lack of independence between cows in the same herd.

Factors such as season, region, age-group and different management practices were tested in both the logistic and the negative binomial part of the model. Factors from the management questionnaires were selected through a screening process where subsets of management variables were tried in a backward stepwise negative binomial regression model. First, all the factors regarding heifer management were run through the model with a liberal cutoff for inclusion (P=0.1), then factors pertaining to lactating animals and finally dry cows were screened in the same way. The zero inflated negative binomial model was fitted manually, testing factors that had been identified as significant

predictors through the stepwise regression screening process. Cut off for keeping a variable in the final model was set to $p<0.05$.

To assess the breakdown of the variance present, a four-level random effects negative binomial model was built. The four levels in the hierarchy were: province, herd, cow and observation. The same independent variables as were used in the ZINB model were included and an exchangeable correlation structure was assumed. The purpose of this exercise was to determine in which hierarchical level most of the variability in the data was located. The software package MLwiN 1.1 (32) was used to build this multilevel model.

3 Results

3.1 Test animals

Table 1 summarizes the number of samples taken from the four study sites. A total of 315 cows were included in the longitudinal part of the study. The number of samples taken from each cow varied from 1 to 12. Some cows left the herd after only one sample, these were replaced at the second visit. This explains why the total number of animals is greater that eight times 38 (=304). The number of cows in the longitudinal study was decreasing in the PEI and Quebec farms, partially due to the fact that these herds were enrolled in a clinical trial where 50% of the animals received an anthelmintic drug at calving and hence were excluded from further analyses.

The questionnaire results revealed some differences in the use of pasture between provinces, with the use being more widespread in herds from the two eastern locations. Each producer was asked how heifers, lactating and dry cows were managed. Table 2

summarizes the management of these three groups of animals by region. Herd size was another parameter that showed variability across regions, with the Saskatchewan herds in this study being larger than the rest. See Table 3.

3.2 Fecal examinations

3.2.1 Fecal egg counts (FEC)

Raw fecal egg count results are reported as trichostrongyle type eggs per 5 grams of feces (ep5g). The range of egg counts was from 0 to 419 ep5g. Table 4 summarizes the range, mean and median counts for the four regions. In 46% of the samples, no trichostrongyle type eggs were present upon analysis, leading the data to being strongly right-skewed with many zero counts and a wide range of values among the non-zero count samples. The average number of trichostrongyle type eggs per 5 grams of feces among the non-zero counts was 17. Standard transformations failed to normalize the data, as can be seen in Figure 1. However, a ln(ep5g + 1) transformation was used to illustrate the seasonal variability in FEC as it will reduce some of the impact of single large observations on the mean. Figure 2 shows the fluctuation in FEC through the year for the four study sites.

The cows were classified in age groups as either 1st lactation or 2nd lactation and greater. Figure 3 shows the mean ln(ep5g+1) for primiparous and multiparous cows as a function of season.

3.2.2 Larval cultures

Out of 236 pooled larval cultures performed, 161 (68%) yielded trichostrongyle larvae. Fecal egg counts based on the same samples gave 216 positive results. The number of fecal egg count positive/ larval culture negative samples was 59 or 27.3%, and the number of negative fecal egg counts (n=20) that yielded positive larval cultures was 4 or 20%. The majority of L_3 larvae identified in the cultures were either *Ostertagia ostertagi* or belonged to the group of *Cooperia* species. Table 5 summarizes the breakdown between the five species identified, and it can be seen that more than 99% of the larvae belonged to one of these two groups. From Ontario and Saskatchewan, all larvae were *Ostertagia* or *Cooperia*. The samples from Quebec yielded small numbers of *Haemonchus* and *Trichostrongylus* as well, while the identified larvae from PEI also consisted of some *Oesophagostomum*. Figure 4 shows the proportion of *Ostertagia* and *Cooperia* of the total number of larvae broken down by region, and figure 5 shows the relative number of *Ostertagia* to *Cooperia* larvae broken down by the age of the animals and by province.

Each pooled age group sample was classified as being either predominantly *Cooperia* or predominantly *Ostertagia*. The groups in which most of the identified larvae were *Cooperia* had significantly higher mean FEC on the day of sampling(27.4 ep5g) than the *Ostertagia* groups (16.0 ep5g), based on a t-test ($P<0.03$).

The fact that all the larval cultures were performed at one single location led to different storage times for the samples. Table 6 summarizes the time it took from fecal samples from each site were collected until they were read. All samples were cultured for 14 days, this interval is included in the storage time variable. A t-test comparing the

dichotomized proportion of larvae (ie mostly *Ostertagia* or mostly *Cooperia)* failed to detect an effect of storage time on the species distribution (P=0.54).

3.3 Data analysis - multivariable methods

The fecal egg count data were heavily skewed to the right, and contained a high proportion (46.2%) of zero counts. The overall variance was 998.7 and the mean 9.8, hence the Poisson model was not considered appropriate since it assumes the mean and the variance to be approximately the same.

A zero-inflated negative binomial model was fit to the data. The Vuong test (26) had a high positive value, 8.43 ($p<0.001$), indicating that the zero inflated model fit the data better than a regular negative binomial model. Herd was included in the logistic part of the model as a fixed effect, and a robust variance estimator was used to account for the clustering of tests within each cow. A number of herd level variables from the management questionnaires were tested in the model, along with province, season and age of the animal.

Coefficients for the final model are shown in Table 7, separated into the logit and the negative binomial part of the model. Samples taken from October 2000 and up until September 2000 were included in the model to give one full year of results, which accounts for the fact that 1840 observations are used when a total of 1946 samples were available.

Parity group was significant in the logistic part of the model, together with herd. The coefficient for age was 1.50 with 1st lactation as the baseline. Since the probability of a zero count is what is being modelled, the interpretation of the coefficient is that cows

from second lactation and older have a higher probability of a zero fecal egg count. By exponentiation of the coefficient, the odds ratio can be estimated to 4.48. In other words, second lactation and older animals were approximately 4.5 times more likely to have a zero count. The 37 coefficients for the dummy variables for each herd are nuisance parameters that are of little interest, and were not included in Table 7.

In the negative binomial part of the fecal egg count model, the variables age group, season and region were included together with the three management variables that were found to be significant. As can be seen from Table 7, second lactation animals had a negative coefficient meaning that older animals have lower expected egg counts. Both season and region were overall significant variables using a likelihood ratio test. When fall was used as a baseline, the winter yielded lower expected fecal egg counts than all of the other seasons. Consequently, in the spring (April to June) counts were higher than in the winter months. When pasture exposure, season and age was accounted for the Quebec herds had lower expected fecal egg count numbers than herds from PEI (the baseline). Mechanical spreading of manure on pastures used by heifers contributed to a lower expected fecal egg count, while mechanical spreading of manure on pastures used by lactating cows had the opposite effect. If lactating cows were on pasture the expected number of trichostrongyle type eggs was increased compared to non-pastured animals.

Continuous versus rotational grazing, grazing heifers with other age groups, clipping, cutting or dragging of pastures together with various parasite treatment practices were not found to have an impact on the magnitude of fecal egg counts in this model. Nor did a predominance of Ostertagia in larval cultures from the initial visit in the fall of 1999.

A multilevel negative binomial model with province, herd, cow and observation included as random effects was built. The proportion of variance found at each of the four levels was province 1.57%, herd 3.65%, cow 22.83% and observation within cow 71.93%. The estimate of between province variance was smaller than the estimated standard error of this variance, hence the level was removed leaving a three level model. Province was tested as a fixed effect in this model but not found to be significant ($P>0.05$). The coefficients and standard errors from the four level model are presented in Table 8.

4 Discussion

In the results section the raw fecal egg counts were reported in eggs per 5 grams (ep5g), because this was the unit of measurement in the sugar flotation technique used. In order to facilitate comparison to results from previous studies, this discussion will use eggs per gram (epg) as the unit. The fecal egg counts in the current study ranged from 0 to 419 ep5g, with a mean of 9.8 ep5g among all cows and mean among positive samples only being 17.0 ep5g. Expressed as eggs per gram this translates to a range from 0 to 83.8 epg, a mean of 1.96 epg and mean for positive samples only of 3.4 epg.

The fecal egg count results from this study tended to be slightly lower than previous work done in North America. A trial in Georgia reported an average fecal egg count of 5 eggs per gram (5). Maine has similar climatic conditions as Atlantic Canada, and a study performed there found average fecal egg counts of 1.1 epg in January/February and 14.2 epg in May/June. This study also described a small increase in the number of *Ostertagia* eggs recovered in the spring (23). An increase in the fecal egg

counts in spring, before animals were exposed to pasture, was also seen in a study performed in Quebec (17). In that one-year study, 50 percent of the adult animals had trichostrongyle type eggs present on the day of analysis and seven percent had between 100 and 1000 epg. Smith (18) suggested 200 epg as the cutoff value for significant counts in bovine yearlings, and all the results from the present study are well below that(18). Other reports of fecal egg count levels in adult cattle have also typically been in the area of 3 - 5 epg (33-35).

Compared to the study by Agneessens et al. (19) from Belgium, we found more positive fecal egg counts; 54% compared to 14%. However, in positive cows the Belgian researchers found a mean of 89 eggs per gram of feces compared to our 3.4 epg. The fact that two different techniques of trichostrongyle egg detection were used can explain these differences, as the detection limit for the McMaster assay used by the Belgian researchers is higher than the Wisconsin sugar flotation used in the current study (15;19).

More than 99% of the larvae identified in this study were either *Ostertagia* or *Cooperia*. Reports in the literature tend to suggest that *O. ostertagi* is commonly found, but finding 56% of the larvae being *Cooperia* spp. was somewhat surprising (2). The high number of young animals included in the pooled larval cultures may account for this over representation of *Cooperia*, together with the fact that an overwhelming majority of larvae recovered from Saskatchewan belonged to this species. Based on experiments with larval cultures, Berrie and others (36) concluded that larval recoveries were disproportionate and that the technique should only be used to identify the species present, not to draw conclusions about the relative proportions.

The proportion of known positive fecal samples that failed to detect larvae after

culturing was 27.3%, while the number of samples that were negative on fecal egg count and positive by larval culture was 4 out of 20 (20%). Because all larval culturing was performed at the same location and by the same people, fecal samples had to be shipped and stored for some time before they were read. The effect of previous cold storage on larval recovery was discussed by McKenna (37). In fecal samples from sheep it was discovered that the susceptibility of trichostrongyle eggs to a period at 4°C depended on the species, and that the number of *Cooperia* larvae recovered after exposure to low temperatures declined more rapidly than the number of *Ostertagia*. For our samples the majority of the storage time happened after culturing but before larval identification, and the cultures from PEI were subject to the longest post-culture storage periods as can be seen in Table 6.

The species breakdown between the larvae was similar for PEI, Quebec and Ontario while the Saskatchewan herds had the highest relative number of *Cooperia* to *Ostertagi* L_3 larvae recovered. Considering the fact that all non-PEI samples had to be shipped on ice and hence were exposed to comparable pre-culturing cold storage, and that Saskatoon samples overall had the shortest handling and storage time it is hard to explain the predominance of *Cooperia* in samples from this region based on exposure to cold storage. Larval cultures from Alberta beef cattle have showed a 60 (*Cooperia*)/40 (*Ostertagia*) breakdown between the two species (38), and Saskatchewan could be expected to have comparable climatic conditions as those in other prairie provinces. Gibbs et al. (39) states that the adult lifespan of *O. ostertagi* is 25 to 50 days, which is shorter than for the other trichostrongyles. This leads to a low expected number of egg shedding *O.ostertagi* females present from about a month or two after animals are taken

off pasture. Fecal samples for larval culturing were collected at approximately the same time in all provinces. However, if the animals in Saskatchewan were taken off pasture earlier than in the three other provinces, an adult worm population consisting mainly of *Cooperia* spp. could explain the dominance of this species in the larval cultures from Saskatchewan.

Multivariable models

Fecal egg count observations were not statistically independent as the data set contained repeated measures on the same individuals over time, and cows were clustered in herds. However, software to fit multilevel zero inflated negative binomial models is currently not available. Consequently, a multilevel negative binomial model and a zero-inflated negative binomial model, generating a separate logistic model for the probability of a zero outcome, were considered for these analyses. Because the high proportion of tests with zero counts was a prominent feature of the data, the ZINB model was selected. The multilevel model yielded similar results, but was theoretically less suited for dealing with the high proportion of zero counts.

Based on the analysis of the crude variance components from the multilevel model, most of the variance (73%) was found between samples within a cow. Applying the robust variance estimator at the cow level, along with the negative binomial error distribution, should have prevented the dependency among observations from having a substantial effect on the standard errors in the ZINB model. Some variation between cows in a herd was also detected using the multilevel approach, and the clustering on herd was accounted for by including herd as a fixed effect in the ZINB model. Both herd and

province were significant predictors in the ZINB model based on the likelihood ratio test. However, province was not significant in the multilevel model once factors such as age, season and pasture use were controlled for and herd was included as a random effect.

Age was dichotomized into 1st lactation or 2nd lactation and older animals. The ZINB model uses 1st lactation as a baseline, and since group 2 has a positive coefficient in the logistic part, cows from 2nd lactation or older were more likely to be in the group that had negative fecal egg counts. In the negative binomial part of the model parity group had a negative coefficient, meaning that older cows had lower expected fecal egg counts than the 1st lactation group. If we assume that a similar biological mechanism (i.e. host immunity) is involved in increasing the probability of a zero count and in lowering the magnitude of a positive outcome, these two coefficients must always have opposite signs.

The study period was categorized into four seasons, based on the patterns seen in the graphical presentation of egg output through the year. The winter fecal egg counts were significantly lower than all the other seasons, hence the April/June egg counts were higher than the January/March. It is interesting to note that the fecal egg counts in PEI and Quebec rise as early as April or May, even though the animals were not let out on pasture until late May or early June in 2000 (Figure 2). The observation that the counts start rising even before any possible re-exposure could have occurred can be attributed to hypobiotic L_4 larvae maturing to adult fecund females at this time. Although the magnitude of this increase in fecal egg counts is low, it was statistically significant using both methods of analysis (multilevel negative binomial and zero inflated negative binomial). This apparent *spring-rise* is a well know phenomenon in small ruminants, and has previously been reported in cattle although in this species it is seen on a much smaller

scale (40).

Visual assessment of the data suggested that PEI and Quebec herds had higher fecal egg counts than the western herds included in the study (Figure 2). Animals from farms that used pasture for lactating cows had a higher number of trichostrongyle type eggs per 5g of feces than the ones that used pasture to a lesser extent. Based on the questionnaire results we know that the use of pasture was less widespread amongst our group of study herds in the two western locations, even though all herds had to have used some degree of pasture or outdoor exercise. One of five (20%) of the herds in both Ontario and Saskatchewan kept their lactating cows totally confined, while none of the monitored herds in PEI and Quebec did so. Approximately 70 % of the PEI and Quebec herds in this trial used pasture to meet some of the nutritional requirements of their lactating animals, compared to 20% in Ontario and Saskatchewan. Based on a questionnaire of 239 herds in Nova Scotia, this proportion is expected to be representative of the situation under Maritime conditions (41). The Nova Scotia study revealed that 70 % of both heifers and lactating cows in this region had pasture exposure, and also that pasture exposure was associated with higher ELISA optical densities, a diagnostic test of parasitism based on host antibodies in serum, individual or bulk tank milk samples. A similar relationship between pasture use and optical densities was seen by Caldwell and others in Quebec (35).

The herds from Ontario and Saskatchewan appeared to have much lower fecal egg counts than the PEI and Quebec herds upon visual assessment, but once use of pasture and other factors were accounted for the differences were less pronounced. This indicates that the apparent difference between PEI and the western provinces is likely to

be due to variation in the degree of pasture exposure rather than to climatic or geographic differences. A prevalence study of gastrointestinal nematodes in dairy heifers from three western Canadian provinces, concluded that the farm average fecal egg count in the included Saskatchewan herds was 10.3 epg. This was lower than for the investigated herds from Alberta and Manitoba, which could be explained by the dryer climate in Saskatchewan (42). Overall, there is no reason to believe that parasite burdens should be substantially different between herds in Saskatchewan, Ontario, Quebec and PEI.

Mechanical spreading of manure on pastures used by heifers in the summer of 1999 contributed to lower fecal egg counts in the lactating animals through the fall of 1999 to 2000. Meanwhile, spreading of manure on pastures used by lactating cows in the summer of 1999 lead to higher counts in this age group for the duration of the study. Trichostrongyle eggs have been shown to survive in slurry tanks, and spreading of manure has previously been identified as a potential source of pasture contamination (43). The opposite direction of the effect in the two age groups can possibly be attributed to heavy exposure of young animals to parasites if manure is spread on heifer-pastures, leading to better immunity and lower egg counts when these animals go back out on pasture as lactating cows.

5 Conclusions

The number of gastrointestinal nematodes detected by the use of fecal egg counts was low in all the cows of this trial. Variation in the magnitude of output was seen between seasons, age groups and herd management characteristics, both in the ZINB and in the multilevel model. Whether lactating animals had access to pasture or not was an

important factor in determining levels of parasite burden in these animals. A small, but statistically significant, rise in the fecal egg counts was seen in the early spring.

Due to the large degree of variability in fecal egg counts both between cows in a herd and between observations within a cow, there was relatively little variation between herds once factors such as exposure to pasture, age, etc. were known. If fecal egg counts are to be applied as a herd level measure of gastrointestinal parasitism in adult cows, it is recommended to perform a series of tests on multiple animals from each herd.

Reference List

(1) Georgi JR, Georgi ME. Helminths. Parasitology for Veterinarians. Philadelphia: W.B. Saunders, 1990: 103-223.

(2) Armour J. The influence of host immunity on the epidemiology of trichostrongyle infections in cattle. Vet Parasitol 1989; 32(1):5-19.

(3) Anderson N, Armour J, Jarrett WF, Jennings FW, Ritchie JS, Urquhart GM. A field study of parasitic gastritis in cattle. Vet Rec 1965; 77(41):1196-1204.

(4) Gutierres V, Todd AC, Crowley JW, Jr. Natural populations of helminths in Wisconsin dairy cows. Vet Med Small Anim Clin 1979; 74(3):369-72, 374.

(5) Ciordia H. Occurrence of gastrointestinal parasites in Georgia cattle. Am J Vet Res 1975; 36(4 Pt.1):457-461.

(6) Borgsteede FH, Tibben J, Cornelissen JB, Agneessens J, Gaasenbeek CP. Nematode parasites of adult dairy cattle in the Netherlands. Vet Parasitol 2000; 89(4):287-296.

(7) Smith HJ. On the persistence of infective Ostertagia ostertagi, Cooperia oncophora and Nematodirus helvetianus on pastures. Can J Comp Med Vet Sci 1972; 36(4):333-338.

(8) Armour J, Duncan M. Arrested larval development in cattle nematodes. Parasitol

Today 1987; 3(6):171-176.

(9) Gross SJ, Ryan WG, Ploeger HW. Anthelmintic treatment of dairy cows and its effect on milk production. Vet Rec 1999; 144(21):581-587.

(10) Stromberg BE. Environmental factors influencing transmission. Vet Parasitol 1997; 72(3-4):247-256.

(11) Stromberg BE, Averbeck GA. The role of parasite epidemiology in the management of grazing cattle. Int J Parasitol 1999; 29(1):33-39.

(12) Borgsteede FH, Eysker M. Strains of cattle parasites in the Netherlands with different propensities for inhibited development. Vet Parasitol 1987; 24(1-2):93-101.

(13) Eysker M, Ploeger HW. Value of present diagnostic methods for gastrointestinal nematode infections in ruminants. Parasitology 2000; 120 Suppl:S109-S119.

(14) Kloosterman A, Borgsteede FH, Eysker M. The effect of experimental Ostertagia ostertagi infections in stabled milking cows on egg output, serum pepsinogen levels, antibody titres and milk production. Vet Parasitol 1985; 17(4):299-308.

(15) Burrows RO, Davison CC, Best PJ. Survey of abomasal parasitism of culled dairy cows in southern Britain. Vet Rec 1980; 107(12):289-290.

(16) Ploeger HW, Kloosterman A, Bargeman G, von Wuijckhuise L, van den BR. Milk yield increase after anthelmintic treatment of dairy cattle related to some

parameters estimating helminth infection. Vet Parasitol 1990; 35(1-2):103-116.

(17) Frechette JL, Gibbs HC. Studies on the incidence of gastrointestinal helminths of cattle in Quebec. Can Vet J 1971; 12(11):207-210.

(18) Smith HJ. On the development of gastrointestinal parasitism in bovine yearlings. Can J Comp Med Vet Sci 1970; 34(4):303-308.

(19) Agneessens J, Claerebout E, Dorny P, Borgsteede FH, Vercruysse J. Nematode parasitism in adult dairy cows in Belgium. Vet Parasitol 2000; 90(1-2):83-92.

(20) Overend D. Abomasal trichostrongylidiasis of dairy cattle grazing irrigated pastures. Aust Vet J 1984; 61(4):124-126.

(21) Cox DD, Todd AC. Survey of gastrointestinal parasitism in Wisconsin cattle. J Am Vet Med Assoc 1962; 141:706-709.

(22) Gasbarre LC, Leighton EA, Bryant D. Reliability of a single fecal egg per gram determination as a measure of individual and herd values for trichostrongyle nematodes of cattle. Am J Vet Res 1996; 57(2):168-171.

(23) Yazwinski TA, Gibbs HC. Survey of helminth infections in Maine dairy cattle. Am J Vet Res 1975; 36(11):1677-1682.

(24) Wilson K, Grenfell BT. Generalized Linear Modelling for Parasitologists. Parasitol Today 1997; 13(1):33-38.

(25) McDermott JJ, Schukken YH. Study design and analytic methods for data

collected from clusters of animals. Prev Vet Med 1994; 18:175-191.

(26) Long JS. Count Outcomes: Regression Models for Counts. Regression Models for Categorical and Limited Dependent Variables. Thousand Oaks: Sage Publications, 1997: 217-250.

(27) Chang B, Pocock S. Analyzing data with clumping at zero. An example demonstration. J Clin Epidemiol 2000; 53(10):1036-1043.

(28) Statacorp. Stata Statistical Software. Relase 7 Manual. College Station (TX) USA: Stata Corporation, 2001.

(29) Kennedy MJ, Mackinnon JD, Higgs GW. Veterinary Parasitology Labaratory Procedures. Edmonton, Alberta: Alberta Agriculture, Food and Rural Developement, 1998.

(30) MAFF. Manual of Veterinary Parasitological Laboratory Techniques. 3rd ed. London: Ministry of Agriculture and Food, 1986.

(31) Stata Statistical Software. College Station (TX) USA: Stata Corporation, 2001.

(32) Goldstein H, Rasbash J, Plewis I, Draper D, Browne W, Yang M et al. A user's guide to MLwiN. 1.1 ed. Institute of Education, University of London, 1998.

(33) Nansen P, Jorgensen P, Soulsby EJL, editors. An evaluation of anthelmintic treatment in a dairy herd.: The commission of the European Communities., 1981.

(34) Nansen P, Jorgensen P, Soulsby EJL, editors. Observations on the epidemiology

and pathogenicity of nematode infections in adult dairy cattle in Great Britain. The commission of the European Communities: 1981.

(35) Caldwell V. Gastro-intestinal nematodes in dairy cattle: Prevalence, level of infection estimated by bulk tank milk ELISA testing and related risk factors. Faculte de Medicine Veterinaire de l'Universite de Montreal, Canada., 1997.

(36) Berrie DA, East IJ, Bourne AS, Bremner KC. Differential recoveries from faecal cultures of larvae of some gastro- intestinal nematodes of cattle. J Helminthol 1988; 62(2):110-114.

(37) McKenna PB. The effect of previous cold storage on the subsequent recovery of infective third stage nematode larvae from sheep faeces. Vet Parasitol 1998; 80(2):167-172.

(38) Piché CA. The epidemiology of gastrointestinal nematode infections if two beef cow-calf herds in Alberta and Quebec. MSc Thesis. The University of Calagary, Department of Biological Sciences., 1992.

(39) Gibbs HC, Herd RP. Nematodiasis in cattle. Importance, species involved, immunity, and resistance. Vet Clin North Am Food Anim Pract 1986; 2(2):211-224.

(40) Burrows RO, Best PJ, Preston JM. Trichostrongylid egg output of dairy cows. Vet Rec 1980; 107(17):399-401.

(41) Guitian FJ, Dohoo IR, Markham RJ, Conboy G, Keefe GP. Relationships between

bulk-tank antibodies to Ostertagia ostertagi and herd-management practices and measures of milk production in Nova Scotia dairy herds. Prev Vet Med 1999; 47(1-2):79-89.

(42) Cox WR, Lemiski D. Prevalence of gastrointestinal nematodes in dairy heifers in western Canada. Can Vet J 1989; 30:666-668.

(43) Nansen P, Jorgensen P, Soulsby EJL, editors. Trichostrongylid nematode infections associated with the handling of cattle slurry - a survey of Danish studies.: The commission of the European Communities, 1981.

Table 1. The number of fecal egg count samples by month, from adult Holstein cows at four different sites in Canada. September 1999 to October 2000.

	1999			2000										
Province	**S/O**	**N**	**D**	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**Total**
PEI	84	82	95	107	101	99	84	80	64	64	64	61	31	1016
Quebec	90	99	85	79	68	56	51		41		41			610
Ontario		40			42			43			38			163
Saskatchewan			40		39			39			39			157
Total	177	221	220	186	250	155	135	162	105	64	184	61	31	1946

Table 2. The number of herds using each housing system at 38 farms across four different regions of Canada.

	Confined [a]	Exercise, yard [b]	Exercise, grass [c]	Pasture [d]
PEI (n=14)				
Heifers	1	0	1	12
Lactating cows	0	1	3	10
Dry cows	1	1	1	11
Quebec (n=14)				
Heifers	1	1	2	10
Lactating cows	0	0	4	10
Dry cows	0	0	3	11
Ontario (n=5)				
Heifers	0	2	2	1
Lactating cows	1	0	2	2
Dry cows	0	1	3	1
Saskatchewan(n=5)				
Heifers	0	1	1	3
Lactating cows	1	2	2	0
Dry cows	0	0	2	3
Overall (n=38)	5	9	26	74
	(4.4%)	(7.9%)	(22.8%)	(65.0%)

a- "Confined" -animals kept inside 24 hours per day.

b- "Exercise, yard" - use of a gravel, dirt or concrete exercise yard.

c- "Exercise, grass" -animals were let out on a smaller grass covered area with the main purpose being exercise, not nutrition.

d- "Pasture" -animals obtained part of or entire nutritional requirement through grazing.

Table 3. The mean and (range) for the number of lactating cows, dry cows and heifers for herds from each province, based on questionnaire data.

	PEI	Quebec	Ontario	Saskatchewan	Overall
	n=14	n=14	n=5	n=5	n=38
Lactating cows	60 (27 - 140)	41 (22 - 64)	48 (31 - 65)	76 (50 - 170)	53 (22 - 170)
Dry cows	10 (3 - 25)	6 (4 - 9)	9 (4 - 15)	13 (6 - 35)	9 (3 - 35)
Heifers	25 (10 - 75)	17 (2 - 35)	24 (14 - 30)	30 (23 - 75)	23 (2 - 75)

Table 4. Summary statistics for 1946 fecal egg counts of Trichostrongyle-type eggs per 5 grams of feces (ep5g) from samples collected from 38 Canadian dairy herds.

	Range	Mean	Median	Std. Dev.	Variance	n
PEI	0 - 419	12.8	2	37.8	1428.5	1016
Quebec	0 - 241	7.8	1	23.6	558.7	610
Ontario	0 - 48	2.2	0	6.1	37.2	163
Saskatchewan	0 - 189	5.6	0	25.5	652.7	157
Overall	0 - 419	9.8	1	29.0	998.7	1946

Table 5. The number and relative proportion of different species of bovine L_3 nematodes, derived from 161 positive larval cultures from Canadian dairy herds in the fall of 1999.

	Ostertagia	*Cooperia* spp.	*Oesophagostomum*	*Haemonchus*	*Trichostrongylus*
n	2676	3479	9	8	14
Proportion of total	43.25%	56.24%	0.15%	0.13%	0.23%

Table 6. A summary of the mean, range and standard deviation for combined storage and culture time (days from sample date to test date) of larval cultures taken in the fall of 1999 from dairy cattle in 4 different regions of Canada.

		Storage and culture time (days)			
Region	Observations	Mean	Std.Dev.	Min	Max
PEI	85	56.4	33.5	19	146
Quebec	89	63.2	31.2	27	125
Ontario	34	74.0	27.1	31	133
Saskatchewan	23	43.7	4.2	37	49

Table 7. Factors related to the number of trichostrongyle type eggs per 5 grams in fecal samples from adult Holstein cows in four different regions of Canada from October 1999 to September 2000. Zero inflated negative binomial model based on 1840 fecal egg counts.

Part of model	Variable	Level	Coefficient	Std Err	p-value
Count / ep5g	Lactation	*1st*	baseline		
		2nd+	-.94	.23	<0.001
	Season	*Oct-Dec -99*	baseline		
		Jan - Mar00	-.70	.18	<0.001
		Apr - Jun00	.36	.22	0.107
		Jul - Sep00	.08	.26	0.766
	Province	*PEI*	baseline		
		Quebec	-.56	.27	0.042
		Ontario	-.33	.54	0.553
		Sask.	.47	.81	0.555
	Pasture, lact cows [a]		.93	.33	0.006
	Manure, heifers [b]		-.88	.27	<0.001
	Manure, lactating [c]		.60	.28	0.035
Logit / inflate*	Lactation	*1st*	baseline		
		2nd+	1.50	.53	0.005

a "Pasture, lact cows": lactating animals met some of their nutritional requirement from pasture

b "Manure, heifers": manure was mechanically spread on pastures used by heifers, summer of -99

c "Manure, lactating": manure was mechanically spread on pastures used by lactating cows, summer of -99

*Note: One variable per herd was included in the logit part of the model. These nuisance parameters are not listed in the table.

Table 8. A hierarchical, negative binomial model of fecal egg counts from 1840 samples taken from adult Holstein cows in four different regions of Canada from October 1999 to September 2000.

Variable	Level	Coefficient	Standard Error
Intercept*		1.926	0.451
Season	*Fall*	baseline	
	Winter	-0.824	0.172
	Spring	0.283	0.198
	Summer	0.151	0.217
Age group	*1st lactation*	baseline	
	2nd + lactation	-0.699	0.207
Pasture, lact. cows		1.491	0.339

* The intercept was allowed to have random variation at four levels. The variance components (standard error) and % variation at each level were: Province 0.168 (0.197) 1.57% / Herd 0.391 (0.222) 3.65% / Cow 2.440 (0.348) 22.83% / Test 7.688 (0.288) 71.93 %.

Figure 1. Frequency distribution of fecal egg counts as ep5g, ln(ep5g + 1) and square root(ep5g). Data from 1946 samples collected form 38 Canadian dairy herds between October 1999 and September 2000.

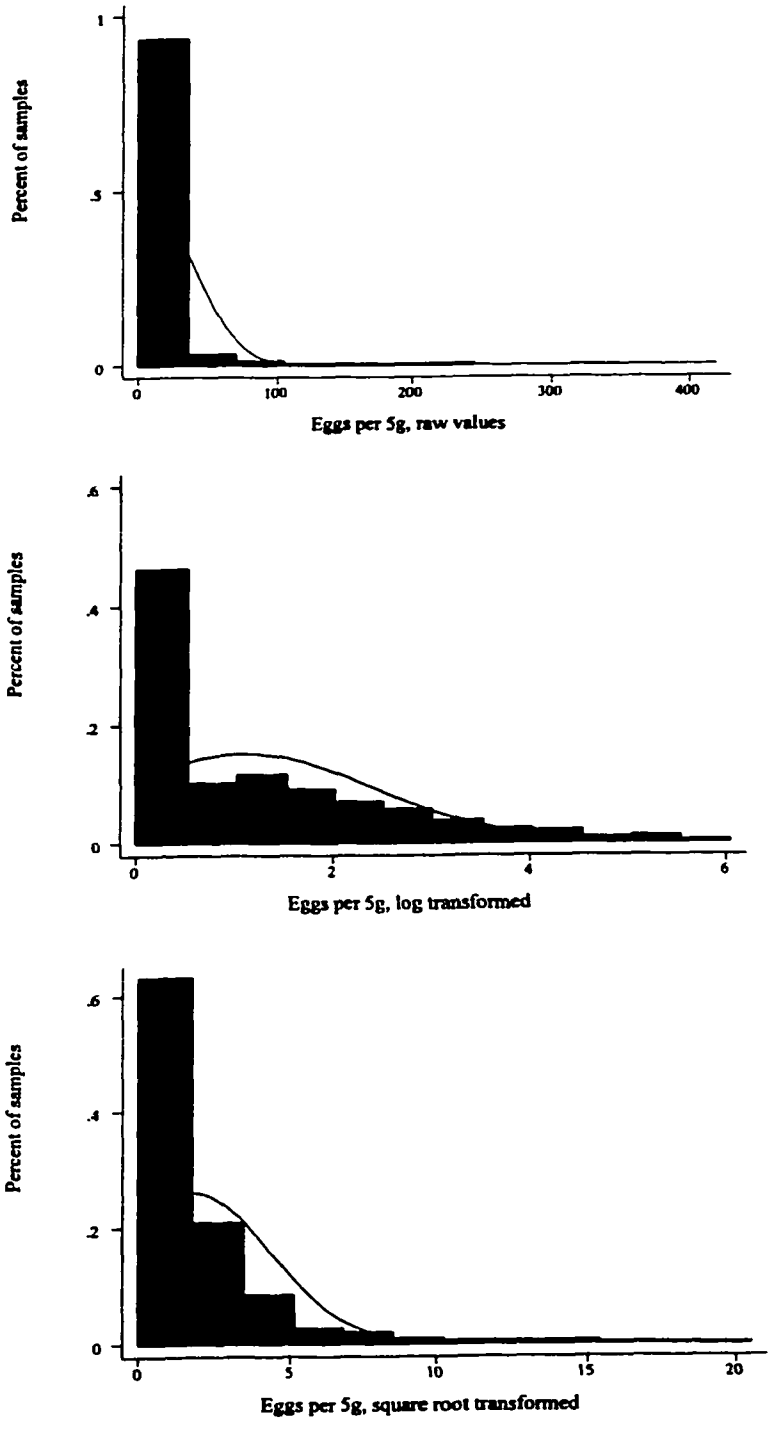


Figure 2. Variation in fecal egg counts from cows in 4 different regions of Canada, October 1999 to September 2000. Data from 1946 samples collected form 38 herds.

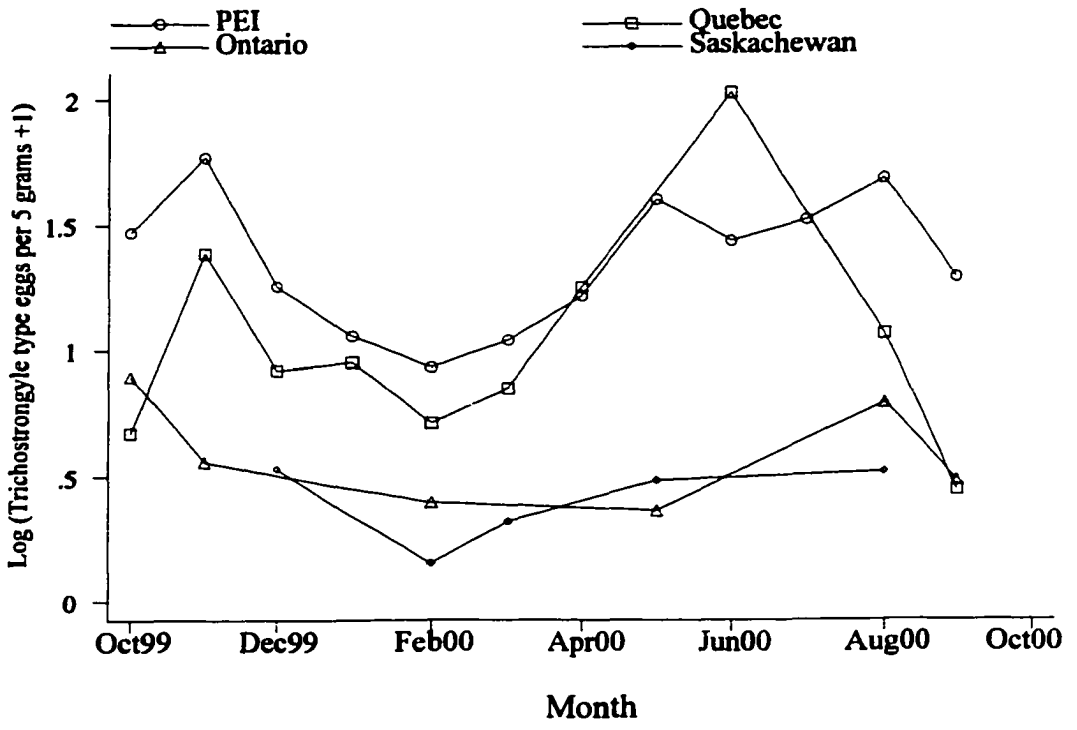


Figure 3. The variation in fecal egg count for cows from primiparous and multiparous cows in Canada, October 1999 to September 2000. 1946 samples taken from 38 dairy herds.

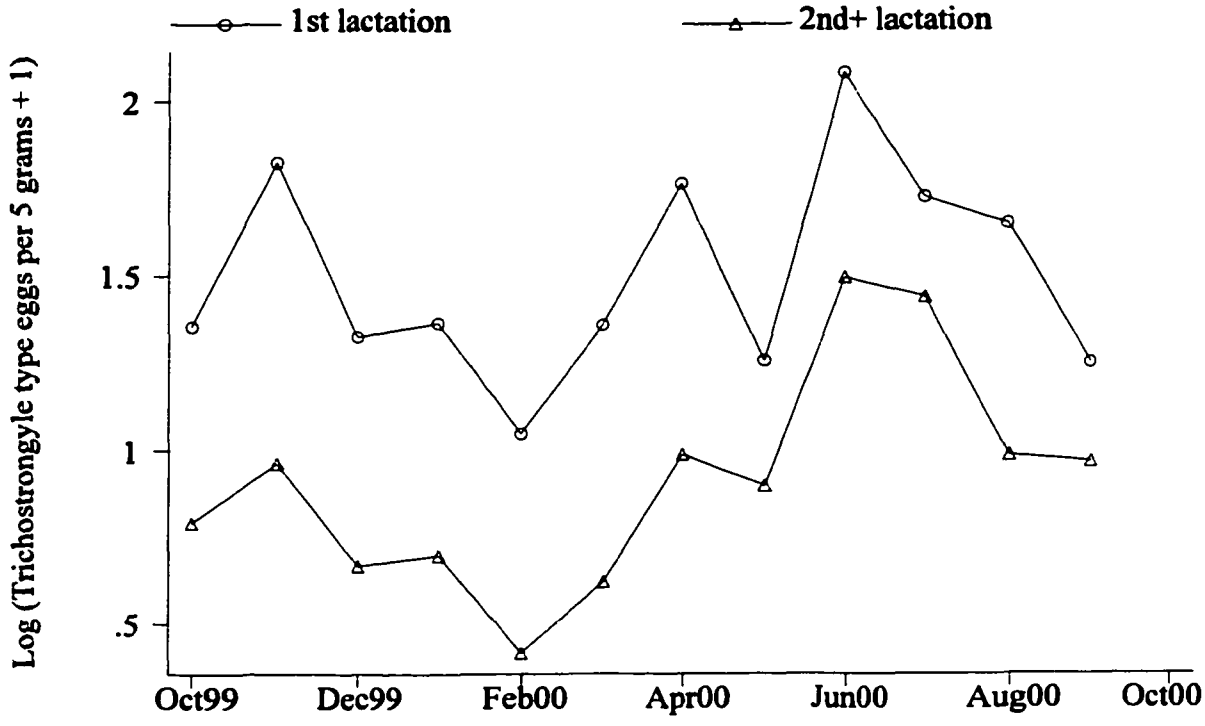


Figure 4. The proportion of Ostertagia and Cooperia larvae derived from pooled fecal samples obtained in the fall of 1999, by Province.

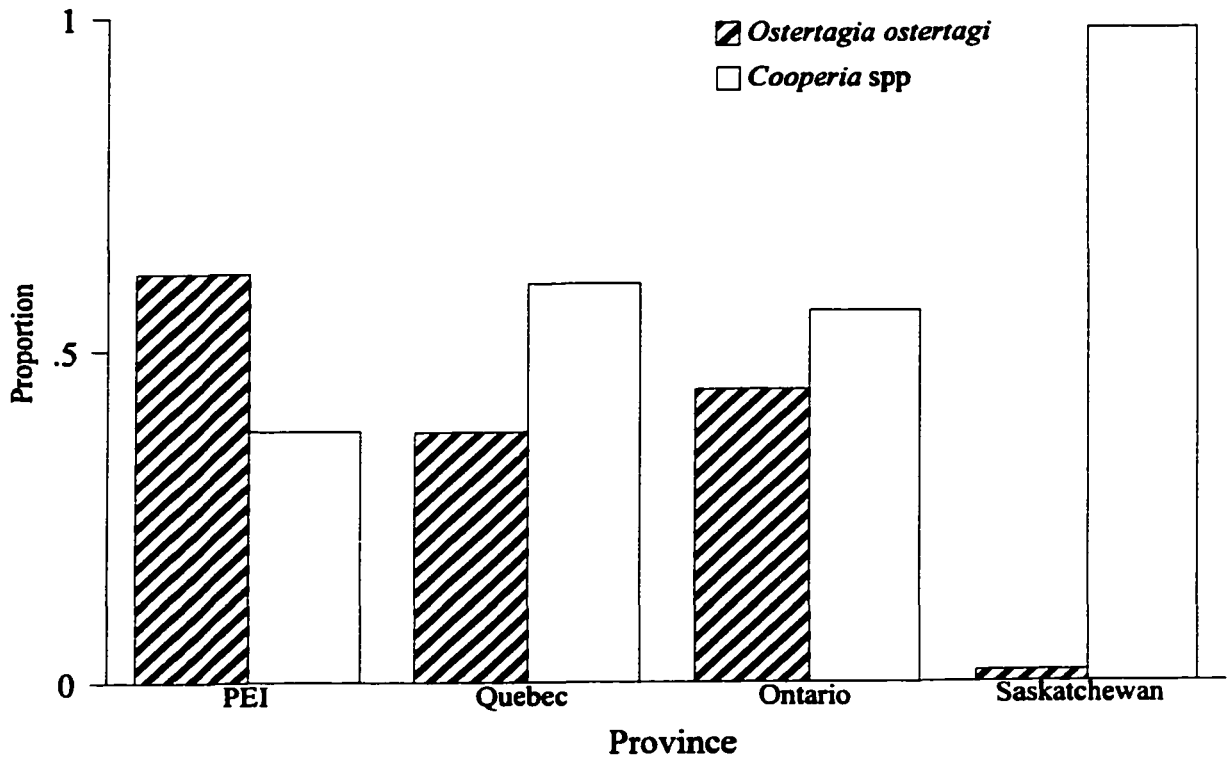
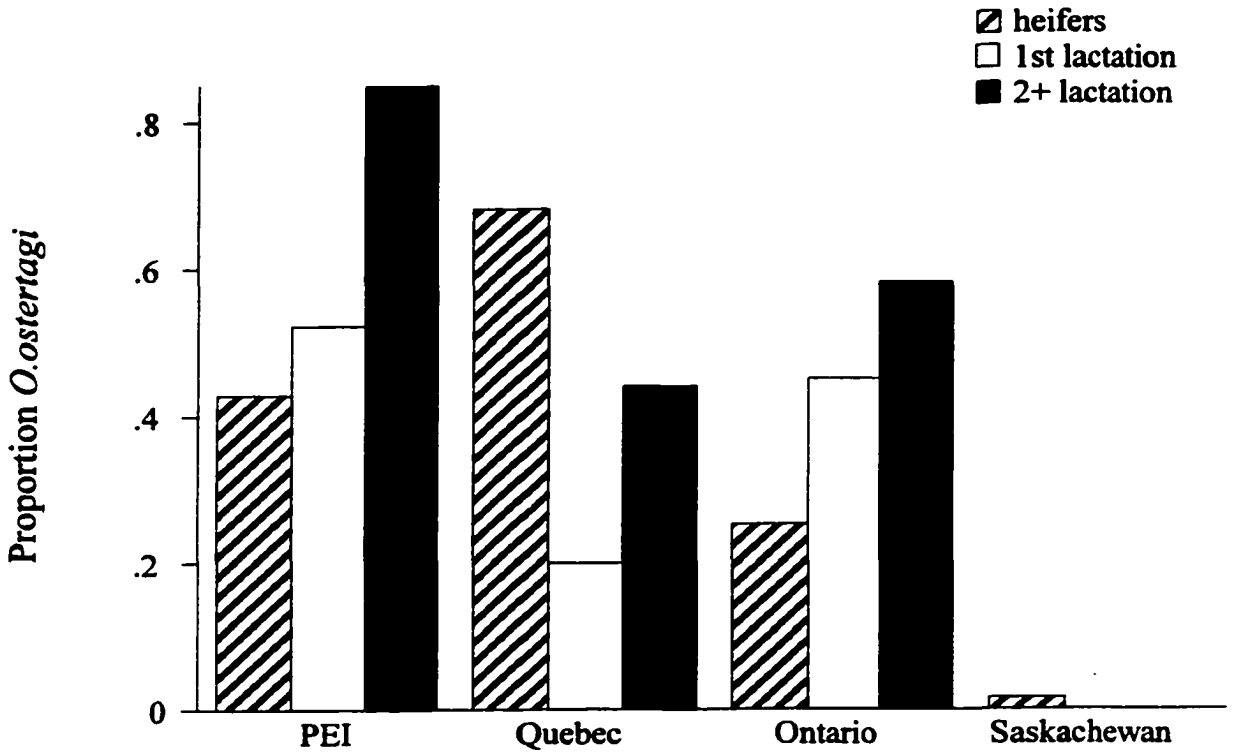


Figure 5. The proportion of Ostertagia of the total number of larvae, from cultures taken in the fall of 1999. Presented by age group and province.



3. The effect of eprinomectin pour-on solution in lactating cattle - a clinical trial in pastured dairy herds

1 Introduction

Gastrointestinal parasitism in adult dairy cows is a topic that has been the focus of much research effort over the last three decades. Gross et al.(1) reviewed the results of more than 80 different clinical trials assessing the effect of anthelmintic treatment in dairy cows and its potential effect on milk production, and concluded that overall there was a benefit from such treatment. Various study designs and anthelmintics were applied in the studies assessed, and it was concluded that the median increase in milk production across all the trials was 0.63 kg of milk per day. The majority of trials in which the anthelmintic treatment was applied during the dry period or at calving showed an increase in milk production for treated compared to non-treated cows in the following lactation. For this group of studies the median increase in production per cow was estimated to 0.42 kg/ milk/ day (1).

Previous studies performed in Atlantic Canada (2;3) and Quebec (4) have suggested that nematode infections are widespread among dairy cows in these regions, and that this has the potential of affecting milk production in adult cattle. However, assessing parasite burdens in adult dairy cows is complicated by the low correlation between worm burden and trichostrongyle type eggs obtained by fecal egg counts from adult cows (5-7) . Serology parameters, such as gastrin and pepsinogen have been used to diagnose parasite burdens as an alternative to the traditionally applied fecal egg counts.

An ELISA test based on either recombinant or crude parasite protein has shown

promise as a tool for herd level monitoring, but further epidemiological studies are needed before any of these tests can be implemented on a routine basis (8). Therefore, the most commonly measured outcomes in clinical trials of nematodiasis in adult cattle are indirect indicators of the parasite burden, such as milk production response following anthelmintic treatment. The relationship between level of parasitism and the size of such a treatment response has been hard to prove because of the mentioned lack of adequate diagnostic tests for parasitism in this age group (9). Ploeger at al. (9) indicated that high yielding dairy cows showed a greater increase in projected 305-day milk production following treatment with ivermectin than low producers. This effect was not found to be significant when herds were the unit of analysis instead of individual animals. Another Dutch study reported a similar trend; higher producing cows seemed to gain more in milk production relative to lower producers. However, this interaction between production level and treatment effect was not statistically significant (10) .

Recent advances in the development of new and improved anthelmintics within the group of macrocyclic lactone endectocides provide opportunities for more effective control of parasites in adult dairy cattle. Some of the benefits of these new drugs are persistent effects for at least three weeks after application (11) and effectiveness against immature *O.Ostertagi*, which is the most economically important of the bovine gastrointestinal nematodes in temperate parts of the Northern hemisphere (12). The fact that some of these newer anthelmintics require no withdrawal of milk after application contributes to an "at-calving" treatment scheme being feasible under field conditions.

In addition, recent developments in dairy information processing and statistical

analysis techniques make large multi-centre clinical trials more feasible. Test day milk yields for all Canadian dairy cows in a production recording program are now stored in the Canadian Dairy Herd Management System (CDHMS) database. By accessing this information, series of repeated measurements of milk production can be obtained from any given animal. Test day models provide a more accurate way of analysing short term differences in milk production than models based on 305-day milk production (13;14).

The assumption of independent observations is violated in test day models due to the fact that there are repeated observations within each cow, and that the cows are clustered in herds (13). The dependence between measurements from the same individual can be taken into account by modelling the within-cow error variance-covariance matrix (15) in a mixed effects model containing both fixed and random effects (16). By utilising a mixed model the clustering of cows in herds can be accounted for by including a random term for herd, in addition to modelling the within-cow correlation as a random effect. The clustering of cows in herds can alternatively be corrected for by including dummy variables for each herd, except one, as fixed effects in the model. The choice between including herd as a random effect, and hence adding another level of organisation to the model, or to use the fixed effect approach should be based on the design of the study and the outcome of interest (17).

The objective of the current study was to determine the effect of treatment with eprinomectin pour-on solution (Ivomec-Eprinex®, Merial) at calving on milk production, parasite burdens and selected health parameters in lactating dairy cattle from herds that utilize pasture for adult animals to some extent.

2 Materials and methods

2.1 Test animals

Holstein cows from 28 herds in two different regions of Canada were included in the clinical trial. Of these herds, 14 were in Prince Edward Island and 14 in Quebec. The selected herds were a convenience sample based on expected compliance from the producer and proximity to the Farm Service Units at the Veterinary Colleges of the University of Prince Edward Island and the University of Montreal, respectively. The herd selection criteria for the trial included some exposure of adult cattle to pasture, participation in CDHMS, on-farm recording of health events and a history of no use of broad spectrum endectocides in lactating animals in the six months before the onset of the study. The minimum exposure to pasture required was that the lactating cows had access to a grass-covered exercise area, but for the majority of the herds adult animals met some of their nutritional requirements from pasture (See Table 2, Chapter 2). All the included herds had cows calving throughout the year.

2.1.1 Treatment protocol

All cows due to calve within 12 months of the start of the trial on PEI and within 6 months in Quebec, were eligible for inclusion in the study. The study was a double blind randomized clinical trial, with anthelmintic and placebo being delivered in indistinguishable bottles labelled only with a number. The placebo consisted of the

vehicle for Ivomec-Eprinex® without the active ingredient. Because the sample size on Prince Edward Island was increased during the study, additional placebo was obtained by using mineral oil which was delivered in identical bottles and could not be distinguished from the anthelmintic drug. As they calved, cows would be randomly allocated to treatment with eprinomectin pour-on solution or placebo within blocks of 10, to ensure that both treatment groups would have a balanced distribution of animals calving through the duration of the study. Application was done according to label use for Ivomec-Eprinex® (5 mg eprinomectin/ ml) by each producer. The weight of the animal was estimated using a weight tape provided by the researchers, and eprinomectin pour-on solution applied at 1 ml/10 kg (500 μg per kg body weight) by the producer on the day of calving. Treatment date and dose applied was recorded by the person performing the treatment. The maximum cow number cut-off for inclusion in the trial was 80 cows from any single farm, in order to prevent the larger herds from having too much influence in the data analysis.

2.2 Milk production data

Individual test day (24-hour) milk yields were obtained from the CDHMS database in Montreal. Information on milk composition, days in milk, projected (or actual) 305 day production, calving date, lactation number and date of first breeding were also available through the same source. The database contained data that were recorded and updated monthly by field-technicians at each farm. Because the last cows that were

included in the study calved in September 2000, production data up until March 2001 were downloaded to ensure that a minimum of 6 test day measurements were available for the majority of the trial participants.

2.3 Fecal examinations

In addition to measuring milk production for all animals that were included in the clinical trial, a subset of cows were selected in order to be able to monitor the pre- and post-treatment output of parasite eggs using fecal egg counts. Four first lactation and four second lactation or older animals at each of the 28 farms were selected using a random numbers table, and randomly assigned to receive either eprinomectin or placebo at calving. Each farm was visited monthly for twelve months on PEI and fecal samples were collected rectally from the eight monitored cows. Monthly samples were collected from the herds in Quebec for six months, after which they were sampled bi-monthly. Using a modified Wisconsin sugar flotation technique (18) the number of trichostrongyle type eggs per 5 grams of feces was determined. See Chapter 2 for a detailed description of the fecal egg count results.

2.4 Health data

Cases of retained placenta, milk fever, ketosis, lameness, acute mastitis (defined as a mastitis case that required medication), cystic ovaries and abortion were recorded by

the producer or the herd health veterinarian at each participating farm. For all the included herds at the Quebec location, computerized health records were stored at the Farm Service Unit at the Faculte de Medicin Veterinaire in St.Hyacinthe. Six of the herds on PEI kept similar computerized records, while for the remaining herds disease was recorded manually by the researchers upon the monthly monitoring visit. The number of cases of each disease were summarized and unconditional associations between treatment with eprinomectin pour-on and the selected health parameters were evaluated.

2.5 Data analysis

2.5.1 Descriptive statistics

Mean herd size, number of treated cows by lactation, distribution of calving dates, average production by province, number of cows that received anthelmintic or placebo and occurrence of selected health events were summarized. Unconditional associations were tested using the Student t-test for continuous outcomes and the chi-square test for categorical outcomes, using the software package Stata version 7 (19).

2.5.2 Multivariable methods

Daily milk production will vary depending on factors such as parity, calving season, month of test, herd and days in milk, hence all these factors were included in a model to be able to determine the effect of anthelmintic treatment on production. Animals

were classified as belonging to one of three parity groups; 1st lactation, 2nd lactation or 3rd lactation and older. Each cow would also fall into a calving season category based on her calving date. The study period was divided into four calving seasons; Fall (October to December 1999), Winter (January to March 2000), Spring (April to June 2000) and Summer (July to September 2000). Month of test was included in the model to account for the fact that milk production shows a distinct seasonal pattern, and herd was included to explain some of the variation that can be attributed to different management and feeding practices between herds. Length of time, in months, until re-exposure to pasture after treatment was identified as an additional factor that could affect the size of the production effect and was also tested in the model.

In order to be able to correct for the effect of days in milk on test day yield, the shape of the lactation curve must be accounted for. According to Wilmink's function, a linear term for days in milk was included in the model as well as an exponential function to the power -0.05 times days in milk (14) . Appendix B shows the fitted values for a model of test day milk yield for the first 6 tests after calving. The figure is based on a model that includes only an intercept, days in milk and the exponential transformation of days in milk and is included here to demonstrate how Wilmink's function fits the lactation curve.

In the initial steps of the data analysis, generalized estimating equations (gee) were used to build separate population averaged models for individual test days one to eight. All the above mentioned fixed effects were evaluated in these models, and an exchangeable within-herd correlation structure was assumed. The software package Stata

version 7 was used for this analysis (19).

The PROC MIXED command in SAS version 6.12 (20) was utilized to build a random effects regression model of the overall effect of the anthelmintic treatment on individual test day milk production for the first six tests after calving/ treatment. Cow was identified as a clustering variable and various structures for the within-cow correlation were tested (independent, exchangeable, first order auto regressive, toeplitz). Comparison between models with different correlation structures was based on log likelihood. Herd was included as a random effect, and this model was compared to a model with dummy variables for each herd as fixed effects. All factors and potential first order interactions with anthelmintic treatment were evaluated in the model with the cut-off for inclusion being $P<0.05$.

The log of the somatic cell count, percent fat and percent protein were substituted for daily milk production as the outcome in three separate population averaged models where the explanatory variables were the same as in the overall production model. Herd was included as a fixed effect in these models, and a first order auto regressive within-cow correlation was assumed. This analysis was performed using Stata version 7 (19).

3 Results

3.1 Test animals

A total of 942 cows were treated as part of the clinical trial, 662 from the Prince

Edward Island herds and 280 from Quebec. For 901 of these animals complete test day milk yield records were retrieved, and the proportion of animals that were treated with the eprinomectin pour on solution versus placebo was 49.6 to 50.4%. Approximately half the cows in the trial were in their first or second lactation. Figure 1 shows the parity distribution of the treated cows by region. The overall parity distribution was 28.1% 1st lactation, 22.5% 2nd lactation and 49.4% 3rd lactation and older animals. The cows from the PEI farms calved between October 7th 1999 and September 27th 2000, with a mean calving date of February 26th and median February 13th. In Quebec, cows enrolled in the study calved between October 30th 1999 and July 16th 2000 with the mean and median calving dates being the 30th and 27th of January 2000, respectively. The calving distribution for the two locations is summarized in Table 1. The number of animals from each farm that contributed to the study, or the cluster size, ranged from 6 to 79 individuals. (Table 2.)

3.2 *Milk production data*

Figure 2 illustrates that the overall distribution of test day milk yields for all observations included in the analysis were approximately normally distributed. The somatic cell count observations were log transformed (ln(scc)), to make the distribution closer to normal but it was still somewhat skewed to the right. The distributions of percent fat and protein were approximately normally distributed.

Average test day milk production obtained from the CDHMS database was 27.75

kg/cow for individual tests taken from the enrolled animals, in the 28 study herds between August 1999 and March 2001. A t-test showed a significant difference ($P<0.001$) between the two regions with the average daily milk production for the PEI animals being 2.16 kg above that of the herds from Quebec. For multiparous cows previous lactation 305 day milk production was compared and revealed similar results; the average production for the PEI herds was 9040 kg per cow and for the Quebec herds it was 8360 kg per cow. The difference of 680 kg was found to be highly significant using a t-test ($P<0.001$). No difference in previous lactation milk production was found between the placebo and the treated group, the average production for the two groups in the previous lactation being 8831.7 kg per cow and 8832.4 kg per cow, respectively.

Figure 3 shows the variation in average daily milk production through the year for all eligible animals in the included herds that calved during the study. Data from both regions were pooled, and a distinctive seasonal pattern was seen.

3.3 Fecal examinations

Of the cows from which feces were obtained, 172 calved and were treated with either anthelmintic (51.3%) or placebo (48.7%). The number of trichostrongyle type eggs per 5 grams (ep5g) of feces obtained from the 8 monitored cows at each farm ranged from 0 to 419. The mean was 9.2, the median 1.0 and the standard deviation was 30.0. These numbers only include samples up to the day of treatment for animals that received the active substance, and samples for the entire study period for placebo treated animals. In

general the fecal egg counts from the PEI cows were somewhat higher than the numbers found in Quebec. The fecal egg counts were not normally distributed, and the counts were log(ep5g + 1) transformed before graphical assessment in order to remove some of the effect of single high observations on the means. Figure 4 shows the log transformed number of trichostrongyle type eggs obtained in the period from 200 days before to 200 days after calving for the 8 monitored cows on each farm, comparing cows that were treated with placebo to cows that received eprinomectin pour-on solution. The apparent delay in the drop in fecal egg count numbers for animals that were treated with the active drug is an artifact, and can be explained by the fact that the graph is based on average counts over 30 day periods. The fecal egg count numbers for the treated animals actually decreased immediately after application, and remained very low for at least the first 100 days after calving.

3.4 Health data

Disease records were obtained from 942 animals that were treated as part of the clinical trial, even though the analysis of effect on milk production only included 901 cows due to incomplete records for some individuals. Table 3 summarizes the number of cases and the p-values from chi-square tests of the association between each disease and treatment. No effect of treatment with eprinomectin on the occurrence of the selected health parameters was detected.

3.5 Data analysis - multivariable methods

Population averaged models with exchangeable within-herd correlation structure were built for each test number in the initial steps of the analysis. The explanatory variables found to be significant in these models were: anthelmintic treatment, days in milk, parity group and test month. The models are summarized in Table 4. The first six individual test day models yielded estimates of the effect of eprinomectin pour-on treatment on milk production which were significantly different from placebo treated animals. Estimated effects at tests number seven and eight were based on fewer individuals since the number of animals from which test day milk production values could be obtained decreased with increasing time from treatment. It was therefore decided to restrict the analysis of milk production effect to include tests one to six only post calving.

The factors that were found to be significant in the mixed model of overall test day milk production were anthelmintic treatment at calving, parity group, month of test, herd, days in milk (dim) and the exponential function for days in milk ($dim^{-0.05}$). Calving season was only marginally significant but was forced in the model. Table 5 shows the coefficients from an overall model of the anthelmintic treatment versus placebo for the first 6 milk tests after treatment combined. The average increase in milk production for animals in the treated group was estimated to 0.94 kg/day. The model with herd as a fixed effect also estimated the increase in test day milk production following eprinomectin treatment at calving to be 0.94 kg/day. Region was a significant explanatory variable in the fixed effect model, but apart from that the results were comparable.

Interaction terms between the anthelmintic treatment and province, parity group and calving season were tested but found to be non-significant. P-values for the interaction terms were 0.520, 0.142 and 0.484, respectively. While the effect of topical eprinomectin treatment did not seem to vary between provinces and calving seasons, there was a trend towards an increased effect of the treatment in animals from 1st or 2nd lactation compared to the 3rd lactation and older animals.

All of the variance-covariance structures that assumed some degree of correlation between observations within a cow performed much better than if samples from the same animal were assumed to be independent of each other. Likelihood ratios for models with different correlation structures were; independent 16803.4, exchangeable 15961.4, ar(1) 15805.3, toeplitz 15752.5. The first order auto regressive correlation structure (ar(1)) was chosen for the analysis, because it makes sense biologically and is commonly used for longitudinal data. The within-cow correlation coefficient between adjacent test day observations was estimated to be 0.64 when the ar(1) variance-covariance matrix was applied.

None of the three population averaged models evaluating the effect of eprinomectin pour-on treatment on milk composition was able to detect a significant effect. The coefficients for eprinomectin treatment in the models being; log somatic cell count .001 (P=.99), percent fat -.014 (P=.56), percent protein -.003 (P=.77).

4 Discussion

Approximately one third of the animals included in the clinical trial were located in Quebec, and the remainder were in Prince Edward Island. This was because the study period lasted for a full year at the latter site, and only six months in Quebec. This also explains why the study animals in Quebec tended to calve earlier. Comparing the distribution of animals in each lactation group in Figure 1, PEI herds had a marginally higher proportion of primiparous cows than those in Quebec. The late summer/ fall drop in milk production appears more pronounced for the Quebec herds that were included in the study, than for the participating herds from PEI. This difference in seasonality of milk production can be explained by the fact that all the included animals from Quebec calved during the first six months of the trial. Hence their milk production would peak in a more synchronized way, as all animals would be in early lactation more or less at the same time. Consequently the production will appear to drop more dramatically in the fall when the animals are approaching the termination of the lactation.

Even though the monitored animals in this trial showed lower outputs of nematode eggs than what has been reported from previous studies, the production effect was higher than the overall median effect reported by Gross of 0.63 kg/day across all study types (1). The overall average fecal egg count in this trial was 9.2 ep5g, which was marginally lower than previous reports of 15 to 25 ep5g (4;5;21). This supports the observation of low correlation between worm burden, fecal egg counts and production response in adult cattle (5-7) . Figure 4 shows that the average log transformed fecal egg counts for the treated group stay below that of the placebo group for at least 100 days.

Both the duration of the production effect and, in particular, the effect of eprinomectin pour-on on fecal egg counts are expected to be under-estimated in this study compared to a study of whole herd anthelmintic treatment. This is because treated animals co-mingle with untreated animals and hence get re-exposed to a higher parasite pressure than what might be expected if the whole herd had been treated at the same time. However, months until re-exposure to pasture was not significant when included in the model. If endectocide or placebo had been applied to all animals in the herd at the same time, the control animals would be expected to benefit from the treatment because the overall level of infectious worms in the pasture would be expected to decrease following elimination of the worm burden in the animals that received treatment.

Health records from the Quebec herds could be expected to be of better overall quality because computerized records were already an established part of the herd health monitoring program at this site. This may potentially lead to information bias since the follow-up for the two locations differed. However, when the data were stratified by region there was still no significant relationship between treatment with eprinomectin pour-on and the diseases recorded. If a hypothesized effect of the treatment on the selected health parameters can be expected to be the same for both study sites, this difference in record keeping is of minor importance. Non-differential mis-classification is likely to be a more important source of bias in this study. Not all disease cases are expected to be recorded, but because the design was double blind it is unlikely that producers or veterinarians would have a different probability of recording disease for the treated and the control group. Non-differential mis-classification will bias the estimates

towards the null.

The overall increase in test day milk production estimated by this study was 0.94 kg/day for the first six tests following treatment with topical eprinomectin. The effect persisted for at least the first six test days after application at calving, which translates to between 180 and 200 days in milk. Based on the gee models used to explore the data it can be assumed that the effect of eprinomectin would persist for an even longer period of time, and the consistent high effect has potential economic significance for dairy producers. In order to give the reader an idea of the magnitude of this economic benefit, the cost of an eprinomectin pour-on treatment will be calculated and expressed as milk litre equivalents. In Canada, the cost of treating an adult cow with Ivomec-Eprinex® is approximately Can $11. The producer receives approximately Can $0.5 for a litre of milk delivered, which means that a treatment costs 22 (11*0.5) milk litre equivalents. An increased production of 0.94 litres per day for 180 days corresponds to nearly 170 litres, hence the net gain for the producer can be expressed as 148 (170 - 22) milk litre equivalents. This is a conservative estimate of the benefit of treatment, because the effect on milk production is estimated to persist for 180 days at the least, while it is most likely prolonged. This crude calculation does not take cost of labour into account, because the animals in this study were treated topically at calving and there was little extra time and effort involved. The potential cost of extra feed intake in the treated animals is also ignored in this simplified analysis, which gives an estimated cost/ benefit ratio of (170/22) = 7.73 or a net gain of Can $74 .

The detected increase in milk production is larger than that estimated by Gross et

al. (1) in their review paper (0.63kg/day). The difference could be due to the use of one of a new generation of anthelmintics, or also to changes in management and improved genetic potential for milk production over the past decades (1). A common feature of subclinical nematode infections in ruminants is a decrease in voluntary feed intake (22;23). It can be hypothesized that negative energy balance in the transition cow can be further influenced by decreased appetite caused by abomasal nematodes, and that the elimination of this additional stress factor early in the lactation might contribute to a higher production throughout the lactation.

The interaction between treatment and calving season was not significant in the model, nor was time until re-exposure to pasture after treatment. It must be pointed out that due to a small sample size, there may not have been sufficient power to detect effects during the summer. Ploeger et al. (10) showed a similar lack of interaction between month of calving and treatment as well as between treatment and age or breed, in a study that yielded an overall benefit of treatment with albendazole of 132.9 kg on 305-day milk production (=0.44 kg/day). The production model also failed to detect a significant interaction between anthelmintic treatment and parity group in this dataset, even though the trend was towards a better response in first and second lactation animals compared to older cows. Abattoir studies have shown similar worm burdens across age groups (6;7) in culled dairy cattle, so there is reason to assume that the actual number of parasites present in animals from the three groups would be comparable. None of the three interaction terms were significant when tested in the model, even though the effects appeared quite marked when the data were split and separate models were fitted. There may not have

been enough power present in the current study to detect interactions, if in fact there were any.

Carrier (24) conducted a study in Quebec evaluating the relationship between eprinomectin treatment of adult cows at fall housing and milk production, but failed to detect any production effect. However, the trial included only 290 animals and may have lacked the power to detect the differences one would expect with this treatment protocol. In the random effects model, no difference in the change in milk production between the two regions was noted, which indicates that this was not an important explanatory variable. On average, the cows from the Quebec study herds had a lower milk production than the ones in PEI. Previous work in the Netherlands suggested that a greater production response after anthelmintic treatment can be expected in higher producing animals (10;25) , but the current analysis did not detect any such effect.

The lack of detected effect of anthelmintic treatment on milk composition is in agreement with earlier work done in the Netherlands. Ploeger et al. found an increase in milk production but no effect on the percentage fat or protein in two trials using albendazole (10;25) and one trial using ivermectin (9) as the anthelmintic. However, the review done by Gross et al.(1) reports that an increase in the fat percentage was seen in 26 of 35 clinical trials where milk composition was reported.

It is important to account for the lack of independence between cows in a herd and between repeated samples from the same cow. Under the first order auto-regressive correlation structure it is assumed that the correlation between observations will decay

geometrically based on the absolute difference in time between them (26). This correlation structure is commonly utilized when repeated measures are a feature of the data (13). An unstructured correlation structure may have given a better fit of the model since it estimates a parameter for every correlation. However, this requires the estimation of a very large number of correlation coefficients and is computationally challenging compared to the ar(1) structure, where only one correlation parameter is estimated (15). It also makes sense, biologically, to assume that the correlation between samples decreases as the time period between them increases, which is what the first order auto regressive structure assumes.

Herd was included in the final multi-level model as a random effect, but a mixed model using herd as a fixed effect produced very similar estimates of the effect of treatment with eprinomectin pour-on solution at calving. It was decided to utilize the random effects model even though the 28 herds in the study were based on a convenience sample instead of being chosen randomly from an underlying population (16;26). The fact that 28 herds were included in the trial meant that 27 herd dummy-variables were needed in the model with herd as a fixed effect, and it was therefore more convenient to include herd as a random effect and fit only one overall error term for the herd effect (27). Both herd and all the other factors included in the model were nuisance parameters that needed to be corrected for in order to obtain an accurate estimate of the effect of the anthelmintic treatment on milk production. Hence, it made little difference whether random or fixed effects is chosen for herd, and both options can be justified.

In a review paper from 1995, Reinemeyer (28) concluded that anthelmintic

treatment of adult dairy cows was controversial and that insufficient evidence existed for the recommendation of mass treatment under North American conditions. The main arguments against treating this age group was that adult animals harbour few worms as detected by fecal egg counts or in abattoir studies, and that clinical signs of gastrointestinal nematodes are rare in adult cows. Also, the cost of treatment was too high compared to the gain in production for the trials reviewed. The final point made by Reinemeyer was that the production response following anthelmintic treatment is highly variable between animals within a herd and that a test for identifying animals that would potentially respond to treatment is lacking. The results from the current study proved that a large group of animals responded positively to the elimination of subclinical levels of gastrointestinal parasite burdens using eprinomectin pour-on solution. Because there is no withdrawal of milk following treatment, and very little labour involved in the application when cows are treated at calving, a strong economical benefit was shown when cows that had some exposure to pasture were treated.

It is reasonable to assume that the results of the trial can be generalized to a broader group of dairy cows in temperate zones that meet some of their nutritional requirements from pasture. The increase in test day milk yields shown in this study occurred across various management schemes, degrees of pasture exposure and two geographic locations.

5 Conclusions

The study showed a consistent increase in daily milk production of 0.94 kg/day in the first 6 months of lactation for 901 adult Holstein cows treated with Ivomec-Eprinex® at calving, compared to the placebo group. A positive production response was evident across calving seasons, age groups and provinces. Although all animals in the study had experienced some exposure to pasture, the intensity of such exposure varied to some degree. More work needs to be done to assess the potential benefits of treating animals with no, or low levels of, pasture exposure.

References

(1) Gross SJ, Ryan WG, Ploeger HW. Anthelmintic treatment of dairy cows and its effect on milk production. Vet Rec 1999; 144(21):581-587.

(2) Guitian FJ, Dohoo IR, Markham RJ, Conboy G, Keefe GP. Relationships between bulk-tank antibodies to Ostertagia ostertagi and herd-management practices and measures of milk production in Nova Scotia dairy herds. Prev Vet Med 1999; 47(1-2):79-89.

(3) Hovingh E. An investigation into factors affecting summer/fall milk production and profitability in PEI dairy herds. PhD thesis. University of Prince Edward Island, Canada., 1998.

(4) Caldwell V. Gastro-intestinal nematodes in dairy cattle: Prevalence, level of infection estimated by bulk tank milk ELISA testing and related risk factors. MSc thesis. Faculte de Medicine Veterinaire de l'Universite de Montreal, Canada., 1997.

(5) Thomas RJ, Rowlinson P. An evaluation of anthelmintic treatment in a dairy herd. Epidemiology and Control of Nematodiasis in Cattle (CEC meeting) 1981.

(6) Agneessens J, Claerebout E, Dorny P, Borgsteede FH, Vercruysse J. Nematode parasitism in adult dairy cows in Belgium. Vet Parasitol 2000; 90(1-2):83-92.

(7) Borgsteede FH, Tibben J, Cornelissen JB, Agneessens J, Gaasenbeek CP. Nematode parasites of adult dairy cattle in the Netherlands. Vet Parasitol 2000; 89(4):287-296.

(8) Eysker M, Ploeger HW. Value of present diagnostic methods for gastrointestinal nematode infections in ruminants. Parasitol 2000; 120 Suppl:S109-S119.

(9) Ploeger HW, Schoenmaker GJ, Kloosterman A, Borgsteede FH. Effect of anthelmintic treatment of dairy cattle on milk production related to some parameters estimating nematode infection. Vet Parasitol 1989; 34(3):239-253.

(10) Ploeger HW, Kloosterman A, Bargeman G, von Wuijckhuise L, van den BR. Milk yield increase after anthelmintic treatment of dairy cattle related to some parameters estimating helminth infection. Vet Parasitol 1990; 35(1-2):103-116.

(11) Eddi C, Muniz RA, Caracostantogolo J, Errecalde JO, Rew RS, Michener SL et al. Comparative persistent efficacy of doramectin, ivermectin and fenbendazole against natural nematode infections in cattle. Vet Parasitol 1997; 72(1):33-41.

(12) Georgi JR, Georgi ME. Helminths. Parasitology for Veterinarians. Philadelphia: W.B. Saunders, 1990: 103-223.

(13) Grohn YT, McDermott JJ, Schukken YH, Hertl JA, Eicker SW. Analysis of correlated continuous repeated observations: modelling the effect of ketosis on milk yield in dairy cows. Prev Vet Med 1999; 39(2):137-153.

(14) Schaeffer LR, Jamrozik J, Kistemaker GJ, Van Doormaal BJ. Experience with a test-day model. J Dairy Sci 2000; 83(5):1135-1144.

(15) Singer JD. Using SAS PROC MIXED to Fit Multilevel Models, Hierarhcical Models, and Individual Growth Models. J Ed Beh Stat 1998; 24(4):323-355.

(16) Snijders T, Bosker R. Multilevel Analysis. 1 ed. London, UK: SAGE Publications Ltd, 1999.

(17) McDermott JJ, Schukken YH. Study design and analytic methods for data collected from clusters of animals. Prev Vet Med 1994; 18:175-191.

(18) Cox DD, Todd AC. Survey of gastrointestinal parasitism in Wisconsin cattle. J Am Vet Med Assoc 1962; 141:706-709.

(19) Stata Statistical Software. College Station (TX): Stata Corporation, 2001.

(20) SAS. Cary, NC, USA: 2001.

(21) Fox MT, Jacobs DE. Observations on the epidemiology and pathogenicity of nematode infections in adult dairy cattle in Great Britain. Epidemiology and Control of Nematodiasis in Cattle (CEC meeting) 1981.

(22) Rew R. The risky business of underestimating Cooperia infection of cattle. Topics Vet Med 1999; 9(1):9-18.

(23) Dimander SO, Hoglund J, Sporndly E, Waller PJ. The impact of internal parasites on the productivity of young cattle organically reared on semi-natural pastures in Sweden. Vet Parasitol 2000; 90(4):271-284.

(24) Carrier J. Effect of an Eprinomectin Treatment at Fall Housing on Production and Reproduction of Dairy Cattle. Master of Science Thesis, Faculte de Medicine Veterinaire de l'Universite de Montreal, Canada., 2001.

(25) Ploeger HW, Kloosterman A, Borgsteede FH. Effect of anthelmintic treatment of second-year cattle on growth performance during winter housing and first lactation yield. Vet Parasitol 1990; 36(3-4):311-323.

(26) Liang KY, Zeger SL. Regression analysis for correlated data. Annu Rev Public Health 1993; 14:43-68.

(27) McDermott JJ, Schukken YH. A review of methods used to adjust for cluster effects in explanatory epidemiological studies of animal populations. Prev Vet Med 1994; 18:155-173.

(28) Reinemeyer CR. Should you deworm your clients' dairy cattle? Vet Med 1995; 90:496-502.

Table 1. The number of cows calving in a clinical trial of eprinomectin pour-on solution, by season and region.

	Season				
Region	October / December -99	January / March -00	April / June -00	July / September -00	All seasons
PEI	176	250	143	93	662
Quebec	103	148	29	-	280
Both	279	398	172	93	942

Table 2. The minimum, maximum and mean number of cows enrolled at each farm during a clinical trial involving Holstein cows in two Canadian regions, October 1999 to September 2000.

	Number of cows per herd			
Region	minimum	maximum	mean	Herds
PEI	24	79	47.3	14
Quebec	6	45	20	14
Overall	6	79	33.6	28

Table 3. Summary and chi-square p-values of the number of recorded disease cases in the treated and placebo groups in a clinical trial of the effect of eprinomectin pour-on treatment at calving from 942 Holstein cows in two different Canadian provinces.

	Number of cases (n)			
Disease	**Total**	**Eprinomectin**	**Placebo**	**P-value(chi-sq)**
Retained placenta	61	28	33	0.558
Milk fever	24	11	13	0.714
Ketosis	4	2	2	0.985
Lameness	68	38	30	0.277
Mastitis	123	57	66	0.449
Cystic ovaries	47	22	25	0.702
Abortion	6	4	2	0.4
Total cows	942	467	475	n.a.

Table 4. A summary of eight individual, population averaged models (gee) of test day milk production. Treatment with eprinomectin, days in milk (dim), dim $^{-.05}$, age group and test month were included as fixed effects. An exchangeable within-herd correlation structure was assumed.

Test number	Coefficient (eprinomectin)	n	95 % c.i.	P-value
1	0.93	901	[.04 - 1.82]	0.04
2	0.7	897	[-.90 - 1.48]	0.08
3	1.02	874	[.23 - 1.81]	0.01
4	1.33	858	[.83 - 1.83]	<.001
5	1.14	837	[.49 - 1.78]	0.001
6	1.07	787	[.57 - 1.57]	<.001
7	0.57	727	[-.31 - 1.46]	0.21
8	0.85	662	[.02 - 1.68]	0.05

Table 5. Coefficients with standard errors and p-values from a test day mixed model of the effect of eprinomectin on 24 hour milk production in kg, for the first six tests after treatment. Herd is included as a random effect, and a first order auto-regressive (ar1) within-cow correlation structure was assumed. 5007 observations from 901 cows in 28 herds.

Factor	Level	Coefficient	Standard Error	P-value
Eprinomectin	yes	0.94	0.31	0.002
	no	0	-	-
Province	PEI	1.78	1.52	0.2038
	Quebec	0	-	-
Calving Season	Fall -99	1.07	0.63	0.0643 [1]
	Winter -00	1.63	0.63	
	Spring -00	1.1	0.66	
	Summer -00	0	-	
Lactation	1^{st}	-9.13	0.38	0.0001 [1]
	2^{nd}	-2.73	0.4	
	3^{rd} +	0	-	
Days in milk (dim)		-0.12	0.01	0.001
$(Dim)^{-.05}$		-129.4	5.67	0.001
Month of test	-2	-	-	0.001

[1] For categorical variables, p-value reflects test of overall significance, not individual levels of the variable.

[2] Dummy variables for each of the 12 months not quoted in table.

Figure 1. The parity distribution for 942 Holstein cows from 14 PEI and 14 Quebec dairy farms included in a clinical trial from October 1999 to September 2000.

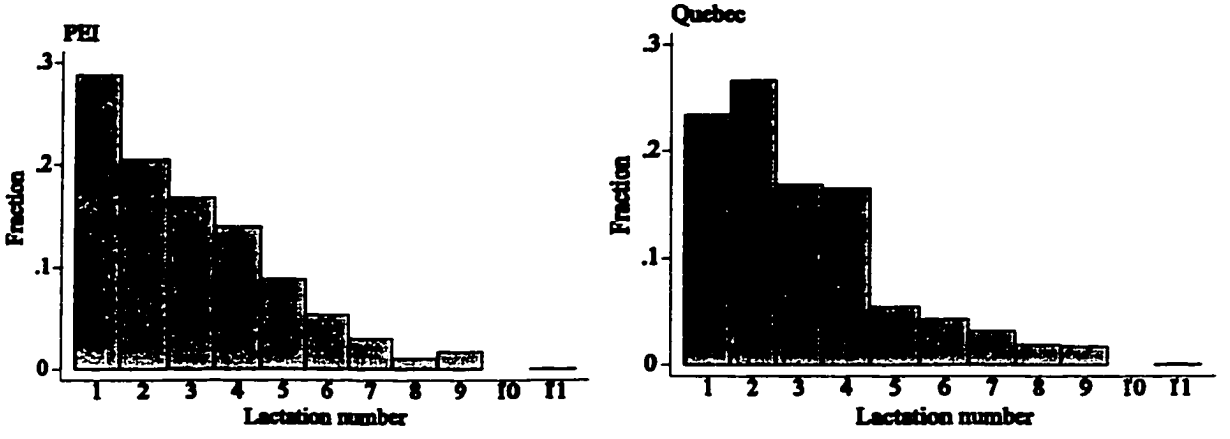


Figure 2. The frequency distribution of test day 24 hour milk yield, log somatic cell count, percent fat and protein for 5007 observations from 942 Holstein cows in a clinical trial of the effect of anthelmintic treatment on milk production. Canada, October 1999 to September 2000.

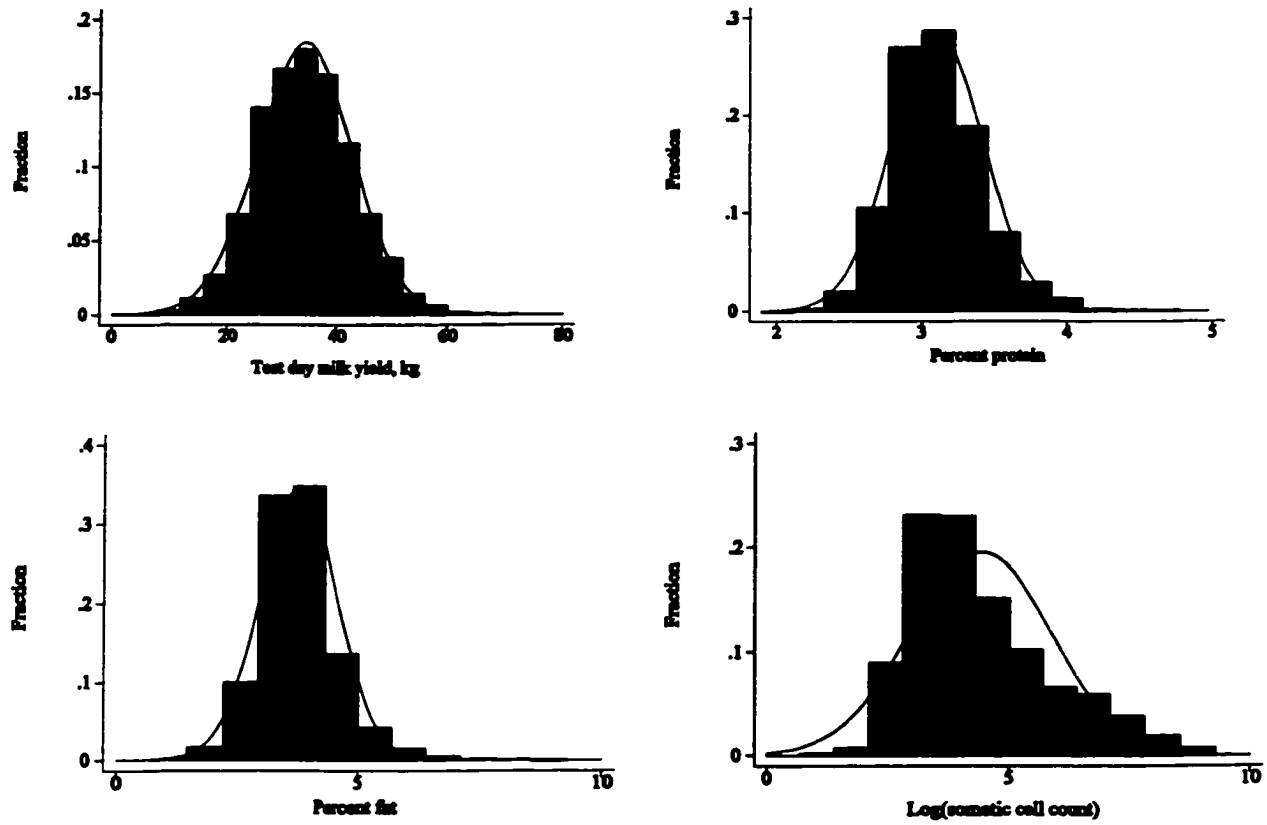


Figure 3. The seasonal variation in average daily milk yield in 14 dairy herds from PEI and 14 from Quebec. Graph based on 13202 test day milk production observations from 1117 cows.

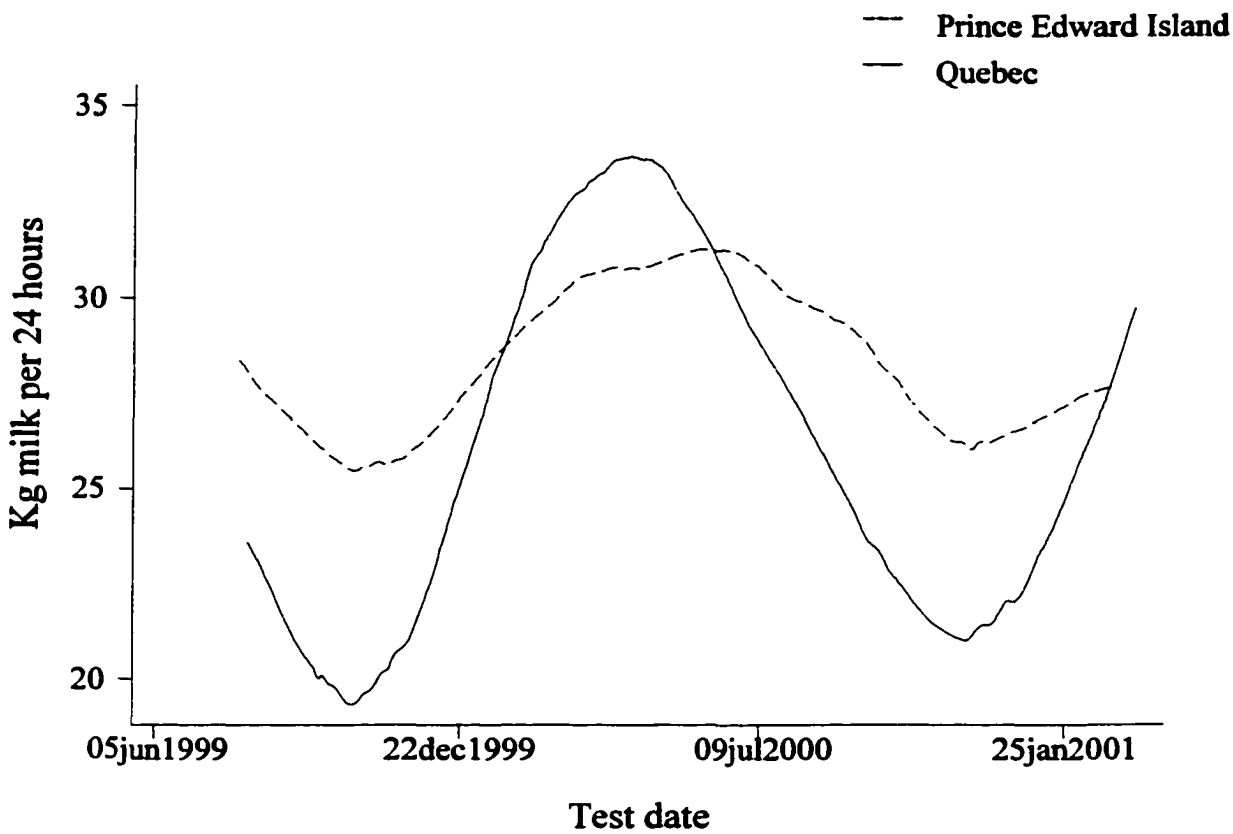
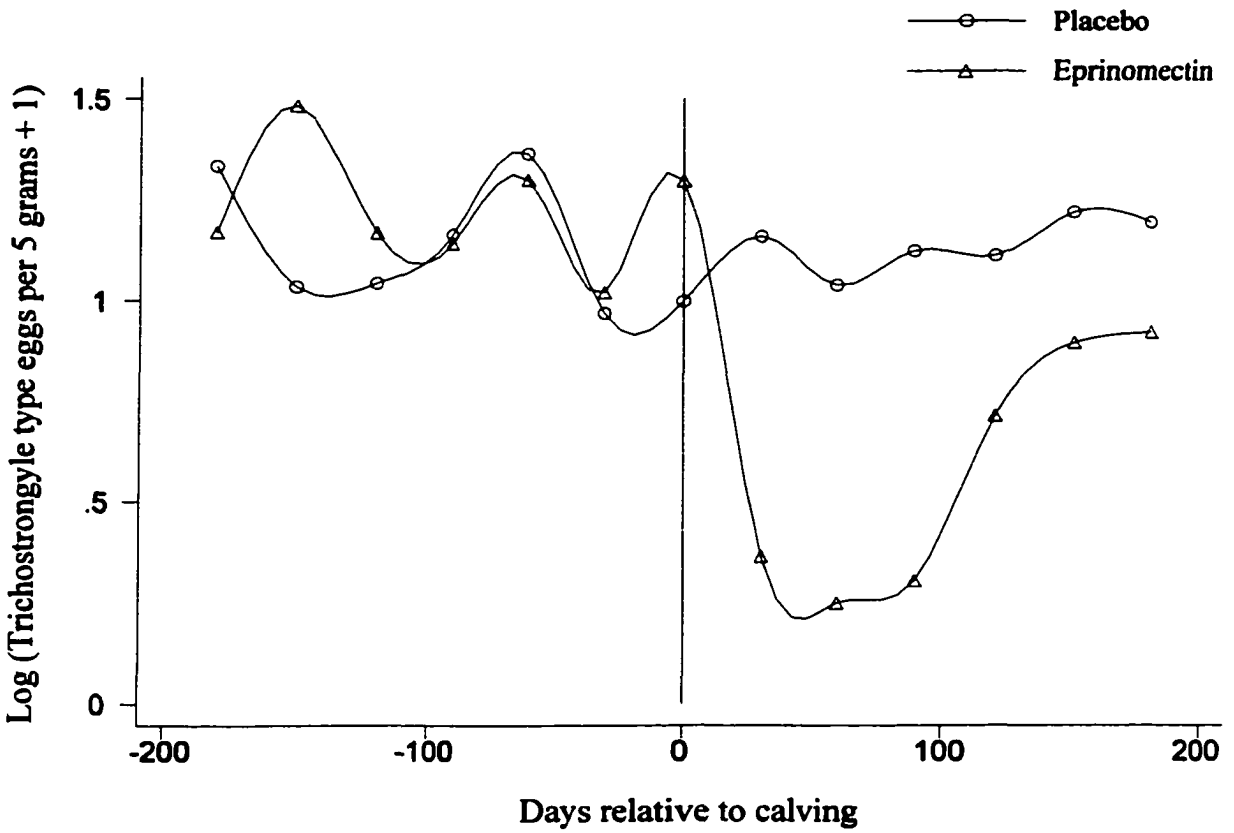


Figure 4. The log transformed number of trichostrongyle type eggs per 5 g of feces by treatment group in the time before and after calving. Anthelmintic treatment was administered on the day of calving. Observations from 224 Holstein cows in 28 Canadian dairy herds.



4. A survey of mange lesions (*Chorioptes bovis*) in Canadian dairy cows

1 Introduction

Ectoparasites in cattle are common, particularly in confined animals. The sucking louse, *Linognathus vituli*, causes itching and rubbing and heavy infestations have been associated with both weight loss and decreased milk production (1). Mange infestations in cattle have on the other hand mainly been seen as an esthetic problem, capable of causing dermatitis but often passing unnoticed (2). However, Leslie and others (3) documented a 4.5 kg increase in first test day milk production when animals with mange lesions were treated with an endectocide to eliminate the parasites before calving.

Sarcoptes scabiei and *Psoroptes ovis* cause serious mange lesions that are reportable in cattle, but both are rare in North America (2). The most common form of mange in cattle is caused by the chorioptic mite; *Chorioptes bovis*. Clinical signs of infestation with this arthropod typically consist of small crusty scabs starting at the base of the tail and spreading to the perineum, udder and thighs. The mites will feed on epithelial debris and cause itchiness in the affected areas (2). Definitive diagnosis is made by skin scrapings from affected areas, however the lesions are characteristic and a tentative diagnosis can be made based upon clinical appearance (1). Direct animal to animal transmission is the primary route of infestation and clinical signs of chorioptic mange in cattle usually peak in the late winter when the animals are kept inside. The lesions are reported to disappear shortly after animals are let out on pasture in the spring (2).

Limited information is available about the prevalence of mange lesions in Canadian dairy cows. Leslie et al. (3) reported that in a cohort of 22 Ontario dairy herds, five herds (23%) had animals with clinical evidence chorioptic mange. Within these positive herds, 123 of 461 cows (27%) had mange like lesions. Kennedy and Kralka (4) investigated the occurrence of ectoparasites in Alberta cattle and found that out of 113 animals (beef and dairy) submitted to the Veterinary Diagnostic Laboratory in Edmonton, 21 percent tested positive for *C. bovis*. Both skin scrapings and visual assessment of five selected areas were performed in this study, and the highest probability of recovering *C. bovis* was obtained by skin scrapings from the cranio-ventral part of the chest. Slocombe (5) compiled records from the teaching hospital at the Ontario Veterinary College from 1965 to 1970, and concluded that *C. bovis* was found in 10% of the bovine skin samples submitted to the parasitology laboratory. Prevalence of disease based on specimens submitted to diagnostic laboratories are not necessarily representative of the situation in the field, hence larger scale epidemiological studies of the occurrence of chorioptic mange in Canada are needed.

The objective of this study was to summarize the occurrence of lesions caused by *C. bovis* in Canadian dairy herds across various regions, and through the year.

2 Materials and methods

As part of a larger study of the epidemiology of gastrointestinal nematodes in adult cattle, 38 Canadian dairy herds were monitored through a year from October 1999 to September 2000. All herds were selected based on the use of pasture, and animals had to have had some degree exposure to an outdoor area. The herds were located in Prince

Edward Island (n=14), Quebec (n=14), Ontario (n=5) and Saskatchewan (n=5). Because the herds from PEI and Quebec were part of a clinical trial of endectocides and gastrointestinal nematodes, all herds in these two provinces had their lactating animals meet some of their nutritional requirements from pasture or have access to an outdoor exercise area.

The number of lactating animals with lesions suggestive of *C. bovis* infestation was recorded approximately quarterly, and the severity of the individual lesions were scored based on a scale developed by Leslie et al. (3). The four levels of severity recorded were:

0	No lesions
1	Small nodules/ thickened skin/ itchy on palpation
2	Coalesced lesion/ serum crusts/ intense itching
3	Heavy scabs/ exuding serum/ intense irritation

Mange scoring charts with the definitions and colour photographs of typical lesions (3) were taken by the researchers to each farm, to ensure similar scoring criteria across study sites. No confirmation of diagnosis by skin scrapings were performed, as the mange-like lesions are considered characteristic of *C. bovis*.

The results were summarized and visually assessed. A hierarchical generalized linear model with a binomial error distribution, logit link and herd as a random effect was built. The outcome variable in this model was the number of cows with mange-like lesions at each sample date for each herd, with the total number of animals in the herd as the denominator. The two explanatory variables included were province (PEI, Quebec,

Ontario or Saskatchewan) and season of sampling. The study period was categorized into four seasons; Fall (September to November 1999), Winter (December 1999 to February 2000), Spring (March to May 2000) and Summer (June to August 2000). All data manipulation, graphing and analysis was performed using the software package Stata version 7 (6).

3 Results

During the one year period of observation, 128 whole-herd evaluations of the prevalence of mange lesions were performed. The number of farms scored for lesions each month is summarized in Table 1, and within-herd chorioptic mange prevalence was calculated for 37 herds in total. One herd in Quebec was dropped from the study due to incomplete records. In each of the regions Ontario and Saskatchewan, 15 herd prevalences were obtained meaning that each herd included in the study was visited three times. For Quebec the number of whole-herd evaluations recorded was 47 and for PEI 51 herd level observations were included.

The study period was categorized into fall, winter, spring and summer for the graphical presentations of the data. Figure 1 shows the prevalence of affected animals through the four seasons for each study site. Out of the 37 herds that were evaluated for mange lesions, 28 had at least one positive animal at one point during the year giving an overall herd prevalence of 75.7 percent. Overall within-herd prevalence for the study was 6.1%. The average within-herd prevalence in PEI, Quebec and Ontario was 3.6% for the study period seen as a whole, ranging from zero to 29 percent. The within-herd prevalence based on the 28 mange positive herds only was 4.7 percent for PEI, 6.0

percent for Quebec and 5.4 percent for the Ontario herds. The herds in the Saskatoon area included in this study all fell into the mange positive group and had an average within-herd prevalence of mange-like lesions of 24.9 percent, ranging from zero to 63.5 percent. Across all four sites, the mean within-herd prevalence for positive herds was 8.4 percent.

The pasture management and housing characteristics from all herds included in the study were obtained by use of a questionnaire. Table 2, chapter 2 summarizes the use of pasture by province. It was seen that the majority of herds based on Prince Edward Island and in Quebec used pasture as a source of nutrition for their lactating animals, while none of the Saskatchewan herds did. Table 3, chapter 2 summarizes the size of the included herds by region and it can be seen that the herds from Saskatchewan on average were larger than herds from the three other study sites.

Coefficients from the two-level generalized linear model are presented in Table 2. Both season and region were overall significant determinants of mange prevalence based on Wald's tests. The predicted number of mange positive animals was significantly higher for the Saskatchewan herds than for herds from the three other provinces. Also, the number of animals with mange lesions was significantly higher for both winter, spring and summer of 2000 when comparing to the fall of 1999. A separate analysis was performed excluding the herds from Saskatchewan, and season was still a significant predictor.

4 Discussion

Due to the fact that the sampling window differed somewhat between the four locations, the number of samples included for each season was variable. No samples from

Saskatchewan were included in the Fall of 1999 and no samples from Ontario were obtained from the Summer of 2000 (Table 1 and Figure 1).

The herd and cow level prevalences reported in this study are comparable to previous studies performed in Canada. Leslie et al. (3) found a herd level prevalence of mange lesions of 23% in the 22 herds included, and a within-herd prevalence in these five positive confinement housed herds of 27 percent. The overall proportion of affected herds was lower than was seen in the present study, while the within-herd prevalence was in the same range as seen in the herds from Saskatchewan. However, the time of year was not reported by Leslie et al. and this is a factor that might potentially affect the observed prevalence.

The other two Canadian studies looked at individual animals only, and did not report overall herd prevalence (4;5). The Alberta study performed by Kennedy et al. (4) in 1986 observed that 21 percent of individual animals were affected with *C. bovis*, but not all of these animals displayed clinical signs of mange infestation. Diagnosis in the current study was based on skin lesions in the tail/udder/hind limb area, while the Alberta study also included skin scrapings and the assessment of five separate locations on each animal. Due to these differences in diagnostic technique, more subclinical cases were likely to be detected in the earlier study.

The two main findings in this trial were that the average within-herd prevalence of *C. bovis* like lesions was significantly higher (25%) in the included herds from Saskatchewan than in herds from the three other provinces, which might be explained by a higher degree of confinement of lactating animals for the selected herds in this region. Secondly, the only significant difference in seasonal mange prevalence was between the

fall of 1999 and the three other seasons. This was also the case if the Saskatchewan herds were excluded from the analysis. Yeruham et al. (7) reported clinical signs of chorioptic mange in 37 of 109 dairy herds (33.9%) followed through a year in Israel, and failed to detect any seasonal variation at all. The study performed by Kennedy et al (4) in Alberta found a similar lack of seasonality in the prevalence of chorioptic mange. These results are in contrast with the situation observed clinically, where *C. bovis* lesions are claimed to disappear shortly after the animals are let out on pasture in the spring (1;2). The results from the current study indicate that the prevalence of mange lesions does decrease when animals are let outside, but that this effect may be somewhat delayed hence not giving lower prevalence until the late summer/fall.

The results from this study are based on a small number of herds, particularly from Ontario and Saskatchewan, and cannot necessarily be generalized to represent a broader group of all herds in these four regions. Also, the selection criteria regarding pasture use was not the same across the four study sites with herds in PEI and Quebec generally having a higher level of pasture exposure. Differences in management factors may explain the observed regional differences in the prevalence of chorioptic mange. For example, all the Saskatchewan herds followed in this study were larger operations that had free stall barns, and none of them depended on pasture as a source of nutrition for their lactating animals. The possibility of between animal transmission of the parasite is larger when animals are confined in a limited area. Additionally, direct sunlight is also thought to have a detrimental effect on the development of the mites (7). More studies need to be done in order to give a better estimate of both overall and within-herd prevalence in dairy herds where the animals are kept confined.

5 Conclusion

Lesions suggestive of *C. bovis* were observed in 75.7 percent of dairy herds monitored from September 1999 to August 2000. A significant seasonal difference in the occurrence of mange lesions was noted, with the lowest prevalence being recorded in the fall of 1999 when the study started. Herds from Saskatchewan had a higher within-herd prevalence than the included herds from Ontario, Quebec and Prince Edward Island. The variation in within-herd prevalence can be hypothesized to be due to differences in housing type or density, and other management factors like exposure to an outdoor environment.

References

(1) Radostits OM, Gay CC, Blood DC, Hinchcliff KW. Diseases caused by arthropod parasites. Veterinary Medicine. W.B. Saunders, 2000: 1414-1415.

(2) Bowman DD. Georgis' parasitology for veterinarians. 7th ed. Philadelphia: W.B. Saunders Company, 1999.

(3) Leslie K, Duffield T, Tenhag J. Mange can rob you of milk. Hoard's Dairyman 2000;49.

(4) Kennedy MJ, Kralka RA. A survey of ectoparasites on cattle in Central Alberta, November 1984-July 1985. Can Vet J 1986.

(5) Slocombe JO. Parasitisms in domesticated animals in Ontario. I. Ontario Veterinary College Records 1965-70. Can Vet J 1973; 14(2):36-42.

(6) Stata Statistical Software. College Station (TX): Stata Corporation, 2001.

(7) Yeruham I, Hadani A, Sklar A, Monbaz A. The occurence of chorioptic mange in dairy cattle in Israel. Refuah vet 1981; 38(4):176-179.

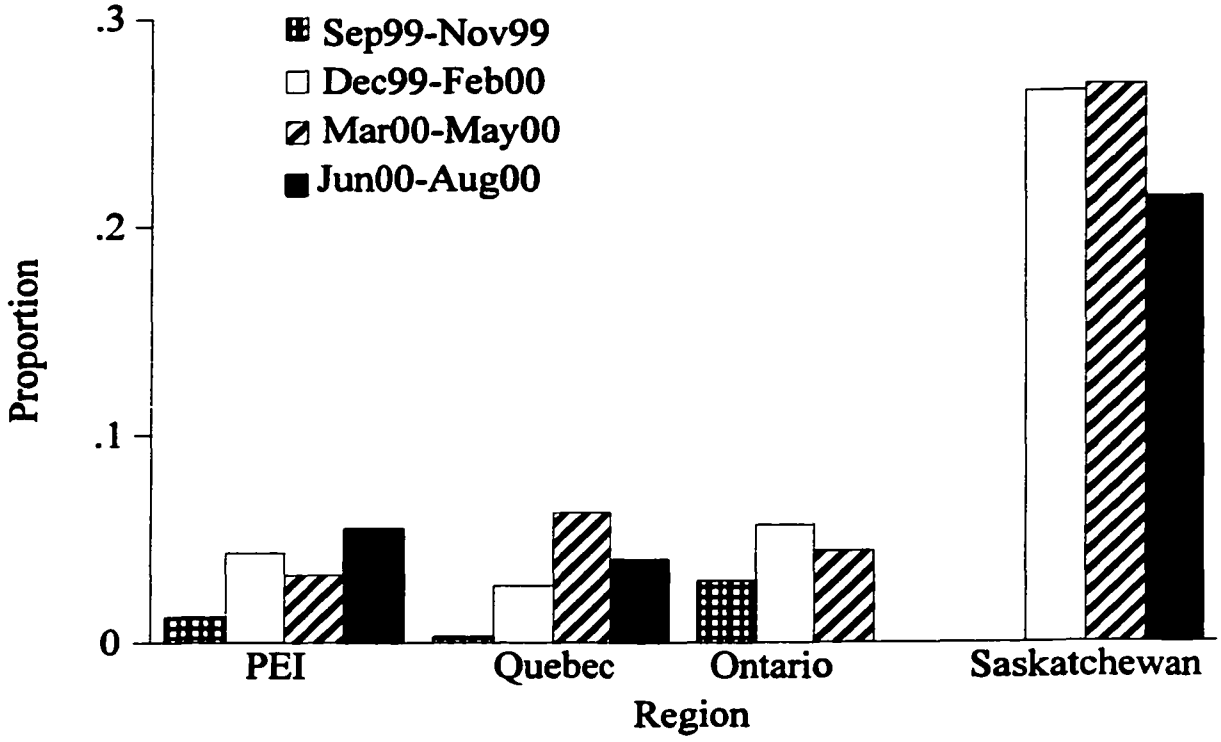
Table 1. The number of herds evaluated for mange lesions each month in a survey of chorioptic mange in four Canadian regions from September 1999 to August 2000.

Season	PEI	Quebec	Ontario	Saskatchewan
Fall	11	13	5	0
Winter	14	12	5	5
Spring	13	12	5	5
Summer	13	10	0	5

Table 2. Coefficients, standard errors and p-values from a generalized linear model of the number of mange-lesion positive animals in 37 dairy herds in Canada from September 1999 to August 2000. Herd was included in the model as a random effect.

Variable	Level	Coefficient (ß)	Std. Err.	P-value
Province	PEI	(baseline)		
	Quebec	-0.22	0.22	0.316
	Ontario	0.44	0.24	0.243
	Saskatchewan	2.11	0.16	<0.001
Season	Sep 99/Nov 99	(baseline)		
	Dec 99/Feb 00	1.25	0.28	<0.001
	Mar 00/May 00	1.27	0.29	<0.001
	Jun 00/Aug 00	1.23	0.29	<0.001
Constant		-4.93	0.31	<0.001

Figure 1. The proportion of lactating animals with lesions suggestive of *C. bovis* in 37 Canadian dairy herds, by season and region. October 1999 to September 2000.



5. Summary

1. The epidemiology of gastrointestinal parasites in adult dairy cows

During the time period from October 1999 to September 2000, 38 Canadian dairy herds were monitored for gastrointestinal parasites. The herds were located in PEI (n=14), Quebec (n=14), Ontario (n=5) and Saskatchewan (n=5). Eight animals from each herd were randomly chosen and fecal samples for egg counts were performed either monthly or quarterly. Four of these longitudinally monitored animals at each farm were in their first lactation, while the other four were in second lactation or older. In addition to the repeated fecal samples from the monitored individuals, a larger group of animals were sampled during the first visit in the fall of 1999 and larval cultures were performed to determine the species breakdown of the trichostrongyles present. All animals were required to have had some degree of exposure to an outdoor grazing area. Based on questionnaire data from the included herds, it was established that the majority of the herds from Prince Edward Island and Quebec used pasture as a source of nutrition while none of the study herds from Saskatchewan and only two of the five Ontario herds did.

The numbers of trichostrongyle-type eggs obtained from the animals followed in this study were low, ranging from 0 to 419 eggs per 5 grams of feces (ep5g) or equivalently; 0 to 84 eggs per gram (epg) . Out of the 1946 collected samples, 46% contained no eggs and the average fecal egg count among the animals with positive samples was 17.0 ep5g. A seasonal variability in fecal egg count numbers was observed, with the number of eggs detected being significantly lower in the winter months January to March compared to the other seasons. An increase in fecal egg counts was observed in

the spring (April/June), compared to the winter months, even though animals were not re-exposed to pasture until late May or early June.

Identification of larvae from the cultures performed in the fall of 1999 showed that more than 99.5% of the parasites present were of the species *Ostertagia ostertagi* or *Cooperia* spp.. By looking at fecal egg counts from the same samples, it was seen that groups of animals with a predominance of *Cooperia* yielded higher counts than with *Ostertagia* as the most common species. There was approximately the same proportion of larvae from the two species in most cases, except for the herds from Saskatchewan where *Cooperia* spp. was found almost exclusively. The reason for this difference is unknown as other studies from western Canada have shown *Ostertagia* to be common in this region.

The fact that fecal egg count data were far from normally distributed posed challenges to the multivariable modelling of such data. In addition to the lack of normality, there was also a very high number of zero counts present in this dataset, because mature animals with some degree of immunity against gastrointestinal nematodes were monitored. While the Poisson model is commonly applied to count data, it assumes the mean and the variance to be the same. In the current study, fecal egg counts variance was much larger than the mean. Negative binomial models allow for additional over-dispersion (i.e. additional variation) of the Poisson model, and for this particular analysis a zero inflated negative binomial (ZINB) model was chosen to account for both the over-dispersion and the large number of zero counts. It simultaneously models the probability of a zero outcome through a logistic process, and the continuous outcome including zeros using a negative binomial model.

Important associations with fecal egg count levels detected in the ZINB model were: young cows had higher expected fecal egg counts than older animals; expected counts were lower in the winter months and increased in the spring; spreading of manure on heiferpastures resulted in lower expected counts in lactating cows while the effect was the opposite if manure was spread on pastures used by lactating cows; and the expected egg counts were higher if lactating cows had access to pasture.

2. The effect of eprinomectin pour-on solution in lactating dairy cows

A double blind, randomized clinical trial involving 28 dairy herds in Canada was performed during the time period from October 1999 to September 2000. The herds were located on Prince Edward Island (n=14) and in the St Hyacinthe area in Quebec (n=14), and all 942 cows were Holsteins. Selection criteria for inclusion in the trial included pasture exposure, participation in the Canadian Dairy Herd Management System (CDHMS) and no treatment of adult animals with endectocides in the last six months before the onset of the trial.

Animals were treated by the producer with eprinomectin pour-on solution (500 μg per kg body weight) or placebo. All animals were treated at calving, and cows were enrolled in the study up until June 2000 in Quebec and September 2000 in PEI. The maximum number of animals included in the analysis from each herd was set to 80. The outcomes measured in the study were 24 hour milk production, somatic cell count and percentage fat and protein. This information was retrieved from the CDHMS database. The association between treatment with eprinomectin pour-on and selected health parameters was investigated for the herds in the study. Finally, monthly fecal egg counts

were obtained from eight animals in each herd and the effect of the treatment on the number of trichostrongyle type eggs recovered was analysed.

A random effects regression model was applied, taking the clustering of animals in herds into account by including herd as a random variable. The fact that repeated milk samples from the same individuals were included, was accounted for by using a first order auto regressive covariance structure to correct for within-cow correlation. Since an individual animal's milk yield follows a distinctive pattern through the lactation, the effect of stage of lactation was corrected for using Wilmink's function ($dim + dim^{.05}$). Other explanatory variables in the model were parity, calving season, province and month of test.

When the first six test day milk yields after calving were included in the model, an increase in milk production of 0.94 kg per cow per day was observed. Interactions between treatment and parity, season and region were tested in the model but not found to be significant. However, there was a trend towards a greater treatment effect in animals from first and second lactation compared to animals from third lactation and older. No effect of treatment with eprinomectin was detected on the percentage of fat or protein in the milk, or on the somatic cell count. Unconditional associations between the treatment and selected health parameters such as retained placenta, ketosis, clinical mastitis and more, revealed no significant effects.

3. The occurrence of mange lesions

In the period from October 1999 to September 2000, the occurrence of lesions suggestive of chorioptic mange was recorded in 37 Canadian dairy herds. All lactating

animals were checked for mange lesions on a quarterly basis and out of the 37 monitored herds, 28 had at least one positive animal giving an overall herd prevalence of mange lesions of 75.7%. The overall within-herd prevalence for the positive herds only was 8.4%.

A hierarchical generalized linear model with a binomial error distribution accounting for repeated measures on herd, was used to evaluate factors affecting the presence of mange-like lesions. Both season and region were found to be significant explanatory variables when the outcome was the number of animals with mange lesions present at each sampling date. The Saskatchewan herds included in the study had higher numbers of affected animals, which can likely be explained by the fact that these herds used confinement of lactating cows to a larger degree. The prevalence of chorioptic mange was lower in the fall of 1999 than in any of the other seasons included in this study.

4. Conclusion

The knowledge gained from these studies shows that gastrointestinal parasites of lactating dairy cows can be an important economic factor in dairy production systems where pasture is used. The parasite burdens detected using fecal egg counts were low throughout the year, yet a seasonal variation was seen. A small but statistically significant increase in egg output in the spring before animals were re-exposed to pasture serves as evidence of an apparent "spring rise" in cattle. Additional factors associated with higher expected fecal egg counts were young animals, spreading of manure on pastures used by cows and access of lactating cows to pasture.

It was shown by the clinical trial that by eliminating the subclinical nematode burden from lactating animals through anthelmintic treatment, milk production can be positively effected. An increase in daily milk yield of 0.94 kg per cow for a minimum of the first 180 days of lactation was detected, resulting in a (conservative) estimated gain of 170 litres of milk per animal. The price of treating an adult cow topically with commercially available eprinomectin can be expressed as 22 milk litre equivalents (Can $ 11 at Can $ 0.5 a litre for milk), hence it can be concluded that individual cow treatment at calving yields a net gain of 148 milk litre equivalents (or Can $ 74) for the producer and a conservative benefit cost ratio is 7.72 (170/22). This is the estimated effect of treating animals that have had some degree of pasture exposure with eprinomectin pour-on solution at calving.

An important direction for future studies will be to assess the effect of endectocide treatment in herds with a broader range of pasture exposures, and also of treating cows at different stages of lactation. Furthermore, it will become important to develop a diagnostic test that can identify the animals that will be most likely to respond positively to anthelmintic treatment.

6. Appendices

Appendix A:
PARASITE SURVEY

Owner Lastname: _____ **DHI-Herd #**_____

A. Herd Size

A1. Average number of lactating cows

A2. Average number of dry cows _____

A3. Average number of heifers (12 mo. - 1st calving) _____

A4. Average number of calves (< 12 mo.) _____

A5. Breed(s) _____

B. Heifers (Breeding Age or Pregnant)

B1. In the Summer of 1999, heifers were (check one):

a. totally confined (in the barn) 24 hrs./day. ☐

b. given access to a concrete or gravel surface exercise yard (outdoors) some time each day. ☐

c. given access to a small field for the purpose of exercise (not primarily for grazing). ☐

d. spent some time grazing and met some of their nutritional requirements from pasture. ☐

B2. If heifers were on pasture, which of the following have also grazed on the same pasture(s)

-during the summer of 1999?

a..Calves ☐

b. Dry cows ☐

c. Lactating cows ☐

-during the summer of 1998?

d. Calves ☐

e. Heifers ☐

f.. Dry cows ☐

g. Lactating cows ☐

Pasture Management for pastures used by heifers (summer 1999)

If heifers were on pasture during the summer of 1999 complete the following questions:

B3. Date animals first turned out on to pasture in 1999(MM/DD) _____

B4. Total pasture area used for grazing heifers. _____

B5. Number of fields/paddocks _____

B6. How long have these pastures been used for grazing since last reseeding :(Check one)

a. < 1 year ☐
b. 2-5 years ☐
c. > 5 years ☐

B7. How were these pastures managed last summer? (Check one).

		Yes	No
a.	continuous grazing (continuous access for pasture season).	☐	☐
b.	controlled access grazing (rotational or strip grazing).	☐	☐

B8. If controlled access grazing is used, how frequently are cattle moved:

a. < 7 days ☐
b. 7 - 14 d ☐
c. > 14days ☐

B9. Has any cattle manure been mechanically spread on these pastures this year? ☐ ☐

B10. Have these pastures been dragged or harrowed this year? ☐ ☐

B11. Have these pastures been clipped this year? ☐ ☐

B12. Was a cut of hay or silage taken off any of these pastures before they were used for grazing? ☐ ☐

B13. If Yes, how many acres were cut? _____

C. Milking cows

C1. In the Summer of 1998, milking cows were (check one):

a. totally confined (in the barn) 24 hrs./day. ☐
b. given access to a concrete or gravel surface exercise yard (outdoors) some time each day. ☐
c. given access to a small field for the purpose of exercise (not primarily for grazing). ☐
d. spent some time grazing and met some of their nutritional requirements form pasture. ☐

C2. If milking cows were on pastures, which of the following have also grazed on the same pasture(s)

-during the summer of 1999?

a.. Calves ☐
b. Dry cows ☐
c. Lactating cows ☐

-during the summer of 1998?

d. Calves ☐
e. Heifers ☐
f. Dry cows ☐
g. Lactating cows ☐

Pasture Management for pastures used by milking cows (summer 1999)

If milking cows were on pasture during the summer of 1999 complete questions:

C3. Date animals first turned out on to pasture in 1999(MM/DD) _____

C4. Total pasture area used for grazing milking cows. _____

C5. Number of fields / paddocks _____

C6. How long have these been pastures been used for grazing since last reseeding :(Check one)

a. < 1 year ☐
b. 2-5 years ☐
c. > 5 years ☐

		Yes	No
C7.	How were these pastures managed last summer? (Check one).		
a.	continuous grazing (continuous access for pasture season).	☐	☐
b.	controlled access grazing (rotational or strip grazing).	☐	☐

C8. If controlled access grazing is used, how frequently are cattle moved:

a. < 7 days ☐
b. 7 - 14 d ☐
c. > 14days ☐

C9.	Has any cattle manure been mechanically spread on these pastures this year?.	☐	☐
C10.	Have these pastures been dragged or harrowed this year?	☐	☐
C11.	Have these pastures been clipped this year?	☐	☐
C12.	Was a cut of hay or silage taken off any of these pastures before they were used for grazing?	☐	☐

C13. If Yes, how many acres were cut? _____

D. Dry cows

D1. In the Summer of 1998, dry cows were (check one):

a. totally confined (in the barn) 24 hrs./day. ☐
b. given access to a concrete or gravel surface exercise yard (outdoors) some time each day. ☐
c. given access to a small field for the purpose of exercise (not primarily for grazing). ☐
d. spent some time grazing and met some of their nutritional requirements form pasture. ☐

D2. If dry cows were on pastures, which of the following have also grazed on the same pasture(s)

-during the summer of 1999?

a. Calves ☐
b. Heifers ☐
c. Lactating cows ☐

-during the summer of 1998?

d. Calves ☐
e. Heifers ☐
f. Dry cows ☐
g. Lactating cows ☐

Pasture management for pastures used by dry cows (summer 1999).

If dry cows were on pasture during the summer of 1999 complete questions

D3. Date animals first turned out on to pasture in 1999(MM/DD) _____

D4. Total pasture area used for grazing dry cows. _____

D5. Number of fields / paddocks. _____

D6. How long have these been pastures been used for grazing since last reseeding :(Check one)

a. < 1 year ☐
b. 2-5 years ☐
c. > 5 years ☐

		Yes	No
D7.	How were these pastures managed last summer? (Check one).		
a.	continuous grazing (continuous access for pasture season).	☐	☐
b.	controlled access grazing (rotational or strip grazing).	☐	☐

D8. If controlled access grazing is used, how frequently are cattle moved:

a. < 7 days ☐
b. 7 - 14 d ☐
c. > 14days ☐

D9.	Has any cattle manure been mechanically spread on these pastures grazing this year?.	☐	☐

D10. Have these pastures been dragged or harrowed this year? ☐ ☐

D11. Have these pastures been clipped this year? ☐ ☐

D12. Was a cut of hay or silage taken off any of these pastures before they were used for grazing? ☐ ☐

D13. If Yes, how many acres were cut? _____

E. Treatments

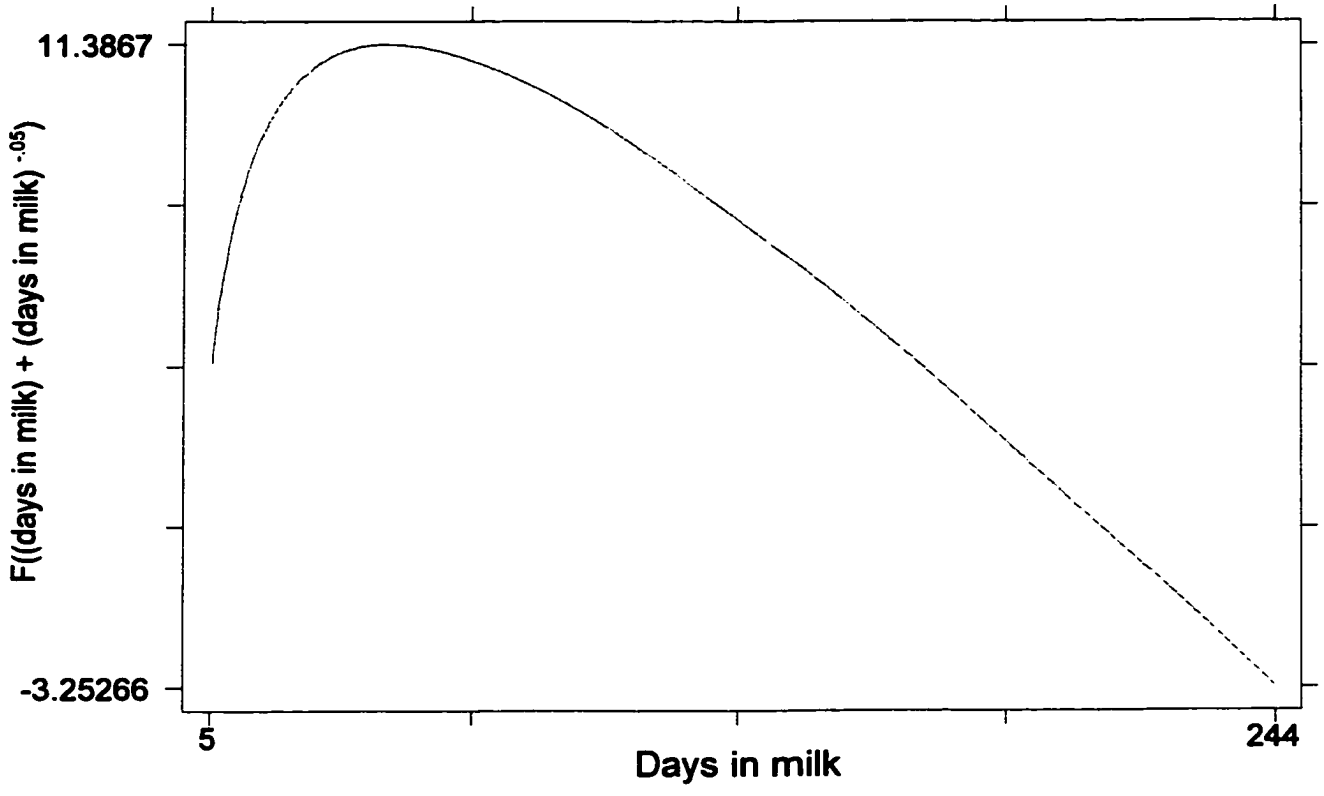
E1. Which of the following treatments have been used for worm control in cows, heifers and calves over the past year? (Check all that apply).

		Cows	Heifers (12 mo - 1st calving)	Calves (< 12 mo)
E2	-Pour on or injectable deworming in Fall 1998			
	b. Ivomec pour on	☐	☐	☐
	c. Ivomec injectable	☐	☐	☐
	d. Dectomax pour on	☐	☐	☐
	e. Dectomax injectable	☐	☐	☐
	f. Cydectin pour on	☐	☐	☐
	g. Levasol / Tramisol pour on	☐	☐	☐
	h. Ripercol pour on	☐	☐	☐
	i. Other (specify)_____	☐	☐	☐
E3	-Pour on or injectable deworming in Spring or Summer 1999			
	a. Ivomec pour on	☐	☐	☐
	b. Ivomec injectable	☐	☐	☐
	c. Dectomax pour on	☐	☐	☐
	d. Dectomax injectable	☐	☐	☐
	e. Cydectin pour on	☐	☐	☐
	f. Levasol / Tramisol pour on	☐	☐	☐
	g. Ripercol pour on	☐	☐	☐
	h. Other(specify)_____	☐	☐	☐
	_____			
E4	-Sustained release bolus in Summer 1999			
	a. Ivomec bolus	☐	☐	☐
	b. Paratect flex bolus	☐	☐	☐
	c. Other(specify)_____	☐	☐	☐
	_____			
E5	-Oral deworming any time in Fall/winter 1998			
	a. Banminth II 20 % Premix	☐	☐	☐
	b. Exhelm E Pellets	☐	☐	☐
	c. Safe-Guard Premix 20%	☐	☐	☐
	d. Other(specify)_____	☐	☐	☐
	_____			

		Cows	Heifers (12 mo - 1st calving)	Calves (< 12 mo)
E6	-Oral dewroming Spring/summer 1999			
	a. Banminth II 20 % Premix	☐	☐	☐
	b. Exhelm E Pellets	☐	☐	☐
	c. Safe-Guard Premix 20%	☐	☐	☐
	d. Other (specify)_____	☐	☐	☐
E7.	No treatments in last 12 months	☐	☐	☐

		Yes	No
E8.	In the last 12 months, have you seen signs of tail head mange (lumpy skin, itchy areas, crusty lesions, open scores) at the tail head or top of udder on any cows?.	☐	☐
E9.	If yes,		
	a. What % of milking cows were affected ? _____		
	b. Did you treat these cows?	☐	☐
	c. What type of treatments did you use? _____		
	d. When did you apply these treatments? _____		
	e. Do you feel these treatments were effective?	☐	☐

Appendix B. The shape of the lactation curve as expressed by days in milk (dim) and days in milk to the power of -0.05 (Wilmink's function). Based on a model of test day milk yield that includes only an intercept, dim and dim$^{-.05}$.



Appendix C. Abbreviations used in thesis:

AR1	first order auto regressive (correlation)
Can	Canadian dollars
CDHMS	Canadian Dairy Herd Management System
ELISA	enzyme linked immunosorbent assay
epg	(trichostrongyle type) eggs per gram
ep5g	eggs per 5 grams of feces
FEC	fecal egg count
gee	generalized estimating equations
OD	optical density
PEI	Prince Edward Island
SCC	somatic cell count
ZINB	zero inflated negative binomial (model)