EPIDEMIOLOGY OF GASTROINTESTINAL NEMATODES IN GRAZING YEARLING BEEF CATTLE IN SASKATCHEWAN

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ABSTRACT

Gastrointestinal nematodes (GIN) in beef cattle can be a concern for cattle producers due to loss in profit associated with anthelmintic treatment costs and reduced production performance. There is limited current information regarding the epidemiology of GIN in grazing yearling beef cattle in western Canada. Hence, the objectives of this research were to: 1) describe the epidemiology of GIN and assess their impact on weight gain (Chapter 2), and 2) conduct a nemabiome study to determine the diversity and abundance of nematode species within Saskatchewan pastured beef cattle (Chapter 3). Seventeen cohorts of pastured yearling beef cattle were processed in the spring and fall of 2019. Animals were individually weighed, and rectal fecal samples obtained for pooled fecal egg count (FEC). A subset of calves (n = 25) in each herd was administered oral fenbendazole (Safeguard®, Merck, Canada) and a parenterally administered extended-release eprinomectin (LongRange®, Boehringer Ingelheim, Canada), while the remaining cohort was left untreated. Eggs per gram of feces (EPG) were determined in pooled fecal samples using the Modified Wisconsin Sugar Flotation Technique, and deep amplicon nemabiome sequencing of the ITS-2 DNA locus was used to describe nematode species diversity and abundance. Across all cattle (n = 867), there were differences between treatment and control groups regarding FEC ($p < 0.01$). In the generalized estimating equations (GEE) model, FEC decreased by 44 times over the grazing season, and FEC were 1.8 times greater on pastures located in black/gray soil *versus* dark brown soil zones. Areas with higher precipitation also had higher FEC. There was no significant difference ($p = 0.41$) in the ADG across all cattle, but differences were found in the ADG between treated and control cattle in five cohorts. *Haemonchus placei* was found in all spring cohorts, accounting for 30% of the L3 species composition. Hence, it was one of the dominant species together with *Ostertagia ostertagi* (40%) and *Cooperia oncophora* (26.2%). *Ostertagia ostertagi* (47.5%) and *C. oncophora* (42.0%) were the most common species recovered at the time of fall sampling. *Haemonchus placei* represented 5.2% of the species diversity at the time of fall sampling, which is higher than previously reported in western Canada. The lack of correlation between FEC and ADG is likely due to differences in farm-specific environmental conditions (rain, temperature), soil type and husbandry factors.

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LIST OF ABBREVIATIONS

°C	Celsius
ADG	Average daily gain
AR	Anthelmintic resistance
BW	Body weight
BZ	Benzimidazoles
CI	Confidence interval
cm	Centimetres
d	Days
DNA	Deoxyribonucleic acid
EPG	Eggs per gram of feces
FEC	Fecal egg count
FECRT	FEC reduction test
FMC	Fecal moisture content
g	Grams
G	Group
GEE	Generalized estimating equations
GIN	Gastrointestinal nematodes
GIT	Gastrointestinal tract
Ig	Immunoglobulin
ITS	Internal transcribed spacers
Kg	Kilograms
Km	Kilometers
lb	Pound
L1	First stage larvae
L2	Second stage larvae
L3	Third stage larvae
L4	Fourth stage larvae
min	Minutes

ML	Macrocyclic lactone
mm	Millimetres
mo	Months
nm	Nanometres
ODR	Optical density ratio
PCR	Polymerase chain reaction
rDNA	Ribosomal DNA
SD	Standard deviation
SK	Saskatchewan
SR	Stocking rate
spp.	Species
Th	T helper cell
Th2	T-helper 2 cell
TST	Targeted selective treatment
TT	Targeted treatment
US	United States of America
UV	Ultraviolet radiation
wk	Week

1. LITERATURE REVIEW

1.1 Economic losses associated with GIN infections

The beef industry is a significant component of the Canadian livestock industry. In 2019, Canada's beef herd was comprised of 12.24 million cattle and calves, with in-country sales of $8.3 billion and $4.4 billion in exports (1). Compared to the previous year, consumption of Canadian beef increased by 1.6 percent to 958.000 tonnes (2). If this trend continues, the national herd will need to increase in size or become more efficient to meet future demands. One factor that has been shown to interfere with production performance in pastured cattle is gastrointestinal nematodes (GIN) (3–7). In North America, GIN costs the cattle industry over $2 billion annually due to treatment costs and suboptimal growth performance (8–10). While the use of anthelmintics has decreased the incidence of clinical disease, subclinical disease remains a concern (3). Furthermore, the inappropriate use of anthelmintics has led to the emergence of anthelmintic resistance (AR), which is a significant challenge for GIN control (11,12). Improving GIN diagnosis and treatment protocols could enhance productivity, leading to a more competitive beef industry (11).

Gastrointestinal nematodes such as *Oesophagostomum* spp., *Cooperia* spp., *Trichostrongylus* spp., *Strongyloides* spp., *Ostertagia* spp. and *Haemonchus placei* have been shown to negatively impact beef production (13). Loss in production occurs through three different mechanisms (9). First, GIN infections cause direct tissue damage to the abomasum and intestinal mucosa, which interferes with nutrient absorption. Secondly, they divert energy and protein from growth and maintenance to the immune system. Finally, GIN reduce feed intake, which is a shared characteristic of all helminth infections due to hormonal fluctuations in the host, making this the principal mechanism of subclinical production loss (11). All three mechanisms reduce average daily gain (ADG), feed efficiency (FE), bodyweight (BW), and fertility. In some cases, it can increase mortality, representing monetary losses along with costs associated with control and treatment (9,13).

In cattle, diagnostic tools such as serum pepsinogen concentrations and *O. ostertagi* antibody assays have been used to quantify GIN burdens. These tools, however, have not been

intensely studied in beef cattle due to a lack of data related to the economic impact of GIN on production (11). However, a recent Canadian study using serum antibody titres to *O. ostertagi* coupled with geographic information systems data (GIS) and a multivariate Bayesian statistical model found a correlation between spatial variability and the risk of infection (14). The researchers reported a higher risk of GIN exposure in animals located in areas with greater average humidity (precipitation) and moderate temperatures *versus* cattle located in drier regions experiencing both warmer and colder average temperatures. The evaluation of financial loss can be challenging in cattle because indirect factors associated with GIN infection are difficult to quantify (11). Furthermore, the presence of farm-specific factors, such as technology, budget, and farm size, confound the effect of GIN on beef cattle production. Hence, consideration needs to be given to a farm's economics (inputs and outputs) when deciding on the use of GIN control measures (15).

1.2 Biology and epidemiology of GIN in beef cattle in North America

1.2.1 Gastrointestinal nematode life cycle

The GIN superfamilies that affect cattle (Strongyloidea, Trichstrongyloidea) have a direct life cycle (Figure 1.1). Most GIN have a prepatent period of approximately 28 d, which is the time between infection and egg production (16). In northern temperate regions, pasture contamination is associated with overwintered larvae. Contamination then amplifies during the summer grazing season as manure contaminated with parasite eggs is deposited on pasture (17,18).

The development of the eggs from a morula to first stage larva (L1) occurs in the manure within 24-48 h of being deposited on pasture. First stage larvae moult into second stage larvae (L2), obtaining nutrition from the consumption of microorganisms (18). The L2 then moult into infective third-stage larvae (L3) while retaining the cuticle of the L2, which helps the L3 to endure adverse environmental conditions (8,19). This process of maturation from L1 to L3 takes approximately 10 d under optimal temperature (25°C) (20). Approximately 1 wk later, the L3 begins its migration from the feces to the surrounding soil and plant coverture. During the grazing season, the L3 are consumed by cattle, exsheath the cuticle within the gastrointestinal

tract (GIT), and depending on the nematode's species, they migrate into the mucosa of the intestines (*Cooperia* spp., and *Nematodirus* spp. and *Trichuris* spp.) or abomasum (*Ostertagia ostertagi* and *Haemonchus* spp.) (17). Approximately 3 wk later, the L4 and L5 emerge from the mucosa and enter the gut lumen, where the adult females begin producing eggs by sexual reproduction for the next 50-80 d (21,22). Heavy parasite loads result in a massive number of larvae emerging from the gastric mucosa, resulting in the destruction of functional gastric glands that are replaced by undifferentiated cells. There is also increased permeability of the GIT, leading to diarrhea and low productive performance (23).

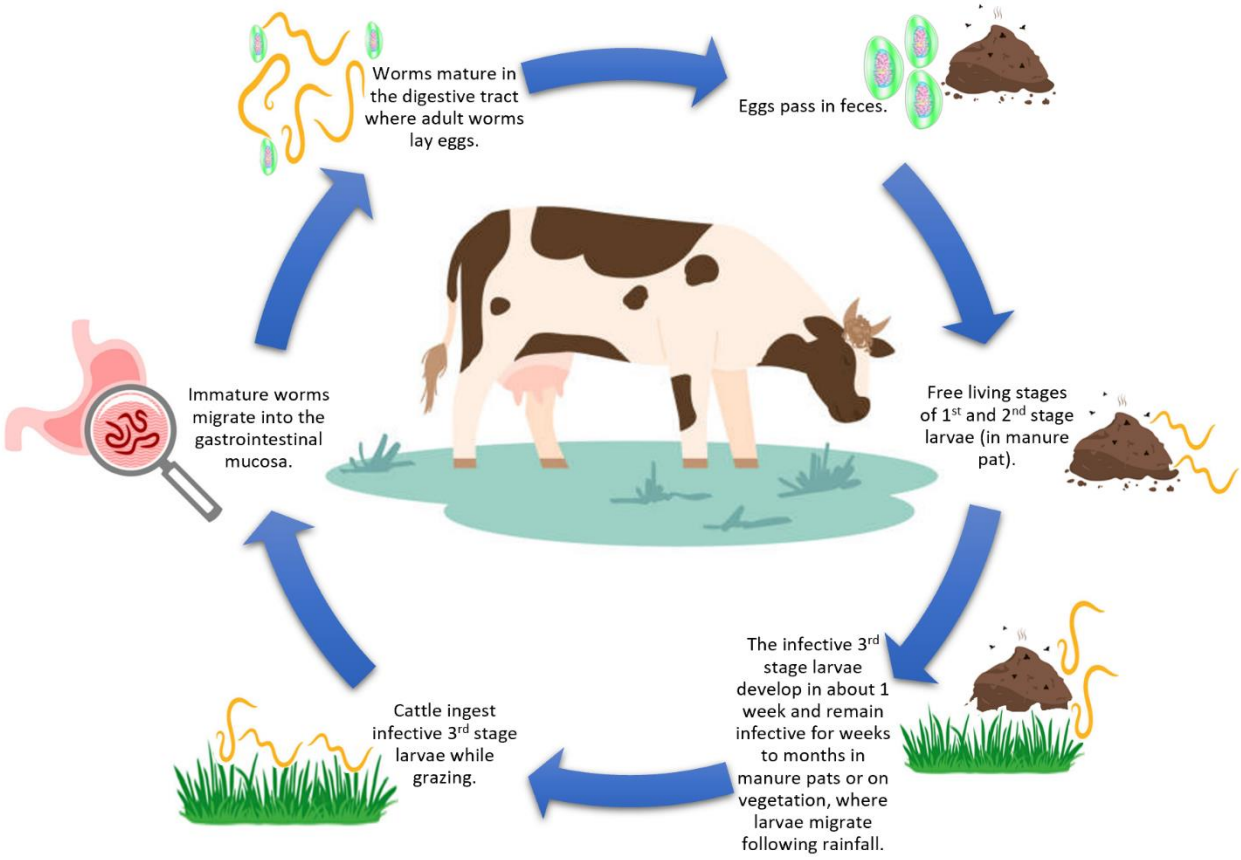

Figure 1.1 Life cycle of gastrointestinal nematodes of cattle (created by Daniel Merchan).

In North America, L1-L3 are affected by extremely cold environmental conditions, which influence the parasite life cycle and fitness. Consequently, most GIN enter a dormant period, also known as arrested development or hypobiosis, during the winter months (22). Although the process of hypobiosis is not fully understood, it is mediated by harsh environmental conditions

and the host's immune status (17). Hypobiosis occurs at the L4 stage, with the larvae decreasing their metabolic activity, allowing them to survive for 5-6 mo until more suitable environmental conditions for transmission develop (24). Hypobiosis also allows the larvae to evade the host's immune system by remaining encapsulated within the mucosal tissues and be less accessible to the immune system (25–27). Nematodes also release chemicals that affect the host's antigen-presenting immune system and stimulate the production of anti-inflammatory molecules such as IL-10 to enhance their survival within the mucosa (28). In North America, dormant larvae emerge in early spring and the progression to adults results in a rise in egg production (29).

An important consideration regarding a GIN's ability to survive is its level of egg production or fecundity. The range in egg production per female varies among nematode species with *Ostertagia* spp. having very low egg production compared to *Haemonchus* spp. and *Cooperia* spp. (29,30). This is particularly important to appreciate when performing diagnostic tests such as fecal egg counts (FEC) because most GIN are morphologically indistinguishable.

Table 1.1 Daily egg production of gastrointestinal nematodes in ruminants.

Nematode species	Daily egg production/female
Cooperia spp.	1,000 – 3,000
Haemonchus spp.	5,000 – 15,000
Nematodirus spp.	50 - 100
Ostertagia spp.	100 - 200

While some larvae survive the winter by entering into hypobiosis, others survive on pasture. An Ontario study found that the L3 of *O. ostertagi, C. oncophora, C. mcmasteri*, and *N. helvetianus* survived winter on pasture (30). Similarly, a recent study in Alberta evidenced high winter survivability for *C. oncophora,* and *O. ostertagi* on pasture. This study also described the overwinter ability of *N. helvetianus* and *T. axei* (31). While snow covering helps to increase L3

survivability (32), post-winter larvae become less infective over time due to energy depletion and will die within weeks in the spring if they fail to find a host (17,22).

1.2.2 Gastrointestinal nematode species in cattle

The GIN that affect the health and performance of cattle are members of the Order Strongylida, with most belonging to the superfamily *Trichostrongyloidea*. The GIN found in the GIT have different niches depending on the species. For instance, *O. ostertagi, H. placei, H. contortus,* and *T. axei* are associated with the abomasum, while *C. oncophora, C. punctata, N. helvetianus, T. colubriformis, O. radiatum* and *Trichuris*. spp. reside in the intestines (27). Worldwide, *H. placei* and *O. ostertagi* are recognized as the most pathogenic nematodes in warm and cold temperatures, respectively (33). However, in North America, *O. ostertagi, C. oncophora*, and *Trichostrongylus* spp. are the most relevant species due to their survivability in the Northern hemisphere (17,22,34).

Regarding GIN infections in Canada, studies have found that *O. ostertagi* and *C. oncophora* are the most common species of grazing beef and dairy cattle (35–37). Using nemabiome metabarcoding, Avramenko *et al* 2017. found that 59.1% and 37.6% of GIN DNA was from *O. ostertagi* and *C. oncophora*, while <5% of the DNA was from *C. punctata, H. placei,* and *O. radiatum* (37). While these latter species occurred at a low prevalence, research in the northern United States found that the presence of *H. placei* and *C. punctata* may be increasing due to global warming (22).

1.2.2.1 Ostertagia ostertagi.

Ostertagia ostertagi is of particular concern because they are the most pathogenic nematodes of cattle in temperate regions, and their ability to enter into hypobiosis allows them to survive the environmental conditions common to North America and western Canada in particular (38–41). This method of survival is of particular importance because this parasite has very low fecundity, producing between 100 to 200 eggs/female/per day (42).

Ostertagia spp. is associated with two different disease manifestations. Type I ostertagiosis is the most common and occurs in young grazing cattle (weaning to 18 mo) that

acquire a substantial burden of larvae in the mid-summer. Upon consumption, the larvae moult into adults within 3 to 4 wk, causing diarrhea, which affects production performance. Type II ostertagiosis is associated with yearling and mature cattle, and generally occurs sporadically in the spring of the year. Type II ostertagiosis occurs when large numbers of arrested larvae emerge from the abomasal mucosa, causing extensive mucosal tissue damage, which may lead to diarrhea, weight loss, and rarely submandibular edema (18,41,43).

1.2.2.2 Cooperia spp.

Cooperia spp. are widespread and are often used in anthelmintic studies as the dose-limiting species. Thus, these nematodes are frequently found on the label claims for anthelmintic products along with AR data (44). The most commonly reported species in the US and Canada are *C. oncophora*, *C. punctata*, and *C. pectinata*, all of which primarily affect cattle under 3 y of age (37,40). Even though *Cooperia spp*. have low pathogenicity, they are very prolific, producing between 1,000 to 3,000 eggs/day (45). There is also an association between higher pathogenicity in *Cooperia* spp. isolates that are resistant to macrocyclic lactone (ML) treatments (7,45).

1.2.2.3 *Haemonchus* spp.

Haemonchus contortus and *H. placei* are avid blood-feeders that affect a broad range of hosts (i.e. cattle, small ruminants, white-tailed deer) and are of particular concern in the southern hemisphere (8,40,46,47). They undergo hypobiosis and are associated with a periparturient rise in FEC before calving and up to 8 wk post-calving. *Haemonchus* spp. has been associated with AR and due to their high fecundity they can produce anemia in young or immunocompromised animals (29,40). *H. placei* is not commonly seen in Canadian beef cattle, only present in <1% of the parasite population within a herd (37). However, it was recently reported to be the predominant trichostrongyle species within five commercial bison herds in Canada (36). This finding suggests that bison may be more susceptible to *H. placei*. This may reflect that this species is emerging as a more common component of the parasite population in the northern hemisphere (36).

1.2.2.4 Nematodirus spp.

Although all GIN share a similar life cycle, *N. helvetianus* has some unique differences. This genus develops its L1 and L2 within the eggs, providing better protection against colder conditions. For this reason, it is often found in considerable numbers of young animals in the northern United States and Canada (22,40). However, these parasitic infections are rarely associated with clinical disease (diarrhea), with calves developing immunity rapidly to *Nematodirus spp*. (18,40). Furthermore, the ova are morphologically distinct from other strongyles, hence they can be distinguished visually from other Trichostrongyloidea eggs. The prevalence of *N. helvetianus* in Saskatchewan ranges from about 4 to 6% in beef and dairy heifers (35,48).

1.2.2.5 Trichuris spp.

Trichuris spp. have a prepatent period of 7 to 9 wk, and their eggs can survive for many years in the environment (47). Infection is common but rarely causes disease. Clinical symptoms of heavily infected cattle include hemorrhagic diarrhea, but generally only a few animals in a herd are affected. Their eggs are morphologically distinct, being lemon-shaped with a plug at each pole (18). In Saskatchewan, the prevalence of *Trichuris* spp. has been reported at 1.7% in beef cattle (48), and approximately 10% of replacement dairy heifers across Canada will be infected (35).

1.2.3 Host immunity

Cattle develop immunity to GIN (8,49), with calves and yearlings being the most susceptible age group (50,51). However, in cow-calf operations, adult cows generally have protective immunity; hence, egg shedding is relatively low. In addition, beef calves engage in relatively low levels of foraging on pasture, which decreases the exposure (ingestion) to GIN (52). Some evidence suggests that administering anthelmintics to calves is counterproductive because it reduces the natural GIN burdens, delaying the development of an immunological response, and this response is needed during the next season when they are grazing as yearling cattle (53).

Innate immunity includes physical barriers, pattern recognition receptors, cytotoxicity, and proinflammatory cells that are the initial host response to GIN; however, this quickly switches to adaptative immunity. Specifically, antigen-presenting cells work with T helper cells (Th) for cell-mediated immunity. While the Th 2 lymphocytes are associated with the production of IgG, IgE, activated complement, along with eosinophils and local nonspecific inflammatory reactions that reduce infection (40). Female cattle generate a better response than males to GIN because estrogens have a positive influence on immune system development (54). However, this advantage is decreased during the peripartum period due to immunosuppression by corticosteroids and prolactin, reducing antibody production (40,54,55). While GIN resistance is heritable (56), it is an inconsistent finding across breeds (57–59).

Concurrent exposure to L3, L4 and adult nematodes stimulates the immune response to GIN in adult cattle (28). Thus, adult cattle can produce a more effective immune response than younger animals (60). The immune response leads to failure of larval establishment, the expulsion of adult worms, altered morphology of adult nematodes, and a reduction in the fecundity of the female parasites (28,52). These immune-mediated mechanisms explain why the shedding of nematode eggs is lower in adult cattle (host resistance). However, adult animals contribute 33% more to pasture contamination than young animals due to both the higher frequency and volume of feces production (61). Thus, management practices like grazing systems and stocking densities influence the risk of disease (62)

Yearling cattle are relatively immunologically naïve to GIN (51). Thus, they amplify the parasite burdens on pasture, particularly in the early months of the grazing season, with FEC decreasing towards the end of the season as immunity increases. However, age-related immunity varies by GIN species with *Cooperia* spp., *Haemonchus* spp., and *Nematodirus* spp. stimulating a rapid immune response, which reduces their burdens 3-4 mo after exposure (51). In contrast, immunity to *O. ostertagi* develops much slower, taking up to 2 y (63,64). This longer period is due to *O. ostertagi* evasion of the host immune system and is the reason why this parasite is of particular concern in temperate regions like western Canada (65).

1.2.4 Environmental factors related to GIN infections

1.2.4.1 Temperature

Temperature and precipitation are the two principal factors affecting the epidemiology of GIN in pastured cattle (14,43,61,66,67). Temperature is crucial for the survival of free-living larvae (68,69), with optimal temperature for larval development varying by species: *O. ostertagi*, 20°- 25°C (14); *N. batus*, 11.5°- 27°C (68); *H. contortus*, 25°- 38°C (69); *C. oncophora*, 17°- 26°C (70); and approximately 25°C for *Trichostrongylus* spp. (71).

Temperature plays an important role in the rate of development, with the egg hatching rate being negatively affected by colder temperatures (69,71). Once hatched, the larvae have a finite energy reserve, with hotter temperatures (40°C) increasing the metabolic consumption of energy, which can result in death. Cool and dry temperatures decrease the mobility of the L3, thereby reducing energy consumption and allowing the larvae to survive for longer periods on pastures (69,72). In North America, the GIN development occurs between 6° and 35°C, with optimal survival between 7° and 25°C (40). A more recent study found that recoveries of the L3 of *C. oncophora* decreased at higher temperatures (20° to 33°C) (70). Generally, cooler temperatures allow for an increase in the longevity of free-living larvae on pasture, except for *H. placei*, which is more prevalent in the southern warmer regions (40). A study in Maine, USA, found that *Nematodirus* spp. larvae persisted on pasture for > 24 mo. Moreover, *O. ostertagi* larvae persisted at low levels for at least 14 mo on pastures naturally contaminated during the summer (73). The persistence of *Nematodirus* spp. for a longer period might be related to its development to L3 within the egg (69).

Saskatchewan is located in the northern temperate zone, where climatic conditions support the free-living L3 stages (74). Temperatures range from an average of – 10°C in winter to 15°C in summer, with extreme temperatures resulting in a 65°C range (75). The number of days exceeding 30.8°C in July varies by 1.6 d in the northeast and 4.3 d in the southwest of the province (76), with these provincial variations encompassing the optimal development temperatures for nematodes.

It is presumed that climate change may alter patterns of GIN transmission and parasite-host dynamics (74,77). This association arises because GIN has free-living stages in which fitness and development are subject to environmental conditions. Hence, adverse climatic conditions on the free-living stages reduce the refugium, which is the proportion of a nematode population on the field (supra-population) or inside animals that have not been exposed to anthelmintics (infra-population) and contribute to the following generation (78,79). This reduction of the refugia along with anthelmintic treatments, may increase the selection pressure, which is a concern because accelerated selection pressure may lead to faster development of AR (70,79). These changes might have a direct effect on the host population; Van Dijk *et al* 2010 suggested that climate change has the potential to affect the nutritional status of the hosts and, ergo, their immune status due to fluctuations in food availability, stocking rates, and length of the grazing season (74).

1.2.4.2 Precipitation

Precipitation is the main factor influencing GIN transmission, providing essential conditions for the development of the larval free-living stages (61,80). In a laboratory study, larvae of *H. contortus, T. colubriformis* and *Teladorsagia* spp. were unable to survive a month of desiccation (81). Survival decreased even after an hour of dryness, and this trend was directly proportional to time (81). On the other hand, rainfall plays a significant role in the infectivity of L3 by enhancing larval activity after a drought period and facilitating larval migration from the fecal pad to the local forage by two mechanisms (8,66,82).

Splash droplets translocate the larvae up to 90 cm from the pat, allowing the L3 to migrate through water films or by the run-off from the manure (82). Rain also influences the moisture exchange from the soil to the feces, which benefits the development of L3 populations. However, the real impact of rainwater depends on the evaporation rate. Low (2.1-3.4 mm/d) and high (3.8- 6.1 mm/d) evaporation rates affect the recoveries of L3 from the soil, with less recovery as evaporation rates increase (83). Saskatchewan's annual precipitation varies between 250 mm and 500 mm. Weather modelling predicts that there could be a 39% increase in precipitation in the northwest, with a 2.1% reduction in the southern regions during the next

decades (84,85). Hence, the predicted changes on precipitation might influence the future epidemiology and dynamics of GIN within the province (69).

1.2.4.3 Plant and grass type

Herbage contributes to the creation of a microclimate, which can have a greater impact on parasite migration than the macroclimate (86). For example, feces exposed to sunlight on short-grass can lose 60% of fecal moisture content (FMC) within 24 h, while pats on long grass with shade maintain FMC of > 60% for 48 h (87). Furthermore, migration from the pat to the local herbage also depends on environmental factors such as moisture and precipitation (40,61,80,86). Most L3 are able to move onto grass three h after rainfall in laboratory conditions (86), but only two to three % are usually recovered from the herbage (88).

A German study (89) assessed the influence of plant composition on the recovery of *C. oncophora* L3 on pasture. Ten different types of plants ranging from compositions with no legumes to medium (52%) and high (62%) legume content were assessed. Legumes improved protection from desiccation by the sun and wind but had no protective effect when drought conditions lasted > 4 wk. Another study (90) reported higher recovery rates of *H. contortus* larvae from plants with a thicker leaf blade and more texture, characteristics that improve the retention of water films on the stem, allowing for larval migration.

1.2.4.4 Ultraviolet radiation

Ultraviolet radiation (UV), which is invisible energy in the wavelength range of 100-400 nm, can affect GIN survival (91). UV light is subcategorized into three types: UVA, or long-range, between 320-400 nm; UVB, medium-wave between 280-320 nm and UVC, short-wave between 100-280 nm. About 95% of UVA trespasses the ozone layer, while 95% of UVB is blocked (92). In one study, exposure to UV radiation increased the death rate of *H. contortus, Teladorsagia circumcinta* and *N. battus*, although *H. contortus* had greater survivability than the others (91). Hence, UV should be considered in the prediction models that calculate GIN burdens on pastures (74). UV may also explain the differences in recoveries of L3 between field and in

vitro studies (91). Legumes and cloud cover provide greater protection from UV radiation (70,89).

1.2.4.5 Soil and soil type

Soil type has been suggested as a factor in larval migration, and larval recoveries have been reported as deep as 15 cm (43,61). Under laboratory conditions, soil acts as a reservoir for larvae, allowing them to endure harsh environmental conditions (70) prior to migrating to the plant coverture (80). However, the relationship between soil and GIN has been a subject of debate. Callinan *et al* 1986. reported an eight times greater recovery of L3 from the soil compared to plant coverture; however, temperature and moisture may have confounded these results (93). Krecek *et al* 1991. found that L3 recovery was greater from the herbage than the soil (94), underscoring that where larvae are recovered is highly variable.

It is also possible that soil type may be associated with GIN infections. Saskatchewan's soils are classified into four groups of the Chernozemic Order: brown, dark brown, black, and dark gray. This classification is based on the colour and represents the amount of organic matter in each class (95). The soil's texture in the province varies with regard to the agro-climatic zones (sub-humid, semi-arid and arid), and it is possible to find texture varying from loamy sands to clays. The southwest of the province is considerably drier than the northeast, which is characterized by cool summers and cold winters (85). In one study, researchers found 3.5 times greater FEC in cattle grazed on dark brown *versus* brown soils (47). However, this may have been confounded by uncharacterized management practices or environmental conditions (wetter conditions on dark brown soils).

1.2.4.6 Grazing management

Grazing management, which includes stocking rate (SR) and grazing methods, has a direct impact on the ADG of pastured animals, with SR expressed in animal unit months (AUM) per unit area (96). Pasture management practices directly influence animal performance through nutrition and indirectly by parasitic infections (97). The two main grazing systems in Canada are continuous and rotational (intensive). In continuous grazing, cattle graze freely on pasture, and

under high SR, the regrowth of the most palatable plant species is affected due to overconsumption. Under light SR, the pasture plants persist before being consumed, which explains why the pasture's nutritional value decreases, resulting in reduced animal performance (96). In rotational or intensive grazing systems, the animals are rotated for short periods of time in paddocks within the pasture, with all plant species being consumed, which enhances the plant's recovery (96).

The effects of the grazing methods on cattle performance are conflicting, which might be explained by the confounding effect of SR within the grazing systems (80-82). A study assessing biocontrol strategies for *O. ostertagi* found that the highest recoveries of overwintering L3 were on pastures under high SR. For this reason, the beneficial effects of the control strategy were evident under high infection pressure. In contrast, under low SR, the parasitic burdens were too low to show the positive effects of antiparasitic control measures (99). In short, "The higher the stocking rates, the greater the propensity for parasite acquisition" (40). This finding is attributed to the animals having to graze closer to the ground and fecal pads, encouraging the ingestion of L3 larvae (97). However, it is important to clarify that properly managed intensive grazing systems rotate the cattle before they overconsume the pasture (no allowing the animals to eat close to the ground) which results in lower exposures to feces.

Gastrointestinal parasitism has been associated with the reduction of food intake by cattle and, ergo, a reduction in ADG (97)**.** Hence, pasture management programs such as rotational grazing have been used as parasitic prevention and control strategies (100,101). However, rotational grazing does not necessarily reduce the parasitic burden by itself since infective larvae are able to survive on pasture, especially in northern temperate regions (40,73). Therefore, rotating animals from paddock to paddock will not control L3 infections (102) if SR are high or if the conditions for nematode survival on the pasture are still optimal.

1.3 Diagnosis and identification of gastrointestinal nematodes

1.3.1 Fecal egg counts

A fecal egg count (FEC) is a simple and inexpensive method for indirectly assessing parasitic load within an individual animal (103). FEC have been used in the estimation of GIN in grazing animals and are measured in eggs per gram (EPG) of feces. This method has been used to assess parasite control programs, evaluate levels of pasture contamination, and assess the efficacy of anthelmintics (29). The technique relies upon differences in the specific gravity of the parasite eggs and the flotation media (i.e. sugar or salt). As a result, the less dense parasite eggs float to the top of the media, where they adhere to a cover slide and are then assessed under a microscope both qualitatively and quantitatively. In cattle, FEC are often lower than in small ruminants, which has led researchers to use the more sensitive Modified Wisconsin (MW) sugar flotation technique (104). This technique has a sensitivity of < 10 EPG, while the McMaster Flotation (MF) salt technique has a sensitivity of > 50 EPG (105). A study of the MW technique found that the test detected < 10 EPG without a false negative rate when the true EPG was > 2.65, and also that the false negative rate was as low as 7% when the true EPG were lower than 1.44, confirming the usefulness of this method for enumerating low FEC (106). Thus, the MW technique is sensitive enough to detect low egg counts from nonprolific nematodes such as *O. ostertagi*. Moreover, the MW technique does not affect the morphology of the eggs, allowing the identification of unique nematode species. In addition, the sugar solution does not crystallize on the slide, which means they do not have to be read immediately. Researchers can also use this method to visualize tapeworm eggs (*Monieizia),* lungworm larvae (*Dictyocaulus*) and coccidia (*Eimeria* and *Isospora*), making it a tool for the diagnosis of a broad spectrum of parasites (104).

The main limitation of FEC is the poor correlation between the number of eggs in the feces and adult parasite worm burdens within the GIT. Therefore, they should be used with caution as an estimator of clinical and subclinical disease (8,16,35,107,108). Another disadvantage of the FEC is that most strongyle eggs look morphologically similar, apart from *Nematodirus* spp. and *Trichuris* spp. (29,48) (Figure 1.2). This is important because *Cooperia* spp. are very fecund but have low pathogenicity, whereas the opposite is true of *O. ostertagi*. Thus, there may not be a

direct association between animal performance and FEC. Also, EPG values are not normally distributed, but are overdispersed. Thus, proper sampling and statistical techniques are essential for estimating the true mean EPG in a group of cattle (109). Hence, it is difficult to compare studies due to sampling methods and discrepancies in the EPG presentation (arithmetic *versus* geometric mean) (35).

FEC are best used for individual *versus* pooled samples. Pooled samples might not account for individual animals that shed a high number of nematode eggs, which are the primary source of pasture contamination. Therefore, if pooled sampling is required, collecting between 15-20 individual fecal samples is recommended to increase the chances of getting at least one sample from animals with high FEC (48,110). However, the inclusion of several 'super shedders' may inflate the true level of shedding occurring over a larger group of animals (29).

The FEC reduction test (FECRT) is used to assess the efficacy of an anthelmintic. This test can be performed on individual animals or pooled fecal samples (111). It is recommended that a minimum of 10 animals be tested, with this number increasing when EPG are low (< 50 eggs under the microscope). The FECRT procedure involves collecting rectal fecal samples before and after treatment (14-21 d). The dosage of the anthelmintic must follow the label guidelines. Results are expressed as a percent reduction in FEC using the following formula ((mean pre-treatment FEC – mean post-treatment FEC)/mean pre-treatment FEC × 100). A percent FECRT of > 95% is considered highly effective (no evidence of anthelmintic resistance), while a reduction of < 80% is suggestive of inappropriate usage or anthelmintic resistance. The pooled sample protocol follows similar recommendations, formula, and interpretation with a minimum of three slides or as needed to count 50 EPG (total number of eggs counted using the microscope). The EPG on each slide are counted and then the average EPG for all the slides are calculated. The same animals and number of slides in the pre-treatment must be used for the post-treatment FEC (111).

Figure 1.2 Morphology of gastrointestinal helminth eggs of ruminants. Note the eggs of *Ostertagia ostertagi*, *Haemonchus* spp., *Cooperia* spp. are indistinguishable from each other, whereas Trichuris spp. and *Nematodirus* spp. are very distinct. Taken from © Bowman, 2014 Georgis' Parasitology for veterinarians, Elsevier Inc (18).

1.3.2 Other diagnostic methods

Other techniques have also been developed for determining parasitic burdens in cattle. One such approach is the enzyme-linked immunosorbent assay (ELISA) (112). ELISA has been used to detect antibodies to *O. ostertagi* in milk, blood, and meat juice samples. ELISA results have also been correlated to production parameters such as weight gain in pastured yearling beef cattle and reproductive performance and milk production in the dairy cattle (113–116). An Irish study reported combining ELISA with an electrochemical sensor to perform a sensitive electrochemical immunoassay (e-ELISA) to rapidly detect *O. ostertagi.* The e-ELISA was specific and capable of detecting a 16-fold dilution of positive serum samples in only 25 min; the incubation period for the commercial ELISA kit is 150 min. This method shows potential as a very quick and sensitive screening tool and deserves further investigation (117). A recent study of western Canadian beef calves found that serum antibody levels might be a better indicator of GIN exposure than FEC (14). However, a limitation of the ELISA is the chance of cross-reaction with other helminths (107,118), and the presence of serum antibodies only provides an indication of past exposure.

Diagnostic tools have also been developed that estimate nematode burdens based upon serum gastrin and pepsinogen concentrations (47). These techniques are specific to GIN that damage the abomasal mucosa. Serum pepsinogen concentrations increase when the parasites damage the abomasal parietal cells, leading to a decrease in acid production. Hence, the transformation from pepsinogen to pepsin decreases, causing the accumulation of pepsinogen that enters the blood system, resulting in an increase in serum and milk pepsinogen concentrations (119). Abomasal nematodes also increase the secretion of gastrin by stimulation of G-cells located in the antral pyloric region (120). The serum pepsinogen method, however, has shown contradictory results with *O. ostertagi* infections (118,120,121), with results from adult cattle being poorly reproducible among laboratories (119,122). The same issues arise with serum-gastrin concentrations, where a large number of larvae (100,000 of *O. ostertagi* L3) did not increase the gastrin level in adult animals (123). This method has other challenges, such as the instability of the enzyme in the serum and the cost of the technique, making it a poor option for herd-health monitoring (118).

1.3.3 Speciation of gastrointestinal nematodes

The identification or speciation of GIN can be performed by hatching the eggs and growing the larvae to the L3 state, in which, unlike the eggs, the larvae are morphologically distinct. Larval features used for identification include the "head" (cranial extremity) or the length of the sheath "tail," which extends from the tip of the larval caudal extremity to the end of the tail of the sheath (Figure 1.3). Although accurate, this is a very time-consuming and technically challenging procedure (124). For this reason, molecular techniques have been developed to distinguish the GIN species (125). Pioneers in this approach used polymerase chain reaction (PCR) to the internal transcribed spacers (ITS-1 and ITS-2) of nuclear ribosomal DNA to speciate the nematode larvae (126). These markers have a low level of intraspecific variation in their sequence (<1%) but significantly higher (>1.5%) variation between species (127). A more recent molecular technique targets an internal transcribed spacer (ITS-2) of the rDNA region, which is common to all roundworm species but contains enough genetic variation to speciate nematodes (128). Thus, from a single fecal sample, this technique will amplify and quantify the DNA of a diverse population of GINs, providing the 'nemabiome', which quantifies the relative diversity and abundance of GIN species within a fecal sample (128).

Briefly, generating the nemabiome relies on DNA from L3 larvae previously cultivated from fecal samples. Following DNA extraction and purification, PCR is used to amplify the larvae's ITS-2 regions, followed by next-generation sequencing (NGS). The result is DNA sequences or 'reads' which are then assembled into longer lengths of DNA (contigs). These DNA sequences are then compared to known genetic sequences unique to each GIN species. The proportions of nematode species are calculated by dividing the number of species-specific reads by the total number of reads per sample, allowing for the relative quantification of the different species within the sample (128). Thus, it provides a profile of the diversity and relative abundance of GINs within a fecal sample. This method was first reported in 2015 and used for beef and dairy cattle fecal samples and in L3 collected from grass and soil (31,35,37). This technique does not provide information on the absolute magnitude of the GIN infection but rather the relative abundance of each species. This tool enables a better understanding of the epidemiology and ecology of GIN, which can be used to design sustainable control strategies.

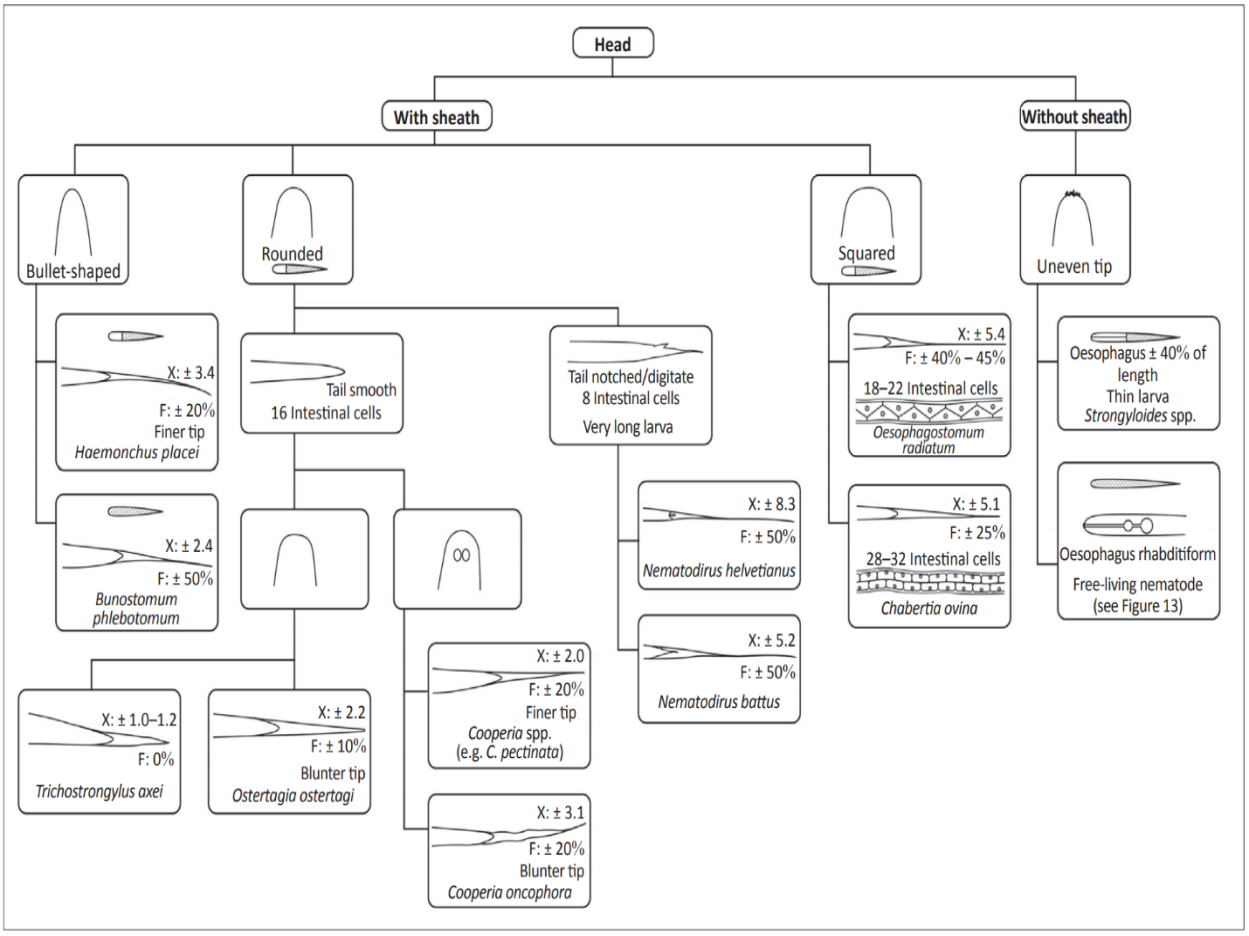

Figure 1.3 Organogram for morphologic identification of GIN larvae in cattle from © Wyk and Mayhew 2013, AOSIS OpenJournals (129).

1.4 Anthelmintic treatment and resistance

There are many strategies for the prevention and control of GIN infections in cattle. Several are a combination of anthelmintics and management practices, such as varying stocking densities within different grazing systems (47). Anthelmintics are primarily used at the herd level to improve the economics *versus* treating individual animals with clinical diseases. However, the improper use of these medications due in part to a lack of epidemiological information has led to the development of AR (34).

Worldwide, more than 40 commercial cattle anthelmintic products are available. This market is mainly represented by three main classes: imidazothiazoles (levamisole),

benzimidazoles (oxfendazole, albendazole, and fenbendazole), and macrocyclic lactones (ivermectin, doramectin, eprinomectin, and moxidectin) (40). A recent western Canadian study found that only macrocyclic lactones (ML), benzimidazoles (BZ) or a combination of the two were being used (130). ML was used by 99% of the producers regardless of the size of the cattle operation, and 16 producers used at least one drench or in-feed/mineral mix BZ product (131). The main reason for using ML was to control ectoparasites (i.e. lice) rather than as an endoparasiticide. This explains why most anthelmintics are applied in the fall (131). However, this timing is suboptimal for GIN control and can lead to AR (16). Furthermore, administering anthelmintics in the fall to young cattle may slow the development of immunity (53). This is a concern because these animals may then harbour a population of hypobiotic larvae that will reactivate in the spring, increasing the contamination of pastures.

Macrocyclic lactones are produced by *Streptomyces* microorganisms and are the most commonly used class of anthelmintics in North America (18). These molecules bind to the glutamate receptors, causing chloride influx, which hyperpolarizes neurons and stops the transmission of normal action potentials, causing parasite paralysis and subsequent death (18). This medication class has been used for several decades in stocker cattle (22,132). A multicentric American study reported a 43 lb weight gain in cattle administered an extended-release eprinomectin *versus* untreated cattle (133). In another study, increases in ADG of 0.12-0.46 lb/d were observed in yearlings grazing over 140 d after treatment with doramectin pour-on compared with untreated controls (134). Similarly, topical moxidectin provided better control of the parasitic burden for all common roundworms (> 90% efficacy) in a study that compared this group with other ML and control groups (135).

Other antiparasitic medications in North America include benzimidazoles and imidazothiazoles. Benzimidazoles bind tubulin molecules with a stronger affinity to the nematode's tubulin *versus* mammalian tubulin. This inhibits microtubule formation, disrupting cell division. Benzimidazoles also inhibit fumarate reductase, which blocks mitochondrial function, leading to the parasite dying from a lack of energy (18). Regarding imidazothiazoles, levamisole is the only product available on the market, and it acts as a nicotinic antagonist to disrupt the neuromuscular system, causing spasm, tonic paralysis, and death. This anthelmintic

has also been associated with interfering with the fumarate reduction system, affecting the mitochondria's energy production (18,29). A study in Canada assessed the administration of levamisole, morantel and thiabendazole in untreated animals. The three anthelmintics groups were effective in removing the adult nematodes, but there were no significant differences in ADG between the anthelmintics and the control group (136).

Combinations of different anthelmintics classes are often used, showing improved outcomes in many cases (137,138). Edmonds *et al*, 2018 assessed the efficacy of three different drug protocols plus an untreated control group to reduce ML-resistant nematodes: 1) a combination of injectable ML having activity up to 28 d (doramectin) and oral BZ (albendazole), 2) injectable ML (doramectin), 3) extended-release ML (eprinomectin) having activity up to 100 d, and 4) untreated control animals. The combination of ML + BZ was nearly 100% effective in reducing FEC to zero, resulting in a 20% increase in BW gain compared to the control group by d 32. At the end of the study (d 118), the improvement in BW gain was 23% and 29% for the ML + BZ combination and the extended-release eprinomectin, respectively. There was no statistical difference between both therapies, suggesting that both treatments provided protection over the length of the grazing period (139).

A western Canadian study found that animals treated with a combination of ivermectin and fenbendazole had a greater percentage of AAA carcasses compared to the ivermectin and nontreated control groups. Both groups of anthelmintic treated cattle had improved carcass quality and profit compared to the control cattle, but there was no significant difference in the profits between the two anthelmintic treatment groups (140).

Anthelmintic usage has led to AR worldwide, a concern for the cattle industry (16,139). Resistance is defined as "when a greater frequency of individuals in a parasite population, usually affected by a dose or concentration of a compound, are no longer affected, or a greater concentration of drug is required to reach a certain level of efficacy" (141). Resistance is likely present if the application of anthelmintics results in a < 80% reduction in FECRT (111). Gasbarre *et al*. were the first to report AR in American cattle with a significant number of *H. contortus* resistant to ML and BZ and *H. placei* and *Cooperia* spp. resistant to ML (142). *C.oncophora* is

the species with the most reports of AR, likely caused by underdosing protocols (143). Stromberg *et al*. noted that anthelmintics alter the frequency of resistant nematodes (*C. oncophora* and *Haemonchus* spp.). Once AR occurs, more significant production impacts will be observed (7). A recent western Canadian feedlot study reported a substantial increase in *Cooperia* spp. and a reduction in *O. ostertagi* after ML protocols (37). De Seram *et al* 2021. confirmed ivermectin resistance in *C. oncophora* and possibly resistance in *H. placei, C. punctata* and arrested larvae of *O. ostertagi* within western Canada (140). These findings should be a concern to the Canadian beef industry.

1.5 Hypothesis and objectives

This research focuses on the epidemiology and production impact of GIN in yearling beef cattle in western Canada. Yearlings are at higher risk of GIN parasitism because they are placed on pastures when their immunity is still developing, and parasitic burdens are increasing over the grazing season. These conditions facilitate exposure to GIN and a potential loss in production. The aims and scope of this thesis were:

- Objective 1: Provide current information on the epidemiology of gastrointestinal nematodes (GIN) in Saskatchewan yearling beef (stocker) cattle (Chapter 2). This objective was met by performing fecal egg counts (FEC) and associating these with environmental conditions (temperature, precipitation, and soil type).
- Objective 2: Evaluate the production impact of GIN in stocker beef cattle in Saskatchewan (Chapter 2). This objective was met by measuring bodyweight (BW) gain and average daily gain (ADG) between control and treated animals (parenteral extended-release eprinomectin + fenbendazole oral suspension) in17 groups of stocker beef cattle.
- Objective 3: Determine the prevalence and species diversity of GIN in fecal samples from stocker beef cattle in Saskatchewan (Chapter 3). This objective was met by performing fecal egg counts (FEC) and ITS-2 nemabiome metabarcoding sequencing assays on fecal samples collected from 17 cohorts of cattle in the spring (turn-out) and fall (round-up).

This research provides contemporary data on the epidemiology of GIN in grazing beef stocker cattle and the effects of parasite burdens on production parameters.

2. DESCRIPTION OF GASTROINTESTINAL NEMATODE FECAL EGG COUNTS AND THEIR RELATIONSHIP TO PRODUCTION PERFORMANCE IN STOCKER CATTLE IN SASKATCHEWAN.

2.1 Abstract

Gastrointestinal nematodes (GIN) affect the beef industry due to the loss in profit associated with reduced production performance and the cost of anthelmintic treatments. There is a paucity of information regarding the epidemiology of GIN in western Canada. Hence, the purpose of this study was twofold: to describe the epidemiology of GIN in pastured yearling beef cattle in the province of Saskatchewan and to assess the impact of internal parasitism on weight gain. The study included 17 cohorts of yearlings located on 13 farming operations. Each operation was visited twice during the grazing season (spring and fall), at which time animals were individually weighed, and rectal fecal samples were obtained for pooled fecal egg counts (FEC). A subset of calves (n = 25) in each herd were administered anthelmintics, while the remaining cohort was left untreated. Of the untreated animals, 25 head were tagged and weighed, and these formed the control group. The anthelmintic treatment group received both oral fenbendazole (Safeguard®, Merck, Canada) and a parenterally administered extended-release eprinomectin (LongRange®, Boehringer Ingelheim, Canada). FECs were estimated using generalized estimating equations (GEE) with precipitation and soil type as independent variables. A total of 867 cattle were enrolled (n = 446 controls; n = 421 treatments). Across all cattle, there were differences between treatment and control groups with respect to FEC ($p < 0.01$). FEC decreased by 44 times over the summer grazing season, and counts were 1.8 times higher on pastures located in black/gray soil *versus* dark brown soil zones. There was no difference ($p = 0.41$) in the ADG between cases and controls across all 867 cattle, but differences were found in the ADG of the treatment and control cattle on five farms ($p < 0.05$). The variation in the effects of GIN on ADG is likely due to differences in environmental conditions (rain, temperature), soil type and husbandry practices. This project provides contemporary data on parasite burdens and their production impacts on grazing yearling beef cattle in western Canada.

2.2 Introduction

Gastrointestinal nematodes (GIN) threaten the profitability of the cattle industry worldwide (34). These infections negatively affect dairy and beef cattle production parameters by reducing feed intake, causing direct tissue damage, and diverting energy to the immune response, all of which lead to weight loss, infertility, decreased weight gain, and a loss in milk production (9,13). For instance, *Ostertagia ostertagi* causes damage to the gastric glands, resulting in increased abomasal pH, which adversely affects digestion. This impaired nutrient digestion and absorption leads to inappetence, resulting in weight loss (144). In an Argentinean study, heifers treated with anthelmintics reached puberty 4 wk sooner than untreated heifers. This difference was attributed to improved body condition and weight gain (145). In dairy cattle, GIN have been associated with milk production losses of 0.35 kg/cow/d, with this reduction attributed to the indirect effect of lower feed intake (146). Other detrimental effects of internal parasitism on the productivity of cattle are the disruption of protein metabolism, interference with water and electrolyte balance, intestinal stasis, and localized inflammation of the gut (147).

The main GIN superfamilies that affect cattle are Strongyloidea and Trichostrongyloidea, with infection occurring on pasture when susceptible animals are grazing. In the Northern hemisphere, the infection begins in the spring with the ingestion of overwintered free-stage infective larvae, with pasture contamination increasing during the grazing season (17,18). The distribution and severity of the infection with GIN differs widely between regions and depends on several factors, such as host immunity status, parasite species, management practices, and environmental conditions (14,35,61). Temperature plays an important role in the development of eggs and in the survival and migration of the free-living stages (148). For instance, the egg hatching rate is negatively affected by cold temperatures (71), while hot temperatures cause larval death by increasing the metabolic consumption of energy (69). Precipitation is also an important factor for GIN transmission by preventing desiccation and facilitating the spread of the larvae from the fecal pads by splash droplets (up to 90 cm) and water run-off from the manure (82). Furthermore, precipitation improves the vertical migration of the larvae onto the surrounding plant coverture (93). Under laboratory conditions, third-stage larvae (L3) use the soil as a reservoir to endure harsh environmental conditions (70). This supports the premise that L3

may use soils as a shelter at some point during their development before migrating to the plant coverture (80).

Haemonchus placei and *Ostertagia ostertagi* are recognized as the most pathogenic nematodes in warm and cold temperatures, respectively (33). In the Canadian context, *O. ostertagi* and *C. oncophora* are considered to be the most prevalent species in grazing beef and dairy cattle (35–37). Using ITS-2 nemabiome metabarcoding, Canadian researchers found that 59.1% and 37.6% of DNA collected from larvae recovered from beef cattle were associated with *O. ostertagi* and *C. oncophora*, respectively (37). Nonetheless, there is a lack of data regarding the epidemiology of GIN in Western Canada's yearling beef cattle, specifically its effects on production parameters (16,44,74,149).

Recent Western Canada studies found associations between FEC and temperature, soil type, moisture and precipitation (14,48). A study in Alberta concluded that the rate of GIN transmission was higher in central *versus* southeastern and northwestern Alberta. This difference was attributed to higher average moisture, rainfall and moderate temperatures in central Alberta compared with the hot, dry and cold dry regions in southeastern and northwestern Alberta, respectively (14). Furthermore, a study in Saskatchewan reported 3.5 times greater FEC in animals grazing on dark brown *versus* brown soils, suggesting a possible association between the soil type and GIN burdens. However, this relationship may have been confounded by management practices or environmental conditions (wetter conditions on dark brown soils) (48).

Most studies assessing production impacts in beef cattle have used average daily gain (ADG) as a parameter for cattle performance (150). One study in South Dakota compared cattle treated with ivermectin sustained release product to nontreated control animals. Treated animals had an increased gain of 0.05 kg/head/d compared to the nontreated animals, equal to an increase of 14.5 lb over a 143 d grazing season (151).

The last comprehensive Saskatchewan study involving grazing yearling beef cattle that looked at the epidemiology of GIN was completed in 1987 (152). Therefore, the objectives of this study were to provide current data regarding the epidemiology of GIN in western Canadian pastured yearling beef cattle and to evaluate the impact of GIN on production performance.

2.3 Materials and methods

2.3.1 Farm selection and study design

Twenty cohorts (herds) of yearling Angus and cross-bred beef cattle, distributed over 15 farms, were recruited from across the province of Saskatchewan, Canada, in 2019. Inclusion criteria included: minimum beef cattle cohorts of ≥ 100 yearlings; cattle had not been administered an anthelmintic within 2 mo prior to the start of the study; and the producer had to commit to supporting the study's research activities.

At the time of the spring sampling (day 0), animals from each cohort were randomly assigned to treatment (n = 25) and control (n = 25) groups. Treated cattle were administered fenbendazole oral suspension (Safe-guard® Merck, Canada) at 5 mg/kg BW and a subcutaneous injection of extended-release eprinomectin product (LongRange ® Boehringer Ingelheim, Canada) at 1 mg/kg BW under the loose skin in front of the shoulder; control animals and the remaining cattle on each herd were left untreated to allow for natural contamination of the pastures. The 50 animals used from each cohort were individually identified with ear tags; weighed; and sampled (rectal fecal sample). At fall sampling (day ≥ 90 d), animals were weighed again, and a second fecal sample was collected from each animal.

The University of Saskatchewan's Animal Research Ethics Board approved this study (protocol number: 20190024)

2.3.2 Fecal sample collection and egg counts

Fecal samples (spring and fall) were collected by rectal palpation from individual animals (>20 g of feces). Samples were placed in sealable plastic bags with the air removed to ensure an anaerobic environment and then stored in insulated coolers with ice packs until they could be processed within 24-48 h post-collection.

Fecal samples were individually weighed, and those with < 20 g of feces were discarded. Individual samples (20 g) were then pooled within either the 25 treated or control cattle providing two pooled samples/farm/sampling points. The pooled samples were mixed to a homogenous

consistency, and a 5 g aliquot of fecal material was used for the Modified Wisconsin sugar floatation technique (104). This procedure was repeated four times for each pooled sample. Due to the similarity of the eggs of *Ostertagia* spp., *Haemonchus* spp., *Oesophagostomum* spp., *Trichostrongylus* spp., and *Cooperia* spp. these were reported as Strongyle-type eggs. The morphologically distinct *Nematodirus* spp. and *Trichuris* spp. eggs were reported separately. All FEC were converted to eggs per gram (EPG) of feces.

2.3.3 Environmental and soil data

The 2019 climatological data (temperature and precipitation) were downloaded from Environment Canada's central database, which has stations across the province. The land location for each farm was used to identify and obtain the information from the nearest station (153). The temperature from April to September was measured in growing degree days (GDD), with a base temperature of 0 °C, where GDD was computed by subtracting the base temperature from the daily mean temperature (negative values were set to 0) and then summing these values. Similarly, the precipitation was measured in mm/day and summed to obtain the values within the April to September interval.

Farm coordinates were used to plot the cohorts on the Saskatchewan chernozemic soil type map, which classifies soil into four different categories (brown, dark brown, black, and dark gray) (95).

2.3.4 Data analysis

Data were entered on a commercial spreadsheet program (Excel 365; Microsoft, Redmond, Washington, USA) and imported into a statistical software program (IBMMB SPSS Statistics for Windows, version 25, Armonk, New York, USA) for analysis. ADG was selected as the performance parameter and was calculated by subtracting the initial BW at the spring sampling from the final BW at the fall sampling and dividing it by the number of grazing days). Descriptive statistics were performed for the spring and fall FEC, ADG and BW data. Students t-test assessed for differences in the mean BW between treatment and control animals across all groups and at the herd level to determine if the cattle had been properly randomized. The same

analyses were performed for the mean final BW and ADG to look for differences in these productive parameters among groups. The FEC were not normally distributed, and for that reason, the Mann-Whitney U Test was used to compare the FEC between treated and control animals. The Wilcoxon Signed-Rank Test was applied to compare the initial and final FEC within each treatment group.

The effects of environmental factors, treatment, and season on FEC were estimated using generalized estimating equations (GEE), negative binomial distribution with log link function, and adjusting for clustering of samples within each cohort for each sample using bootstrap variance estimation with a minimum of 5,000 repetitions. The GEE assessed for differences in FEC counts across treatment groups, soil types, cumulative precipitation, GDD, and length of the grazing season. Variables were screened with an unconditional analysis, and those with $p < 0.20$ were considered for inclusion in the final model. The final multivariable model was developed using a manual stepwise backwards elimination process in which variables with $p < 0.05$ were kept. The removed variables were considered confounders if adding the variable back into the model changed other effect estimates by more than 20%. The effect estimates were reported as relative differences in FEC with a 95% confidence interval (CI). The level of significance for all analytical tests was $p < 0.05$.

A linear mixed model with normal error distribution and identity link function using the farm ID as a random effect was performed to understand the relationship of the parasitic burdens, length of the grazing season, and environmental factors on the ADG. The univariate analysis was performed to select the variables within the initial model ($p < 0.20$). The final multivariable model was obtained through a manual stepwise backwards elimination process using all the covariates and their interactions in which variables were kept if $p < 0.05$.

2.4 Results

2.4.1 Descriptive summary of farms and sampling

Overall, 17 cohorts from 13 different beef operations, representing 867 cattle (446 control and 421 treated), completed the study. Figure 2.1 shows the location of the cattle cohorts, which

covered a wide geographical area of the province. Five groups were steers, 11 were heifers, and one group was a mix of both. The first (spring) samplings occurred between April 30 and June 19, 2019, and the fall samplings were between August 14 and November 28, 2019. Grazing seasons varied from 91 to 198 d (Appendix A Table 1).

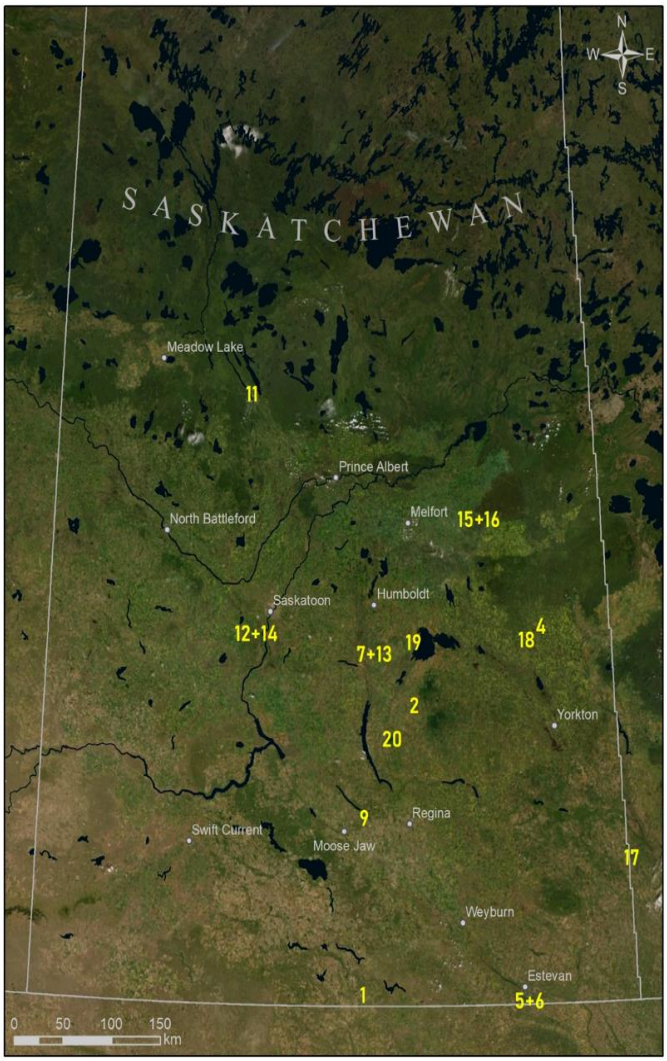

Figure 2.1 Distribution of the beef cattle yearling cohorts within the province of Saskatchewan during the 2019 sampling season.

2.4.2 Fecal egg counts

A total of 272 FEC were performed on 68 pooled fecal samples, 136 for each of the spring and fall samplings. From the spring sampling, at least one strongyle-type egg was detected in 97% (66/68) and 98.5% (67/68) control and treated groups, respectively. During this period, > 1 EPG of *Nematodirus* spp. eggs were detected in 20.6% (14/68) and 26.5% (18/68) of the treated and control samples. In contrast, only 7.3% (5/68) of the control and 8.8% (6/68) of the treated group samples had > 1 *Trichuris* spp. eggs. There was a general reduction in EPG of the treated cattle, with 45% (31/68) having > 1 strongyle-like egg in the fall, compared to 98.5% (67/68) of the control samples with > 1 EPG. Similarly, there were no *Nematodirus* spp. eggs, and only 1.5% (1/68) of the samples had *Trichuris* spp. eggs in the treated group. However, that reduction was not that evident in the control group, where *Nematodirus* spp. and *Trichuris* spp. eggs were present in 14.7% (10/68) and 5.9 (4/68) of the samples respectively (Table 2.1).

Table 2.1 Percentage of pooled samples from the 17 cohorts of Saskatchewan grazing yearling beef cattle having at least one Trichostrongyloidea egg by treatment groups in 2019 spring and fall (n = 272 FEC total, 68 FEC in each sampling period by treatment).

	Spring		**Fall**	
	April 30 to June 19		August 14 to November 28	
Nematode species	**Treatment**	**Control**	**Treatment**	**Control**
Strongyle-like	98.5% (67/68)	96% (66/68)	45% (31/68)	100% (68/68)
Nematodirus spp.	20.6% (14/68)	26.5% (18/68)	0% (0/68)	14.7% (10/68)
Trichuris spp.	8.8% (6/68)	7.3% (5/68)	1.5% (1/68)	5.9% (4/68)

The FEC varied widely between herds and between sampling periods (Figure 2.2). All cohorts that had a significant difference in ADG between control and treated groups (Figure 2.3) were cohorts in which the FEC decreased in control cattle between spring and fall. In the overall analysis, the treated cattle had lower FEC in the fall compared to the controls ($p < 0.01$) (Appendix A, Table 2).

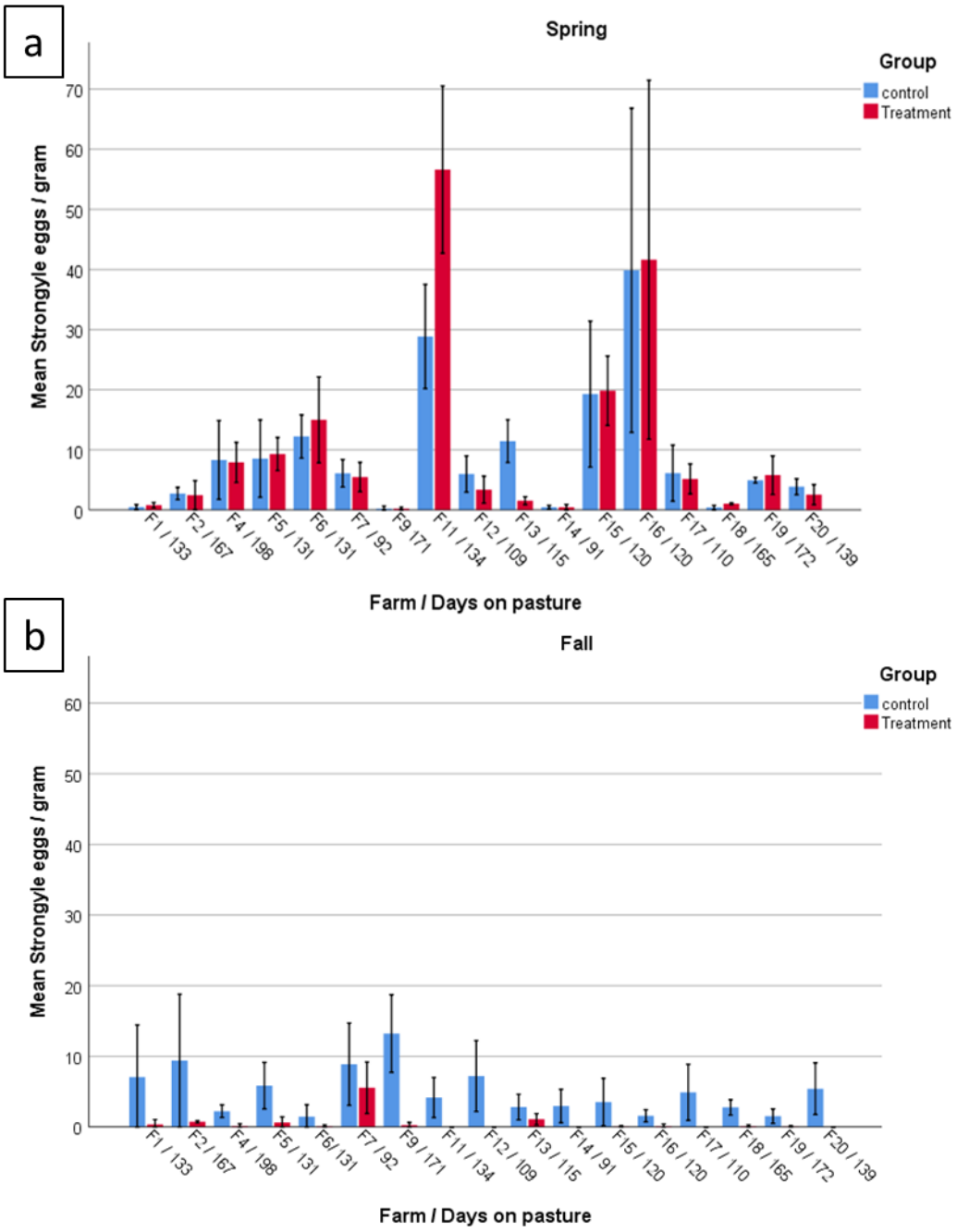

Figure 2.2 a-b. Variation in fecal egg counts (FEC) of strongyle-like eggs taken from each of the 17 cohorts of Saskatchewan's grazing yearling beef cattle in the treated and control group at the two different sampling periods: spring (a) and fall (b). The number of days on pasture for each cohort is noted beside the farm ID. Error bars represent the standard deviation.

2.4.3 Bodyweight and performance parameters

There was no difference in the mean initial BW of the treated and control groups within each cohort ($p > 0.05$) and in the overall analysis ($p = 0.13$). There was, however, a difference in the mean final BW in the overall analysis ($p = 0.002$), where the average weight of all treated cattle (1004 lb) was 10 lb more than the control cattle (976 lb) after calculating the difference between the initial and final BW (Table 2.2) and (Appendix A, Table 3). While there was no difference in the mean ADG between control and treated groups in the overall analysis ($p = 0.41$), differences ($p < 0.05$) in ADG were found in five cohorts (4, 11, 13, 15 and 16) (Figure 2.3) and (Appendix A, Table 3).

Table 2.2 Mean initial and final body weights (lbs) and their comparison between treatment (oral fenbendazole 5 mg/kg plus subcutaneous eprinomectin 1 mg/kg) and control groups in each of the 17 cohorts of Saskatchewan grazing yearling beef cattle. lbs = pounds, S = steer, H = heifer

Farm ID	Treatment Group	Sex	Mean Initial Weight (lbs)	*p*-value	Mean Final Weights (lbs)	*p*-value
1	Control (n = 25)	S	762	0.38	1018	0.10
	Treatment (n = 21)		778		1052	
2	Control (n = 23)	H	730	0.74	881	0.62
	Treatment (n = 23)		720		868	
4	Control (n = 22)	H	825	0.32	1097	0.01
	Treatment (n = 24)		840		1149	
5	Control (n = 25)	H/S	658	0.52	903	0.26
	Treatment (n = 23)		673		931	
6	Control (n = 25)	H	669	0.52	898	0.95
	Treatment (n = 24)		653		900	
7	Control (n = 34)	S	737	0.69	932	0.42
	Treatment (n = 23)		728		915	
9	Control (n = 45)	H	1148	0.44	1196	0.55
	Treatment (n = 49)		1180		1209	
11	Control (n = 24)	S	657	0.97	951	0.02
	Treatment (n = 23)		657		1000	
12	Control (n = 23)	H	676	0.80	824	0.71
	Treatment (n = 23)		680		817	
13	Control (n = 30)	S	746	0.68	973	0.26
	Treatment (n = 25)		737		1001	
14	Control (n = 25)	H	732	0.47	894	0.51
	Treatment (n = 24)		743		903	
15	Control (n = 21)	H	668	0.16	907	0.01
	Treatment (n = 20)		697		975	
16	Control (n = 25)	H	694	0.79	917	0.08
	Treatment (n = 23)		698		956	
17	Control (n = 25)	S	746	0.02	877	0.02
	Treatment (n = 24)		773		909	
18	Control (n = 25)	H	959	0.87	1084	0.09
	Treatment (n = 24)		963		1125	
19	Control (n = 25)	H	878	0.10	1037	0.01
	Treatment (n = 25)		901		1081	
20	Control (n = 24)	H	930	0.24	1031	0.34
	Treatment (n = 23)		957		1049	
Total	Control (n = 446)		794	0.13	976	0.002
	Treatment (n = 421)		812		1004	

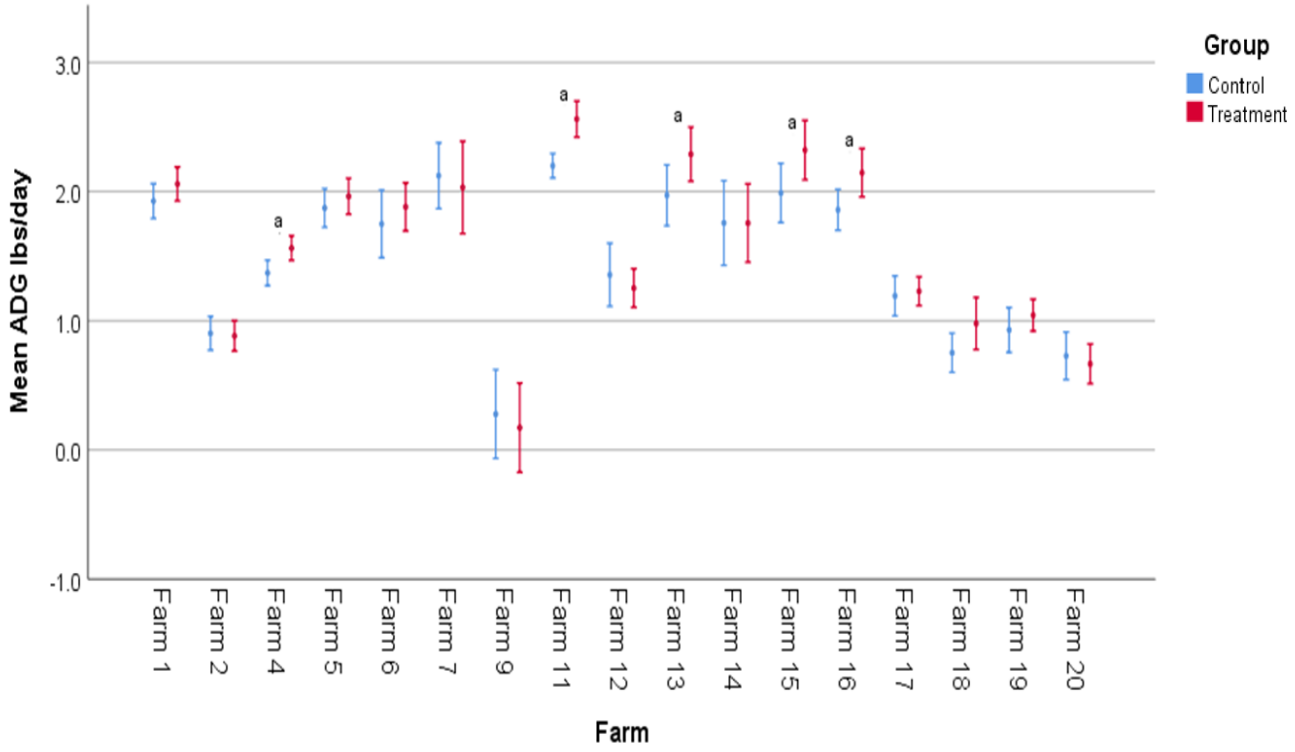

Figure 2.3 Mean and 95% confidence interval (CI) for average daily gain (ADG) in pounds/day of control (n = 443) and treated (n = 421) Saskatchewan grazing yearling beef cattle in 17 cohorts during the 2019 grazing season. [a] – Superscript indicates a significant difference between treatment and control groups within a farm ($p < 0.05$).

2.4.4 Effects of sampling, treatment, environmental factors and soil type on FEC

Temperature (GDD) and cumulative precipitation were calculated from April 1 to September 30. The five cohorts that had a significant difference in ADG were in areas that received between 300 – 350 mm of cumulative precipitation from April to September. Some farms received < 300 mm, especially in the west-central area, while farms in the southeast of the province received > 400 mm (Figure 2.4). Nine of the cohorts were in the chernozemic gray and black soils, seven in the dark brown soil zone, and one in the brown soil zone (Figure 2.5). Due to the low number of cohorts on each soil type, they were categorized into two categories: black/gray and dark brown. The single farm in the brown soil type was combined with the dark brown soil. All five farms with a significant difference in ADG between treated and control cattle were in the black/gray zone.

The final GEE model for the FEC included the sampling period, treatment groups, soil type and cumulative precipitation (Table 2.3). The FEC were 46.1 times higher in the spring than the fall sampling after accounting for all other factors in the model. The total strongyle-like FEC were 3.3 times higher in control *versus* the treated group (Table 2.3). FEC were lower in the dark brown soil than in the black/gray soil ($p = 0.029$). Moreover, the relative increase in the FEC was proportional to the increase in the cumulative precipitation (Table 2.3). Although the grazing season's temperature and length of the grazing season were significant in the unconditional analysis (Appendix A, Table 4), they were left out of the model after the backwards elimination process because there was no evidence of them as confounders in the model.

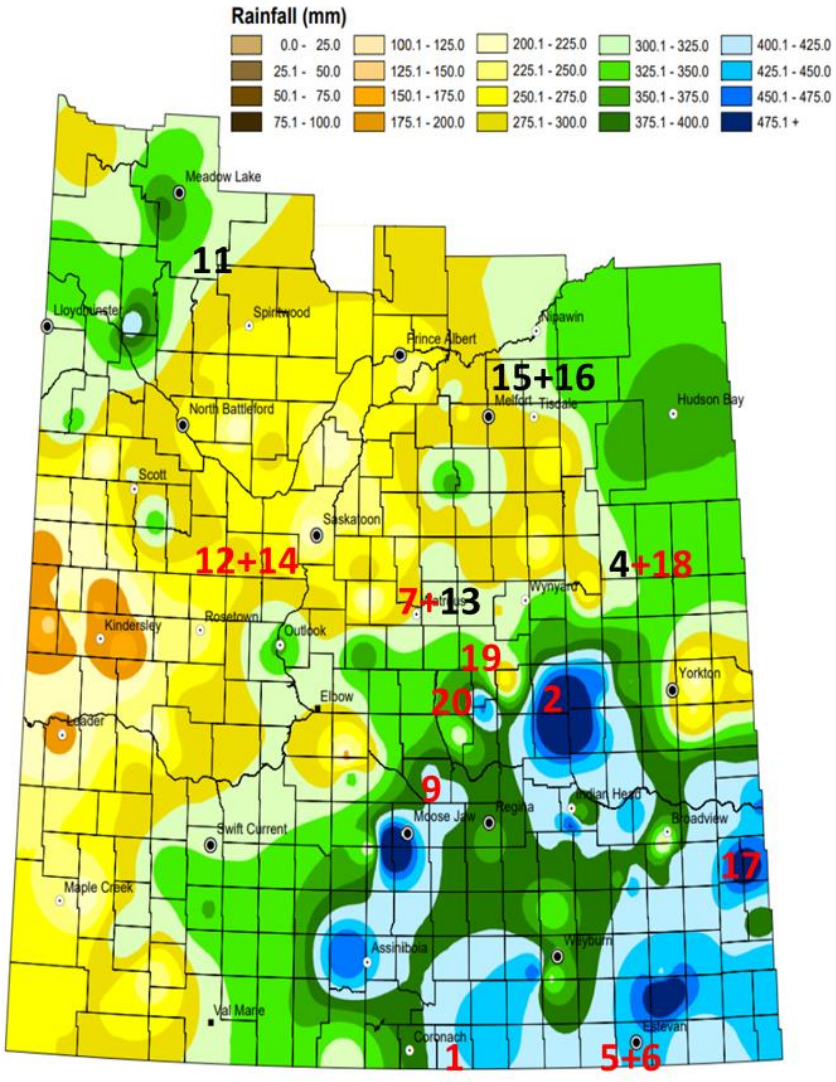

Figure 2.4 Saskatchewan map with the 17 cohorts of grazing yearling beef cattle in relation to cumulative precipitation (mm) from April 1st to September 30th in 2019. The cohorts with a significant difference ($p < 0.05$) in ADG are highlighted in black. Map adapted from © Saskatchewan Agriculture Crop Report 2019, Government of Saskatchewan (154).

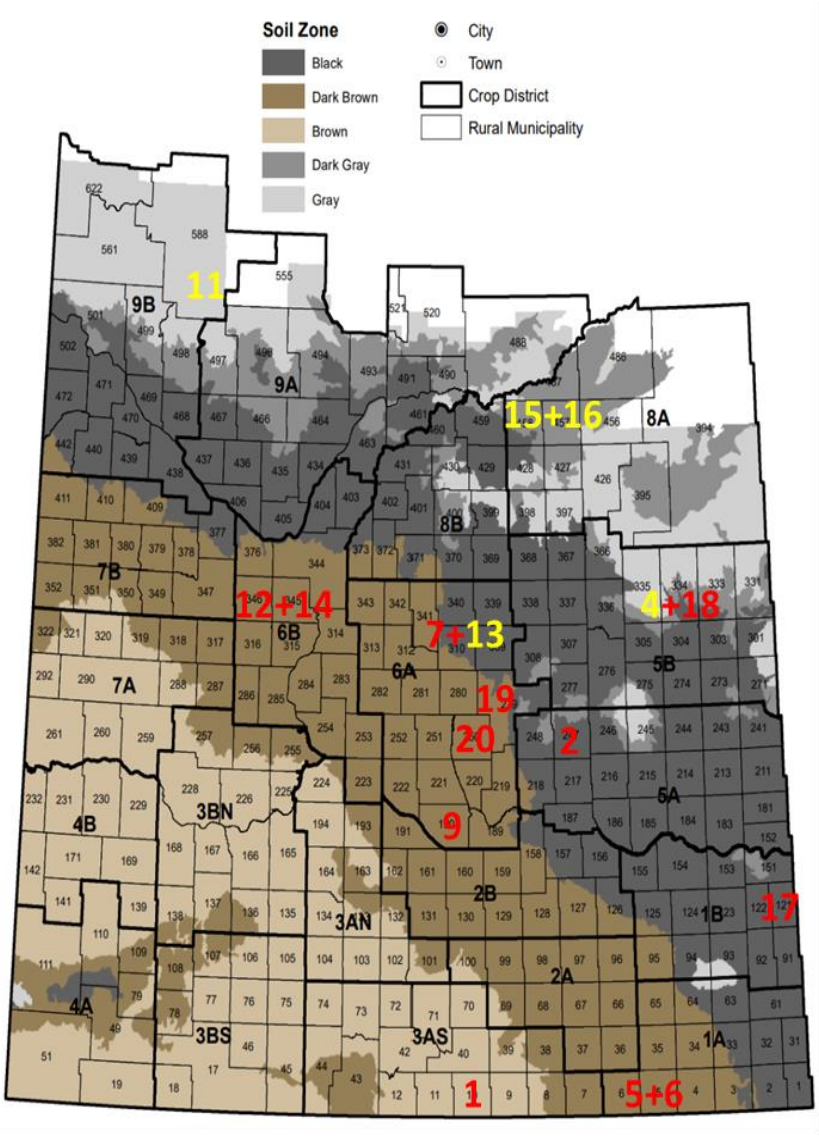

Figure 2.5 Map of Saskatchewan chernozemic soil zones with the location of the 17 cohorts of grazing yearling beef cattle. The cohorts with significant difference in ADG between treated and control cattle ($p < 0.05$) are highlighted in yellow. Map adapted from © Saskatchewan Agriculture Crop Report 2019, Government of Saskatchewan (154).

Table 2.3 Summary GEE model of total strongyle-like fecal egg counts by sampling period, treatment group and soil type after adjusting for precipitation, length of the grazing season, and clustering within management group for each sample collection (n = 34 sample collections from 17 management groups of Saskatchewan grazing yearling beef cattle within the two sampling periods).

Variable	**Relative increase in egg counts**	**Lower 95% CI**	**Upper 95% CI**	***p*- value**
Intercept	0.15	0.034	0.64	0.01
Sample period				
Spring	46.1	14.8	143.8	<0.01
Fall		Reference category		
Treatment Group				
Control	3.3	2.2	4.9	<0.01
Treatment		Reference category		
Soil Type				
Black/Gray	1.85	1.06	3.2	0.029
Dark Brown		Reference category		
Precipitation (mm)				
314 – 440 mm	39.3	8.4	185	<0.01
212 – 313 mm	22.4	4.5	110.6	<0.01
30 – 211 mm	8.3	3.7	18.5	<0.01
4 – 29 mm		Reference category		

2.4.5 Effects of parasitic burdens, treatment, and length of the grazing season on the ADG

The linear mixed model (Table 2.4) for GIN burden and impact, along with the length of the grazing season, found a reduction of 0.08 lb/d in the control animals compared to the treated cattle. Moreover, the model evidenced a 0.01 lb/d gain in ADG for each unit increase in EPG during spring and a 0.01 lb/d reduction for every day on pasture.

Table 2.4 Predicted ADG and 95% confidence interval (CI), measured in pounds per day (lbs/day), derived from a linear mixed model with treatment, spring parasitic burden (EPG) and days on pasture as covariates and farm ID as a random effect in 867 Saskatchewan grazing yearling beef cattle distributed over 17 different cohorts in 2019.

Effect	Estimate	Standard error	95% CI	*F*-value	*p*-value
Intercept	2.91	0.54	1.75; 4.06	28.03	<0.01
Treatment group					
Control	-0.08	0.04	-0.16; -0.004	4.28	0.04
Treatment		Reference category			
Total spring nematodes (EPG)	0.01	0.005	0.002; 0.02	5.96	0.02
Days on Pasture	-0.01	0.004	-0.02; -0.003	7.91	0.01

2.5 Discussion

This is the first comprehensive study to assess the effects of anthelmintics on FEC and production parameters in pastured yearling beef cattle in Saskatchewan since 1987 (152). In that study, Polley and Bickis (1987), reported the prevalence of GIN for cows, yearlings, and calves to be 53%, 44% and 65%, respectively (152). The current study found strongyle-like eggs in almost all the pooled control animal samples during both sampling periods. *Nematodirus* spp. and *Trichuris* spp. were found in 26.5% and 7.3% of the spring control samples, respectively. However, unlike the strongyle-like eggs, the number of positive samples for these two species declined over time. This is similar to what was reported for yearling beef cattle in Saskatchewan

by Jelinski *et al.* in 2017 (48), where the prevalence of strongyle-like, *Nematodirus* spp. and *Trichuris* spp. of was 79.5%, 5.7% and 1.7%, respectively. This is probably due to the nature of these parasites affecting younger animals in most cases, with the older yearling cattle having acquired immunity (14,48).

The higher numbers in this research compared to the 2017 study may be related to several factors. For instance, differences in environmental conditions such as precipitation and temperature are relevant factors in nematode transmission (61). Another reason for this difference may be an overestimation in egg counts because the FEC were performed on pooled samples (> 20 individuals) compared to single animal FEC in the Jelinski *et al.* study in 2017 (48). This comparison is relevant because the shedding of eggs by GIN is not normally distributed, and a few animals (15-20%) may have excreted most of the eggs. Therefore, few animals are responsible for most pasture contamination and infection in cattle groups (29,109,110).

The spring parasitic intensities found in this research were highly variable between cattle groups. The strongyle FEC in the spring sampling ranged from 0 to 63.9 EPG, with a mean of 9.4 EPG for the control animals. FEC were generally considered to be low (<10 EPG) or moderate (10-30 EPG) (104) in control and treated cattle with the exception of groups 11 and 16. A recent Canadian study of beef calves from 50 beef operations found strongyle FEC ranged from 0.5 to 54 EPG (mean = 12.7 EPG) (37). It is generally assumed that GIN intensities in temperate regions are low during the spring and build up during the grazing season (14). Also, in the current study the low FEC could be also associated to the very dry spring that caused the withdraw of 3 cohorts during the study period. However, FEC of > 10 EPG were found in five different control groups in the spring, suggesting the emergence of hypobiotic larvae (29). Only one control group with a mean FEC > 10 EPG was observed in the fall. These differences in parasitic intensities between sampling periods in control groups can be associated with the development of immunity by the end of the grazing season (28,60).

Regarding the treated cattle, there was a > 50% reduction in the prevalence of strongyle-like and *Nematodirus* spp. eggs between sampling periods. Similarly, a reduction in the parasitic

burden was evident for the same nematode species between spring *versus* fall and treated *versus* control animals. These findings are evidence of the appropriate performance of the anthelmintic protocol used in this study along with the immune development of the cattle. This reduction was not evident in farms #7 and #13, which may indicate the development of macrocyclic lactone resistance. This is of concern because macrocyclic lactones are the best drug class for treating inhibited larvae and the benzimidazoles are not available in long-acting formulations.

There was no significant association regarding the ADG between control and treated groups in the crude overall analysis. However, the ADG differed in five farms with FEC in controls with > 10 EPG in spring. Presumably, the higher FEC are indicative of higher GIN burdens, which would suppress performance. Therefore, an anthelmintic used in such circumstances would conceivably result in improved ADG. Moreover, the ADG is a parameter that could have been influenced by several factors like length of the grazing season (-0.01 lbs/d for every day on pasture, $p = 0.01$) and stocking densities that varied widely between farms. Thus, some factors could have adversely affected beef operations during the grazing season. For instance, some farms had over 150 d on pasture, and by that point, in late October and November, the nutritional value of the pastures was likely poor, resulting in suboptimal ADG (75,154). Suboptimal performance in both the treatment and control animals would make it more difficult to find differences in ADG in the under-performing cattle.

Previous research using similar protocols in seven different locations in North America demonstrated the protective effect of extended-release eprinomectin, where treated animals were 43.9 lb heavier than control cattle after grazing for 120 d. However, the contrast should be made carefully since the US study animals had a wider age range compared to the present study (133). In the overall analysis within the current study, the treated animals gained only 10 lb more than the control animals. According to the October 2019 cattle market update from the Government of Saskatchewan, the price in Canadian dollars per hundredweight ($/cwt) in animals > 900 lb was, on average, was $181.17 and $179.2 for feeder steers and heifers, respectively (155). Thus, treated steers and heifers within this study potentially earned an additional $18.1 and $17.9, respectively. These results were lower than those reported in a previous investigation (133), and after deducting the medication costs ($13.1 per animal), the economic benefit was not as great as

expected. However, for the animals within the five farms with significant differences in ADG, the treated cattle gained, on average, 37 lb more than the control cattle. Representing a potential revenue of $53.9 and $53.2 after deducting the study's anthelmintic treatment costs for each steer and heifer, respectively.

The linear mixed model found that treated cattle gained approximately 0.1 lbs/d more than the control cattle, regardless of the farm factor. As expected, the anthelmintic treatment increased ADG, which is consistent with previous studies (138,156–159). A US study comparing extended-release eprinomectin against short-term anthelmintic found that beef animals treated with eprinomectin had increased marbling scores and improved average quality grades; however, there were no significant differences in weight gain or ADG. (160). On the other hand, a recent western Canadian study under similar circumstances, with an elevated statistical power found that animals treated with extended-release eprinomectin had significantly greater final BW and 11% ADG increase compared to topical ivermectin treated cattle. The economic analysis determined that treatment resulted in increased weight gain worth about $7 per head in the extended release eprinomectin group *vs* the topical ivermectin animals. (161). Hence, the recent and current studies evidenced the ability of long acting medications in reducing the detrimental effects of GIN in yearling pastured beef cattle that resulted in economical profit under wester Canadian conditions.

Even though parasitic burdens in Canada are typically considered low, some cohorts had >10 EPG in the FEC, and this can result in subclinical disease affecting the performance of the cattle (104,133). The linear mixed model also found a negative relationship between the ADG and the length of the grazing season, which may be related to extending the grazing season to late October and November when the nutritional value of these pastures was likely very poor.

The final GEE model found an epidemiological pattern of decreasing FEC during the grazing season, which has not been customarily described for cattle in temperate regions (35,162). Also, as expected, there was a 3.3-fold increase in the FEC in the control animals compared with the treated ones. Furthermore, the model pointed out that FEC were 1.8 times higher in the black and gray soil *versus* the dark brown soils. This is slightly different from what

was reported previously (47), where cattle grazing dark brown soils had higher FEC compared with those on brown soils (48). This may be confounded by the fact that black and gray soils are in regions that receive more precipitation and hence also have a higher content of organic material. Black soils might allow for the growth of different plant species that may have provided a better microenvironment with more access to shelter and food for the free larvae (61,70,95). Another explanation is that soil type may have been confounded by different husbandry practices that led to more or less parasitism within different regions of the province. For instance, producers in the southwest may tend to use more extensive grazing because of the poorer soils. Whereas in the northern region, the more fertile soils presumably allow for greater stocking densities. The model also showed a proportional increase in egg counts with cumulative precipitation. This result was expected as it has been reported in many studies that wetter conditions and rain have a positive influence on the fitness of free-living nematode stages (61,80,82,87).

The study had several limitations, including the abnormally dry conditions during the 2019 spring that caused the withdrawal of three cohorts in the middle of the study. The abnormally dry conditions would have also reduced parasite transmission, making it more difficult to find a treatment effect. Another limitation was the difficulty of recruiting producers from the southwest of the province, which is mainly dominated by brown soils. Moreover, the presence of more than one person reading the slides may have introduced some bias that explains the apparent laboratory error on the difference in the FEC of treated and control groups within farm 11 in spring.

In conclusion, the diversity in the FEC and significant differences in production parameters between cohorts might be associated with several factors that deserve future attention. Most of the variations may have been due to environmental conditions, livestock management practices, and GIN species diversity. For this reason, the use of anthelmintics depends on the factors within each farming operation. Even though most farms did not have a significant benefit on ADG with the treatment under the generally dry spring, the performance impact of subclinical parasitism was observed in five cohorts within the province. Therefore, the study results suggest that subclinical parasitism is present, causing economic losses in yearling cattle within Saskatchewan.

Therefore, producers could use FEC as a decision-making tool for anthelmintic treatment during spring processing in Saskatchewan. It may be recommended to collect samples from 20 to 25 animals before the spring processing to perform pooled FEC and consider the delivery of anthelmintic treatments to the groups with mean FEC >10 EPG (104).

Despite its limitations, the study provides current information about the epidemiology and production impact of GIN in yearling beef cattle in western Canada that are relevant for the development of sustainable control programs.

3. FECAL EGG COUNTS AND SPECIES IDENTIFICATION OF GASTROINTESTINAL NEMATODES IN PASTURED YEARLING BEEF CATTLE IN SASKATCHEWAN

3.1 Abstract

Identification and quantification of GIN from yearling beef cattle in Saskatchewan was the main objective of this study. Pooled fecal egg counts (n= 272) from 17 different cohorts of pastured yearling beef cattle were obtained in the spring and fall of 2019 in Saskatchewan. Rectal fecal samples were obtained from cattle administered anthelmintics and from nontreated control cattle. The modified Wisconsin sugar flotation technique was used to quantify fecal egg counts (FEC). Nematodes eggs were hatched to the infective larvae (L3) stage and then underwent deep-amplicon nemabiome sequencing of the internal transcribed spacer-2 rDNA locus. The nemabiome provided the relative quantity and diversity of GIN obtained from cattle over two sampling points. The ITS-2 nemabiome metabarcoding analysis was limited to the control samples since there was not enough L3 for a reliable analysis in the treated group. The analysis reported nine different species within the nematodes diversity in which *Haemonchus placei* was found in all cohorts during spring, and overall accounted for 30% of the species composition. Hence, it was one of the dominant species, together with *Ostertagia ostertagi* (39.9%) and *Cooperia oncophora* (26.1%). In the fall *O. ostertagi* and *C. oncophora* increased to 47% and 42%, respectively, while *H. placei* decreased to 5%. This is the first report describing the presence of *H. placei* within pastured yearling beef cattle in western Canada. It is a concern since the high pathogenicity and prolificity of *H. placei,* and because this nematode is generally associated as a problem in warmer regions in the south.

3.2 Introduction

Although gastrointestinal nematodes (GIN) have been associated with reduced production performance in cattle (150), the epidemiology of GIN in western Canadian pastured yearling beef cattle is lacking. GIN parasitism is a concern in grazing yearling cattle having a naïve immune system due to low parasitic challenge during the previous grazing season (50,51). Moreover,

GIN's epidemiology may be changing in western Canada. Husbandry practices targeting productivity and efficiency have allowed producers to increase cattle stocking densities, which facilitates GIN contamination and transmission (97,98). Suboptimal anthelmintic strategies has probably contributed to anthelmintic resistance (AR) in different GIN species (53,130). Climate change may also have altered the patterns of parasitic intensities and species distribution within Canada (74,77,163). Hence, there is a need for data describing the current state of internal parasitism in western Canadian yearling beef cattle, which is needed for the development of sustainable GIN control strategies.

GIN infections in cattle invariably involve a coinfection with a broad diversity of species, each varying with respect to epidemiology, pathogenicity, and resistance to anthelmintics (6,37,164). Current methods for diagnosing and quantifying parasite loads have low sensitivity and specificity, especially under low parasitic burdens (35,37). Furthermore, they are time-consuming and require experienced personnel to be performed appropriately (165). Thus, the development and use of the ITS-2 nemabiome metabarcoding technique, a novel deep sequencing assay of the ITS-2 rDNA gene that is common to all GIN, provides a robust and quantitatively accurate method to identify the diversity and abundance of parasitic species in fecal samples (128). Recently, the ITS-2 nemabiome metabarcoding method has been used in several bison and cattle studies (35–37).

In North America, GIN are highly prevalent in cattle, while generally causing infections of low to moderate intensity (22,166). *Ostertagia ostertagi* and *Cooperia oncophora* are considered to be the most common species, with *O. ostertagi* being the most pathogenic (39,40). However, *C. oncophora* populations may be increasing due to AR (45,167). In a recent study in Canadian weaned beef calves, these two species were the most prevalent and accounted for 96.7% of the parasite community. However, coinfection with other species (*Cooperia punctata*, *Haemonchus* spp., *Oesophagostomum* spp. and *Trichostrongylus* spp.) was also observed, but in much lower percentages (37). Similarly, a Canadian nemabiome study of pastured replacement dairy heifers found *O. ostertagi* and *C. oncophora* were most prevalent, comprising >50% of the nemabiome in 90% of the samples, with the exception of a few samples in which *Oesohagostomum radiatum* was the dominant species (35). *O. ostertagi* and *C. oncophora* were

also identified as the most dominant species within the nemabiome in an Alberta study of L3 larvae collected from grass surrounding fresh fecal pats (166).

GIN diversity has not been studied in Canadian pastured yearling beef cattle. This paucity of information regarding the epidemiology and infection intensities within this population must be addressed. ITS-2 nemabiome metabarcoding provides an opportunity to use this novel approach to generate species-specific epidemiological data to further our understanding of GIN in yearling beef cattle. The objective of this study was to use a combination of fecal egg counts and nemabiome analysis to describe the species-specific prevalence of GIN in stocker beef cattle in Saskatchewan.

3.3 Materials and Methods

3.3.1 Fecal sampling and fecal egg counts (FEC)

The study design, materials, and methods for how the fecal egg counts (FEC) were obtained have been previously described in Chapter 2. Briefly, the study involved 20 cohorts of yearling Angus and beef-cross cattle distributed across 15 Saskatchewan beef farms. Fecal samples were collected in the spring and fall from 25 heads from each cohort treated with anthelmintics and 25 nontreated control cattle. Fecal samples were individually weighed, and those weighing < 20 g were discarded. For each sample, 20 g of feces were taken and pooled with the respective group (treatment and control). The modified Wisconsin Double-Centrifugation Floatation Technique (104) was used to enumerate the eggs. Due to the similarity in egg morphology of Trichostrogyloidea eggs, it is not possible to distinguish the eggs of *Ostertagia* spp., *Haemonchus* spp., *Oesophagostomum* spp., *Trichostrongylus* spp., and *Cooperia* spp. Therefore, these eggs were all labelled as strongyle-type eggs. However, both *Nematodirus* spp. and *Trichuris* spp. have unique egg morphologies and were enumerated and reported separately.

The University of Saskatchewan's Animal Research Ethics Board approved this study (protocol number: 20190024).

3.3.2 Larval coprocultures

To isolate the 3rd stage larvae (L3) of GIN for molecular species identification, the fecal material from each pool was divided into 60 g samples and then added to culture glasses along with vermiculite (Premier Tech Home & Garden Inc., Brantford, Ontario, Canada). The eggs were incubated for 21 d at room temperature (22-24 °C), with the cultures being checked daily and misted to ensure the presence of adequate moisture in the incubation process. After 21 d, the L3 were collected using the modified Baermann technique (168). Briefly, the culture glasses were filled with water, inverted onto a petri dish, and left for > 12 h. The L3 were collected from the supernatant in the petri dish, quantified under a stereoscope and stored in 2-mL cryotubes with 70% ethanol at 4°C.

3.3.3 ITS-2 nematode species identification

Deep-amplicon sequencing of the ITS-2 nematode ITS-2 rDNA gene locus, as described by Avramenko (128), was used to describe the GIN species composition of the fecal parasite communities, otherwise known as the "nemabiome". The analysis was limited to the control group because an insufficient number of L3 were recovered from the treated coprocultures. To obtain the DNA lysates from each farm, the L3 were added into 0.2 mL tubes containing ten µL of lysis buffer (50 mM KCl, ten mM Tris (pH8.3), 2.5 mM $MgCl_2$, 0.45% Nonidet P-40, 0.45% Tween 20, 0.01% (w/v) gelatin, and Proteinase K 120 µg/mL (Thermo Scientific, Waltham, Massachusetts, USA). After incubation, the tubes were heated to inactivate proteinase K, and the lysates were diluted in molecular-grade water (1:10). Then, the lysates were used to perform the initial PCR amplification of the ITS-2 rDNA locus under the following cycling conditions: 95°C for 3 min, followed by 98°C for 20 s, 62°C for 15 s, 72°C for 15 min for 35 cycles, and the final of 72°C for 2 min. Subsequently, the instructions of the manufacturer were followed to do magnetic bead purification of the PCR products with AMPure XP Magnetic Beads (Beckman Coulter, Brea, California, USA), and agarose gel electrophoresis confirmed the amplification.

A second limited-cycle PCR amplification was performed under the following thermocycling conditions: 98°C for 45 s, followed by 98°C for 20 s, 63°C for 30 s, 72°C for 2 min for seven cycles and a final extension kept at 10°C to combine each amplicon with unique

mixtures of Illumina index tags (Illumina Inc., San Diego, California, USA). Next-generation sequencing was performed on an Illumina MiSeq Desktop Sequencer with the 2×250 Reagent kit (Illumina Inc., San Diego, California, USA). Using the Mothur bioinformatic tool version 1.36.1 (169), each sequence read was designated to the nematode species identity with the same methodology as described by Avramenko (37). Further details of the analysis methods can be found at www.nemabiome.ca.

3.3.4 Data analysis

The sample metadata were entered into a commercial spreadsheet program (Excel 365; Microsoft, Redmond, Washington, USA) and then analyzed with the statistical software IBM SPSS Statistics for Windows (version 25, IBM Corp, Armonk, New York, USA). Summaries of descriptive statistics, which included the arithmetic mean fecal egg count of strongyle-type eggs (EPG; ± standard deviation (SD), range and median), were performed for the spring and fall samplings. The Mann-Whitney U test was used to compare the pooled FEC between treated and control animals. Wilcoxon Signed-Rank test was applied to compare the spring and fall pooled FEC within each treatment group and to assess differences in the species proportion between each nematode species during the two sampling periods in the control group. All levels of statistical significance were $p < 0.05$.

3.4 Results

3.4.1 Prevalence and infection intensity in Saskatchewan's yearling beef cattle

The results of the fecal collection and FEC have been previously described in Chapter 2. Briefly, three of the 20 cohorts of cattle enrolled in the study were lost to follow-up sampling FEC for Strongyle-type eggs, *Trichuris* pp. and *Nematodirus* spp. were performed for every treatment group during the two sampling periods for the 17 remaining cattle cohorts, which were owned by 13 different cattle producers. A total of 272 FEC were completed: 136 each in spring an fall, with 68 in each treatment/control group. Strongyle-like eggs were detected in most samples (98.5% (67/68) and 96% (66/68) for FEC from pooled samples from treated and control

cattle, respectively) during the spring, while *Nematodirus* and *Trichuris* eggs were less abundant, as presented in Table 2.1 in Chapter 2.

The FEC between individual farms varied widely (Appendix B Table 1). It was more evident in the spring sampling, where the strongyle-like FEC range was (0.0; 63.9 EPG) and (0.0;65.9 EPG) for the control and treated groups respectively (Table 3.1).

Table 3.1 Overall arithmetic mean, standard deviation, range and median FEC for strongyle-like, *Nematodirus* spp. and *Trichuris* spp. eggs from Saskatchewan grazing yearling beef cattle during each sampling for treatment and control groups in 2019 (n = 272 FEC total, 68 FEC in each sampling period by treatment).

	Sampling/Treatment	**Mean EPG**	**SD + (Range)**	**Median EPG**
Strongyle-like	Spring / Control	9.40	11.4 (0.0; 63.9)	5.46
	Spring / Treatment	10.53	16.0 (0.0; 65.9)	4.65
	Fall / Control	5.00	3.96 (0.6; 18.0)	3.50
	Fall / Treatment	0.54	1.40 (0.0; 8.22)	0.00
***Nematodirus* spp.**	Spring / Control	0.09	0.19 (0.0; 1.0)	0.00
	Spring / Treatment	0.09	0.24 (0.0; 1.2)	0.00
	Fall / Control	0.04	0.12 (0.0; 0.8)	0.00
	Fall / Treatment	0.00	na	0.00
***Trichuris* spp.**	Spring / Control	0.02	0.07 (0.0; 0.4)	0.00
	Spring / Treatment	0.02	0.06 (0.0; 0.2)	0.00
	Fall / Control	0.01	0.06 (0.0; 0.4)	0.00
	Fall / Treatment	0.003	0.02 (0.0; 0.2)	0.00

There were no differences in FEC for strongyle-like ($p = 0.59$), *Nematodirus* spp. ($p = 0.48$) and *Trichuris* spp. ($p = 0.78$) between the treatment and control cattle at the start of the study. Meanwhile, in the fall, there was a reduction in FEC between control and treatment animals for strongyle-like ($p < 0.01$) and *Nematodirus* spp. eggs ($p < 0.01$). Moreover in the

spring *vs* fall comparison both control ($p = 0.03$) and treated ($p < 0.01$) groups had lower FEC for strongyle-like eggs. (Table 3.2).

Table 3.2 Comparisons of medians of FEC between spring and fall samplings in each treatment/control group and among control and treated Saskatchewan grazing yearling beef cattle during each sampling period (n = 272 FEC total, 68 FEC in each sampling period by treatment). * = Statistical significance $p < 0.05$

Comparison	**Spring *vs* Fall**				**Control *vs* Treatment**			
	Treatment		**Control**		**Spring**		**Fall**	
Nematodes	***Z*-value**	***p***	***Z*-value**	***p***	***Z*-value**	***p***	***Z*-value**	***p***
Strongyle-like	-6.71	<0.01*	-2.15	0.03*	-0.53	0.59	-9.27	<0.01*
***Nematodirus* spp.**	-3.29	<0.01*	-1.70	0.09	-0.69	0.48	-3.27	<0.01*
***Trichuris* spp.**	-1.86	0.06	-2.96	0.77	-0.27	0.78	-1.36	0.17

3.4.2 Relative abundance of gastrointestinal nematodes species in commercial yearling beef cattle in Saskatchewan

The L3 nemabiome data from control animals obtained during the two sampling periods included nine trichostrongylid species Figures 3.1 and 3.2. Overall, *O. ostertagi*, *H. placei* and *C. oncophora* accounted for 40.0, 29.7% and 26.2% of the species, respectively, in the spring. Moreover, these three species were present in all sampled yearling groups. The less abundant trichostrongylid species that were not identified in all cattle groups in the spring included *O. radiatum* (2.43%), *C. punctata* (1.68%), *T. axei* (0.002%) and two unknown *Trichostrongylus* spp. (< 0.001%).

A similar trend was observed in the species composition in the fall sampling, with *O. ostertagi* and *C. oncophora* again representing the greatest proportion of the nemabiome. An increase in the overall species proportions compared to the spring for *Cooperia oncophora* (42.19%) and *Ostertagia ostertagi* (47.05%) was observed, but it was only significant for *C.*

oncophora ($p = 0.01$). These two species were found in all cohorts and together accounted for almost 90% of the species composition. The opposite was true for *H. placei*, which decreased to a proportion of 5.01% in the fall and was not present in all cohorts ($p < 0.01$). The remaining species were again at low proportions and rarely differed significantly from the values obtained during the spring sampling, as presented in Table 3.3. *O. radiatum* tended to increase ($p = 0.08$) from 2.43% in the spring to 3.60% in the fall. A similar trend was observed for two *Trichostrogylus* spp. that were found at low levels in different cattle groups and only accounted for maximum of 1% of the nematodes diversity.

Table 3.3 Mean and 95% CI of relative species abundance of gastrointestinal nematode species determined by ITS-2 nemabiome metabarcoding, in sampling-level pools (spring and fall) of L3 harvested from pooled coprocultures (n= 20 to 25 animals per pool) of 17 cohorts of Saskatchewan grazing yearling beef cattle with the results of the comparison between the two sampling periods by nematode specie.

Nematodes Species	**Spring species proportion % and 95% CI**	**Fall species proportion % and 95% CI**	***Z*-value**	***p*-value**
Ostertagia ostertagi	39.96 (31.56; 48.37)	47.05 (40.67; 53.44)	-1.21	0.22
Cooperia oncophora	26.16 (19.05; 33.28)	42.19 (33.40; 50.99)	-2.44	0.01
Haemonchus placei	29.70 (18.49; 40.91)	5.16 (2.37; 7.94)	-3.52	<0.01
Oesophagostomum radiatum	2.43 (0.43; 4.45)	3.60 (0.45; 6.76)	-1.77	0.08
Cooperia punctata	1.69 (0.37; 3.01)	1.94 (0.13; 3.75)	-0.40	0.69
Haemonchus contortus	0.01 (-0.0; 0.03)	0.02 (-0.01; 0.05)	-0.63	0.53
Trichostrongylus axei	0.02 (-0.01; 0.05)	0.001 (-0.00; 0.00)	-1.07	0.28
Trichostrongylus unknown 1	0.0006 (-0,00; 0.00)	0.10 (-0.00; 0.20)	-1.99	0.05
Trichostrongylus unknown 2	0.0002 (-0.00; 0.00)	0.06 (-0.01; 0.14)	-2.02	0.04

The parasite communities varied by cohort. For instance, the proportions of *H. placei* differed widely in spring, ranging from 1.44% in group 5 to 94.16% in group 9. Similarly, *O. radiatum* was absent on several farms while it comprised a substantial proportion (21%) in group 11, which

also had a considerable proportion of *C. punctata* during the fall (14.55%), as observed in (Figure 3.2).

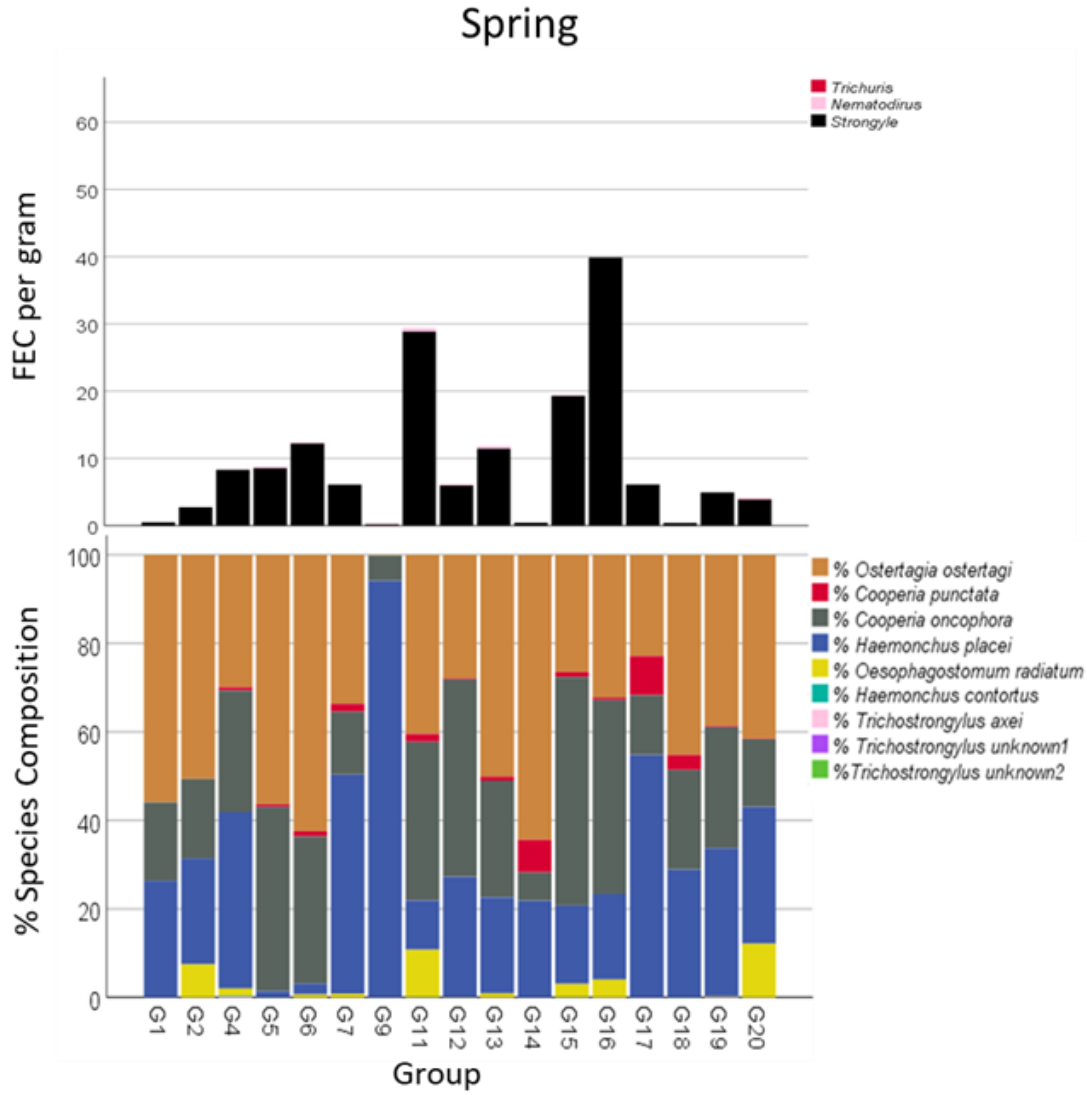

Figure 3.1 Relative species abundance of gastrointestinal nematode species determined by ITS-2 nemabiome metabarcoding, in cohort-level pools (n= 20 to 25 animals per pool) of L3 harvested from pooled coprocultures (n= 6 to 8 coprocultures per cohort) of 17 cohorts of Saskatchewan grazing yearling beef cattle in spring of 2019. The arithmetic mean of FEC of strongyle-type, *Nematodirus* spp., and *Trichuris* spp. are presented on top of each cohort with their respective stacked bar chart for percentage species composition. G = cohort

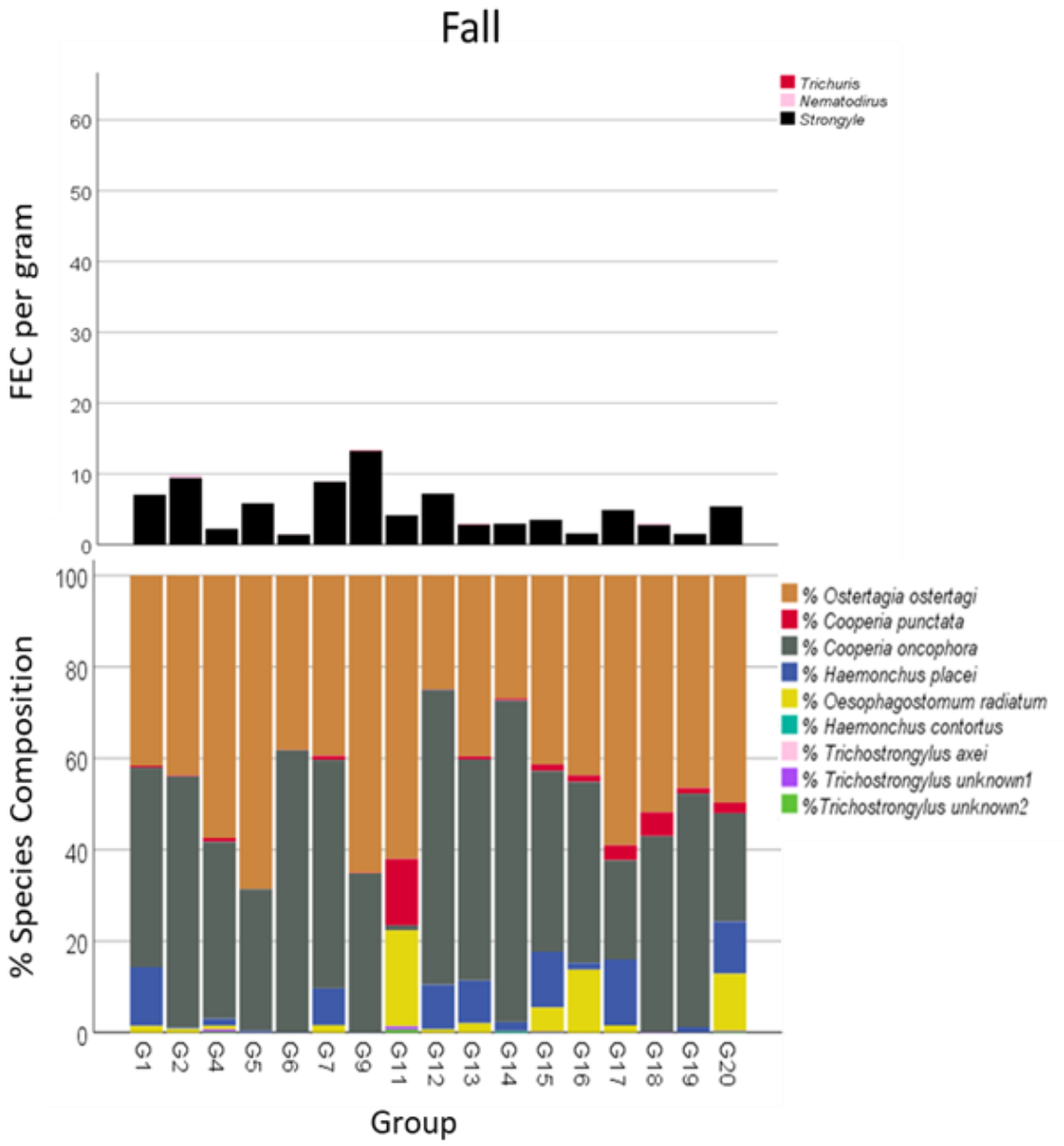

Figure 3.2 Relative species abundance of gastrointestinal nematode species determined by ITS-2 nemabiome metabarcoding, in cohort-level pools (n= 20 to 25 animals per pool) of L3 harvested from pooled coprocultures (n= 6 to 8 coprocultures per cohort) of 17 cohorts of Saskatchewan grazing yearling beef cattle during the fall of 2019. The arithmetic mean of FEC of strongyle-type, *Nematodirus* spp., and *Trichuris* spp. are presented on top of each cohort with their respective stacked bar chart for percentage species composition. G = cohort

3.5 Discussion

The dynamics of GIN species in yearling pastured western Canadian beef cattle using a combination of FEC, and deep-amplicon nemabiome sequencing assay of the ITS-2rDNA locus has not been previously reported; this approach has been used in other GIN cattle research (128). In Canada, ITS-2 nemabiome metabarcoding profiles were generated from L3 collected from replacement heifers (35), beef calves (37), bison (36) and grass surrounding fecal pads (166). However, interpretation of the nemabiome can be problematic if samples have very low FEC intensities (treated cattle) since a subsequently low number of L3 in coprocultures will lead to insufficient material for DNA extraction. For that reason, we limited our analysis to the control samples. Another caveat is that the interpretation of the results must simultaneously consider the FEC because nematodes differ in fecundity and pathogenicity (35). For instance, a FEC of 10 EPG with *O. ostertagi* counting for most of the species diversity would be more worrisome than the same FEC but dominated by *C. oncophora* because *O. ostertagi* has a greater pathogenicity and produce less eggs than *C. oncophora* (38,41,42).

GIN infections in cattle are characterized by the presence of several species that coinfect the host (40). Avramenko *et al* (37) reported lower species diversity within Canadian beef cattle compared with southern regions like the United States and Brazil. Furthermore, they described *O. ostertagi* and *C. oncophora* as the dominant species, with 59.1% and 37.6% of the species composition, respectively, within 50 beef operations in Canada. Similar dominance of species composition was also observed in a Canadian study of dairy replacement heifers where these two species accounted for > 50% of the nematode diversity in more than 90% of the farms (35). The current study found a similar breakdown in diversity, with *O. ostertagi* (39.96%) and *C. oncophora* (26.16%) being the main species in spring fecal samples. But unexpectedly and probably the most relevant finding in this study, *H. placei* was observed as one of the dominant species in the spring, accounting for almost 30% of the species diversity and present in all 17 groups of cattle. Recent research using a similar approach reported *H. placei* as the third most abundant specie in commercial bison herds across western Canada. In Saskatchewan, Alberta, Manitoba, and British Columbia, *H. placei* represented 16.6%, 9.2%, 17% and 0.03% of the

nematode species diversity. However, it was not detected in all bison groups involved in the study (36).

Finding *H. placei* in substantial amounts is concerning. This highly pathogenic GIN is a very prolific blood feeder and has been reported to be resistant to avermectin and moxidectin (37,142). Even though this nematode is usually associated with warmer temperatures located in the south (8,37,170), the high prevalence of *H. placei* might be explained by more suitable environmental conditions caused by climate change. It might have allowed the survival and infection of this parasite within Canada, as per increases of *C. punctata* in the cattle populations in the US (22). However, the dominance and distribution of *H. placei* decreased to 5.16% in the fall sampling, whereas *C. oncophora* and *O. ostertagi* increased to 42.19% and 47.05%, respectively. Moreover, the dominance of *O. ostertagi* and the reduction of *H. placei* could be associated with cattle's slow immune response against *O. ostertagi*. It can take up to two years to develop immunity while it is comparatively faster for other nematodes such as *Cooperia* spp. and *Haemonchus* spp. (51). This slow rise in immunity is significant because *O. ostertagi* is described as the most pathogenic species of cattle in temperate regions (171,172). The fact that *O. ostertagi* is the most dominant nematode within the species composition, regardless of its low fecundity compared to other nematodes, is a sign that a substantial number of mature worms are infecting the animals (173).

The frequency of *C. oncophora*, especially in the fall, is a significant finding due to AR reported worldwide for this nematode (44). The same applies to *C. punctata*, which has been associated with AR (7,22) and was an important contributor to the species composition of some of the cohorts in the current study, specifically, group nine, where *C. punctata* accounted for >10% of the species diversity in the fall. Among other species identified in the study, *O. radiatum* was also detected. It accounted for 2.5% and 3.8% of the nematode diversity in spring and fall. This percentage is greater than the previous results in Canada and deserves further attention because *O. radiatum* has been reported to be more common in the US and Brazil, and hence this may be a sign of a change in the dynamics of this parasite (37).

This study found strongyle-like eggs in 96% of the spring FEC of the control cattle. However, *Nematodirus* spp. and *Trichuris* spp. were found in lower proportions, 26.5% and 7.3%, respectively in the same period. In the fall sampling, the proportion of samples infected with strongyle-like eggs increased to 100%. The proportions were comparable to the reported by a yearling beef cattle study in 2017 within the province. In which the prevalence for strongyle-like eggs, *Nematodirus* spp. and *Trichuris* spp. were 79.5%, 5.7% and 1.7%, respectively (48). The nematode FEC intensities in the control cattle within the current study were very variable among the beef operations, ranging between 0 to 63.9 EPG, and a mean 9.4 EPG. This is similar to the range (0.5 to 54 EPG) and mean (12.7 EPG) reported in another recent Canadian beef cattle study (37). Furthermore, most cohorts had low FEC (<10 EPG) to moderate (10-30 EPG) in spring which is similar to what has been commonly reported for northern temperate climates (104).

Nevertheless, caution must be used in interpreting FEC due to the poor correlation between the number of eggs detected and the adult worms within the gastrointestinal tract (8,16,35,107,108). Pooled samples might not account for individual animals that shed many nematode eggs, which are the primary source of pasture contamination (29). Experimental designs should include between 20 and 25 samples within the pools to ensure at least one sample comes from a high FEC individual (47,111). On the other hand, pooled FEC can include several of these "super shedders" that may inflate the actual level of shedding over a larger group of animals (29). Thus, pooled FEC must be interpreted with caution to avoid overestimations.

Some of the limitations of this study include sampling in a year characterized by a very dry spring (153). As a result, three cohorts of cattle were sold before the fall fecal collection period. The dry weather invariably would have decreased the intensity of the GIN infections. Secondly, treated animals had very low FEC in the fall, and hence it was challenging to obtain enough L3 to perform reliable deep-amplicon sequencing assays. As a suggestion for future investigations aiming to observe the parasitic dynamics within treated animals, it would be recommended to collect larger fecal samples to generate more coprocultures and, ergo, a greater amount of L3.

Despite the limitations, this is the first study to describe the nematode dynamics in yearling pastured beef animals in Saskatchewan using a combination of deep-amplicon sequencing essays and FEC. It allowed us to determine that *O. ostertagi*, *C. oncophora* and *H. plac*ei are the most dominant species within this population at risk. The presence of high levels of *H. placei* in the nematode's diversity is problematic. It should be the baseline for further investigations aiming to improve the understanding of *H. placei* dynamics within the yearling beef cattle. Moreover, the parasite's intensities were overall considered low, ranging from 9.40 to 5.46 EPG in spring and fall, respectively. The data is valuable since it helps to close the informational gap about the epidemiology of nematodes in pastured yearling animals and will contribute to developing more evidence-based control strategies within western Canada.

4. GENERAL SUMMARY & CONCLUSIONS

This research aimed to update the information on the epidemiology of GIN in yearling beef cattle on pasture, enhancing the understanding of the environmental factors involved in nematode transmission along with their prevalence, FEC intensities, effect on production performance and species diversity within the province. These objectives were approached by two different studies performed and presented in this manuscript in chapters 2 and 3. Chapter 2 focused on describing the parasitic challenge of GIN in yearling beef cattle during the grazing season of 2019 in Saskatchewan. Moreover, chapter 2 aimed to improve the understanding of some environmental factors related to the FEC within cattle in various geographic areas and the impact that these nematodes have on Saskatchewan grazing yearling beef cattle. The main objective of chapter 3 was to describe the GIN species affecting yearling beef animals across Saskatchewan.

The rationale for performing this study was to improve and update the knowledge of the epidemiology of GIN in pastured yearling beef cattle in Saskatchewan. Young animals, such as yearlings, are more susceptible to GIN infections due to their immature immunological system (50,51,150). There is a paucity of data relating to the epidemiology of GIN in pastured beef cattle in western Canada, with many of the initial studies having been conducted decades ago (152). This is an issue because several factors involved in GIN distribution, infection intensities and transmission might have changed over the years. Some factors that might have changed include climatic conditions, species distribution, anthelmintic resistance, and husbandry practices adopted by the producers. Therefore, it is essential to generate contemporary data that allows producers and animal health professionals to develop and implement measures against parasitic infections.

Climate is particularly important since precipitation and temperature directly affect the free-living stages of the nematodes (61,166). For instance, precipitation has been proven to increase the survival rate and mobility of the L3 on the pastures (80). Also, climate change, particularly global warming, is changing species distribution patterns, making less frequently observed nematodes now being identified in Canadian cattle production (14). Over time, some producers aiming to increase the profitability of their operations have adopted different strategies,

like improved pastures to increase the stocking densities (97,98). These practices may increase the parasitic challenge on the fields because more animals are grazing in the same area. In some cases, if an adequate rotation is not performed, the cattle may graze closer to fecal pads which facilitates the ingestion of infective stages (61).

4.1 Discussion

The current study helped to update the epidemiology and species distribution information for pastured yearling animals in SK. The overall prevalence for strongyle-like egg, *Nematodirus* spp. and *Trichuris* spp. was 96%, 26.5% and 7.3%, respectively. These results are higher than a previous study that found a prevalence of 79.5% for strongyle-like eggs, 5.7% for *Nematodirus* spp. and 1.7% for *Trichuris* spp. (48). As expected in both studies, the prevalence of *Nematodirus* spp. and *Trichuris* spp. was lower since these parasites are associated with younger animals (14,48). Moreover, the methodology of performing the FEC might explain the difference between the two studies. This study used pooled samples to perform the FEC, while the previous one performed individual FEC for each animal. At least 20 animals were used to perform the pooled FEC, which improves the chance of obtaining at least one "super shedder" since few animals within a herd are responsible for most of the contamination. However, it might be possible that several of these highly contaminated animals were taken into the pooling, which might have caused an overestimation in the number of infected samples and the reported FEC intensities (29,110,111).

The nematode intensities were considered from low (<10 EPG) to moderate (10-30 EPG) in most cohorts and varied widely among them. The mean FEC was 9.4 and 10.5 EPG during the spring for control and treated animals, respectively. At the end of the grazing season, the FEC decreased for both groups with a clear difference in the mean FEC, with control cattle having a mean of 5 EPG while the treated animals had a mean of 0.5 EPG. This finding proves that the anthelmintic protocol used in this study is helpful as a control measure against the GIN in Saskatchewan. The GEE model evidenced that FEC were 3.3 times higher in control cattle compared to treated animals, likely because of the anthelmintic use. The model also determined a 1.8 increase in FEC from samples in black and gray soils compared to dark and brown soil while

controlling for other factors. This can be explained since black and gray soils are located in regions that received more precipitation and have different plant coverture that might enhance the microclimatic conditions for nematode survival (70,95). It can also be associated with varying beef cattle husbandry practices associated with the two kinds of soils. Poorer soils in the southwest are used more commonly for extensive grazing, while the better pastures in the north are grazed more intensively and hence might have increased pasture contamination with GIN larvae. Finally, as expected, the model confirmed that precipitation is an essential factor for parasite transmission within Saskatchewan since FEC increased accordingly with the amount of rainfall, and it has been previously reported in several studies(61,80,82).

However, even with the evidence of reducing the parasitic burdens using the anthelmintic protocol, there was no overall difference ($p = 0.41$) in ADG. In fact, it was only significantly demonstrated in five cohorts, all of which were cattle groups that had > 10 EPG at the beginning of the grazing season. Parasite FEC intensities of > 10 EPG can cause subclinical disease and, ergo, economic detriment (104). Therefore, the use of this protocol in such conditions can provide better performance in the animals. But the absence of a significant difference in the ADG between treated and control animals in the overall analysis can not only be associated with the low parasitic burdens on the other cohorts. The ADG can also be affected by several different factors, as shown in the linear mixed model in Chapter 2. The model demonstrated that treated animals gained 0.1 lbs/d more than control cattle and that the longer grazing seasons negatively impacted the ADG (-0.1 lbs/d). Stocking densities could have also had an important role in this aspect but were not assessed in this study.

The beginning of the grazing season during this study period was abnormally dry compared with the 30 y average for SK, but was compensated with a very wet fall (Figure 4.1). This may explain why many producers extended the time on pasture, in some cases until November. The very long grazing seasons might have compromised the performance of the animals since the nutritional value of those pastures late in the grazing season might have been suboptimal, decreasing the ADG in all groups, making it harder to assess differences between treatment groups. Moreover, the drought likely caused the animals to graze closer to the fecal pads they would generally avoid in searching for food. A longer time on pasture could have also

changed the animals' immunity, which might explain why the nematode intensities also decreased in the control animals by the end of the grazing season.

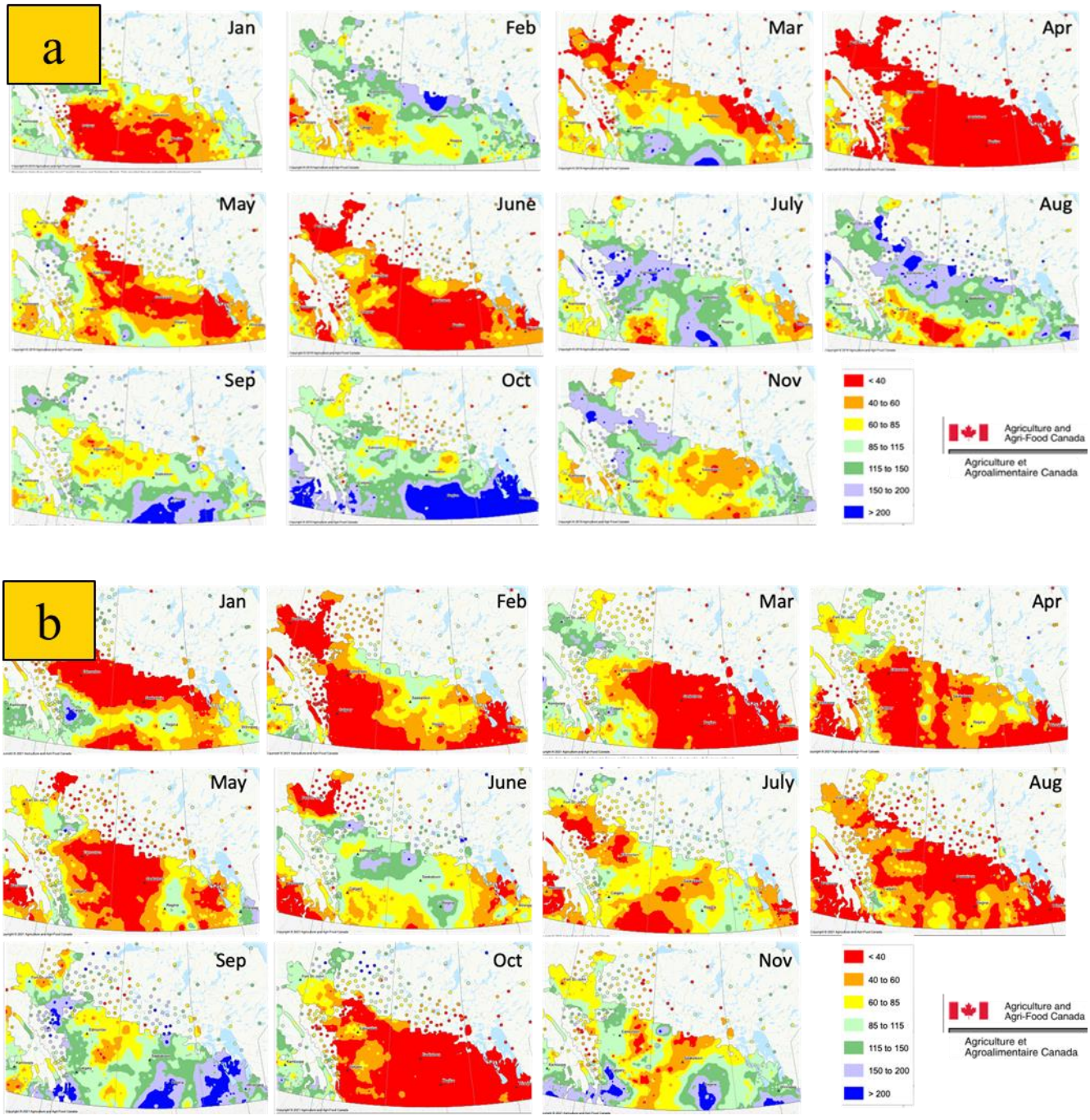

Figure 4.1 Comparison of Saskatchewan precipitation maps (mm/month) for each month during 2019 (a) and for the 30-year average (b) Agriculture and Agrifood Canada 2019 (175).

Since GIN infections have several species that coinfect the host (40), chapter three focused on identifying the species diversity of nematodes in grazing yearling beef cattle across the province. A next generation deep-sequencing nemabiome assay was used for this purpose. This method was developed recently by Avramenko *et al*. in 2015 and has been used satisfactorily in studies involving cattle in Canada, the USA and Brazil (37,128). Up to the date of this manuscript, this technique has not been used in pastured yearling beef cattle, representing an opportunity to assess its use in identifying the species profile within this population. The information provides insight into the species causing detrimental effects within this specific population and improves our understanding of GIN dynamics.

Previous studies determined *Ostertagia ostertagi* and *Cooperia oncophora* were the predominant species accounting for 59.1 % and 37% of the species in Canadian beef cattle (37). As expected, in spring control cattle, these two nematodes were described as the predominant species, with 39.9% for *O. ostertagi* and 26.1% for *C. oncophora*. However, *Haemounchus placei* accounted for the second most dominant species, with almost 30% of the species diversity and was present in all 17 cohorts. This was likely the most unexpected finding within this chapter since *H. placei* is a parasite mostly reported in warmer regions in the south. Furthermore, although it was previously reported in some bison herds within the country, *H. placei* has not been detected in such a large proportion before in Canadian beef cattle (36).

The unexpected presence of *H. placei* could potentially be problematic since this parasite is highly pathogenic and very prolific. Also, this species is characterized as an avid blood feeder, and resistance to moxidectin and avermectin has been reported (37,142). The presence of *H. placei* may have had changes in its distribution and dynamics as a consequence of climate change that might have favoured the environmental condition for establishment in a new area (77,166). Fortunately, its dominance decreased by the end of the grazing season, only accounting for 5.1% of the nemabiome profile of control cattle. This reduction may have been likely associated with the host developing immunity, which is relatively fast for both *Haemonchus* spp. and *Cooperia* spp (51).

In contract, the immune response can take up to two years for *O. ostertagi* and might be why the percentage of this species increased in the control cattle to 47% within the nemabiome profile in the fall (51). *O. ostertagi* is the most pathogenic species reported for temperate regions, and even though it was expected, it was found in a high proportion within the species diversity. This can be translated to high amounts of mature worms within the animals because this nematode is not very prolific (172,173). *C. oncophora* also increased its dominance during the fall and is probably associated with the development of anthelmintic resistance reported by this species worldwide (44).

Some of the limitations within this thesis included the abnormally low precipitation during the spring period. Which caused three cohorts to withdraw from the study due to poor pasture conditions. There were also difficulties recruiting producers from the southwest of the province, so the study did not have a representation in the GEE model of farms on brown soils. Moreover, there was limited or no reliable information about the stocking densities and the historical data from the herds, so this valuable information was not considered within this study. Moreover, FEC must also be examined cautiously since a poor correlation between the number of eggs and adult worms inside the animals has been reported (8,35,108) and the pooled FEC may mislead the interpretation if high shedding animals are present or not (29). Similarly, the nemabiome results must be interpreted carefully along with the FEC since the nematodes within a determined species profile differ in their prolificity and pathogenicity (6,37). Furthermore, the treated group samples did not produce enough larvae to perform a consistent ITS-2 nemabiome metabarcoding, so it was not possible to properly compare the species diversity among treatment groups. Finally, there is always the chance of human mistakes when reading the FEC slides, and this increases when more than one person performs them.

4.2 Future Research

Currently, there is no information regarding the anthelmintic resistance issue for pastured yearling beef cattle in Canada. This is a subject that deserves future attention since anthelmintic resistance has been described widely for most of the dominant species found in this research. Even though this was not the main objective of this manuscript, there was some evidence that

anthelmintic resistance might be present in some cohorts. This represents a significant threat to adequate performance and profitability within beef operations in western Canada. It would also be very valuable to know the nematode species profile within the nemabiome for animals that have received treatment to have a better idea of which species are more likely associated with anthelmintic resistance in Canadian beef cattle.

Further investigations should also determine the role of the different husbandry practices, like stocking densities, in the parasite dynamics within the province. This would be of great interest to complete the picture for GIN epidemiology and might help to elucidate if husbandry practices confound the findings about soil type. A similar study should also be performed in a more regular grazing season since the results in this thesis might be different due to the abnormal precipitation conditions. As described, the parasite's dynamics and impacts depend on multiple factors in which the climatic conditions play a protagonist role. Hence, it would be of great value for producers and animal health practitioners to fully understand what happens within a regular grazing season to develop the proper control strategies.

4.3 Conclusions

This research helps to improve the understanding of GIN epidemiology and its impact on pastured yearling beef cattle. Even though the impact of nematodes and their burdens varied widely among cohorts and were generally low, the selected anthelmintic protocol effectively reduced the egg counts and, in some cases, significantly improved the ADG, in those cohorts where the FEC were > 10 EPG. The variation in results in this thesis were a consequence of several risk factors. The parasite burden differed according to geographical and climatic regions. It was also observed that the anthelmintic treatment seemed to have a greater effect in geographic areas with higher rainfall resulting in significant differences in ADG between treated and control animals in those cohorts.

A take-home message for producers, assessing the climate forecast and performing pooled FEC of 25 animals before the animals go to pasture in spring would be recommended. If the predictions show increased precipitation and the FEC are > 10 EPG, they should consider applying this anthelmintic protocol as a control measure. By looking at the historical precipitation

map, the treatment of the animals would be advisable for operations in the north and southeast of the province. On the other hand, operations with FEC < 10 EPG and lower levels of precipitation should avoid the indiscriminate use of anthelmintic medications since it might hasten the development of anthelmintic resistance within their operations. Moreover, the use of medications when not needed is an extra expense that can affect the profitability of producers.

Finally, *Haemonchus placei* is a potential threat to the Canadian beef industry and deserves further attention. This is the first time such a parasite has been described as one of the most predominant species within the species diversity of nematodes for beef cattle in Canada. The description of this finding and the other species involved in GIN infection in pastured yearling beef cattle will be the baseline for many further studies that will try to understand the dynamics of these species in Canada to develop the proper sustainable roundworm control strategies.

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APPENDIX A - CHAPTER 2: RAW DATA TABLES

Table 1. Description of farm location, sampling period, treatments, gender (H = heifer and S = steer), and initial and final body weight for 17 cohorts of Saskatchewan grazing yearling beef cattle.

Farm ID	Location	Study Period	Treatment/Number animals per treatment	Sex	Mean Initial Weight (lbs)	*p*-value	Mean Final Weights (lbs)	*p*-value
1	Big Beaver	April 30 – Sep 10	Control (n= 25)	S	762	0.38	1018	0.10
			Treatment (n= 21)		778		1052	
2	Kelliher	May 7 – Oct 21	Control (n= 23)	H	730	0.74	881	0.62
			Treatment (n= 23)		720		868	
4	Preeceville	May 14 – Nov 28	Control (n= 22)	H	825	0.32	1097	0.01
			Treatment (n= 24)		840		1149	
5	Estevan	May 13 – Sep 21	Control (n= 25)	H/S	658	0.52	903	0.26
			Treatment (n= 23)		673		931	
6	Estevan	May 13 - Sep 21	Control (n= 25)	H	669	0.52	898	0.95
			Treatment (n= 24)		653		900	
7	Lanigan	May 14 – Aug 14	Control (n= 34)	S	737	0.69	932	0.42
			Treatment (n= 23)		728		915	
9	Bethune	May 17 – Nov 4	Control (n= 45)	H	1148	0.44	1196	0.55
			Treatment (n= 49)		1180		1209	
11	Big River	May 20 – Oct 1	Control (n= 24)	S	657	0.97	951	0.02
			Treatment (n= 23)		657		1000	
12	Delisle	May 27 – Sep 13	Control (n= 23)	H	676	0.80	824	0.71
			Treatment (n= 23)		680		817	
13	Lanigan	May 27 – Sep 19	Control (n= 30)	S	746	0.68	973	0.26
			Treatment (n= 25)		737		1001	
14	Delisle	May 28 – Aug 27	Control (n= 25)	H	732	0.47	894	0.51

			Treatment (n= 24)		743		903	
15	Tisdale	Jun 5 – Oct 3	Control (n= 21)	H	668	0.16	907	0.01
			Treatment (n= 20)		697		975	
16	Tisdale	Jun 5 – Oct 3	Control (n= 25)	H	694	0.79	917	0.08
			Treatment (n= 23)		698		956	
17	Moosomin	Jun 5 – Sep 23	Control (n= 25)	S	746	0.02	877	0.02
			Treatment (n= 24)		773		909	
18	Preeceville	7 Jun – Nov 19	Control (n= 25)	H	959	0.87	1084	0.09
			Treatment (n= 24)		963		1125	
19	Raymore	Jun 8 – Nov 27	Control (n= 25)	H	878	0.10	1037	0.01
			Treatment (n= 25)		901		1081	
20	Duval	Jun 19 – Nov 5	Control (n= 24)	H	930	0.24	1031	0.34
			Treatment (n= 23)		957		1049	
	TOTAL	April 30 – Nov 28	Control (n= 446)		794	0.13	976	0.002
			Treatment (n= 421)		812		1004	

Treatments received Fenbendazole - Safe-guard® (5mg/kg/PO); and Eprinomectin - ERI LongRange®(1mg/Kg/SC) during the first sampling – Steer (S); Heifer (H)

Table 2. Description Strongyle-like FEC in each sampling within the respective treatment group by farm and its associated statistical analysis**.**

Farm ID	Location	Study Period	Mean FEC		Median FEC		Z statistic / *p*-value Wilcoxon test Spring vs Fall FEC in each Tx group	*p*-value MWU test C vs Tx FEC in Fall sampling
			Control	Treatment	Control	Treatment		
1	Big Beaver	Spring Fall	.496 (n=23) 7.07 (n=22)	.796 (n=22) .348 (n=20)	.49 6.44	.89 .19	-1.82 / 0.07 -1.60 / 0.10	0.02*
2	Kelliher	Spring Fall	2.74 (n=16) 9.40 (n=22)	2.46 (n=21) .744 (n=22)	2.71 7.74	1.87 .79	-1.82 / 0.07 -1.82 / 0.07	0.02*
4	Preeceville	Spring Fall	8.31 (n=21) 2.24 (n=21)	7.91 (n=22) .148 (n=21)	9.06 2.28	7.74 .09	-1.82 / 0.07 -1.82 / 0.07	0.02*
5	Estevan	Spring Fall	8.55 (n=25) 5.85 (n=25)	9.30 (n=23) .642 (n=24)	8.48 6.75	9.76 .69	-7.30 / 0.46 -1.82 / 0.07	0.02*
6	Estevan	Spring Fall	12.2 (n=23) 1.44 (n=18)	14.9 (n=24) .099 (n=22)	12.34 1.09	14.5 .09	-1.82 / 0.07 -1.82 / 0.07	0.02*
7	Lanigan	Spring Fall	6.10 (n=20) 8.88 (n=25)	5.47 (n=22) 5.55 (n=22)	6.30 7.43	5.63 5.58	-1.46 / 0.14 -0.36 / 0.71	0.2
9	Bethune	Spring Fall	.197 (n=22) 13.2 (n=25)	.200 (n=20) .247 (n=25)	.10 12.34	.20 .19	-1.82 / 0.07 0.00 / 1.00	0.02*
11	Big River	Spring Fall	28.8 (n=22) 4.20 (n=3)	56.6 (n=24) 0.65 (n=6)	27.71 3.82	55.84 .00	-1.82 / 0.07 -1.82 / 0.07	0.01*
12	Delisle	Spring Fall	5.98 (n=21) 7.21 (n=20)	3.38 (n=23) 0.00 (n=20)	5.92 7.91	3.36 .00	-0.73 / 0.46 -1.82 / 0.07	0.01*
13	Lanigan	Spring Fall	11.4 (n=25) 2.82 (n=25)	1.53 (n=24) 1.09 (n=22)	11.94 2.67	1.48 1.19	-1.82 / 0.07 -1.46 / 0.14	0.02*

14	Delisle	Spring Fall	.447 (n=25) 2.96 (n=23)	.448 (n=25) 0.00 (n=24)	.49 2.39	.39 .00	-1.82 / 0.07 -1.82 / 0.07	0.01*
15	Tisdale	Spring Fall	19.2 (n=24) 3.54 (n=19)	19.8 (n=22) 0.49 (n=17)	20.21 3.10	18.59 .00	-1.82 / 0.07 -1.82 / 0.07	0.02*
16	Tisdale	Spring Fall	39.8 (n=22) 1.58 (n=22)	41.6 (n=23) .099 (n=21)	34.4 1.78	46.84 .00	-1.82 / 0.07 -1.82 / 0.07	0.02*
17	Moosomin	Spring Fall	6.12 (n=23) 4.90 (n=23)	5.16 (n=24) 0.00 (n=22)	7.36 4.06	5.56 .00	-0.36 / 0.71 -1.82 / 0.07	0.01*
18	Preeceville	Spring Fall	.396 (n=20) 2.78 (n=22)	1.04 (n=21) .099 (n=19)	.39 2.77	.99 .09	-1.82 / 0.07 -1.82 / 0.07	0.02*
19	Raymore	Spring Fall	4.95 (n=24) 1.53 (n=24)	5.79 (n=24) .049 (n=24)	4.85 1.48	5.93 .00	-1.82 / 0.07 -1.82 / 0.07	0.02*
20	Duval	Spring Fall	3.87 (n=20) 5.41 (n=24)	2.55 (n=25) 0.00 (n=23)	3.75 4.99	2.65 .00	-0.73 / 0.46 -1.82 / 0.07	0.01*
	TOTAL	Spring Fall	9.40 5.00	10.5 0.54	5.46 3.50	4.65 0.00	-2.15 / 0.03 -6.70/ 0.00	<0.001*

Table 3. Description of Farms, treatments with the differences in body weight, the length of the grazing season and the average daily gain in each group.

Farm ID	Location	Treatment/Number animals per treatment	Δ BW (lbs)	Length of grazing season (d)	Mean ADG (lbs)	Mean difference C vs T (95% CI)	Mean difference for ADG
1	Big Beaver	Control (n =25)	256	133	1.92	-0.132	0.15
		Treatment (n=21)	274		2.06	(-0317, 0.052)	
2	Kelliher	Control (n=23)	151	167	0.90	0.020	0.81
		Treatment (n=23)	147		0.88	(-0.150, 0.191)	
4	Preeceville	Control (n=22)	271	198	1.37	-0.191	0.01
		Treatment (n=24)	309		1.56	(-0.324, -0.059)	
5	Estevan	Control (n=25)	245	131	1.87	-0.090	0.37
		Treatment (n=23)	257		1.96	(-0.290, 0.109)	
6	Estevan	Control (n=25)	229	131	1.75	-0.132	0.40
		Treatment (n=24)	246		1.88	(-0.447, 0.182)	
7	Lanigan	Control (n=34)	195	92	2.12	0.090	0.66
		Treatment (n=23)	187		2.03	(-0.326, 0.507)	
9	Bethune	Control (n=45)	47	171	1.14	0.105	0.66
		Treatment (n=49)	29		1.20	(-0.376, 0.587)	
11	Big River	Control (n=24)	295	134	2.19	-0.361	<0.001
		Treatment (n=23)	343		2.56	(-0.524, -0.196)	
12	Delisle	Control (n=23)	148	109	1.35	0.102	0.46
		Treatment (n=23)	137		1.25	(-0.177, 0.382)	

13	Lanigan	Control (n=30)	227	115	1.97	-0.318	0.05
		Treatment (n=25)	263		2.28	(-0.632, -0.004)	
14	Delisle	Control (n=25)	160	91	1.75	0.0005	0.99
		Treatment (n=24)	160		1.75	(-0.436, 0.435)	
15	Tisdale	Control (n=21)	239	120	1.98	-0.331	0.04
		Treatment (n=20)	278		2.32	(-0.646, -0.175)	
16	Tisdale	Control (n=25)	223	120	1.86	-0.287	0.02
		Treatment (n=23)	257		2.14	(-0.524, - 0.498)	
17	Moosomin	Control (n=25)	131	110	1.19	-0.036	0.70
		Treatment (n=24)	135		1.22	(-0.222, 0.149)	
18	Preeceville	Control (n=25)	124	165	0.75	-0.226	0.07
		Treatment (n=24)	161		0.98	(-0.47, 0.017)	
19	Raymore	Control (n=25)	160	172	0.93	-0.113	0.27
		Treatment (n=25)	179		1.04	(-0.32, 0.093)	
20	Duval	Control (n=24)	101	139	0.73	0.062	0.59
		Treatment (n=23)	93		0.67	(-0.171, 0.296)	
	TOTAL	Control (n=446)	182		1.43	-0.049	0.41
		Treatment (n=421)	192		1.48	(-0.16, 0.068)	

ADG, Average Daily Gain - Δ BW, Difference in body weight (Final weight – Initial weight)

Table 4. List of different variables assessed in the unconditional analysis for the GEE model with their corresponding *p* value and 95% C.I.; Walt test was used for the categorical variables only and the table also contain the values of QIC (Quasi likelihood under independence model criterion) and QICC (Corrected quasi likelihood under independence model criterion) to check the goodness of fit of the variables in the unconditional analysis.

Variable	Unconditional association	p-value	95%CI	p- value Walt test	QIC QICC
Treatment Treatment = base	0.33	0.046	0.005, 0.651	0.002	701.3 671.9
Sampling Fall = baseline	1.3	0.002	0.5, 2.1	0.027	602.2 573.5
Tapril-may	-0.01	0.004	-0.017, -0.003		640.3 620.9
Tapril-june	-0.007	0.004	-0.013 , -0.002		630.8 611.7
Tapril-july	-0.005	0.012	-0.01 , -0.001		633.1 611.1
Tapril-aug	- 0.004	0.039	-0.007 , 0.0		651.7 625.3
Tarpil-sept	-0.003	0.029	-0.006, 0.0		644.1 618.8

Tjune-sep	-0.004	0.071	-0.009 , 0.00		652.4 621.7
Soil type Brown= baseline	1.4 (Black/Gray) 0.6 (Dark Brown	<0.01 <0.01	0.82, 1.9 0.25, 0.95	<0.01	338.7 331.3
Papril-may	0.023	<0.001	0.015 , 0.03		610.8 596.5
Papril-june	0.01	0.132	-0.003 , 0.02		702.2 662.0
Parpil-july	0.01	0.002	0.004, 0.016		637.7 617.1
Parpril-aug	0.008	0.004	0.003 , 0.014		637.7 615.2
Parpil- sept	0.004	0.041	0.0 , 0.007		689.1 659.4
Pjune-sept	0.001	0.55	-0.002 , 0.004		707.8 676.2
Soil type 2 categories D Brown/Brown = baseline	0.82 (Black/Gray)	0.012	0.17 ,1.5	0.050	659.1 634
Soil type no farm 1 Dark Brown = baseline	0.76 (Black/Gray)	0.021	0.16 , 1.4	0.064	626.1 601.7
Length Grazing Season	-0.007	0.08	-0.016, 0.001		699 667
Length Grazing Season R	0.56 (91-115d) 1.089 (116-131d)	0.79 <0.001	-0.35, 0.5 0.56 , 1.6	0.07	680 627

166-198 d= baseline	0.59 (132-165d)	0.32	-0.6, 18		
Temperature 2 intervals (Aug)	-0.001	0.002	-0.002, 0.00		597 569
Temperature 2 intervals (Aug) Categorical baseline = 4	1.2 (1) -0.48(2) -0.55(3)	0.018 0.26 0.21		<0.001	571 521
Precipitation 2 intervals (Aug)	-0.006	0.006	-0.01 , -0.002		669 628
Precipitation 2 intervals Categorical (Aug) baseline = 4	0.58 (1) 1.9 (2) 0.58 (3)	0.15 <0.001 0.15	-0.2, 1.4(1) 0.9 , 3 (2) -0.2 , 1.4 (3)	0.001	568 534
Temperature 2 intervals (Sep)	-0.001	0.002	-0.001, 0.000		598 571
Temperature 2 intervals (Sep) Categorical Baseline=4	1.6 (1) 0.25(2) -0.11(3)	0.003 0.6 0.8	0.5, 2.7(1) -0.9 , 1.4(2) -0.9 , 0.7(3)	0.002	592 540
Precipitation 2 intervals (Sep)	-0.004	0.01	-0.007 , -0.001		656.6 618.5
Precipitation 2 intervals Categorical (Sep) baseline = 4	-0.22 (1) 1.4(2) -0.8(3)	0.56 <0.001 0.11	-0.9, 0.5 (1) 0.7 , 2 (2) -1.8, 0.2 (3)	<.001	597 550

APPENDIX B - CHAPTER 3: RAW DATA TABLES

Table 1. Description arithmetic mean and media for strongyle-like FEC from 17 cohorts of Saskatchewan grazing yearling beef cattle in each sampling period within the respective treatment and their associated statistical analysis**.**

Farm ID	Location	Study Period	Mean FEC		Median FEC		Z statistic / *p*-value Wilcoxon test Spring vs Fall FEC in each Tx group	*p*-value MWU test C vs Tx FEC in Fall sampling
			Control	Treatment	Control	Treatment		
1	Big Beaver	Spring	.496 (n=23)	.796 (n=22)	.49	.89	-1.82 / 0.07	0.02*
		Fall	7.07 (n=22)	.348 (n=20)	6.44	.19	-1.60 / 0.10	
2	Kelliher	Spring	2.74 (n=16)	2.46 (n=21)	2.71	1.87	-1.82 / 0.07	0.02*
		Fall	9.40 (n=22)	.744 (n=22)	7.74	.79	-1.82 / 0.07	
4	Preeceville	Spring	8.31 (n=21)	7.91 (n=22)	9.06	7.74	-1.82 / 0.07	0.02*
		Fall	2.24 (n=21)	.148 (n=21)	2.28	.09	-1.82 / 0.07	
5	Estevan	Spring	8.55 (n=25)	9.30 (n=23)	8.48	9.76	-7.30 / 0.46	0.02*
		Fall	5.85 (n=25)	.642 (n=24)	6.75	.69	-1.82 / 0.07	
6	Estevan	Spring	12.2 (n=23)	14.9 (n=24)	12.34	14.5	-1.82 / 0.07	0.02*

		Fall	1.44 (n=18)	.099 (n=22)	1.09	.09	-1.82 / 0.07	
7	Lanigan	Spring	6.10 (n=20)	5.47 (n=22)	6.30	5.63	-1.46 / 0.14	0.2
		Fall	8.88 (n=25)	5.55 (n=22)	7.43	5.58	-0.36 / 0.71	
9	Bethune	Spring	.197 (n=22)	.200 (n=20)	.10	.20	-1.82 / 0.07	0.02*
		Fall	13.2 (n=25)	.247 (n=25)	12.34	.19	0.00 / 1.00	
11	Big River	Spring	28.8 (n=22)	56.6 (n=24)	27.71	55.84	-1.82 / 0.07	0.01*
		Fall	4.20 (n=3)	0.65 (n=6)	3.82	.00	-1.82 / 0.07	
12	Delisle	Spring	5.98 (n=21)	3.38 (n=23)	5.92	3.36	-0.73 / 0.46	0.01*
		Fall	7.21 (n=20)	0.00 (n=20)	7.91	.00	-1.82 / 0.07	
13	Lanigan	Spring	11.4 (n=25)	1.53 (n=24)	11.94	1.48	-1.82 / 0.07	0.02*
		Fall	2.82 (n=25)	1.09 (n=22)	2.67	1.19	-1.46 / 0.14	
14	Delisle	Spring	.447 (n=25)	.448 (n=25)	.49	.39	-1.82 / 0.07	0.01*
		Fall	2.96 (n=23)	0.00 (n=24)	2.39	.00	-1.82 / 0.07	
15	Tisdale	Spring	19.2 (n=24)	19.8 (n=22)	20.21	18.59	-1.82 / 0.07	0.02*
		Fall	3.54 (n=19)	0.49 (n=17)	3.10	.00	-1.82 / 0.07	
16	Tisdale	Spring	39.8 (n=22)	41.6 (n=23)	34.4	46.84	-1.82 / 0.07	0.02*
		Fall	1.58 (n=22)	.099 (n=21)	1.78	.00	-1.82 / 0.07	
17	Moosomin	Spring	6.12 (n=23)	5.16 (n=24)	7.36	5.56	-0.36 / 0.71	0.01*
		Fall	4.90 (n=23)	0.00 (n=22)	4.06	.00	-1.82 / 0.07	
18	Preeceville	Spring	.396 (n=20)	1.04 (n=21)	.39	.99	-1.82 / 0.07	0.02*

		Fall	2.78 (n=22)	.099 (n=19)	2.77	.09	-1.82 / 0.07	
19	Raymore	Spring	4.95 (n=24)	5.79 (n=24)	4.85	5.93	-1.82 / 0.07	0.02*
		Fall	1.53 (n=24)	.049 (n=24)	1.48	.00	-1.82 / 0.07	
20	Duval	Spring	3.87 (n=20)	2.55 (n=25)	3.75	2.65	-0.73 / 0.46	0.01*
		Fall	5.41 (n=24)	0.00 (n=23)	4.99	.00	-1.82 / 0.07	
	TOTAL	Spring	9.40	10.5	5.46	4.65	-2.15 / 0.03	<0.001*
		Fall	5.00	1.36	3.50	0.00	-6.70/ 0.00	