SURVIVAL-TIME FOR POST-WINTER THIRD-STAGE JUVENILE TRICHOSTRONGYLES OF CATTLE IN THE U.S.A. NORTHERN GREAT PLAINS

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ABSTRACT

Throughout the U.S.A. Northern Great Plains, cattle are infected with trichostrongyle nematodes, particularly *Ostertagia ostertagi* and *Cooperia oncophora*. When populations are high, these nematodes cause production losses such as reduced weaning weights in calves. Cattle acquire trichostrongyles by ingesting their third-stage juveniles (J3s) located on pasture vegetation. *Ostertagia ostertagi* and *C. oncophora* overwinter as hypobiotic tissue-dwelling fourth-stage juveniles (J4s) in cattle tissue, or as hypobiotic J3s in the soil of pastures. In most cases, pasture-dwelling J3s are thought to be the more important mechanism for winter survival. To successfully complete their life-cycle, these post-winter J3s must survive long enough during the spring to be ingested by susceptible cattle. Spring survival times for J3s are limited by the presence of a juvenile sheath which prevents the enclosed juvenile from ingesting any new nutrients, and infectivity gradually decreases as the stored nutrients become depleted. Modern strategic control programs are based upon protecting cattle from infectious J3s at times when the J3s are most vulnerable as they overwinter in cold winter climates. Knowing how long post-winter J3s survive on pastures can help cattle producers optimize the efficacy of their trichostrongyle control efforts. This study measured the infectivity-decline of J3s during the early grazing season under natural conditions on an eastern South Dakota pasture. The study was conducted over two consecutive years in a pasture that was divided into 12 (2001) or 15 (2002) paddocks. To measure post-winter J3 infectivity, we used uninfected tracer-calves that were grazed for two weeks in groups of five on the paddocks that had been purposely contaminated (trichostrongyle-infected cattle were grazed from August to October in the paddocks) the previous fall. The two-week grazing periods were staggered such that three groups were assessed for viability over the two-week blocks from May 31 to July 27 (2001) or May 13 to July 22 (2002). After grazing, calves were moved to a concrete-floored drylot until the juvenile trichostrongyles had matured to egg-laying adults (21 days for 2001 and 30 days for 2002). Fecal samples were then collected from the drylotted calves for three

consecutive days, and the number of trichostrongyle egg were counted as a way of estimating the relative number of adult worms. Necropsy and coproculture of one drylotted calf from each grazing period showed that *O. ostertagi* and *C. oncophora* were the only trichostrongyles present. Fecal egg counts (FECs) from all groups during both years showed a very significant decline in pasture infectivity for the overwintered juveniles as the grazing season progressed. By the second half of June, infectivity had decreased by 69% for both years, and by the second half of July, there were virtually no infective J3s left on the paddocks. Overall for both years combined, FEC averaged 55.9% of the prior period (a 44% decline each 14-day period). These results suggest that the survival half-life averages 15 days in this climate. Therefore, nematode control programs designed to protect cattle until the last couple of weeks in June should significantly impact trichostrongyle population levels in the herd.

Keyword

Cattle, *Cooperia*, hypobiosis, Nematoda, Northern Great Plains, *Ostertagia*, South Dakota, Trichostrongyles, winter juvenile survival

INTRODUCTION

The United States Northern Great Plains contains extensive grasslands used for beef cattle production and extends from western Minnesota through the Dakotas into eastern Montana and Wyoming. In this region, internal parasitism by trichostrongyle nematodes is largely subclinical, yet does produce production losses, especially in young grazing cattle (Wohlgemuth et al. 1990; Stromberg et al. 1997; Epperson et al. 2001; Mertz et al. 2005). Surveys of cattle parasites from this area have shown that *Ostertagia ostertagi* and *Cooperia oncophora* are the most common species of trichostrongyle, though species of *Trichostrongylus*, *Nematodirus* and *Haemonchus* have also been reported (Stromberg et al. 1991; Malczewski et al. 1996; Harmon et al. 2009; Avramenko et al. 2017).

Cattle acquire trichostrongyle infections by ingesting third-stage juveniles (J3) located on pasture vegetation. The intensity of trichostrongyle infections in grazing cattle is largely determined by the number of J3s available in a pasture. Overwinter survival of *Ostertagia* spp. and *Cooperia* spp. J3s has been observed in previous studies located in cold climates (Smith and Archibald 1969; Smith 1972; Taylor et al. 1973; Slocombe 1974; Gibbs 1979; Stromberg et al. 1991). Most trichostrongyle species infecting cattle can also overwinter as tissue-dwelling fourth-stage juveniles (J4), but Gibbs (1979) found that at least in the ocean-moderated, humid continental climate of Maine, over-winter survival of juveniles in pastures was more important than juvenile survival in tissues. With proper use, most modern cattle anthelmintics can kill tissue-dwelling J4s, and so winter survival of trichostrongyles, especially in fall-treated cattle, appears to be largely dependent on the survivability of J3s within the pasture (Gibbs 1979). These J3 juveniles migrate into the soil as temperatures cool during the fall season, undergo hypobiosis during the winter and reemerge back up onto vegetation

as temperatures warm the following spring. To successfully complete their life-cycle, these post-winter J3s (PW-J3s) must survive long enough during the follow spring to be ingested by susceptible cattle.

A juvenile sheath from the remnant J2 cuticle completely encloses the J3 stage and is thought to aid in the survivability of the juveniles during adverse environmental conditions; however, this sheath also prevents the enclosed juvenile from ingesting any nutrients, and therefore limits the survival-time of the PW-J3s, especially as increasing temperatures increase the metabolic rates of the juveniles (Stromberg 1997). Therefore, the infectivity of PW-J3s gradually decreases during the spring and summer months as the stored nutrients become depleted. Most modern strategic control programs are based upon protecting cattle from infectious J3s at times when they are most vulnerable. These vulnerable times vary by climate, but in regions with cold winters, focus is generally on PW-J3s (Rew and Vercruysse 2002). Most macrocyclic lactone anthelmintics provide persistent activity ranging from about 20 to 120 days (Vercruysse and Rew 2002; Kunkle et al. 2013). Therefore, knowing how long PW-J3s survive on pastures can help cattle producers optimize the efficacy of their trichostrongyle control efforts.

In the moderate cold climate of New Brunswick, Canada, the infectivity of PW-J3s was measured with tracer calves during four 19-day-periods (June 22-July 10, July 24-Aug. 11, Aug. 21-Sept. 8 and Sept. 18-Oct. 6) in 1967 (Smith and Archibald 1969). Very large numbers of *O. ostertagi* and *C. oncophora* worms were recovered from calves grazing during the first time-period, but worm numbers decreased by 88% and 60% respectively in calves grazed from July 24 to August 11. By the third time period, juvenile infectivity had decreased by 99% for *O. ostertagi* and 96% for *C. oncophora*. Calves grazed in the final period (Sept. 18-Oct. 6) contained very few *O. ostertagi* worms (almost a 100% reduction) and few *C. oncophora* worms (99% reduction). The following year, a similar study was conducted for the first and last grazing periods, and these results were consistent with those of the preceding year. A similar tracer-calf study was also conducted in Maine, U.S.A. (similar region to the preceding studies) about ten years later that started assaying pasture infectivity earlier (June 1), and included monthly time-periods (of two weeks) through the summer and fall (Gibbs 1980). During the first two weeks in June, both tracer calves were heavily infected with *C. oncophora* and moderately infected with *O. ostertagia*, but by the first two weeks of July, mean worm numbers had decreased by 92.5% and 99.6% respectively. In August, worm numbers for both species had decreased by about another 50% compared to July. By September, no worms from either species were found in the two tracer calves. While the Gibbs (1980) results are consistent with the earlier Canadian study (Smith 1972), their sample schedule allowed them to demonstrate that the decreasing survivability of PW-J3s is occurring in the early part of summer.

There have been no similar attempts to measure the decreasing infectivity rates of PW-J3s in cattle within the colder and drier pastures of the U.S.A. Northern Great Plains. The objective of this study was to measure this infectivity-decline during the early grazing season under natural conditions on an eastern South Dakota pasture. This study was conducted over two consecutive years (2001

and 2002), and the study design was based upon the tracer-calf study by Gibbs (1980).

Previous tracer-calf studies have determined the number of juveniles infecting calves by waiting 20-30 days, and then counting the number of adult worms and fourth-stage inhibited juveniles by necropsying each animal (Smith and Archibald 1969; Smith 1972; Gibbs 1979; Gibbs 1980). In these previous studies, the inhibited juveniles constituted a minor portion of the total population in the tracer calves during the spring and summer, and so it is possible to access the relative number of adult worms by counting the number of eggs being excreted from the calves at uniform times. Fecal egg counts (FECs) do not always closely correlate with the number of adults present in each animal when comparing trichostrongyle population densities of different species in cattle herds with varying ages, however, it is a reliable approach to comparing densities in young immunologically naïve calves infected with the same species of trichostrongyles, especially when FECs are measured for at least three separate days (Gasbarre et al. 1996). Therefore, FECs were used to estimate adult worm populations in the present study, and only three calves were necropsied for the purpose of confirming the identity of trichostrongyle species present.

METHODS

***Pasture and Paddocks*—**A single 2.17 ha pasture was divided into 12 (Year 1) or 15 (Year 2) paddocks of approximately 0.18 ha and 0.12 ha, respectively. Paddocks generally contained cool season grasses, and forage production on these paddocks was of modest quantity. Paddocks at one end of the 2.17 ha pasture tended to be slightly wetter, featuring soil with a slightly spongy consistency, while paddocks at the opposite end tended to be drier with firmer soil. Because of this inconsistency, paddocks were subjectively blocked into 3 groups (A [wet], B [intermediate], and C [dry]), and turnout period was randomly assigned within blocks. Block designation was consistent across years.

***Pasture contamination*—**Paddocks were purposely contaminated the fall prior to each evaluation period by daily rotating a group of 8 (year 2000) or 12 (during year 2001) naturally infected yearling cattle through each paddock. Animals were obtained from the South Dakota State University livestock facility and had relatively low trichostrongyle FECs, typical for this region. Grazing of these contaminator animals started in early August and ended by late October. Total grazing days were 78 (year 2000) and 75 (year 2001) days. For the first-year study, a single fecal sample was obtained from all 8 heifers on Oct. 15, 2000, and the mean FEC was 2.38 eggs per gram (EPG) with a standard deviation (SD) of 1.69. For the Year 2 study, mean FECs were determined at three different time-points: 4.50 EPG (SD = 2.00) on August 8; 0.72 (SD = 0.31) on September 6 and 1.11 EPG (SD = 1.34) on October 6, 2001.

***Measurement of over-wintered pasture infectivity*—**Pasture infectivity from PW-J3s was measured by grazing nematode-free tracer calves within the paddocks for 14-day periods, moving them to a concrete-floored drylot until the ju-

veniles had matured to egg-laying adults, and then performing FECs for multiple days as a way of estimating the relative number of adult worms. Each paddock was grazed by five Holstein calves born during the previous fall and early winter (6-8 months old) and raised in confinement without pasture access so that they were naïve to any nematode infection. The group of 68 (Year 1) and 85 (Year 2) calves were randomly assigned to 4 (Year 1) or 5 (Year 2) turnout periods. The grazing periods for each study year are given in Table 1; and while turnout dates for Year 2 were four days earlier than for Year 1, these grazing periods were considered equivalent for comparison purposes. Year 1 did not include the first grazing period.

To ensure that the tracer calves were not excreting trichostrongyle eggs prior to turnout, we administered to each calf approximately a double dose (20 mg/kg) of albendazole (Valbazen®, Zoetis Animal Health) on 2 separate occasions, separated by at least seven days and at least one day prior to turn-out onto the paddocks. Fecal samples (3 gram) from all tracer calves were also evaluated for the presence of trichostrongyle eggs immediately prior to turnout, and no eggs were observed in any of these calf samples. In addition, two calves from each grazing-period-group were assigned as dry-lot controls; these animals remained in the dry-lot for the entire duration of the project. All control animal FECs were evaluated as described for the grazing calves.

At each turnout period, the 15 allocated tracer calves were randomly divided among three paddocks. Paddocks were assigned to turnout period *a priori* within the blocking structure previously described, with one paddock/block used at each 14-day turnout period. Trace mineral salt and water was provided *ad libitum*. It was expected that paddocks would be heavily grazed at the stocking rate utilized. At the conclusion of each grazing period, animals were transferred to dry lot housing, fed conventional diets of grain and stored hay.

Fecal samples were obtained on 3 consecutive days (post-grazing days 21, 22, and 23 in Year 1; days 30, 31, and 32 in Year 2), and trichostrongyle FECs were determined using a modified Wisconsin sugar flotation technique (Cox and Todd 1962). For each animal, arithmetic means were calculated among the three days and used to calculate the mean for each grazing period. Differences in infection intensity between time periods were determined by analysis of variance using GraphPad InStat version 3.05. Further analysis was conducted (Proc GLM, SAS 9.4) to estimate the linear rate of FEC decline between periods. FEC were log

Table 1. Grazing time periods for each group of 15 sentinel calves.

14-Day Time Period	Year 1 (2001)	Year 2 (2002)
Period 1: 2nd Half May	NA*	May 13-May 27
Period 2: 1st Half June	May 31-June 14	May 27-June 10
Period 3: 2nd Half June	June 14-June 29	June 10-24
Period 4: 1st Half July	June 29-July 13	June 24-July 8
Period 5: 2nd Half July	July 13-July 27	July 8-22

*This grazing period was not attempted during 2001

transformed and modelled as a linear function of period, year, and period•year. *P*-values greater than 0.05 were not considered to be statistically different.

In Year 1, coprocultures were conducted on four fecal egg samples obtained 21 days post-grazing from tracer calves in the first pasture turnout group (grazing period 2, May 31-June 4). Juveniles in coprocultures were identified to the genus level as described by Bowman (1999). In Year 2, the calves excreting the highest number of trichostrongyle eggs for the first three grazing periods were necropsied at the end of the fecal sampling period to determine the species of trichostrongyles present. For each calf, abomasum and small intestine were immediately removed, and the intestine was divided into three equal segments. The gastrointestinal contents were recovered from each, and aliquots were visually inspected with a stereomicroscope. Adult worms were harvested and identified to the genus level as described by Bowman (Bowman 1999).

Determination of soil temperatures—Daily soil temperatures at 2 inches were obtained from weather station 391076/99999, BROOKINGS 2 NE, South Dakota, which is approximately 3.2 kilometers east of the study site. Data were recorded and tabulated by the National Climatic Data Center (http://www.lwf.ncdc.noaa.gov/oa/ncdc.html). To compare soil temperature conditions (below freezing) of the 2000-2001 winter to that of the 2001-2002, we determined the area above the curve below 0 °C (in Figure 2) for both winters and compared these using the FIJI version of ImageJ (Schindelin et al. 2012; Rueden et al. 2017). In a similar way, soil temperatures from grazing period 1 through period 5 were compared by measuring the area under the curve (summer grey area in Figure 5) for both years using the FIJI version of ImageJ.

RESULTS

Prior to turnout, no nematode eggs were observed in the 3-gram fecal samples from any tracer calf. In addition, nematode eggs were also not found among any of the samples taken from the 18 (8 from Year 1 and 10 from Year 2) drylot-control-calves. By the end of each grazing period, the sentinel calves had removed greater than 80% of the forage in each paddock as estimated by photography and visual appraisal by the investigators. During Year 1, the coprocultures from calves for grazing period 2 contained about 60% *Ostertagia* spp. juveniles and about 40% *Cooperia* spp. During Year 2, both *Cooperia* spp. and *Ostertagia* spp. adults were recovered from the most heavily infected tracer calf of grazing period 1; however, there were only 53 *Ostertagia* adults (0.65% of total), but 8,100 *Cooperia* adults. Only *Cooperia* adults were found in necropsied calves from time periods 2 and 3.

During both years, FECs among the tracer calves progressively decreased as the grazing season progressed, though at differing rates (Figure 1). By the last grazing period, mean FECs were not statistically different between the two years and were below one egg per gram among all calves from both years. For the earlier grazing periods, FECs for Year 1 were significantly higher than those for Year 2. During Year 2, the mean FECs had decreased by 36.1% from grazing period 1 to period 2, and by period 3 (ending June 24) pasture infectivity had decreased by

a total of 69.0%. By July 8, very few juveniles were still infective in the paddocks as illustrated by the 97.4% decrease in FECs measured over the entire grazing period (Figure 1). Pasture infectivity was not measured during grazing period 1 in Year 1 (Table 1); however, during period 2, FEC decreased by 69.4%, similar to the decrease measured during the first two periods in Year 2 (Figure 1). FEC reduction appeared to decrease from period 3 to 4, with infectivity of paddocks dropping 88.8% compared to the beginning of the grazing. During the final period of Year 2, FECs suggest survival of only 1.4% of the J3s. Overall for both years combined, FECs averaged 55.9% of those of the period prior (a 44% decline each 14-day period). Years varied in the rate of decline. The average FEC compared to the prior period was 39.4% in 2001 (an average decline of 60.6%) or 59.8% in 2002 (decline of 40.2%).

Daily temperatures at 2 inches below the soil surface are shown in Figure 2 for the time period involving the two studies (July 1, 2000 to July 31, 2002). These temperatures were measured close enough to the study area that they would reflect the soil conditions associated with the study area. In Figure 2, the area at which the soil temperatures remained below 0 °C were shaded grey for both winter months. This area for the winter preceding the 2002 (Year 2) study was 404% higher than for the same winter months preceding the 2001 study (Year 1). This difference demonstrates that the over-wintering juveniles for the 2002 study were exposed to freezing soil temperatures that were four time lower than

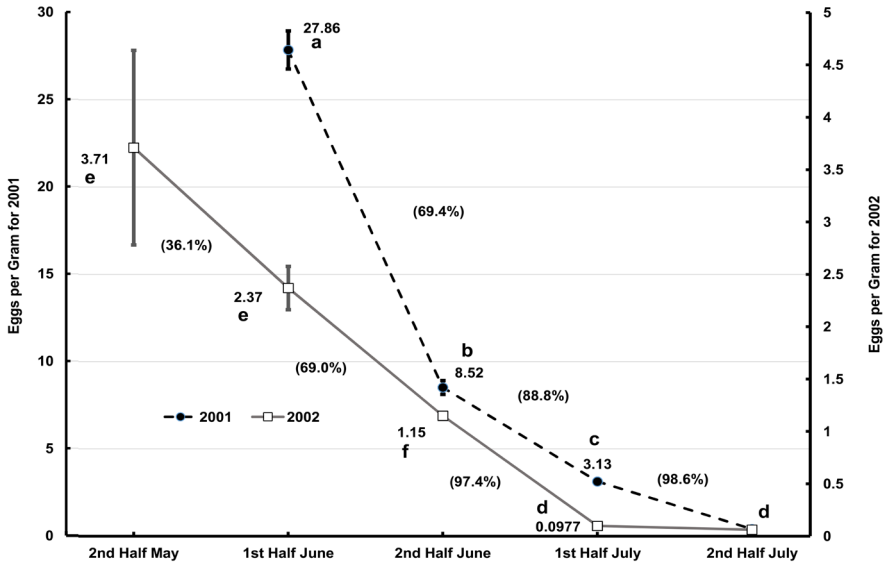

Figure 1. Mean fecal egg counts (in eggs per gram of feces) among sentinel calves within the five grazing time periods in years 2001 (dashed line with black circle markers using the left Y-axis) and 2002 (solid line with white square markers using the right Y-axis). Numbers associated with each marker show the mean eggs per gram with the error bars representing the standard error of each mean; mean values associated with similar letters are not statistically different. Percentage values in parentheses show the percent decrease occurring between a grazing period relative to the first period evaluated.

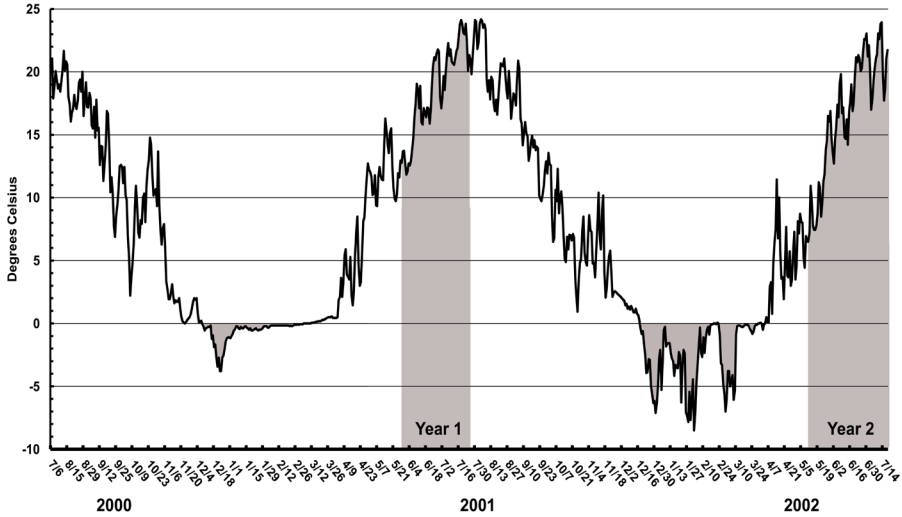

Figure 2. Daily soil temperatures (in degrees Celsius) at 2 inches below the surface from July 1, 2000 to July 31, 2002. Shaded areas during the winter months show days when temperatures were below 0 °C. Shaded bars during the summer months show that days during which the sentinel calves graze in the paddock.

juveniles for the 2001 study. A similar approach comparing summer soil temperatures between the two study periods (from period 1-5 for both years) yielded an area under the curve for Year 1 that was only 3.85% larger than that for Year 2. This demonstrates that the summer soil temperatures were very similar between the two study periods.

DISCUSSION

The *Ostertagia* spp. and *Cooperia* spp. adults and juveniles identified in this study were almost certainly *O. ostertagi* and *C. oncophora*. These two species are the predominant trichostrongyles in the region, and very few other species from these two genera have been described from the area (Stromberg et al. 1991; Malczewski et al. 1996; Harmon et al. 2009; Avramenko et al. 2017). Coproculture results from Year 1 showed slightly higher numbers of *Ostertagia* spp. than *Cooperia* spp., but necropsies during Year 2 showed *Cooperia* spp. was the predominant species.

The present study confirms the ability of *O. ostertagi* and *C. oncophora* J3s to survive through winters on pastures located in the U.S.A. Northern Great Plains; these results are consistent with previous studies from cold winter climates (Smith and Archibald 1969; Smith 1972; Taylor et al. 1973; Slocombe 1974; Gibbs 1979; Stromberg et al. 1991). Winter temperatures during the two years of this study were not out of the normal range for this area, however, freezing soil temperatures (at 2 inches below the surface) during the 2001-2002 winter were

about four times lower than the previous year. At three separate time periods during the 2001-2002 winter, soil temperature dropped to below -7 °C. Lab studies involving *Teladorsagia circumcincta* (a close relative of *O. ostertagi*) found that about 50% of its third-stage juveniles were not viable after 70 days of exposure to weekly freeze/thaw cycles at -10°C/3°C (Jasmer et al. 1987). A similar sensitivity to temperatures below -10 °C for *O. ostertagi* and *C. oncophora* may help explain why beginning FECs were so low for the 2002 study. Soil temperatures measurements are becoming more available to cattle producers, and they might be useful for making parasite control decisions. Further field studies are needed to better understand the effects of prolonged freezing soil temperatures on the survivability of each trichostrongyle species that infect cattle.

Results from the two-season tracer-calf study showed a very significant decline in pasture infectivity for the PW-J3s as the grazing season progressed, but that most of the decrease occurred before July. Infectivity was not measured for the first year until May 31; for the second-year-study, an additional grazing period was added for the second half of May. Results from the first grazing period for Year 1 showed a 36% decrease in infectivity, which would likely have occurred during Year 1 if measured. These results indicate that future studies should start evaluating infectivity prior to May 13, and maybe prior to May 1 in order to better evaluate the temporal availability of PW-J3s in a region. Because our South Dakota study evaluated infectivity during consecutive two-week periods, it was possible to measure the decrease infectivity more precisely than was reported in the Atlantic maritime studies (Smith and Archibald 1969; Smith 1972; Gibbs 1980). The South Dakota results were consistent with these previous studies and showed a very significant population collapse for the PW-J3s on pastures. In the South Dakota study, most of this collapse had occurred prior to the second week in June, and virtually none were infectious by the second half of July. These results suggest that the survival half-life averages 15 days in this climate. Summer soil temperatures were similar during the grazing period for both years of the South Dakota study, which may explain why the diminishing infectivity rates were fairly similar for the two years. Assuming average pasture survival of 55.9% between 14-day periods, pasture infectivity would be expected to be only 5.4% of original infectivity following five (two week) intervals. The initiation of infectivity decline is probably dependent on temperature, moisture, and microenvironment. Average yearly precipitation decreases from about 65 cm/year on the eastern edge of the Northern Great Plains to under 40 cm/year in some of the more western areas, and this decreasing precipitation correlates with decreasing trichostrongyle populations in cattle (Hildreth et al. 2007). Future studies are needed to better understand the effects of weather conditions on the infectivity of each juvenile species across this natural precipitation gradient. Understanding such provides framework for improved productivity, while optimizing anthelmintic use.

Despite the rather short window of survival for the overwinter juveniles, virtually all cattle from the Northern Plains region are infected with trichostrongyles (Hildreth et al. 2007), and they have been show to diminish weight-gains in South Dakota yearling cattle by 0.1 lb/head per grazing-day (Epperson et al. 2001; Mertz et al. 2005). Springtime deworming programs, designed to pre-

vent exposure to post-winter juveniles, can reduce these losses (Stromberg and Gasbarre 2006). Results from this study demonstrate that nematode control programs designed to protect cattle until the last couple of weeks in June should significantly impact trichostrongyle population levels in the herd.

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