

Ecology/Physiology Workgroup

Importance of Nematode Parasites in Cattle Grazing Research

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1.0 Introduction

Grazing cattle research ranges from investigating univariate processes to entire systems of production. In spite of the extensive land and animal requirements of many grazing experiments, resources are often limiting; consequently, compromises are made in design and measurements. Every researcher constantly battles with variability that is unexplained. Not only is it important to avoid confounding of effects, but it is also important to minimize experimental error, i.e., that variation among observations of experimental units treated alike (Stuedemann and Matches, 1989)

Gastrointestinal parasites are ubiquitous among grazing animals and can potentially confound and/or add to variation when studying other effects. The impact of parasites on animal production can be influenced by many factors (Stuedemann et al., 1995). Consequently, the researcher conducting grazing experiments must be constantly concerned with how to deal with internal parasites, i.e., how to avoid confounding the effects of parasitism and the treatments under study as well as how to avoid differential effects of parasitism among experimental units within treatments. The purpose of this paper is to discuss the impact that nematode parasites may have on grazing research and how researchers may deal with them.

2.0 Impact and species involved

Favorable environmental conditions, particularly abundant moisture and relatively warm temperatures, enable parasite development and survival on a year-round basis in the Southeastern U. S. In this region, researchers may need to use more caution in avoiding confounding of effects and increased variation caused by parasites.

Craig (1988) suggested that internal parasitism could be divided into three categories (infection, economic, and clinical) based on the effects of the parasites in the host. The first category is simply when animals are infected, but do not show measurable adverse effects. The second is known as economic because the level of infection is such that it causes reduced production. The third category, or clinical parasitism, occurs when there are obvious abnormal signs such as

anemia, diarrhea, and lack of appetite and poor growth or other noticeable changes. The first two categories of parasitism are the most difficult to detect and manage because they do not result in obvious signs nor are they easily measured.

Parasite species and their relative pathogenicity has been presented by Miller et al. (2005) earlier in this series of presentations. *Haemonchus placei* and *Ostertagia ostertagia* are two of the most pathogenic nematodes in cattle with *Ostertagia* being of major concern during the cool season and *Haemonchus* during the warm season. Numerous species of *Cooperia* also very commonly infect cattle, and though of significantly less pathogenicity than either *Haemonchus* or *Ostertagia*, *Cooperia* may be present in very large numbers and in such cases can be quite important. In addition, *Cooperia* spp. are often major contributors to fecal egg counts.

3.0 Importance of immunity or differences in classes of cattle

Believe it or not, the economic impact of parasites has not been precisely determined. A common technique used to assess the impact of parasites is to administer an anthelmintic to grazing cattle and then measuring response criteria that assess production or performance. Craig (1988) cited 13 studies that used this technique. In those studies the reduction in weight gain ranged from 13 to 33 % with the greatest reduction occurring in young, non-suckling cattle, which typically have no or poor immunity to nematode parasites. This technique probably underestimates the impact of parasites because it does not account for re-infection. Animals may be re-infected from almost immediately to several weeks post-treatment depending upon the persistent effect of the anthelmintic used.

The impact of nematode parasites on cow-calf production is less clear because it is well documented that mature cows develop excellent immunity to nematode parasites. Profitability of cow-calf production systems can largely be accounted for by the units of weaned calf produced and the herd pregnancy rate, coupled with the rate of live births. The economic benefit of cow weight or gain beyond that necessary to sustain optimum suckling calf growth and to assure rebreeding is difficult to determine even though it is well recognized that this gain can provide nutrients to the cow when feedstuffs are lacking. Reinemeyer (1992) reviewed the literature relative to anthelmintic treatment of mature cows and suggested that deworming of mature beef cows continues to be a controversial issue. Little has changed on this point through the present time but it appears there is less need to be concerned about nematode parasites in grazing research involving mature cows so long as high planes of nutrition are maintained in all experimental groups. However, it is important to note that anything that significantly stresses the cow or compromises her general health and nutritional status may also lead to a significant decline in levels of immunity to internal parasites.

4.0 The importance of drug resistance in nematodes of cattle and the potential role of forage/livestock researchers

Drug resistance is a major threat to small ruminant production throughout the world (Kaplan, 2004). Reports of anthelmintic resistance in nematodes of cattle have been less common. It is generally thought that resistance is not yet an important issue in cattle, and this is particularly

true in the U.S. However, no studies have been conducted to investigate the prevalence of resistance in nematodes of cattle in the U. S. This past year, the first case of AM (ivermectin-milbemycin) resistance in cattle nematodes in the U.S. was reported on a large background operation in Wisconsin (Smith and Gasbarre, 2004). This report is very significant because multiple species of nematodes were found to be resistant, and the worms were resistant to multiple anthelmintic classes. Importantly, many of these calves originated from Georgia, a state in which large numbers of weaned calves are raised in background and stocker operations prior to shipment to feedlots or other backgrounders, usually in the Midwest. Also important is the fact that this occurrence of resistance was only investigated because the calves had lower than expected weight gains. This is not surprising given the fact that failure of treatments to prevent clinical disease usually only occur once resistance has reached high levels. In contrast, low to moderate levels of resistance will not produce outright disease symptoms, but will have an important impact on animal productivity. In light of these recent findings, anthelmintic resistance in nematodes of cattle in the U. S. may be considerably more common than is currently recognized.

How important is the issue of drug resistance to grazing cattle researchers? It is difficult to answer that question other than to say that if parasites are not monitored in your research animals, it will be impossible to answer that question relative to your specific situation. However, given the recent rapid rise in parasite drug resistance in small ruminants, is likely that the issue of resistance in cattle parasites will become increasingly more important in the next 5-10 years.

There are serious questions being raised by parasitologists throughout the world about resistance and prevalence of resistance in cattle. The seriousness of ivermectin-milbemycin resistance in *Cooperia* spp. is evident in many parts of the world. Should it be a major concern everywhere? Parasitologists are calling for research to address mechanisms of resistance and the need for addressing strategies that will preserve the effectiveness of different available drugs (Coles 2002, 2005; Kaplan 2004). This concern is based on the fact that levels of resistance can develop rapidly and there are few anthelmintics currently being developed. According to Kaplan (2004), two new classes of anthelmintics have emerged in the post-ivermectin-milbemycin years: the cyclooctadepsipeptides and the oxindole alkaloid, paraherquamide. However, at the present time there is no public information on the plans for development and marketing of these drugs.

Because very few parasitologists have access to cattle and production facilities that are actively handling animals under controlled, well characterized conditions, it appears that forage/livestock researchers are in the perfect position to form cooperative relationships with parasitologists. These relationships will enable the forage/livestock researchers to accomplish their objectives while facilitating parasitologists in detecting and characterizing resistance. Plus, it will enable parasitologists and forage/livestock researchers to evaluate methods for prolonging the development of resistance and for developing alternative strategies for parasite control while accomplishing forage/animal research objectives.

Maintaining adequate levels of refugia, (parasites unselected by drug treatments, e.g., eggs and larvae on pastures and worms in animals left untreated), has been proposed as a key element for avoiding resistance (Van Wyk, 2001; Coles, 2002). It is theorized if a sufficient number of drug-

susceptible parasites are maintained that they will dilute any resistant worms that are present. This should significantly reduce the rate with which resistance develops. For example, on the farm on which one of the first field cases of resistance of *Haemonchus contortus* to ivermectin was recorded, the sheep had been drenched a mean of every 23 days over the preceding 5 months, thus giving susceptible worms almost no chance to reproduce (Van Wyk, 2001). Forage/livestock researchers are in a key position to assist parasitologists in testing alternative strategies for maintaining susceptible parasites as well other strategies that might reduce the potential for the development of parasite resistance in cattle.

5.0 Statistical considerations

In grazing research, the group of animals on a pasture or the pasture is usually the experimental unit. If one defines the experimental unit as the animal on the pasture rather than the pasture, there is an erroneous increase in the degrees of freedom, which increases the likelihood of declaring a significant difference; however, the test will be invalid. When treatments are applied to an entire pasture, e.g. a forage species or herbage mass treatment, each animal is a subsample and the group of animals is the experimental unit. There could be exceptions to this rule such as administration of a medication, other than an anthelmintic, that is applied as a split plot to one or more animals in a given pasture. Split-plot designs actually have more than one estimate of experimental error, and it varies because there are, by definition, multiple sizes or types of experimental units within the design, but you assume that the variance is homogeneous for all experimental units in split-plot designs (Fisher, 1999; Stuedemann and Matches, 1989)

The use of an anthelmintic could technically fit the split plot definition but confound the definition of the experimental treatment. This is because nematode parasites have a free-living stage that is present on the pasture. If treated and untreated animals are mixed then effects on the treated and untreated animals are no longer independent, which is a basic assumption when conducting analyses of variance. The anthelmintic treatment, in this case, is anthelmintic treatment while treated and untreated animals are grazing the same pasture. This means that animals could be re-infected immediately after treatment and this re-infection varies depending upon the number of parasite larvae on the pasture, plant morphology, plant species, etc. Plus, treatment with an anthelmintic could increase appetite resulting in not only an increase in forage intake, but potentially a simultaneous increase in larval intake. In other words, the experimenter is not simply testing the difference between treatment with an anthelmintic and no anthelmintic treatment. Consequently, the hypothesis tested is not the hypothesis the experimenter desired to test. For studies of anthelmintic treatment of cattle on pasture, the design should make the pasture the experimental unit by avoiding the mixture of treated and untreated animals on the same pasture.

6.0 Importance and measurement of nematode parasites in grazing research

Miller et al. (2005), in this series of presentations, have presented response variables and methodologies for determining or estimating parasites in the grazing animal. They will not be reviewed or presented in this paper.

Animal scientists or forage/livestock researchers have largely ignored internal parasites in grazing experiments by making one or more of the following assumptions:

Assumption 1. Treat all animals alike.

It is common for researchers involved in grazing research to ignore the potential presence of gastrointestinal nematodes and their subsequent effects on animal performance. Many researchers simply deworm animals at the beginning of a grazing experiment and assume that they have removed the parasites for the duration of their studies. The justification for this is that "all of the animals were similarly treated" and thus would not be a variable in the results of their research. This is not a safe assumption because parasites are over-dispersed in their hosts meaning that a small percent (20%) of cattle harbor most of the parasites (80%). Thus when exposed to pastures containing infective larvae, re-infection will not be normally distributed. Instead, a minority of cattle will be heavily infected relative to the rest of the herd, which is likely to lead to animals within different treatment groups harboring dissimilar infection levels. Also, it is possible for a few animals to be missed or under dosed during the deworming process, especially with large number of animals. Thus, a few animals with relatively high infection levels may influence, from the beginning, the results if assigned to the same treatment group or experimental unit. Finally, if drug resistant worms are present, the response to treatment will not be uniform among cattle. Consequently, following treatment some cattle will be left with many more parasites than others.

Assumption 2. All animal and pasture parasite burdens are the same.

Another common assumption is that all pasture parasite burdens are similar; however, no two pastures are exactly alike. This may be particularly true if dissimilar (in terms of parasite burdens) groups of cattle or different management options were employed on pastures used in a preceding experiment. Even if pasture parasite burdens are similar, in studies using different stocking rates, animals grazing the more intensely stocked pastures will consume more larvae than those grazing at a lower stocking rate. If there is a forage mass difference among treatments, it could be postulated that the cattle grazing the pastures with the lower forage mass could have a greater potential for intake of larvae and result in a higher parasite infection rate. Consequently, performance differences at different stocking rates or forage mass may be related to differences in forage quality and/or amount, but also would be confounded by pasture parasite load, number of infective larvae consumed and subsequent effects of the parasites in the animal. This could be of particular concern when grazing animals that are more susceptible to infection, i.e., usually non-suckling, growing cattle that are less than 18-24 months old are employed as the experimental animal. This is often the type of animal used in grazing experiments.

Assumption 3. It is unnecessary to monitor parasite burdens.

It is commonly assumed that if cattle are dewormed prior to a grazing experiment, monitoring infection level during the experiment is not necessary because treatments and animals were randomly assigned and parasite affects will therefore be random and not confound results or their interpretation. This may be a critical fallacy, particularly if stocking rate or forage mass is a variable, if forages of differing morphological characteristics are being studied, or if pastures have differing infectivity levels. Under all of these conditions it may be critical for the researcher to carefully plan a parasite treatment regimen that avoids confounding or at least monitors the parasite load of the cattle and/or pastures.

The methodology that the forage/livestock researcher uses to adjust or account for differences in parasite levels in animals will depend upon a variety of factors. The first factor of major concern would be the homogeneity of variance associated with the parasite response variables. Most parasite data is not normally distributed and would most likely need some transformation in order to be statistically tested. If statistical analyses of parasite response variables, reveals some treatment differences, then the forage/livestock researcher has a good basis for further investigating the potential for confounding effects of parasitism and forage-animal treatments. However, just because there are forage-animal treatment differences relative to the parasite variable measured does not mean that it is of concern or of biological importance. For example, in one of our studies involving restoration of degraded land with grazing cattle, which included treatments of different forage mass and fertilization, we found that after summarizing 5 years of results we had significant treatment differences in fecal egg counts at the end of the summer grazing season when egg counts were the highest (Table 1). However, even though there were statistical differences, the mean eggs per gram of feces or differences were so small that they were of little biological importance (Stuedemann et al., 2004).

7.0 Techniques and methodologies that may aid in interpreting results, as well as reduce confounding or differential effects of parasites in grazing research

Monitoring fecal egg counts (FEC): Collecting fecal samples, per rectum, is relatively simple. However, in cattle, FEC is only a general indicator of the animals' worm burden because the number of eggs present will depend upon such factors as species of parasites present, egg laying efficiency of the parasite, fecal consistency, and methodology, which vary in sensitivity and precision, used for enumeration of the eggs. It is recommended that several samples be obtained over time. This will enable the researcher to determine trends and give some indication of infection level within and between experimental groups. Obviously, sampling more animals per experimental unit will increase the precision of the estimate of the worm burden. Examination of FEC from one of our studies over a five-year period where all steers were treated with two different anthelmintics prior to being placed on pasture, revealed a significant negative (-0.128, $P < .01$) correlation between FEC and yearling steer ADG. This correlation was computed on an experimental unit basis and not on an individual animal basis.

In another experiment, in each of two years, lactating cows (calves were not treated) were treated in late April or early May. The winter calving cows were either treated with ivermectin, doramectin, or served as untreated controls. There were five replications of each treatment or 15 pastures each containing six cow-calf units. Although this was a study designed to examine the effect of anthelmintic treatment of cows, it showed that treatment can be beneficial in terms of calf ADG. It revealed that calves suckling control cows had a high negative correlation (-0.73, $P < .0001$ in year one: and -0.52, $P < .0033$ in year two) between FEC and ADG. In this case there was a highly significant relationship between FEC and ADG. In year one, for every 100 eggs per gram of feces there was a 0.3 kg decrease in ADG. In year two, for every 100 eggs per gram of feces there was a 0.1 kg decrease in ADG. This illustrates that the nematodes can affect ADG and that the effects may vary greatly.

Although there may be a significant relationship between FEC and ADG, because this will vary with conditions of each experiment, the worm burdens present and other factors, it would be difficult to adjust performance or production response variables based on FEC. Probably the most useful aspect would be to “raise a flag” to the researcher that other animal response variables may be compromised by the parasite burdens in the cattle and that other corrective measures may be needed to avoid confounding or differential effects among experimental units treated alike. For example, if ADG was lower in one out of five experimental units within a treatment and the FEC were greatest in animals in that unit, it would suggest that parasite burden in the animals in that unit could have affected the ADG above the forage/animal treatments being evaluated. The more obvious situation occurs when FEC differ among or between forage/animal treatments, suggesting that a confounding of animal performance or production data might have occurred. If no differences in FEC occur, the researcher’s confidence that parasites are not influencing the interpretation of data is strengthened.

Rotation of parasite-infected cattle through pastures before experiments begin: Use of this technique may help overcome differential pasture parasite burdens potentially caused by a variety of factors including previous use of the pastures. However, it simply assumes that all pastures will then be infected equally. This is not necessarily true since larvae may survive for extended periods of time.

Treatment with anthelmintics: Treatment of cattle before placing them on pastures coupled with monitoring of FEC and perhaps in combination with rotation of infected cattle through pastures may be a useful tool. However, treatment, by itself, provides little reassurance that nematode parasites are not affecting cattle performance or production response variables.

8.0 Summary

Gastrointestinal parasites are associated with all grazing animals and can potentially confound and/or add to variability in results. Consequently, the researcher conducting grazing experiments must be constantly concerned with how to deal with internal parasites and how to avoid confounding the effects of parasitism with the treatments under study as well as how to avoid differential effects of parasitism among experimental units within treatments. Animal scientists or forage/livestock researchers have largely ignored internal parasites in experiments by making one or more of the following assumptions: (1) if all cattle are treated with an anthelmintic before the experiment begins, parasites should not be of concern, after all “all animals were treated similarly”, (2) all cattle and pasture parasite burdens are the same, and/or (3) it is not necessary to monitor parasite burdens during an experiment because treatments and animals were randomly assigned and parasite affects will therefore be random and not affect results or their interpretation.

It is important that forage/livestock researchers monitor parasite fecal egg counts or other pertinent parasite related response variables in grazing research because it could “raise a flag” to the researcher that other animal response variables may be compromised by the parasite burdens in the cattle. The researcher could then employ corrective measures to avoid confounding or differential effects among experimental units treated alike.

Anthelmintic resistance in nematodes of cattle appears to be on the horizon in the U. S. This could have major effects on how forage/livestock researchers execute grazing experiments and how they interpret results. It appears that forage/livestock researchers are in the perfect position to form cooperative relationships with parasitologists that will enable accomplishment of their objectives while facilitating parasitologists in detecting and characterizing resistance as well as assisting parasitologists in evaluating methods for delaying the development of resistance. Plus, this cooperative relationship would provide opportunities for development and study of alternative strategies for parasite control while accomplishing forage/animal research objectives.

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Table 1. Mean fecal egg counts (eggs/gram) for October by fertilizer treatment and grazing intensity.

Year	Fertilizer treatment			Grazing intensity	
	Clover	Litter	Mineral	High	Low
1994	.7	4.1	6.1	2.6	4.7
1995	0	.7	.4	.7	.1
1996	.9	4.2	4.0	3.1	3.0
1997	.4	3.6	2.4	.6	.7
1998	1.3	2.6	1.7	.8	2.9
Mean	.7a	3.0b	2.9b	1.6c	2.9d

Values with different letters (a,b) across nitrogen treatments differ, $P < .05$. Values with different letters (c,d) across grazing intensity differ, $P < .05$. Year x grazing intensity was significant, $P < .0251$.