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# Shedding and characterization of gastrointestinal nematodes of growing beef heifers in Central Texas



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# ABSTRACT

Gastrointestinal nematodes (GIN) do not detrimentally affect cattle to the extent of small ruminants. However, they are developing resistance to drugs used to treat them. Genetic strategies to control the nematodes and/or their detrimental effects could be a sustainable alternative to treatment with drugs. An essential first step in development of such a strategy is characterization of nematode populations in commonly used breed types of cattle in local conditions. Fecal egg counts (FEC) were obtained every two months on a cohort of 53 crossbred Nellore-Angus heifers grazing Central Texas pastures from an average heifer age of 3 months to approximately 2 years of age. For 10 of those 12 sets of samples, coprocultures were set up to characterize gastrointestinal nematode species present. Heifers were ½ Nellore ½ Angus (n = 18) or ¾ Angus ¼ Nellore (n = 35). They were born in the spring of 2014 to cows that were from 3-5 years old. They were maintained as a group throughout weaning, postweaning, exposure to bulls as yearlings, and as pregnant cows through the birth of their first calves. An interaction of breed group with sampling time (P < 0.0001) highlighted favorable FEC of <sup>1</sup>/<sub>4</sub> Nellore heifers as compared to ½ Nellore in all but two sampling times. Fecal egg count means were, in general, higher for heifers of both groups in sampling times up to one year of age. Season effects on FEC may be important, but the effect of age may have obscured their detection. There were few significant correlation coefficients for FEC traits with a variety of production traits of these females. Average FEC residuals were positively correlated (r = 0.28 and 0.41; P < 0.05) with winter coat shedding score evaluated at approximately 17 and 24 months of age. Residual correlations of average FEC with calf weaning weight and incidence of shedding with calf age at weaning (r > 0.3) may be indicative of the increased susceptibility of females that lactate heavily or longer to internal parasite infection. Proportions of GIN genera by sampling day differed from  $\chi^2$  expectation (P < 0.0001). Cooperia and Haemonchus species were detected in large proportions in sampling dates that corresponded to heifers less than one year of age. Ostertagia and Trichostrongylus species predominated in sampling dates after heifers reached one year of age.

# 1. Introduction

The detrimental gastrointestinal nematodes (GIN) in small ruminants rapidly acquire resistance to drugs used to treat them (Sargison, 2012; Kaplan and Vidyashankar, 2012). The nature and extent of internal parasite problems in cattle is quite different, and has not been an area of research focus to the extent of that in small ruminants. However, there is strong evidence for the emergence of resistance of various GIN of cattle to drugs used for their control (Cotter et al., 2015; Rose et al., 2015; Bullen et al., 2016; Fiel et al., 2017; Kalmobe et al., 2017). It is expected that in the long term, many of the same problems presented by anthelmintic resistance of GIN in small ruminants will be prominent in the production of beef in many parts of the world (Gasbarre, 2014; Cristel et al., 2017; Wang et al., 2017). A goal of sustainable management of GIN is the reduction of treatment with anthelminitics (Knubben-Schweizer and Pfister, 2017). It is responsible and appropriate to commence efforts to develop internal parasite control strategies based on genetics and genomics in cattle (Gilleard, 2013). Characterization is needed at the most coarse level of genetics (breed or sub-species differences) and at the level of the genome as components of a widely applicable genetic intervention strategy. Many beef producers in the Southern United States utilize crossbred *Bos indicus* (cattle whose ancestors originated on the Indian subcontinent)-*Bos taurus* (breeds of European origin) cattle because of large hybrid vigor for almost all traits and superior adaptation to hot, humid, low forage quality environments. However, those cattle have a reputation for lower beef

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quality, such that producers will favor using crossbreds with lower amount of *Bos indicus* background. This results in a large part of the Southern United States herd being less than ½ *Bos indicus*. The objectives of this work were to begin development of such a strategy by 1) longitudinal characterization of fecal egg counts (FEC) in growing heifers (½ *Bos indicus* or ¼ *Bos indicus*) in grazing conditions of Central Texas from 3 months to 24 months of age and 2) characterization of relative proportions of GIN species across that time period.

# 2. Materials and methods

A cohort of 53 heifers born in the spring of 2016 were characterized longitudinally for FEC from approximately 2 months of age (May, 2016) through 25 months of age (April, 2018). These heifers were born to  $F_2$  Nellore (*Bos indicus*)-Angus (*Bos taurus*) cows that were 3–5 years old. They were sired either by Angus bulls (therefore  $\frac{3}{4}$  Angus  $\frac{1}{4}$  Nellore; n = 35) or  $F_1$  Nellore-Angus bulls (therefore  $\frac{1}{2}$  Angus  $\frac{1}{2}$  Nellore; n = 18). They were weaned in October, 2016, and maintained as a group thereafter on native pastures at the Texas A&M AgriLife Research Center at McGregor, Texas. They were exposed to two unrelated Angus bulls beginning May 9, 2017 through July 13, 2017.

Fecal samples collected directly from the rectum were obtained from heifers at approximately 2-month intervals for a total of 12 sampling times (Table 1). Other than for the first two collection times, coprocultures were set up to grow and identify larvae to genus in pooled samples. Eggs per gram of feces of GIN were determined using the Wisconsin Double Centrifugal Sugar Flotation test (modified from Zajac and Conboy, 2012). Heifers were treated a single time (~2.4 ml per 100 kg body weight) with injectable Cydectin (moxidectin 1%; Bayer, Shawnee Mission, KS, USA) on January 6, 2017.

The trait FEC was evaluated in a generalized linear mixed model using the GLIMMIX procedures of SAS (SAS Inst., Inc., Cary, NC) assuming a negative binomial distribution and log link function (Alexander, 2012). Heifer was a random variable in those models. Investigated fixed effects included breed group (two levels: ¼ Nellore, ½ Nellore), sampling date (12 unique sampling dates), initial age of heifers in days as a covariate, age of the dam of the heifers (3 levels: 3, 4, 5 years), and interactions. Residual variance was set at 1. Fecal egg count was also evaluated as binomially distributed in generalized linear mixed models using a logit link function, with any detection given a value of 1, and no detection given values of 0. Production traits of cows and their calves were evaluated using the MIXED procedures of SAS and assuming normal distributions.

Weights of these females were recorded at birth and at pregnancy determination in the fall of 2017. Body condition scores from 1 to 9,

indicating increasing amounts of visually evaluated fat cover (Herd and Sprott, 1986), were recorded on females at the time of pregnancy determination. Calving and weaning rates were constructed as binomiallydistributed traits by assigning values of 1 to females that gave birth and weaned a calf and 0 to those that failed. Disposition score of dams of heifers at the time of calving was recorded as scores from 1 (good) to 5 (wild or mean to excess); this score was later assigned to heifers that eventually calved themselves. Cows that calved also had the birth weight of their calf recorded, her disposition score at the time of calving, weaning weight of the calf, and calf age as indicative of the time in the breeding (calving) season that the cow conceived (gave birth). Winter coat shedding scores (1-5 indicating winter coats from no shedding to complete shedding: Grav et al., 2011) were recorded on females at one or two month irregular intervals from June, 2017 through June, 2018. Relationships of FEC (averaged for each heifer) with all other traits were assessed with Pearson correlation coefficients of unadjusted trait values using the CORR procedures of SAS. Residuals for the production traits were generated applying the general mixed models (either linear or generalized linear mixed model as appropriate for the trait) as described above.

Numbers (proportions) of GIN genera detected in coprocultures unique to sampling dates were assessed against  $\chi^2$  expectation using the FREQ procedures of SAS.

# 3. Results

The interaction of breed group-sampling time (Table 2) was highly significant in analysis of FEC. Means for  $\frac{1}{2}$  Nellore heifers differed (P < 0.05) from  $\frac{1}{4}$  Nellore for all times except August, 2016 (average age 5.5 months) and May, 2017 (average age 15 months); in all cases except one (July, 2016; 4.5 months of age),  $\frac{1}{2}$  Nellore means were larger. These differences were not detected after correction of *P* values for multiple testing.

The majority of the species detected in coprocultures were *Cooperia* and *Haemonchus* (Fig. 1). At later sampling dates, when heifers passed two years of age, *Ostertagia* and *Trichostrongylus* species were a larger percentage of the coprocultures. Sampling date genera percentages differed from  $\chi^2$  expection (P < 0.0001).

Most observed traits expressed as recorded or as residual values were not associated (P > 0.05 of correlation coefficient) with average FEC, average residual FEC, or incidence of shedding (Table 2). Coat score residuals at 17 (July, 2017) and 24 months of age (April, 2018) were positively correlated (r = 0.28 and 0.41; P < 0.05) with residual FEC. This relationship of residuals indicates higher FEC associated with greater amounts of winter coat present on the cow. There was a

#### Table 1

Ambient conditions, unadjusted mean fecal egg count (FEC, reported as eggs per gram) and SEM by breed group and date of sampling<sup>1,2,3</sup>.

Date	Age, d	Temperature, °C		Rainfall,	<sup>1</sup> /4 Nellore			½ Nellore		
		Min	Max	mm	N	FEC	SEM	N	FEC	SEM
5/5/2016	51	16.3	27.0	197.4	18	0.7 <sup>f</sup>	0.24	9	7.6 <sup>d</sup>	5.03
7/1/2016	108	23.8	35.1	15.0	32	$368.2^{a}$	54.61	16	176.3 <sup>b</sup>	46.48
8/26/2016	164	22.7	31.8	170.2	30	62.5 <sup>c</sup>	12.89	13	85.8 <sup>c</sup>	22.13
10/21/2016	219	16.4	28.5	99.8	33	457.9 <sup>a</sup>	85.80	14	778.6 <sup>a</sup>	182.38
1/6/2017	297	7.5	18.2	64.3	32	$105.8^{b}$	24.55	14	168.3 <sup>bc</sup>	34.77
2/2/2017	324	7.5	20.4	70.6	32	27.7 <sup>c</sup>	12.60	16	59.8 <sup>cd</sup>	21.50
3/29/2017	378	13.8	25.8	105.4	33	5.5 <sup>de</sup>	1.57	16	15.5 <sup>d</sup>	4.35
5/31/2017	442	20.0	30.9	107.2	29	8.7 <sup>c</sup>	2.17	13	12.3 <sup>d</sup>	3.72
7/25/2017	497	24.7	35.8	85.9	34	5.1 <sup>de</sup>	1.42	13	16.0 <sup>d</sup>	7.46
10/3/2017	567	17.9	30.4	45.7	33	7.4 <sup>d</sup>	2.44	14	11.4 <sup>d</sup>	3.47
1/12/2018	668	0.0	12.8	5.1	31	3.9 <sup>de</sup>	1.13	13	10.5 <sup>d</sup>	3.80
4/20/2018	766	11.3	24.7	62.0	29	5.8 <sup>de</sup>	2.36	12	17.4 <sup>d</sup>	8.66

<sup>a-f</sup> Means within the same column that do not share a superscript differ (P < 0.05; Tukey adjustment for multiple comparisons of least squares means applied). <sup>1</sup> Average minimum and maximum temperatures for periods of sampling date  $\pm$  15 days.

<sup>2</sup> Total rainfall for same period.

<sup>3</sup> SEM = standard error of the mean;  $\frac{SD}{\sqrt{N}}$ .

#### Table 2

Correlation coefficients of heifer FEC with traits as calves and as dam	IS <sup>a, D</sup> .
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Traits	FEC				
	Actual	Residual	Incidence		
Traits as calves					
Birth weight	-0.14	-0.16	-0.20		
Dam's disposition score <sup>c</sup>	-0.03	-0.03	-0.22		
Weaning weight	-0.02	0.10	0.01		
Coat score <sup>d</sup>					
16 months of age	0.11	0.21	0.21		
17 months of age	-0.03	0.28	0.12		
18 months of age	-0.11	0.02	0.14		
19 months of age	0.05	0.11	0.03		
20 months of age	0.01	0.01	0.16		
21 months of age	0.05	0.05	-0.09		
24 months of age	0.07	0.41	0.13		
26 months of age	0.13	0.04	-0.33		
Traits at 18 months of age					
Weight	-0.02	0.02	-0.11		
Body condition score <sup>e</sup>	-0.16	-0.03	0.15		
Calving status <sup>f</sup>	0.04	-0.09	0.08		
Weaning status <sup>f</sup>	0.07	0.09	0.15		
Traits at first calving					
Disposition score <sup>b</sup>	0.22	0.10	-0.11		
Calf birth weight	-0.03	-0.15	-0.32		
Traits at first weaning					
30 months of age					
Weight	-0.02	0.02	-0.11		
Body condition score <sup>e</sup>	-0.16	-0.03	0.15		
Pregnancy status <sup>f</sup>	0.24	0.20	0.04		
Calf age	0.01	0.13	0.36		
Calf weight	0.25	0.31	0.29		
Calf body condition score <sup>e</sup>	0.10	0.19	0.12		

<sup>a</sup> Correlation coefficients  $|\mathbf{r}| > 0.25$  differ from 0 (P < 0.05) and are bold type.

type. <sup>b</sup> FEC average for each animal across the project duration. Residuals were from appropriate linear mixed models or generalized linear mixed models with breed group, initial heifer age in days as a covariate, sampling time, and random heifer, and were average per animal. Incidence: any FEC given a value of 1 and 0 otherwise.

<sup>c</sup> Disposition scores from 1 to 5 indicating from good to extremely mean or wild temperament of cows at the time of processing their calves.

<sup>d</sup> Coat scores per Gray et al. (2011) where 1 indicates completely shed coat and 5 indicates a full winter coat.

 $^{\rm e}$  Body condition scores from 1 to 9 indicating increasing amounts of fat cover over back and ribs (Herd and Sprott, 1986).

<sup>f</sup> Pregnancy, calving, and weaning status were evaluated as binomially-distributed traits in which values of 1 and 0 were assigned to success and failure, respectively.

significant correlation of residual FEC with weaning weight of the calf raised by these females, and larger values of calf age at weaning were correlated with higher incidence of shedding. Incidence of shedding was negatively correlated with birth weight of calves produced by these females.

# 4. Discussion

Means for FEC within breed groups were higher in October, 2016 and January and February of 2017, suggesting that the winter conditions were favorable for parasite infection. The effect of age as a source of this variation cannot be ruled out as heifers were 8–12 months of age in this time period, and no corresponding differences were noted one year later (20–24 months).

The differences between sub-species of cattle (*Bos indicus* and *Bos taurus*) are substantial across a spectrum of production and adaptation traits (Cartwright, 1980; Turner, 1980). These sub-species are quite different phenotypically although there is no barrier to reproduction between them. *Bos indicus* purebred and crossbred cattle have consistently demonstrated relatively superior adaptation to environments

categorized as harsh in a variety of ways (Prayaga et al., 2009; Riley et al., 2012, 2014a, 2014b, 2015). This adaptation and the heterosis expressed by *Bos indicus-Bos taurus* crossbreds for most important beef production traits are the two primary reasons that *Bos indicus-Bos taurus* crossbreds represent almost one third of the U.S. beef production herd. Small to moderate heritability values for resistance to GIN in cattle have been reported (see Table 1 of May et al., 2017; essentially ranged from 0.04 to 0.36, but a single high estimate of 0.78 in dairy cattle was included in that summary).

Results from the present study between  $\frac{1}{4}$  and  $\frac{1}{2}$  Nellore (a small difference in *Bos indicus* DNA) heifers are consistent with many comparisons among cattle more widely divergent in proportion of *Bos indicus* makeup, for example between straight Angus and Nellore or Brahman. The generally higher FEC in samples in the present study from heifers with a greater proportion of *Bos indicus* (i.e.,  $\frac{1}{2}$  *Bos indicus* heifers) is consistent with South American results indicating greater susceptibility of 100 % Bos indicus (Nellore) relative to 50 % Bos indicus (Suarez et al., 1990; Oliveira et al., 2009) and of  $\frac{3}{8}$  Bos indicus to 100 % Bos taurus (Suarez et al., 1995). O'Kelly (1980) reported minimal differences in FEC among 50 % Bos indicus and 100 % Bos taurus cattle, suggesting that resistance to infection was not different, however, anthelminthic treatment resulted in greater improvements in weight change in the 50 % Bos indicus cattle, suggesting that these cattle were more susceptible to the detrimental effects of infection.

Alternatively, Peña et al. (2000) reported essentially the opposite outcome, where FEC in a <sup>3</sup>/<sub>4</sub> Bos indicus composite breed (Brangus) were lower relative to 100 % Bos taurus (Angus) during a single season in the southeastern United States. Although Nellore is considered to be one of the primary ancestor breeds of Brahman (Sanders, 1980), the source of Bos indicus differs between the work of Peña et al. (2000) and the present study. Australian investigators reported no influence of breed on such traits (Barger et al., 1983). Differential FEC of GIN were detected for different Holstein genetic lines (May et al., 2017). Selection (especially natural selection) for parasite resistance could be responsible for differing results.

These contradicting results may represent environmental specific breed superiority as genotype-environment interactions. O'Kelly (1980) suggested that effects of parasite burden may be masked or exacerbated by concomitant differential response to environmental stressors. In the present study, the presence of *Bos indicus* influence in both groups may have resulted in similar resilience to the production environment while resulting in differential resistance to parasites.

Clarification of these interactions would make breed selection a potentially straightforward GIN management strategy. Heterosis may be important for resistance to or tolerance of GIN (Oliveira et al., 2009). Although this genetic effect could not be characterized in the context of this work, in general, expression of heterosis is proportional to breed heterozygosity (Dickerson, 1973). The expectation for these two groups of females would be approximately the same (0.5) for proportion of heterozygous loci, and therefore for heterosis expression (as a proportion of maximal which would be expressed by first cross animals, that is, the  $F_1$ ). Heterosis is not necessarily responsible for differences in FEC; breed-specific inheritance of susceptibility was observed in advanced generations of crosses of susceptible and resistant sheep breeds (Miller et al., 2006). There remains a need to fully genetically characterize these traits in *Bos indicus* purebreds and crossbreds in production environments.

The positive relationship of residuals indicates higher FEC associated with greater amounts of winter coat present on the cow at two times. In April of 2018, most females were early in their first lactation. This month is when the most productive females achieve lower winter coat shedding scores, indicating a greater degree of winter coat shed (Gray et al., 2011). In July, minimal coat score differences were earlier noted (Riley et al., 2015); however, winter coat regrowth has regularly been observed in that summer month in that research herd of purebred Angus (unpublished results). The significant correlations of average

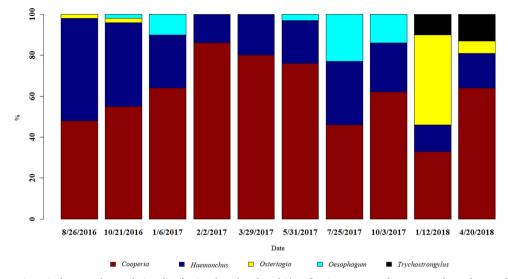


Fig. 1. Gastrointestinal nematode population distribution, based on larval identification to genus, from coprocultures by sampling date.

residual FEC with residual calf weaning weight and FEC incidence with calf weaning age could be indications of higher FEC or incidence corresponding with the stress of heavier or longer lactation or the negative energy balance that females, especially those in their first lactation, have at that time. No explanation seemed apparent for the negative relationship of FEC incidence with calf birth weight.

Cooperia and Haemonchus GIN comprised most of the coprocultures across the sampling times. Ostertagia was evidenced at more advanced ages of heifers. Early (ca. 1980) unpublished work in Central and South Texas suggested that Brahman (Bos indicus) harbored greater numbers of Ostertagia GIN than Bos taurus (unpublished results). Peña et al. (2000) reported higher mean percentage of Ostertagia and lower Cooperia percentage in Brangus cows across sampling times than in Angus mature cows. Otherwise, there were no across-season breed differences in numbers of GIN by species for mature cows, yearling heifers, or calves in that work; however, in a small number of sampling times, inconsistent breed differences were detected in one or more of the three groups (Peña et al., 2000). Purebred and crossbred (with Angus) Nellore heifers were evaluated from 9 months of age for 14 consecutive months in São Paulo state, Brazil, and it was concluded that crossbred heifers were more resistant than purebred Nellore to GIN (Oliveira et al., 2009). Results from that study also indicated higher proportions of Cooperia in coprocultures of the crossbred females.

Species population changes of GIN in cattle are seasonal (Craig, 1979; Peña et al., 2000). Levels of *Ostertagia* were highest in late autumn and winter; *Cooperia* and *Haemonchus* were most numerous in summer and early fall (Craig, 1979; Peña et al., 2000). *Ostertagia ostertagi* appear to be transmitted more in times corresponding to Texas winter and spring (Craig, 1979). Coprocultures or other assessment of GIN populations should be carried out in cattle sub-species groups in this environment to best develop strategies for control.

# 5. Conclusion

This longitudinal characterization of GIN infection of growing beef heifers on Central Texas pastures suggests differential infection by breed group; breed group differences are based upon a small difference in proportion *Bos indicus* (Nellore) and Angus. The differences between *Bos indicus* and *Bos taurus* may be large; those have not been characterized.

# **Declaration of Competing Interest**

None.

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