**Prevalence of anthelmintic resistance on sheep and goat farms in the mid-Atlantic
region and comparison of *in vivo* and *in vitro* detection methods**

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Abstract

Despite strong economic opportunities and incentives for small ruminant production, their health and productivity are often severely affected by parasitic disease. To combat these effects, most farms administer anthelmintics to their animals at frequent intervals, and without consideration to principles of sustainable integrated parasite management (SIPM). This has led to growing problems caused by the development of drug-resistant populations of gastrointestinal nematodes (GIN) in much of the world, particularly in *Haemonchus contortus*. The objectives of this research were to characterize levels of anthelmintic resistance on small ruminant farms located in the mid-Atlantic US and to compare the fecal egg count reduction test (FECRT) and larval development assay (LDA) for detecting resistance. To achieve these objectives, the DrenchRite[®] LDA was used to evaluate resistance status to benzimidazoles, ivermectin, moxidectin, and levamisole on 20 goat and 14 sheep farms in the Mid-Atlantic US over a 2-year period. A FECRT was also conducted on 14 of the same farms and on 2 additional farms in which the LDA was not completed. For the LDA and coprocultures, fecal samples were collected rectally from a minimum of 10 individual animals, pooled, and express-mailed to the University of Georgia for analysis. For the FECRT, albendazole, ivermectin, moxidectin, and/or levamisole were tested on each farm. Animals were allocated randomly based on FAMACHA[®] scores to 2-5 treatment groups, which included an untreated control group. The number of treatment groups on a farm depended on the number of qualified animals present. *Haemonchus contortus* was the most common parasite recovered from fecal cultures; the mean level across all farms was 79%. Results of the LDA indicated resistance to benzimidazoles, ivermectin, moxidectin, and levamisole on 100%, 82%, 47%, and 24% of farms, respectively. Multi-drug resistance to all 3 drug classes was detected for *H. contortus* on 18% of farms (1 sheep and 5 goat farms). Of the

16 farms tested by FECRT, resistance to albendazole was present on 8/10 farms, to ivermectin on 4/4 farms, to moxidectin on 7/9 farms and to levamisole on 2/5 farms tested. Results obtained from the FECRT and the LDA ($p = 0.51$) were similar. The prevalence of resistance found in this study in the mid-Atlantic region of the US is very similar to that reported in an earlier survey of resistance performed in the Southern US, demonstrating that anthelmintic resistance in GIN is a serious problem on small ruminant farms throughout the Eastern US.

Key Words: anthelmintic resistance, FECRT, gastrointestinal nematode, goats, larval developmental assay, sheep

1. Introduction

A recent wave of immigration to the US has placed a new demand for small ruminant meat and other products (McLean-Meyinsse, 2003). Many of these immigrants are from countries that typically consume goat and sheep meat. This has created a new niche market for the small ruminant industry where the demand far exceeds the supply (Knight et al., 2006). Thus, there are excellent opportunities for profitable production of small ruminants. However, infection with gastrointestinal nematode (GIN) parasites, particularly *Haemonchus contortus*, provides a major challenge for efficient production. Traditionally, chemical anthelmintics, which include benzimidazoles, macrocyclic lactones, and nicotinic agonists have been used to treat infections with GIN. However, decades of over and misuse of these drugs has led to the development of anthelmintic resistance, which is now a global problem (Kaplan, 2004; Kaplan and Vidyashankar, 2012).

Several methods are currently available for detecting resistance in GIN of small ruminants; the two most common being the fecal egg count reduction test (FECRT) and larval development assay (LDA). With the FECRT, the effectiveness of an anthelmintic is determined by comparing the fecal egg counts (FEC) of animals both before and after treatment or by comparing the FEC of treated and untreated control groups. This test is suitable for field surveys, however it is very time-consuming and costly. In addition, on small farms there are often inadequate numbers of animals present to test multiple drugs in a single test, and sometimes even to test a single drug. The LDA (DrenchRite[®]) offers a diagnostic alternative to the laborious task of performing a FECRT, and can test for resistance to all anthelmintic groups in a single test without the requirement for large herd size.

Using both the FECRT and LDA, several studies conducted in the southeast region of the U.S. have reported a high prevalence of resistance to all three classes of commercially available anthelmintics (Zajac and Gipson, 2000; Terrill et al., 2001; Mortensen et al., 2003; Howell et al., 2008). However, there are no published data on the prevalence of anthelmintic resistance in the mid-Atlantic region. The mid-Atlantic region is more temperate and has a shorter transmission season, thus fewer treatments with anthelmintics are needed on an annual basis. It remains unknown however, if this translates to a reduced problem with regard to anthelmintic resistance. The objectives of this study were to characterize levels of anthelmintic resistance in GIN of small ruminants located in the mid-Atlantic US, while also comparing the FECRT and LDA procedures for detecting resistance in GIN parasites.

2. Materials and methods

2.1. Farm use criteria

Farms were selected based on the following criteria. All animals had to be raised predominately on pasture, be a minimum of three months of age, and could not have been treated with an anthelmintic for at least 8 weeks prior to testing (Coles et al., 1992). To qualify for testing with the LDA, a minimum of 10 animals that met all criteria were required, while for the FECRT, the farm had to have at least 40 animals that met the criteria.

2.2. Sampling procedures for FECRT, LDA and larval identification

Potential farms were identified and the FAMACHA[®] scoring system (Van Wyk and Bath, 2002; Kaplan et al., 2004b) was used to monitor levels of infection with *H. contortus*. When farmers reported that individual animal FAMACHA[®] scores within participating herds were predominantly ≥ 3 , the initial sampling took place. Producers not FAMACHA[®] certified were asked to send a preliminary representative fecal sample from the herd to Delaware State University (DSU) for FEC to confirm that ≥ 500 GIN eggs per gram were present.

Thirty-four small ruminant farms, 20 goat and 14 sheep, met the study criteria. The DrenchRite[®] LDA was used to evaluate resistance status to benzimidazoles, ivermectin, moxidectin, and levamisole on all 34 farms. In addition, a FECRT was conducted on 14 of the 34 farms and on 2 additional farms in which the LDA was not completed due to the presence of larvated eggs in submitted pooled samples. All goat farms raised Boer and Boer crossbreds, with the exception of one farm that raised Kiko. Sheep farms were more diverse, including Suffolk and Suffolk crosses (29%), Katahdin and Katahdin crosses (21%), Dorper and Dorper crosses (21%), and other miscellaneous breeds (Dorset, Polypay, Romney, Tunis; 29%).

Just prior to performing the FECRT, all animals were FAMACHA[®] scored by one of the investigators from DSU. Animals with FAMACHA[®] scores ≥ 3 were then allocated randomly to treatment groups. The number of treatment groups on each farm depended on the number of qualified animals available, and included one or more of the following: albendazole, ivermectin, moxidectin, and levamisole, plus an untreated control group. Anthelmintics tested were determined based on prior anthelmintic use on the specific farm and the number of animals available. If limited animals were present, the most frequently used anthelminthic was tested which was either albendazole or moxidectin. If two drugs were tested, then anthelmintics tested were albendazole and moxidectin and if three drugs were tested the third drug was ivermectin or

levamisole based on history of previous use. Anthelmintics were administered based on body weight measured at the time of treatment (DS-Livestock scale with digital unit, Frostburg, MD), at the label dose for sheep (7.5 mg/kg for albendazole, 0.2 mg/kg for ivermectin, 0.2 mg/kg for moxidectin, and 8 mg/kg for levamisole) and 2 times the label sheep dose for goats. The FECRT was performed according to methods described in the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Coles et al., 1992) utilizing the modified McMaster technique for FEC (Whitlock, 1948). Fecal samples (1-4 g) were collected directly from the rectum, using exam gloves with lubricating jelly, bagged individually, labeled, and placed on ice until brought to the laboratory. The post-treatment fecal sample for the FECRT were collected at 7 d after treatment for levamisole, 10 d after treatment for albendazole, 14 d after treatment for ivermectin, and 14-17 d after treatment for moxidectin (Coles et al., 1992). For situations in which multiple anthelmintics were tested and levamisole was not included, the final sample was taken at 10-14 d post treatment, and when moxidectin was used, the final sampling occurred 14 d post treatment.

Sub-samples of feces collected pre-treatment in the FECRT were pooled prior to shipment to UGA for LDA and coproculture. For farms where the FECRT was not performed, fecal samples were collected rectally from a minimum of 10 animals for LDA and coproculture analysis. In both instances, fecal samples were placed into vacuum sealable, quart size commercially available vacuum bags (Reynolds[®] Handi-Vac), air was removed from the bags and samples were stored and shipped at room temperature to the University of Georgia (UGA). In cases where the distance to the farm from DSU was too great for DSU personnel to do sampling, or where DSU personnel were not available for sampling, producers known to be competent in fecal collection submitted fecal samples directly to UGA for LDA. Producers were

advised to collect a pooled sample in a labeled zippered bag from at least 10 animals with FAMACHA scores ≥ 3 and evacuate all air prior to shipping overnight to UGA. All samples were received and processed by UGA within 72 hours after collection. Upon arrival at the UGA lab, feces were mixed well and then a FEC was performed.

LDA analysis and resistance determination

Samples were weighed when received at the laboratory, fecal pellets were crushed, and an equivalent volume of water was added to create a fecal slurry. A FEC was then performed on 4 g of the slurry using the modified McMaster technique (Whitlock, 1948). A volume of slurry needed to obtain 50,000 eggs was then used for egg isolation. If the FEC was too low to meet this target, the total available sample was used.

Coprocultures were prepared from each sample with 20 to 30 g of slurry and an approximately equal volume of vermiculite. Cultures were incubated for 12 days $\pm$ 1 day at room temperature, and infective third-stage larvae (L_3) were recovered using the Baermann technique (Dinaburg, 1942) and identified to the genus level (Ministry of Agriculture, Fisheries and Food, 1977). One hundred L_3 larvae were identified, unless < 100 larvae were recovered, in which case all L_3 that were recovered were identified.

The DrenchRite LDA® was performed in accordance with manufacturer's directions (Horizon Technology, 1996) with modifications. Nematode eggs were concentrated by filtering the fecal slurry through a series of sieves (425 μm , 180 μm , 85 μm , and 30 μm pore sizes). Material retained in the 30- μm sieve was added to a sucrose gradient (Marquardt, 1961) and centrifuged at $1300 \times g$ for 7 minutes at 4°C with slow acceleration and deceleration. The egg

layer was retrieved from the sucrose gradient and rinsed well over a 30 μm sieve with deionized water to remove sucrose residue. The eggs were then rinsed into a 15 ml centrifuge tube. The volume of water was then adjusted to yield a final concentration of approximately 3.5 eggs/ μL , and amphotericin B was added (90 $\mu\text{L}/\text{mL}$).

Ninety-six well plates consisting of 8 rows with 12 wells in each row were used for the LDA. The first well in each row contained only agar and served as control wells. Subsequent wells in each row contained anthelmintic (thiabendazole, levamisole, or ivermectin aglycone) in agar, with each well having twice the concentration of the preceding well. Prior to setup, assay plates were removed from the refrigerator, warmed to room temperature, and 20 μL of deionized water was added to each well to ensure adequate moisture content on the agar surface. Twenty microliters of the egg suspension was then added to all wells on the plate. Plates were sealed with a laboratory film (Parafilm, Pechiney Plastic Packaging, Menasha, WI) to prevent drying and placed in a 25°C humidifying incubator. Following a 24 hr. incubation, which permitted the hatching of eggs, nutritive media supplied with the DrenchRite LDA® plates was diluted 50% with deionized water, and 20 μL was added to each well. Plates were again sealed and returned to the incubator for 6 more days. Plates were examined every 2 to 3 days to ensure that a thin layer of moisture remained on the agar surface, and 20 μL of deionized water was added to any wells that appeared dry. Assays were terminated after 7 days by adding 20 μL of 50% Lugols iodine to each well. Larvae were then transferred to a clean, flat-bottom, 96-well plate for counting and identification (Tandon and Kaplan, 2004) with an inverted compound microscope.

Number of eggs, L₁, L₂ and L₃ larvae were counted in each well at 100× magnification and identified to the genus level (Ministry of Agriculture, Fisheries and Food, 1977). Numbers of L₃ vs. Eggs/L₁/L₂ in each well containing drug were compared to control wells to determine

the critical well, defined as the well in which 50% of the larvae were inhibited from developing to the L₃ stage. Critical wells correspond closely to calculated EC₅₀ values (Kaplan et al., 2007), and can be similarly used to make inferences on the resistance status of the worm population for a given farm. In addition, for ivermectin, plates were evaluated to determine the 5% discriminating concentration (syn. delineating dose), defined as the well number where $\geq 5\%$ of larvae developed to the L₃ stage. This well corresponds to the calculated EC₉₅ value (Tandon and Kaplan, 2004).

A table supplied by the manufacturer in the DrenchRite LDA User Guide provides an expected level of FECR for each critical well for benzimidazoles and levamisole; comparisons of these values were then used to determine the anthelmintic resistance status for these drugs on each farm. Based on the DrenchRite User Manual for benzimidazoles and levamisole with *Haemonchus contortus*, critical wells that corresponded to FECR $\geq 95\%$ were classified as susceptible, suspected resistant if estimated fecal egg count reduction was 90% to 94%, and resistant if fecal egg count reduction was $< 89\%$ (Table 3). However, because of the inherent variability associated with bioassays such as the LDA, we then added an additional 0.5 critical well value to the critical well value used as the cutoff threshold (equivalent to a half well or a 100% increase in the anthelmintic concentration), so that estimates of resistant status were conservative as to not overestimate the prevalence of resistance. In this manner, borderline resistant results would be given the benefit of the doubt and called susceptible (Table 3).

The DrenchRite Users Guide does not provide a table for interpreting LDA results for ivermectin, other than the critical well value obtained for a standard drug-susceptible isolate. We therefore used criteria established in a previous study where we performed both DrenchRite LDA and FECRT (Mortensen et al., 2003; Table 3).

Data from ivermectin wells were also used to make inferences on susceptibility/resistance of *H. contortus* to moxidectin, as reported by Kaplan et al., 2007 (Table 3). In this study, all farms categorized as low resistant or resistant were classified as having *H. contortus* that were resistant to moxidectin for purposes of calculating resistance prevalence.

2.3. Statistical analysis

As per the WAAVP guidelines (Coles et al., 1992), anthelmintic resistance was calculated as $FECR\% = 100(1 - (X_t/X_c))$ with X_t and X_c being the post FEC arithmetic mean in the anthelmintic treatment (t) and control groups (c), respectively. If the FECR percentage of an anthelmintic treatment was less than 95% and the lower 95% confidence limit for the reduction was less than 90%, the GIN were considered resistant (Coles et al., 1992).

For the LDA, percentages of farms with *H. contortus* resistant to benzimidazole, levamisole, ivermectin, and moxidectin were calculated, and 95% CIs for the critical wells were determined. Unpaired *t* tests were used to compare critical well values for thiabendazole, levamisole, and ivermectin and discriminating concentration values for ivermectin between sheep and goat farms. The Fisher's Exact Test χ^2 was used to compare resistance status of *H. contortus* to benzimidazole, levamisole, and ivermectin between sheep and goat farms. All analyses were performed with Graph Pad Prism 5.0 (GraphPad Software, San Diego, CA). For all analyses, $p < 0.05$ were considered significant.

3. Results

3.1. Larval ID

Haemonchus contortus was the most common species recovered from coprocultures on 79% of farms (27 of 34) tested in this study. This species was predominant on farms in DE (80% of farms), MD (90% of farms), VA (75% of farms), WV (75% of farms), and PA (60% of farms). On 31 farms some larvae were identified as *Trichostrongylus* and/or *Teladorsagia* however, it is extremely difficult to differentiate these two species. Given this and the fact that the dose response in the LDA differs for these two species, it is not possible to make reliable determinations of resistance status; therefore resistance status was not determined for these species. Other species present in smaller numbers and also not evaluated for resistance included *Oesophagostomum* spp. and *Nematodirus* spp.

3.2. FECRT

Overall, GIN were resistant to albendazole on 8 out of 10 farms tested (80%), including 5 out of 7 goat farms and all 3 sheep farms (Tables 1 and 2). Ivermectin was ineffective on all sheep and goat farms tested (4 of 4; 100%). The related drug, moxidectin, was ineffective on 7 out of 9 farms tested (78%), including 3 out of 4 goat farms and 4 out of 5 sheep farms. However, levamisole was ineffective on only 2 out of 5 farms tested (40%), including 1 out of 2 goat farms and 1 out of 3 sheep farms.

3.3. LDA

Anthelmintic resistance status for *H. contortus* was determined if L₃ present in the control wells of the LDA plate were at least 20% of the total, and this criteria was met on all 34 farms.

Haemonchus contortus were classified as resistant to benzimidazoles on all 34 farms (Table 4), and mean drug concentrations for the critical well ($P = 0.4468$; Figure 1) were not significantly different between sheep and goat farms.

Haemonchus contortus on 28 farms (82%; 9 of 14 sheep and 19/20 goat) were classified as resistant to ivermectin (Table 4). Mean drug concentrations for ivermectin were significantly different between sheep and goat farms ($P = 0.0452$; Figure 1), and percentages of farms with *H. contortus* resistant to ivermectin were significantly different between sheep and goat farms ($P = 0.0084$; Table 4).

Haemonchus contortus on 8 farms (24%; 2 of 14 sheep and 6 of 20 goat) were classified as resistant to levamisole (Table 4). Both mean drug concentrations for the critical well ($P = 0.7311$; Figure 1), and percentages of farms with *H. contortus* resistant to levamisole ($P = 0.4083$) were not significantly different between sheep and goat farms. Multi-drug resistance to all 3 drug classes (benzimidazole, levamisole, and ivermectin) were detected for *H. contortus* on 18% of farms (1 sheep and 5 goat farms).

Haemonchus contortus on 16 farms (47%; 5 of 14 sheep and 11 of 20 goat), were classified as resistant to moxidectin based on both the critical well (EC₅₀) dose concentration and the discriminating concentration (EC₉₅; Table 4). Discriminating concentration values were significantly different ($P = 0.0143$) between sheep and goat farms (Figure 1), but percentages of farms with *H. contortus* resistant to moxidectin did not differ significantly ($P = 0.3151$) between

sheep and goat farms. Total anthelmintic failure to all three classes of commercially available anthelmintics plus moxidectin was present on 1 sheep and 3 goat farms (12%).

3.4. FECRT and LDA comparison

Results of the LDA and the FECRT were in agreement for 12 of 14 farms where both types of data were collected. On 2 goat farms (DE01G and MD03G), the LDA showed resistance to benzimidazoles, whereas the FECRT demonstrated susceptibility to albendazole (Tables 5 and 6). In the LDA, the critical well cutoff for resistance to benzimidazole was 6 (Table 3), and both farms had critical well values above this indicating resistance to this class (11.5 and 9 for DE01G and MD03G farms, respectively). One of the farms tested by the FECRT (DE01G) marginally made the susceptible cutoff being only 1% above the cutoff for both efficacy and lower 95% CI (Table 1). It should be noted that other less potent benzimidazoles were not tested, so it is very possible that resistance was present, but not expressed fully with albendazole treatment. However, Chi square analysis revealed that there was no significant difference between results obtained from the FECRT and the LDA ($P = 0.51$).

4. Discussion

This is the first extensive assessment of the prevalence of anthelmintic resistance on small ruminant farms in the mid-Atlantic region. In the current study, the LDA revealed resistance to anthelmintics in *H. contortus* on 100%, 82%, 47%, and 24% of farms to benzimidazole, ivermectin, moxidectin, and levamisole, respectively. Similar studies to

characterize anthelmintic resistance have also been conducted in the southeast US with resistance reported to all three major anthelmintic classes (Mortensen et al., 2003; Howell et al., 2008). In the more recent study, Howell et al. (2008) found *H. contortus* resistance to benzimidazole, ivermectin, moxidectin, and levamisole on 98%, 76%, 24%, and 54% of farms, respectively. Comparing the mean anthelmintic resistance prevalence and critical well values of both these studies suggests several things. The present study found similar results for benzimidazole resistance prevalence in both sheep and goats compared to the southeast study (Howell et al., 2008). However the critical well means were higher in both species in the current mid-Atlantic study. The overall prevalence and critical well means for levamisole resistance in the mid-Atlantic region were lower than that reported previously by Howell et al. (2008) for both species. This lower prevalence is most likely due to limited use of levamisole in this region. The prevalence of ivermectin resistance was also similar between the two studies, but was higher in sheep in the current study (64% and 37% for mid-Atlantic and southeast studies, respectively). Also, the critical well means were higher in both species in the current study. Moxidectin resistance and critical well means were higher in both species compared to that previously reported in the southeast (Howell et al., 2008). Due to the mid-Atlantic region being more temperate and having a shorter transmission season, it was expected that fewer treatments with anthelmintics were needed on an annual basis and that there would be a lower incidence of anthelmintic resistance reported. However, these comparisons not only suggest a strong trend toward higher prevalence of anthelmintic resistance in the mid-Atlantic US, but also suggest higher levels of resistance in all drugs tested with the exception of levamisole.

Multiple anthelmintic resistance was reported on 18% of farms tested in this study (1 sheep and 5 goat farms) and total anthelmintic failure was detected on 1 sheep farm and 3 goat

farms (12%) to all three classes of commercially available anthelmintics, plus moxidectin. Howell and colleagues (2008) reported that 48% and 17% of farms tested in the southeast US were resistant to three classes and had total anthelmintic failure, respectively (including moxidectin; Howell et al., 2008). Though the percent of farms in the current study with resistance to all three classes and with total anthelmintic failure were lower than in the earlier study, these data indicate that the problem is widespread and probably is worsening. Relevant to this problem is the fact that few new anthelmintic drug classes have been developed in the past few decades and currently none of these are approved for use in sheep or goats in the United States. Monepantel (Zolvix®, Elanco, Greenfield, Indiana) represents a new class of anthelmintics known as amino acetonitrile derivatives, which has shown high efficacy against multiple-resistant strains of parasites and is sold widely throughout the world. However this drug has not yet been approved by the FDA, and it is not clear when or even if this drug will become available in the US. Alarming, several cases of monepantel resistance have already been reported on both sheep and goat farms on three different continents (Mederos et al., 2014; Scott et al., 2013; Van den Brom et al., 2015). Given this and recent past history, it is highly likely that the development of anthelmintic resistance will continue to outpace the development of new drugs.

This study utilized the two most common methods available for detecting resistance in GIN of small ruminants, namely the FECRT and the LDA, and compared the results among 14 farms. Despite differing results between the two tests with regard to albendazole resistance on two goat farms, there was no significant difference between the results reported for the FECRT and LDA in the present study. On these farms, the LDA showed resistance for benzimidazoles, whereas the FECRT demonstrated susceptibility to albendazole. However, one of these goat

353 farms tested (MD03G) had one of the lowest critical well values (9) in this study and a high
354 FECR% (98% (94 – 100)). On the other hand, the second farm (DE01G) had a high critical well
355 value (11.5) but marginally made the susceptible cutoff by being only 1% above the cutoff for
356 both efficacy and lower 95% CI according to the FECRT (Table 1; 96% (91-98). An important
357 note is that the drug analogs used in the LDA are not the same drug analogs used in the actual
358 dewormers. These specific analogs were selected based upon previously published research
359 demonstrating that these analogs perform the best in this in vitro assay. In addition, even though
360 the LDA tests for benzimidazole resistance, more potent drugs could still work on resistant
361 worms when resistance level is not too high. This provides an explanation as to why albendazole
362 was more effective on farm MD03G than on farm DE01G that had a higher critical well value
363 for resistance. Previous studies have only reported conflicting results for LEV resistance
364 between the FECRT and LDA (Maingi et al., 1998), however, the data from the present study
365 indicates that results for the two tests are comparable. Therefore, small ruminant producers can
366 use either of these methods to determine drug efficacy depending on their farm situation and the
367 resources available to them. Fecal egg count reduction tests are more suitable for field surveys,
368 but can be extremely time-consuming and costly to the producer. Also, this method is not an
369 option on small farms due to inadequate numbers of animals present to test multiple drugs in a
370 single test, and sometimes even to test a single drug. On the other hand, although costly and only
371 performed in one lab in the US (UGA), the LDA (DrenchRite[®]) offers a diagnostic alternative to
372 the laborious task of performing a FECRT, and can test for resistance to all anthelmintic groups
373 in a single test without the requirement for large herd size (10 animals minimum). Regardless of
374 what method is used, it is recommended that all small ruminant producers know the resistance

status on their farm in order to effectively utilize strategic deworming and sustainable integrated parasite management (SIPM) strategies on farm.

5. Conclusion

Based on the results of this study, anthelmintic resistance in GIN parasites of small ruminants (particularly *H. contortus*) is a common and important problem in the mid-Atlantic region. These data when viewed together with other recent studies performed in the southern US provide strong evidence that anthelmintic resistance has reached critical levels throughout the entire Eastern United States. Eighty two percent of all farms tested in this study had multiple-resistance to at least two different drug classes. An additional concern is our finding that 12% of farms (1 sheep and 3 goat farms) demonstrated total anthelmintic failure, where resistance was detected to all available anthelmintics. Many factors are responsible for this situation, but those factors of greatest importance likely are the indiscriminate and overuse of anthelmintics and frequent movement and transfer of animals across the Eastern United States. Given this situation, traditional drug-based strategies for parasite control in small ruminants are no longer a viable approach. Rather, sustainable integrated parasite management (SIPM) must become the new paradigm, where anthelmintics are used much less frequently and in a more targeted and strategic manner following the principles of ‘Smart Drenching,’ together with a variety of non-drug-based practices (van Wyk et al., 2006). This will require that testing be done on every sheep and goat farm to determine which drugs are still effective, and that veterinary advice is sought, so that a rational and sustainable plan can be developed.

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Mention of trade names or commercial products in this manuscript is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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Table 1: Results of FECRT on goat farms in DE, MD, WV, and PA.

Fecal egg counts reported in eggs per gram (EPG) and fecal egg count reduction percentages (FECR%) on DE, MD, WV and PA goat farms tested for albendazole (ALB) , ivermectin (IVM), moxidectin (MOX), and/or levamisole (LEV) resistance. Treatment groups included an untreated control group (CON). Anthelmintics were considered effective if the FECR% was ≥ 95 and the lower limit of the 95% confidence interval was ≥ 90 . Bold indicates that the anthelmintic was effective.

Farm ID	Anthelmintic Treatment	Mean Post Treatment – EPG	FECR% (95% CI)
DE01G	CON	953	-
	ALB	35	96 (91-98)
	MOX	0	100 (100)
DE02G	CON	687	-
	IVM	902	-31 (-257-52)
	LEV	8	99 (95-100)
DE03G	CON	565	-
	MOX	88	84 (39-96)
MD01G	CON	1339	-
	MOX	250	81 (54-92)
MD02G	CON	1460	-
	ALB	447	69 (-17-92)
	LEV	231	84 (4.6-97)
MD03G	CON	1333	-
	ALB	23	98 (94-100)
WV01G	CON	467	-
	ALB	181	61 (12-83)
	IVM	681	-46 (-207-31)
	MOX	323	31 (-68-72)
WV02G	CON	1972	-
	ALB	265	87 (74-93)
WV03G	CON	2213	-
	ALB	1416	31 (-36-65)
PA01G	CON	675	-
	ALB	13	98 (79-100)

Table 2: Results of FECRT on sheep farms in MD, VA, and WV.

Fecal egg counts reported in eggs per gram (EPG) and fecal egg count reduction percentages (FECR%) on MD, VA, and WV sheep farms tested for benzimidazole (BZ), ivermectin (IVM), moxidectin (MOX), and levamisole (LEV) resistance. Treatment groups included an untreated control group (CON). Anthelmintics were considered effective if the FECR% was ≥ 95 and the lower limit of the 95% confidence interval was ≥ 90 . Bold indicates that the anthelmintic was effective.

State	Anthelmintic Treatment	Mean Post Treatment – EPG	FECR% (95% CI)
MD01S	CON	3785	-
	MOX	10	100 (99-100)
MD02S	CON	1631	-
	IVM	235	86 (43-96)
	MOX	542	67 (-40-92)
	LEV	119	93 (73-98)
VA01S	CON	6034	-
	ALB	1801	70 (38-86)
	MOX	5943	1.5 (-116-55)
	LEV	27	100 (99-100)
VA02S	CON	2217	-
	ALB	473	79 (-11-96)
	IVM	1490	33 (-199-85)
	MOX	579	74 (21-91)
	LEV	0	100 (100)
VA03S	CON	9521	-
	MOX	2414	75 (-28-95)
WV01S	CON	570	-
	ALB	733	22 (-380-87)

Table 3. Fecal egg count reduction (FECR), critical well (CW) and associated drug concentrations cutoffs used in determining resistance to benzimidazole (BZ), ivermectin (IVM), moxidectin (MOX) and levamisole (LEV; DrenchRite® User Manual Table 1).

CW means and drug concentrations			
	Susceptible FECR $\geq$ 95%	Suspected (Low) Resistant FECR 90 – 94%	Resistant FECR $<$ 89%
BZ	≤ 5.0 $\leq 0.156 \mu\text{g/ml}$	5.5 0.233 $\mu\text{g/ml}$	≥ 6.0 $\geq 0.3125 \mu\text{g/ml}$
LEV	≤ 4.0 $\leq 0.78 \mu\text{g/ml}$	4.5 1.17 $\mu\text{g/ml}$	≥ 5.0 $\geq 1.56 \mu\text{g/ml}$
IVM	≤ 4.5 $\leq 5.85 \text{ng/ml}$	5.0 7.8 ng/ml	≥ 5.5 $\geq 11.74 \text{ng/ml}$
MOX	$\leq 7.0^a$ $\leq 31.25 \text{ng/ml}^a$ $\leq 10.0^b$ $\leq 250 \text{ng/ml}^b$	7.5 - 9 ^a 46.90 ^a - 125 ng/ml^a 10.5 - 11 ^b 375.17 ^b - 500 ng/ml^b	$\geq 9.5^a$ $\geq 188 \text{ng/ml}^a$ $\geq 11.5^b$ $\geq 750 \text{ng/ml}^b$

^a MOX EC50

^b MOX EC95

For MOX, resistance status was classified as susceptible if the critical well value was ≤ 7.0 and the well corresponding to the 5% discriminating concentration value was ≤ 10.0 , as low resistant if the critical well value was 7.5 to 9.0 and the 5% discriminating concentration value was 10.5 to 11.0, and as resistant if the critical well value was ≥ 9.5 and the 5% discriminating concentration value was ≥ 11.5 . Both criteria had to be met to assign a resistance status. If critical well and discriminating concentration values did not match the same resistance status category, resistance status of the farm was considered to be between the 2 categories.

528 Table 4. Summary of resistance status for all farms tested for *Haemonchus contortus* (Hc) based on results of the DrenchRite®
529 Larval Development Assay (LDA).

<i>H. Contortus</i>				
Total Farms (34)	BZ	LEV	IVM	MOX ^a
Susceptible	0	21	5	18
Suspected (Low) Resistance	0	5	1	6
Resistant	34	8	28	10
% Resistant	(100)	(24)	(82)	(47)
Goats (20)				
Susceptible	0	11	0	9
Suspected (Low) Resistance	0	3	1	4
Resistant	20	6	19	7
% Resistant	(100)	(30)	(95)	(55)
Sheep (14)				
Susceptible	0	10	5	9
Suspected (Low) Resistance	0	2	0	2
Resistant	14	2	9	3
% Resistant	(100)	(14)	(64)	(36)

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531 ^a Farms that fell between Susceptible and Low Resistant categories for moxidectin are included in the Suspected
532 Resistant category while farms that fell between Low Resistant and Resistant categories for moxidectin are included
533 in the Low Resistant category.

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544 Table 5. Comparison of larval development assay (LDA) and fecal egg count reduction test (FECRT) results on goat farms
545 tested for benzimidazole (BZ), ivermectin (IVM), moxidectin (MOX), and levamisole (LEV) resistance. Results were reported
546 resistant (R), susceptible (S), low resistance (LR), and suspected resistance (SR).

547

State	Anthelmintic Treatment	LDA Results	FECR% (95% CI)
DE01G	CON	-	-
	ALB*	R	96 (91-98)
	MOX	S	100 (100)
DE02G	CON	-	-
	IVM	R	-31 (-257-52)
	LEV	S	99 (95-100)
DE03G	CON	-	-
	MOX	LR	84 (39-96)
MD01G	CON	-	-
	MOX	LR	81 (54-92)
MD03G	CON	-	-
	ALB*	R	98 (94-100)
WV01G	CON	-	-
	ALB	R	61 (12-83)
	IVM	R	-46 (-207-31)
	MOX	R	31 (-68-72)
WV02G	CON	-	-
	ALB	R	87 (74-93)
WV03G	CON	-	-
	ALB	R	31 (-36-65)

548 *There was disagreement between LDA and FECRT results.

Table 6. Comparison of larval development assay (LDA) and fecal egg count reduction test (FECRT) results on sheep farms tested for benzimidazole (BZ), ivermectin (IVM), moxidectin (MOX), and levamisole (LEV) resistance. Results were reported resistant (R), susceptible (S), low resistance (LR), and suspected resistance (SR).

State	Anthelmintic Treatment	LDA Results	FECR% (95% CI)
MD01S	CON	-	-
	MOX	S	100 (99-100)
MD02S	CON	-	-
	IVM	R	86 (43-96)
	MOX	R	67 (-40-92)
	LEV	SR	93 (73-98)
VA01S	CON	-	-
	ALB	R	70 (38-86)
	MOX	R	1.5 (-116-55)
	LEV	S	100 (99-100)
VA02S	CON	-	-
	ALB	R	79 (-11-96)
	IVM	R	33 (-199-85)
	MOX	LR	74 (21-91)
	LEV	S	100 (100)
VA03S	CON	-	-
	MOX	R	75 (-28-95)
WV01S	CON	-	-
	ALB	R	22 (-380-87)

Figure 1 – Scatterplots of approximate EC_{50} values derived from critical wells for *H. contortus* on goat and sheep farms (34) for benzimidazole (BZ), levamisole (LEV), ivermectin (IVM) and moxidectin (MOX). The solid line represents the cutoff for classifying a farm as susceptible and the dashed line represents the cutoff for classifying a farm as resistant. Farms with values in between the 2 lines are classified as suspected resistant. The dotted line for IVM represents the 1st parameter for evaluating MOX resistance and approximates the EC_{50} value. The solid line on the MOX graph represents the cutoff for susceptible farms using the IVM EC_{95} value, which is the 2nd parameter for evaluation of MOX resistance. Values in between the solid line and the dotted line demonstrated low resistance to MOX and farms on or above the dashed line were resistant to MOX. Horizontal bars represent the mean values for all farms.