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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.

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

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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 was 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., 2004) 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 anthelmintic 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 h after collection. Upon arrival at the UGA lab, feces were mixed well and then a FEC was performed.

2.3. 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