INTRODUCTION

Anthelmintic resistance is defined as “a greater frequency of individuals within a population able to tolerate doses of a compound than in the normal population of the same species.” Anthelmintic resistance of gastrointestinal (GI) nematodes, particularly Haemonchus contortus, to commonly used anthelmintics has become a significant problem in small ruminants (sheep and goats) in parts of the United States and Worldwide. In the United States, the most commonly used anthelmintic classes in small ruminants have included macrocyclic lactones (ivermectin and moxidectin), benzimidazoles (fenbendazole and albendazole), and cholinergic agonists (levamisole and morantel). Resistance to all three classes of drug have been documented in small ruminants in the United States, and on some farms GI parasites have developed resistance to multiple products. These same drugs are frequently used in South American camels (llamas and alpacas) to treat endoparasitism, but until recently anthelmintic resistance was not documented. This presentation will document and outline the problem of haemonchosis in an alpaca herd and discuss case work-up and conclusions.

CASE PRESENTATION

In July and August of 2010, a series of 5 adult alpacas were presented to the University of Missouri, Veterinary Medical Teaching Hospital for severe anemia, hypoproteinemia and weight loss. Quantitative fecal egg counts revealed the presence of high numbers of strongyle-type eggs per gram of feces in 3 of the 5 animals. These findings in conjunction with anemia and hypoproteinemia led to a presumptive diagnosis of haemonchosis. All cases were treated with whole blood transfusions. Additionally, each case was treated with an anthelmintic at or shortly before admission to the hospital. Based on follow-up fecal egg counts performed 6-13 days after hospital admission it appeared that the parasites may be resistant to fenbendazole (Panacur, Intervet-Schering Plough, Summit, New Jersey, USA) in 1 of the cases, whereas the fecal egg count had a >95% reduction in an animal treated with pyrantel (Anthelban, IVX Animal Health Inc., St. Joseph, Missouri, USA) and an animal treated with levamisole (Prohibit, Agri-Laboratories, St. Joseph, Missouri, USA). The remaining 2 animals had insufficient data to draw any preliminary conclusions about anthelmintic efficacy. These preliminary findings suggested that there might be anthelmintic resistance to fenbendazole in this herd.

To further investigate this suspicion, a herd investigation was conducted in September of 2010 to evaluate animal husbandry and efficacy of anthelmintics that were in common use on the farm.

HERD INVESTIGATION

The herd consisted of 28 female (age 2-17 years) and 13 male (age 1-10 years) Huacaya breed alpacas. Adult and yearling males were housed in a 1 acre lot. Females were housed in an adjacent approximately 1300 sq ft dirt lot. Each group was independently allowed part-time access to a 20-acre (8.1 ha) pasture that was shared with llamas (n = 6) and horses (n = 2). Pastures were predominantly tall fescue with some white clover and were visibly overgrazed. While confined to their lots, the alpacas had free-choice access to orchard grass hay, each in a single large round-bale feeder. In addition to hay, a custom grain mix consisting of 40% llama/alpaca pellets (Mazuri Llama Chews, PMI Nutrition International, St. Louis, Missouri, USA), 20% alfalfa pellets, 20% oats, 15% cracked corn, and 5% molasses was fed to all alpacas in the herd at approximately 0.45 kg per head per day along with 0.11 kg of calf manna (Manna Pro, Chesterfield, Missouri, USA) per head per day. Thin animals, lactating dams, and yearlings were additionally fed 0.68 kg of alfalfa hay per head per day.

The deworming protocol on the farm at the time of the investigation included monthly treatments with both injectable doramectin solution (Dectomax, Pfizer Animal Health, New York, New York, USA) and oral albendazole (Valbazen, Pfizer Animal Health, New York, New York, USA). This protocol had been implemented by the owner as a strategy to prevent meningeal worm infection in the herd. While both drugs were not labeled for use in alpacas, the owner reported using the label doses for cattle. During the investigation, fecal egg count reduction (FECR) testing was performed to determine whether anthelmintic resistance was present among the gastrointestinal nematode population in this herd. Given the preliminary evidence for fenbendazole resistance and frequent use of doramectin and albendazole, a fecal egg count reduction trial was constructed to test these drugs. Thirty alpacas (n = 10 per group) of various ages and sexes were systematically assigned to 1 of 3 treatment groups. An untreated control group was not included due to the small number of animals in the herd and the owner’s desire to treat as many animals as possible because of the recent severe clinical cases reported above. The majority of 11 animals not selected for the trial had been recently treated and were therefore disqualified from enrollment. The addition of a control group would have allowed us to interpret the FECR results in the context of naturally occurring changes in fecal egg count on the farm, but was not feasible as explained. Treatments were systematically assigned such that each of the treatments was applied to successive animals in turn until all treatments had been applied once and then the cycle was repeated. The order of systematic assignment were 1) doramectin (0.2 mg/kg, subcutaneously), 2) fenbendazole (10 mg/kg, PO) and 3) albendazole (10 mg/kg, PO). Animal weights were estimated based size, stature, and body condition score. Animals were restrained and monitored to ensure swallowing after oral medications had been administered. Animals were maintained in their regular pens after treatment. Fecal samples were collected per rectum from each animal immediately before and 10 days after treatment. Fecal samples were stored at 4°C (39°F) following collection and processed within 24 hours. Quantitative fecal egg counts (FEC) were performed on an individual animal basis using the centrifugation method described by Cebra et al. The
The detection limit of the test was 5 eggs per gram of feces. The person performing the fecal egg counts was blinded to treatment group assignments at the time of counting.

As part of the herd investigation, the 30 animals used to assess anthelmintic resistance were also evaluated for clinical signs of anemia based on conjunctival mucous membrane pallor. Conjunctival mucous membrane pallor was scored using the FAMACHA® chart, described for use in sheep and goats. Briefly, the ocular conjunctiva were scored on a 1-5 scale, whereby 1 of 5 was pink and 5 of 5 was white. FAMACHA® scoring was performed by a licensed veterinarian familiar with FAMACHA® guidelines and with prior experience applying scoring methods. Due to the systematic assignment to treatment groups, the individual performing the scores was not blinded to treatment group at the pre-treatment time point as the scorer was the veterinarian administering the anthelmintics. At the follow-up time point the scorer was not aware of group assignment.

Mean FECR was calculated within each group using the formula FECR% = 100 (1 - [T2/T1]), where T2 was post-treatment and T1 was pre-treatment arithmetic mean egg count per gram of feces. Nematodirus eggs were not included in the FEC calculations. The correlation between mucous membrane score and fecal egg count was evaluated using the Spearman Rank Order Correlation (P < 0.05). Resistance was defined as FECR <95% with the lower 95% confidence limit of <90%.

RESULTS

Two animals in the herd died (1 in the doramectin group and 1 in the fenbendazole group) prior to the 10-day follow up fecal egg count and were therefore removed from data analysis. Necropsies were performed on both animals. The animal in the doramectin group died following a severe degloving injury to its left hind leg. The necropsy report for the animal in the fenbendazole group revealed moderate numbers of Haemonchus contortus in compartment 3 of the stomach and tissue pallor consistent with anemia. The final diagnosis reported by the pathologist was haemonchosis. Mean FECR% met the criteria for resistance in all groups documenting resistance to all three anthelmintics (Table 1). Across all experimental groups, fecal egg count was not significantly correlated with mucous membrane pallor before treatment (Correlation coefficient = 0.25; P = 0.20). However, when considered across all experimental groups, fecal egg count was significantly positively correlated with mucous membrane pallor 10 days after treatment (Correlation coefficient = 0.45; P = 0.02) suggesting an association between anemia and increasing fecal egg count at that time.

<table>
<thead>
<tr>
<th>Group†</th>
<th>Mean ± SD nematode FEC pre-treatment (eggs/gram)</th>
<th>Mean ± SD nematode FEC 10 d later (eggs/gram)</th>
<th>Mean FECR% (95% CI)</th>
<th>Mean ± SD mucous membrane score pre-treatment</th>
<th>Mean ± SD mucous membrane score 10 d later</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doramectin 0.2 mg/kg SQ ( n = 9)</td>
<td>1104 ± 1018</td>
<td>2328 ± 5024</td>
<td>-111 (-377 to 156)</td>
<td>3.4 ± 1.2</td>
<td>2.8 ± 1.5</td>
</tr>
<tr>
<td>Fenbendazole 10 mg/kg PO ( n = 9)</td>
<td>1291 ± 1422</td>
<td>524 ± 833</td>
<td>59 (3 to 116)</td>
<td>3.9 ± 1.3</td>
<td>3.5 ± 1.0</td>
</tr>
</tbody>
</table>

†Label dosages for Cattle

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‡No significant difference in mean pre-treatment FEC between groups (One-way ANOVA, P = 0.80)

‡No significant difference in mean follow-up FEC between groups (One-way ANOVA, P = 0.35)

DISCUSSION

Although anthelmintic resistance in sheep and goats has been well documented in the literature, to the authors’ knowledge there is only one other report documenting anthelmintic resistance in South American camels. Gillespie, et al. evaluated the efficacy of anthelmintic usage in South American camelids (llamas and alpacas) in Georgia. The report documented resistance to several anthelmintics including ivermectin, fenbendazole, and moxidectin. However, no resistance was documented against the cholinergic agonist levamisole. Similar to Gillespie and co-workers report, data from the herd reported here documents anthelmintic resistance to an avermectin and two benzimidazoles. In the present report, none of the treatment groups achieved a >95% mean FECR and seven animals (Group 1, n = 2; Group 2, n = 4; Group 3, n = 1) actually had increases in fecal egg counts following treatment. When gastrointestinal nematodes develop resistance to one drug in a class of anthelmintics they may develop resistance to all drugs in that class as is documented here where resistance to both fenbendazole and albendazole was found. When resistance occurs, an alternate
class of anthelmintic to which the parasite population is susceptible must be utilized. In this herd, treatment of animals with high fecal egg counts was switched to the cholinergic agonist, pyrantel pamoate at 18 mg/kg, PO. Based on a discussion with the herd owners, the animals in this herd had not been previously treated with this drug and preliminary evidence from one of the clinical cases demonstrated a significant fecal egg count reduction (>95%). Levamisole, another cholinergic agonist, was similarly found to reduce fecal egg count by >95% in one of the clinical cases. However, continued use of levamisole in the herd was not possible because levamisole has currently been withdrawn from the animal health market in the United States. While the fecal egg count technique used could not differentiate between strongylo-type eggs, the presence of high strongyle-type egg counts in conjunction with evidence of anemia suggested the endoparasitism was primarily due to haemonchosis. In addition, post-mortem examination of one of the two animals that died revealed haemonchosis. The inconsistent association between FAMACHA scores and fecal egg count may reflect the fact that the strongylo-type eggs noted on fecal egg count represented parasites other than or in addition to Haemonchus contortus. Alternatively, FAMACHA scores may not be uniformly predictive of haemonchosis in South American camelds. Unfortunately, blood was not collected at the time of the investigation so that hematocrit could be correlated with FAMACHA scores and fecal egg count. Similarly, the actual species of strongyle was not determined in the present case due to limited financial resources of the herd owner. Hence, based on the limited data available here, the authors recommend caution when applying FAMACHA scoring to South American camelds until the appropriate studies have been conducted to evaluate the relationship between presence of Haemonchus contortus in feces, hematocrit, and mucus membrane pallor.

Animals in this herd were intensively managed with many animals per acre. In addition to anthelmintic resistance, opportunity for frequent re-infection was present due to high numbers of animals shedding large numbers of eggs leading to high numbers of parasite larvae on the limited pasture surface area that was visibly overgrazed. It was recommended that the numbers of animals per acre be decreased, or the animals be housed on a dry-lot and fed stored forage in an elevated feeder to decrease ingestion of infective larvae.

Perpetuating generations of parasites with resistant alleles against previously effective deworming medication is a serious concern because parasites which are resistant to a particular deworming medication will continue to exist in the environment even if the drug is removed from the market. Moreover, many parasites that are resistant to one drug will also be resistant to other drugs within the same category. Resistance is primarily selected for in clinical cases, and the existence of resistant parasites is not confirmed without post-mortem examination of the animal. Some common practices that may contribute to the development of resistance to anthelmintics are deworming on a schedule that does not consider FECR testing, and selective breeding of animals with inherent resistance to anthelmintics. According to the authors, the permissive practice of using avermectins monthly as a control measure for meningeal worm (Parelaphostrongylus tenuis) is therefore a concern because the gastrointestinal nematodes of those llamas and alpacas are being exposed to therapeutic doses that could select for resistance among their gastrointestinal nematode population. Prudent use of anthelmintics, especially given the limited number of available treatment compounds, and gastrointestinal parasite control measures centered on herd and environmental management should be emphasized by veterinary practitioners to their South American camelid owning clients.

REFERENCES


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