Case Report Rapport de cas

Anthelmintic resistance in a herd of alpacas *(Vicugna pacos)*

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Abstract — A herd of alpacas was examined because of a history of severe endoparasitism, anemia, hypoproteinemia, and weight loss. Resistance of gastrointestinal nematodes to albendazole, fenbendazole, and doramectin was documented. This report suggests that anthelmintic resistance may be an emerging problem in South American camelids in North America.

Résumé — Résistance aux anthelminthiques dans un troupeau d'alpagas *(Vicugna pacos)*. Un troupeau d'alpagas a été examiné en raison d'une anamnèse d'endoparasitisme grave, d'anémie, d'hypoprotéinémie et de perte de poids. La résistance des nématodes gastro-intestinaux à l'albendazole, au fenbendazole et à la doramectine a été documentée. Ce rapport suggère que, en Amérique du Nord, la résistance aux anthelminthiques peut être un problème émergent chez les camélidés sud-américains.

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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 (Table 1). Quantitative fecal egg counts revealed the presence of high numbers of strongyle-type eggs per gram of feces in 3 of the 5 animals (Table 1). These findings, in conjunction with anemia and hypoproteinemia, led to a presumptive diagnosis of haemonchosis. All of the animals were treated with whole blood transfusions. Each animal had been treated with an anthelmintic at or shortly before admission to the hospital (Table 1). Based on follow-up fecal egg counts performed 6 to 13 d (Table 1) after hospital admission it appeared that the parasites may have been resistant to fenbendazole (Panacur; Intervet-Schering Plough, Summit, New Jersey, USA) in 1 of the cases, whereas there was a $>$ 95% reduction in the fecal egg count in an animal treated with pyrantel (Anthelban; IVX Animal Health, 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 resistance to fenbendazole in this herd. To further explore 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.

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Case description

The herd, located in Howard county, Missouri, consisted of 41 Huacaya breed alpacas comprised of 28 females (ages 2 to 17 y), and 13 males (ages 1 to 10 y). Adult and yearling males were housed in a 1-acre (0.4 ha) pasture lot. Females were housed in an adjacent approximately 120 m^2 dirt lot. Each group was independently allowed part-time access to a 20-acre (8.1 ha) pasture that was shared with 6 llamas and 2 horses. 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. Both drugs were not labeled for use in alpacas and the owner reported using the label doses for cattle. During the investigation, fecal egg count reduction (FECR) testing was performed to determine if 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.

Table 1. Summary of data from patient records for 5 adult alpacas presented for severe anemia and weight loss

Case number	Age (y)	PCV%/ TP g/L[a]	Anthelmintic treatment[b]	Pre-treatment FEC (eggs/g)	Follow-up FEC (eggs/g)	Days to follow-up FEC	FECR
1[c]	3	4/3.7	FBZ	1680	815	13	51%
2	2	4/4.3	PP	> 10 000	485	6	> 95%
3	1	10/4.9	NA	0	NA	NA	NA
4	2	9/5.4	Levamisole	1070	0	7	100%
5	1	7/5.5	Levamisole	5	NA	NA	NA

NA (not available) — information not recorded; FEC — fecal egg count, FECR — fecal egg count reduction.
[a] PCV — packed cell volume (reference range: 27% to 45%); TP — serum total protein (reference range: 51 to 79 g/L).
[b] FBZ — fenbendazole 10 mg/kg BW PO; PP — pyrantel pamoate 18 mg/kg BW PO; levamisole — treated by owner on the day of admission with 8 mg/kg BW, PO.
[c] Animal 1 had a body condition score of 1 of 5; body condition scores for the other 4 animals were not available in the medical record.

Table 2. Mean ± standard deviation (SD) strongyle-type fecal egg count (FEC), mean percent fecal egg count reduction (FECR%), and mucous membrane scores by treatment group and time period

Group[a]	Mean ± SD nematode FEC pre-treatment (eggs/gram)[b]	Mean ± SD nematode FEC 10 d later (eggs/gram)[c]	Mean FECR% (95% CI)	Mean ± SD mucous membrane score pre-treatment	Mean ± SD mucous membrane score 10 d later
Doramectin 0.2 mg/kg BW SQ (n = 9)	952 ± 669	626 ± 720	34 (−16 to 85)	3.0 ± 1.2	2.9 ± 0.9
Fenbendazole 10 mg/kg BW PO (n = 9)	1104 ± 1018	2328 ± 5024	−111 (−377 to 156)[d]	3.4 ± 1.2	2.8 ± 1.5
Albendazole 10 mg/kg BW PO (n =10)	1291 ± 1422	524 ± 833	59 (3 to 116)	3.9 ± 1.3	3.5 ± 1.0

BW — body weight; SD — standard deviation; PO — per os; SQ — subcutaneous; 95% CI — 95% confidence interval; FEC — fecal egg count; FECR — fecal egg count reduction.
[a] Label dosages for cattle.
[b] No significant difference in mean pre-treatment FEC between groups [one-way analysis of variance (ANOVA), P = 0.80].
[c] No significant difference in mean follow-up FEC between groups (one-way ANOVA, P = 0.35).
[d] Two animals in this group had a ≥ 5-fold increase in fecal egg count after treatment, thus impacting overall mean FECR%.

Thirty alpacas (n = 10 per group) of various ages and gender 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. Most of the 11 animals not selected for the trial had been recently treated and were therefore not included. 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 this was not feasible. However, in a study in Argentina, Mejia et al (1) reported that, while FECR% calculation methods can affect the result, similar FECR% were seen with and without a control group entered into the calculation.

Treatments were systematically assigned such that each treatment was applied to successive animals in turn until all treatments had been applied once; the cycle was then repeated. The order of systematic assignment was i) doramectin [0.2 mg/kg body weight (BW), subcutaneously], ii) fenbendazole (10 mg/kg BW, PO), and iii) albendazole (10 mg/kg BW, PO). Animal weights were estimated based on size, stature, and body condition score. The animals were restrained and monitored to ensure swallowing after oral medications had been administered and were maintained in their regular pens after treatment. Fecal samples were collected per rectum from each animal immediately before and 10 d after treatment. Fecal samples were stored at 4°C (39°F) following collection and processed within 24 h. Quantitative fecal egg counts (FEC) were done on an individual animal basis using the centrifugation method described by Cebra et al (2). Briefly, 2 g of feces were mixed with 98 mL of tap water to break up the feces. The mixture was refrigerated (4°C) overnight (approximately 14 h) and processed the following day. Ten milliliters of the mixture were centrifuged at 999 × g for 5 min in 13 mL plastic conical tubes. The supernatant was decanted and the resulting pellet was mixed with a saturated sucrose solution (specific gravity = 1.24) until the tube was filled (~13 mL). The re-suspended pellet was centrifuged at 999 × g for 5 min. Additional sucrose was added to form a meniscus and a 22 mm^2 cover slip was placed on top of each sample. Samples were allowed to stand for 10 min. The cover slip was removed and placed on a microscope slide. Slides were evaluated using a light microscope and 100× magnification. The total number of strongyle-type eggs present under the coverslip was counted and multiplied by 5 to yield eggs per gram of feces. The detection limit of the test was 5 eggs per gram of feces (3). 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. This was scored using the FAMACHA chart, described for use in sheep and goats (4). Briefly, the ocular conjunctiva was scored

on a 1 to 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 = post-treatment, and T1 = pre-treatment arithmetic mean egg count per gram of feces (1). 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% (5).

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 3 anthelmintics (Table 2). Fecal egg count was not significantly correlated with mucous membrane pallor before treatment across all experimental groups (correlation coefficient = 0.25; $P = 0.20$). Fecal egg count, however, was significantly positively correlated with mucous membrane pallor 10 d after treatment (correlation coefficient = 0.45; $P = 0.02$) suggesting an association between anemia and increasing fecal egg count at that time.

Discussion

Although anthelmintic resistance in sheep and goats has been well-documented in the literature, to the authors' knowledge there is only 1 other report documenting anthelmintic resistance in South American camelids. Gillespie et al (6) 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 et al (6), data from the herd reported here documents anthelmintic resistance to an avermectin and 2 benzimidazoles. None of the treatment groups herein achieved a $>$ 95% mean FECR and 7 animals (Group 1, $n = 2$; Group 2, $n = 4$; Group 3, $n = 1$) had increases in fecal egg counts following treatment. When gastrointestinal nematodes develop resistance to 1 drug in a class of anthelmintics they may develop resistance to all drugs in that class (7); resistance to both fenbendazole and albendazole was found in our study. 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 BW, PO (8). The animals in this herd had not been previously treated with this drug and preliminary evidence from 1 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 been withdrawn from the animal health market in the United States.

The fecal egg count technique used could not differentiate between strongyle-type eggs, but the presence of high strongyle-type egg counts in conjunction with evidence of anemia suggested that the endoparasitism was primarily due to haemonchosis. In addition, postmortem examination of 1 of the 2 animals that died revealed haemonchosis. The inconsistent association between FAMACHA scores and fecal egg count may reflect the fact that the strongyle-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 camelids. 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 camelids until the appropriate studies have been conducted to evaluate the relationship between presence of *Haemonchus contortus* in feces, hematocrit, and mucous 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 number 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, exposing the herd to a constant source of resistant parasite larvae. Additionally, resistance is a major concern because there are currently few alternative deworming products available for treatment, thus creating major concerns about the future ability to treat animals debilitated with endoparasitism. Hence, strategies aimed at decreasing exposure to parasite larvae and maintaining adequate levels of refugia are imperative for animal health and productivity. Refugia are the portion of the parasite population that has not been exposed to selection pressure, and thus serve as a potential reservoir of susceptible genes. Refugia

are contributed by untreated animals, stages of the parasite life cycle which are not susceptible to the drug used, and free-living organisms in the environment (7). Some strategies for minimizing infection and thus the need for treatment include maintaining a stocking density that minimizes the likelihood of overgrazing and heavy pasture contamination, maintaining a pasture grass height $>$ 5 cm, targeted selective deworming with an effective anthelmintic (e.g., only treating at risk and affected individuals), periodic assessment of anthelmintic effectiveness using FECR testing, and selective breeding of animals with inherent resistance to parasitism (7).

In conclusion, South American camelids appear to be susceptible to the ill effects of endoparasitism, much like sheep and goats. There is a variety of gastrointestinal parasites found in South American camelids, all of which can contribute to production losses (9). However, certain gastrointestinal nematodes such as *Haemonchus contortus* are more concerning than others because of the clinical outcomes of anemia and hypoproteinemia in the host. The findings presented here in conjunction with a recent report from Georgia (6) suggest that resistance against commonly used anthelmintics is an emerging problem in South American camelids in North America. The common practice of using avermectins monthly as a control measure for meningeal worm *(Parelaphostrongylus tenuis)* (10) 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 clients who own South American camelids. CVJ

References

1. Mejia ME, Fernandez-Igartua M, Schmidt E, Cabaret J. Multispecies and multiple anthelmintic resistance on cattle nematodes in a farm in Argentina: The beginning of high resistance. Vet Res 2003;34:461–467.
2. Cebra CK, Stang BV. Comparison of methods to detect gastrointestinal parasites in llamas and alpacas. J Am Vet Med Assoc 2008;232:733–741.
3. Kaplan RM, Klei TR, Lyons ET, et al. Prevalence of anthelmintic resistant cyathostomes on horse farms. J Am Vet Med Assoc 2004;225:903–910.
4. van Wyk JA, Bath GF. The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. Vet Res 2002;33:509–529.
5. Coles GC, Bauer C, Borgsteede FH, et al. World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet Parasitol 1992;44:35–44.
6. Gillespie RA, Williamson LH, Terrill TH, Kaplan RM. Efficacy of anthelmintics on South American camelid (llama and alpaca) farms in Georgia. Vet Parasitol 2010;172:168–171.
7. Fleming SA, Craig T, Kaplan RM, Miller JE, Navarre C, Rings M. Anthelmintic resistance of gastrointestinal parasites in small ruminants. J Vet Intern Med 2006;20:435–444.
8. Plumb DC. Plumb's Veterinary Drug Handbook. 5th ed. Ames, Iowa: Blackwell Publishing, 2005:678–679.
9. Ballweber LR. Ecto- and endoparasites in New World camelids. Vet Clin North Am Food Anim Pract 2009;25:295–310.
10. Nagy DW. *Parelaphostrongylus tenuis* and other parasitic diseases of the ruminant nervous system. Vet Clin North Am Food Anim Pract 2004;20:393–412.