Reemergence of cyathostomins species demonstrates anthelmintic resistance following drug administration

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Introduction

- Previous research has demonstrated anthelmintic resistance (AR), however, it is unclear if cyathostomin species are equally AR
- Current deworming strategies utilize fecal egg counts (FEC) to identify high shedder horses, therefore preserving refugia populations in low shedder horses.
- With little promise of new drug classes to target cyathostomins being introduced to the market soon, anthelmintic drugs must be administered judiciously to prevent complete AR.

Objective

The objective of this study is to determine the reemergence rate of cyathostomin species following three commercial horse dewormers

Subjects, Methods & Analysis

- Nine horses housed at two locations were enrolled to the study and was repeated June-September 2017-2019.
- Control (no treatment), (n=34); Ivermectin (macrocyclic lactones), (n=6); Moxidectin (macrocyclic lactones) (n=8); and Strongid (tetrahydropyrimidine) (n=8)
- Fecal samples were collected every 14d for 98d.
- Fecal egg counts were performed with a modified McMaster technique
- 18S rRNA profiling of the V5.8 and ITS1 regions.
- Sequences were clustered and taxonomy was assigned against a custom NCBI Blast+ database with the aligned sequences of 19 cyathostomins.
- Horses were removed from the study if sequencing failed due to low egg recovery for more than 50% of the timepoints.
- Data was analyzed using presence/absence methods in R studio.

Results

Table 1: Egg Reappearance Rate

	14 d (%)	28 d (%)	42 d (%)	56 d (%)	70 d (%)	84 d (%)	98 d (%)
Moxidectin	100.00	100.00	98.58	96.45	93.85	83.91*	60.25
Ivermectin	100.00	96.15	81.73*	53.85	67.31	34.62	0.00
Strongid	98.37	72.29*	49.46	7.63	-91.29	-152.69	-167.36

* indicates a shortened ERP using a fecal egg count reduction cut-off of ≤90%.

Figure 1: FEC

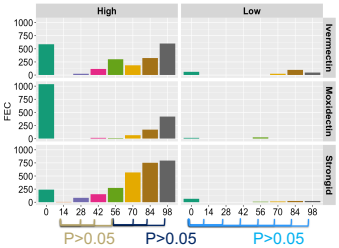


Figure 2: Average # Species Present

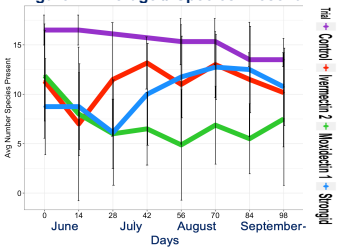
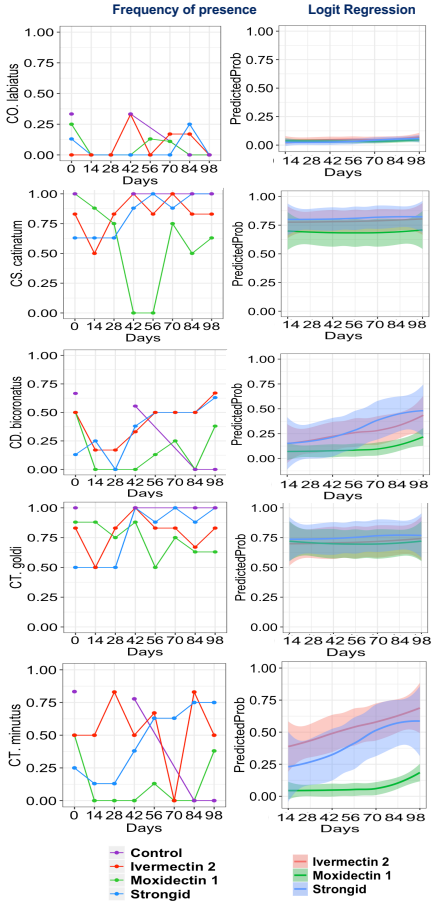


Table 2: ANOVA and Spearman correlations

Worm Species	Con[1]	Mox[2]	Ive[3]	Stro[4]	P –value Treatment	Spearman Correlations Mox	HS[5]	LS[6]
CO. coronatus	1.00[a]	0.20[b]	0.77[ac]	0.64[c]	3.36e-06***	-0.48	0.30	-0.44
CO. labiatus	0.17[a]	0.06[a]	0.08[a]	0.04[a]	**0.403**			
CO. labratum	0.19[ab]	0.24[ab]	0.04[a]	2.7e-17[ab]	0.038*	0.29		
CS. catinatum	1.00[a]	0.56[a]	0.83[a]	0.83[a]	**0.066**		0.30	-0.38
CS. tetracanthum	0.04[a]	0.69[b]	8.3e-17[a]	1.4e-16[a]	9.8e-09***			
CY. ashworth	1.00[a]	0.47[b]	0.83[a]	0.75[a]	0.0004***	-0.34	0.29	-0.40
CY. auriculatus	0.92[a]	0.21[b]	0.31[b]	0.24[b]	1.59e-06***			
CY. insignis	0.94[a]	0.21[c]	0.52[b]	0.46[bc]	9.2e-05***	-0.31		-0.30
CY. leptostomus	1.00[a]	0.27[c]	0.67[b]	0.44[bc]	1.13e-05***	-0.30		
CY. nassatus	1.00[a]	0.47[b]	0.83[a]	0.79[a]	3.04e-05***	-0.35	0.30	-0.41
CY. radiatus	0.97[a]	0.33[b]	0.71[a]	0.69[a]	0.0004***	-0.39		-0.32
CY. elongatus	0.75[a]	0.22[b]	0.31[b]	0.38[ab]	0.025*			
CD. bicoronatus	0.31[a]	0.16[a]	0.42[a]	0.36[a]	**0.108**			-0.35
CT. calicatus	0.97[a]	0.36[b]	0.77[ac]	0.611[bc]	0.0005***	-0.33	0.34	-0.47
CT. goldi	1.00[a]	0.74[a]	0.79[a]	0.78[a]	**0.118**		0.33	-0.42
CT. longibursatus	1.00[a]	0.71[b]	0.83[ab]	0.89[ab]	0.025*		0.31	-0.39
CT. minutus	0.41[a]	0.13[a]	0.54[a]	0.46[a]	**0.074**	-0.39		-0.34

1–Control; 2–Moxidectin; 3–Ivermectin; 4–Strongid; 5–High shedder horse (> 500epg); 6–Low shedder horse (< 200epg). Superscripts with different letters within row demonstrate $P < 0.05$.

Fig 3: Five species demonstrating anthelmintic resistance



Conclusions

- There are species specific differences in AR responses.
- 5 species demonstrate AR and 9 species appear acutely sensitive to Moxidectin.
- Cyathostomin populations differ between high shedder and low shedder horses.

Directions for Future Research

Identifying AR patterns at the species level will enable mechanistic molecular/physiological approaches to determine AR in cyathostomins as well as more targeted treatment approaches.

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