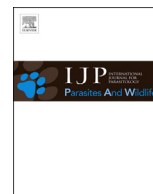




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Fecal parasite identification by microscopy and PCR in scimitar-horned oryx, *Oryx dammah*, managed at two sites

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ABSTRACT

The scimitar-horned oryx, *Oryx dammah*, an endangered species extinct in the wild, is managed in various captive management programs and is the focus of reintroduction efforts. Management variability can contribute to substantial parasite load differences, which can affect deworming programs and potentially transfer parasites to different regions with translocations. Parasite studies in *O. dammah* are lacking. In this study, we determined fecal egg/oocyst counts of *O. dammah* in two captive herds, Fossil Rim Wildlife Center (FRWC) and Kansas City Zoo (KCZ). Fecal egg counts (FEC) were performed on *O. dammah* feces collected seasonally using the modified McMaster method, and microscopy provided additional identification of parasite genera ova and oocysts. To identify parasites to species level, homogenized fecals provided DNA subjected to the polymerase chain reaction (PCR) using genus specific primers. Microscopy and sequencing results indicated the presence of *Strongylus* (*Strongylus vulgaris*, *Angiostrongylus cantonensis*), *Trichostrongylus* (*Haemonchus contortus*, *Camelostrongylus mentulatus*), *Trichuris* (*T. leporis*, *T. ovis*, and *T. discolor*), *Isospora* (*Isospora gryphoni*) and *Eimeria* (*E. zuernii* and *E. bovis*), with *Strongylus* being the most common. *Nematodirus* was identified through microscopy at FRWC. Fecal egg counts were significantly higher in (FRWC) than in (KCZ) in all samplings ($P < 0.001$). No significant difference was seen between parasite load and seasons ($P = 0.103$), nor site and season ($P = 0.51$). Both study sites maintained most animals within commonly accepted FEC levels found in domestic livestock. Individuals with high numbers of EPG or OPG were subordinate males, pregnant females, or neonates. Several significant interactions were found between genera of parasites, age, sex, season, and pregnancy status in the FRWC herd. Sampling limitations prevented further analysis of the KCZ herd. Understanding interactions between parasite loads and physiological, environmental, and regional differences can help determine inter-specific transfer of parasites, and establish appropriate anthelmintic programs for *O. dammah* herds.

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1. Introduction

Captive wildlife programs harbor animals in fenced areas, which can allow parasite infection and re-infection to easily occur. Pasture rotation is not feasible in these programs and therefore, re-infection is often high. Several programs utilize anthelmintics to control parasites; however, in zoological parks parasites can persist regardless of quarantine and anthelmintic treatments (Goossens et al., 2005). Small concentrations of parasites can help animals immunologically build resistance to additional infections via

antibody responses, so exposures can be beneficial (McKay, 2006). Heavy infections can cause health issues, such as a reduction in appetite, gut function, fecundity, decrease body condition (Stien et al., 2002), and can even cause death. Thus, management for minimal parasite loads is important to the entire herd, as well as the crucial consideration of inter-specific interactions if *O. dammah* is housed in areas containing other species.

Understanding parasite genera present in a habitat, along with potential hosts present, is necessary in order for anthelmintic treatments to be effective. Different parasite loads can even exist in animals exposed to different husbandry techniques (Goossens et al., 2005). In addition, parasite loads can be affected by grazing area size, fecal removal from pens or enclosures, animal density, pasture management, as well as inter-species interactions. Inter-

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species interactions must be considered due to the fact that multiple hosts can be infected by the same parasite, allowing transmission of generalist parasites (Ezenwa, 2003). Understanding the types of parasites found within the captive managed artiodactylids is important to establish an effective anthelmintic program to target unwanted parasites, particularly those that are more pathogenic than others. The four classes of anthelmintics utilize different modes of action against nematodes and have varying effects in host species and developmental stages of nematodes (Craig, 2003). Anthelmintic resistance exhibited by parasites also needs to be considered. In North America, *Haemonchus contortus* has shown resistance to three of the four classes of anthelmintics in a captive giraffe and the parasite was still present in captive impala after treatment with the benzimidazole, fenbendazole (Garretson et al., 2009; Nalubamba and Mudenda, 2012). *Haemonchus contortus* is of economic importance in domestic ruminants due to its increased pathogenicity and thus increased host mortality in comparison to other gastrointestinal nematodes.

Surveying and identifying internal parasites of *O. dammah* is important for maintaining the overall health of the animals, and for ensuring the species' productiveness within the different captive management programs. Identification of fecal parasites have economic benefits as well. Human-animal interactions are becoming increasingly important due to emerging zoonotic diseases. Determining parasite species based on morphological characteristics can be difficult and inaccurate; for this reason we utilized polymerase chain reaction (PCR) for higher accuracy than microscopy alone. Currently, parasites documented in scimitar-horned oryx (*O. dammah*) vary regionally, and are considered similar to parasites within domestic cattle (Horak, 1981; Gilbert and Woodfine, 2004). Several species of gastrointestinal nematodes such as *Trichostrongylus* spp., *Haemonchus* spp., *Ostertagia* spp., *Trichuris* spp., *Cooperia* spp., as well as several species of protozoan parasites such as *Isospora* spp. and *Eimeria* spp., are commonly found in cattle. The hypotheses were that concentrations of parasites would vary between *O. dammah* at two locations, and that different parasite genera and species would be found between study sites. The genera of parasites isolated from *O. dammah* was also expected to be similar to those found in domestic cattle. Interactions between age, sex, season, and pregnancy status on parasite loads from specific genera were expected to be significant. Seasonal and regional variances of parasite loads and genera present are important factors to consider in developing treatment programs as well.

2. Materials and methods

2.1. Study sites and animal care

Fossil Rim Wildlife Center (FRWC; Lat 32° 10' 56.64" N, Lon -97° 47' 51.864" W) and Kansas City Zoo (KCZ; Lat 39° 0' 22" N, Long 94° 31' 41" W) maintain their *O. dammah* herds using different management styles. FRWC, a drive-through wildlife park, maintains a free-ranging herd within an approximately 100-acre pasture, and interspecies interactions can occur with white rhinoceros (*Ceratotherium simum*), blackbuck (*Antilope cervicapra*), wildebeest (*Connochaetes taurinus*), roan antelope (*Hippotragus equinus*), 1 blesbok (*Damaliscus pygargus phillipsi*), 3 ostrich (*Struthio camelus*), and native white-tail deer (*Odocoileus virginianus*). The animals free-range year round, with shelters available. The herd is supplemented daily with hay and pelleted commercial feed, containing 14% or 17% protein, depending on the season. FRWC herd is dewormed on an as-needed basis. The climate for this area includes mild winters, and hot summers with temperatures usually above 18°C.

KCZ maintains its herd in an approximately 7-acre pasture

during the day, and within indoor enclosures during the evening. The herd at KCZ can interact with addax (*Addax nasomaculatus*), springbok (*Antidorcas marsupialis*), lesser kudu (*Tragelaphus imberbis*), eland (*Taurotragus oryx*), Grant's zebra (*Equus quagga boehmi*), and giraffe (*Giraffa camelopardalis*). KCZ is located in Kansas City Missouri. Missouri experiences strong seasonality with cold winters that average around 0°C from December to February and hot summers. *O. dammah* are maintained predominantly indoors during cold months, due to lack of adaptation for colder weather. Intact males are housed separately, either individually or in small groups of two or three. Another intact male is occasionally kept with the group that consists of neutered males, juveniles, and females for breeding purposes. The herd is given standardized feed and alfalfa daily, and are dewormed on a six week rotational basis with Safeguard® and Synanthic® liquid.

2.2. Sample collection

Before collection, appropriate animal use and care approval (UCM IACUC number 13–3232) was obtained for working with live vertebrate animals and collection of biological products from the animals. *O. dammah* feces were collected four times, once per season, from October 2013 to July 2014: October (autumn, reproductive), February (winter, lower nutrition), April (spring, rainfall), and July (summer, dry season). During the four collection times, 15–25 samples were collected per herd to ensure at least 75% of the herd was represented. Effort was made to avoid collecting duplicates from the same individual per sampling time. FRWC samples were collected after direct observation of defecation by tagged individuals, and samples at KCZ were collected from different individuals utilizing direct observations of defecation, morphological differentiation of feces, and segregation of individuals. Gloves were changed for each sample in order to avoid cross-contamination. The fecal samples were collected, labeled, placed into sterile plastic bags, and chilled between 0 and 2 h after defecation. The sample date and time were also documented on a data sheet.

2.3. Sample processing

Sample processing began immediately after all samples were collected, and included the modified McMaster method to count for ova and/or oocysts, along with wet mounts to identify potential genera. Samples were processed within a 12–24 h time frame. A small portion of each fecal sample was preserved as homogenized feces in either 10% formalin or 70% ethanol. A flotation solution of magnesium sulfate with a specific gravity of 1.24 was made by mixing 1.5 pounds of Epsom salts into 4 cups of distilled water. Next, 56 mL of flotation solution was added to a leak-proof sterile jar with 4 g of feces and shook for 5 min. A drop of fecal solution was used to make wet mounts, which was used for identification of parasite genera based on morphological characteristics. Microphotographs were taken using a canon T1i SLR camera with an Amscope Canon SLR/D-SLR camera adaptor for microscopes.

2.4. DNA extraction

For DNA extraction of *Eimeria* and *Isospora* genera, 300 mg of homogenized feces was placed into a round bottom cryovial containing 200 µl of lysis buffer. DNA isolation followed methods described by Hauck and Hafez (2012), with slight modifications. Sterile steel beads were added to each suspension and vortexed using a tissue lyser for 3 min, followed by the addition of 20 µl of proteinase K, and incubated for 1 h at 56°C. An additional 200 µl of lysis buffer was added and the suspension was then incubated for an additional 10 min at 70°C.

For DNA extraction of *Trichuris* genera, 300 mg of homogenized feces was placed into a round bottom cryovial with sterile steel beads. The suspension was vortexed using a tissue lyser 5 times with 1 min pauses, which followed methods described by Demeler et al. (2013).

For DNA extraction of *Strongylus* genera, 300 mg of homogenized feces was placed into a round bottom cryovial with 300 µl of lysis buffer and 25 µl of proteinase K. The suspension was incubated at 56°C for 18 h, and then the proteinase K was inactivated by incubating for 10 min at 95°C, which followed methods described by Alberti et al. (2011).

All DNA was isolated using a commercially available DNA isolation kit, QIAmp DNA stool MiniKit (Qiagen, Hilden, Germany), and then quantified with a nanodrop.

2.5. Molecular analysis

Conventional PCR was utilized to amplify all DNA isolates using published genus-specific primers (Table 1). All amplicons were electrophoresed for 1 h at 80 V, in a 1 × Tris-Acetate-EDTA (TAE) 1% agarose gel containing 50 µg/µl of ethidium bromide, and visualized via UV transilluminator. A 1 Kb ladder in each gel served as a standard in which to compare amplicon size. The PCR mixtures for each parasite genera are discussed below and shown in Table 2.

The *Eimeria* and *Isospora* reaction mixtures followed those of Kawahara et al. (2010) and contained a total of 20 µl. The PCR cycles also followed Kawahara et al. (2010) and are shown in Table 3. Next, 10 µl of the amplicons were electrophoresed.

The *Trichuris* reaction mixtures were modified slightly from Demeler et al. (2013). A total reaction of 25 µl contained 12.5 µl of master mix, 0.125 µl of 100 µM primer solutions, 2 µl of DNA template, and 10.25 µl of sterile ddH₂O. The PCR reactions also followed Demeler et al. (2013). Then 20 µl of the amplicons were electrophoresed.

For *Strongylus* genera, a reaction total of 24 µl was used in order to amplify the DNA, and was modified slightly from McLean et al. (2012). The reaction cycles mostly followed McLean et al. (2012), except for an annealing temperature increase to 60 °C. Lastly, 20 µl of the amplicons were electrophoresed.

All bands were extracted from the gel using scalpels, purified, and then sent to Eurofins Genomics (Eurofins MWG Operon LLC, Alabama, USA) for sequencing. All sequences were analyzed utilizing FinchTV Version 1.4.0, 4Peaks, and identified utilizing BLASTn analysis and available sequences in the NCBI GenBank.

2.6. Statistical analysis

All results are presented using descriptive statistics. Samples were grouped and categorized by sampling site in three different sections. The October sampling combined eggs per gram (EPG) and oocysts per gram (OPG), and was grouped and categorized by sampling site. The samplings for February, April, and May were separated by EPG results and OPG results; then categorized by sample sites. All categories were tested for normality using the Studentized Range for Normality Test (SRNT) and all groups were not normally distributed. The October samplings were compared

Table 2

PCR recipes used to identify fecal parasites in Scimitar-horned oryx. Primer quantities listed were for each primer, the forward (F) and reverse (R), added. Quantities are in microliters (µl).

PCR ingredient	<i>Eimeria</i> ^a	<i>Trichuris</i> ^b	<i>Isospora</i> ^c	<i>Strongylus</i> ^d
Mastermix	10	12.5	10	12.5
DNA	1	2	1	2
Primer (each F & R)	0.5	0.125	0.5	1.2
MgCl ₂	—	—	—	2
Sterile ddH ₂ O	8	10.25	8	8.1

^a (Gerhold et al., 2011).

^b (Demeler et al., 2013).

^c (Ruttkowski et al., 2001).

^d (Bisset et al., 2014).

using a Mann-Whitney Rank Sum Test. The other samplings were separated for EPG and OPG, then grouped by location. The locations were then compared between EPG and OPG using a Mann-Whitney Rank Sum Test. The genera were then separated out, tested for normality using the Shapiro-Wilks, and pairwise comparisons using the Hold-Sidak method were used to determine differences for each genera based on month and herd. These analyses were done using SigmaPlot 12.3 (Systat Software Inc., California). Additional analysis was completed for FRWC using a generalized linear model with a Poisson distribution considering ova and oocyst were counted and discrete. This determined significance of interactions between genera of parasites, season, sex, pregnancy status, and age. Due to sampling limitations, the same information was not available from the KCZ herd; therefore, the analysis was only done for FRWC. This analysis was done in SPSS[®] 24 (SPSS Inc., Chicago, IL).

3. Results

3.1. EPG and OPG counts

Summary statistics of EPGs and OPGs for all genera combined were reported for all sampling times including herd size per season (Table 4). October samplings combined EPG and OPG counts per location. For all other seasons, the EPG and OPG are separate for each location. FECs showed a higher EPG and OPG in the FRWC herd. The overall FEC for FRWC were 42.6% higher than KCZ ($P < 0.001$) in October. For the samplings from February 2014, April 2014, and July 2014, the EPG counts for FRWC were 15.9% higher than KCZ ($P < 0.001$) and the OPG counts for FRWC were 15.6% higher than KCZ ($P < 0.001$). For inclusive EPGs, a significant difference was seen between sites ($P = 0.002$), but there was no significant interaction between site and season ($P = 0.103$). For inclusive OPGs a significant difference between sites ($P = 0.002$) was seen, but no significant interaction between site and season ($P = 0.051$) was seen.

Animals with high levels of either EPG (>500) or OPG (>1000) are listed in Table 5. Calves born in the same year as sampling had high levels of OPG and one calf had high levels of both EPG and OPG. Out of 55 McMaster slides viewed during February, April, and July, 56.4% of EPG counts from FRWC were ≤ 200 , with 27.2% between 201 and 500, and only 16.4% above 500. Out of 48 McMaster

Table 1

Genus-specific primer sets designed for conventional polymerase chain reaction to detect species of parasites in scimitar-horned oryx between environments.

Genus	Forward (5'-3')	Reverse (5'-3')	Size
<i>Strongylus</i>	GTGTGACGGATAACGGAGAA	TGCACCACCAACCACTAAA	897 bp
<i>Trichuris</i>	GATGGCCTCGGTCTATTT	GCCAGAGTCTCGTTCGTTATC	525 bp
<i>Eimeria</i> ^a	ATTTCGCTGCGTCTTCA	CCTGCATACTCCTTCAAA	307bp
<i>Isospora</i>	TCTGCACCTCATGAGAAATC	GACACGCAAAGTCCCTCTAA	330bp

^a *Eimeria* primers were obtained from Gerhold et al. (2011).

Table 3
Thermocycler parameters utilized to amplify fecal DNA and identify parasites.

Thermocycler step	<i>Eimeria</i> ^a	<i>Trichuria</i> ^b	<i>Isospora</i> ^c	<i>Strongylus</i> ^d
1	94 °C for 30 s	98 °C for 30 s	94 °C for 30 s	95 °C for 10 m
2	95 °C for 10 s	98 °C for 10 s	95 °C for 10 s	95 °C for 45s
3	50 °C for 30 s	55 °C for 30 s	55 °C for 30 s	60 °C for 45 s
4	72 °C for 30 s	72 °C for 30 s	72 °C for 30 s	72 °C for 45 s
5	72 °C for 2 m	—	72 °C for 2 m	—
# cycles (steps 2–4)	40	40	35	40

^a (Kawahara et al., 2010).^b (Demeler et al., 2013).^c (Ruttkowski et al., 2001).^d (McLean et al., 2012).**Table 4**

Summary statistics for fecal egg counts of gastrointestinal parasites reported as eggs per gram and oocysts per gram in scimitar-horned oryx between two study sites, Fossil Rim Wildlife Center and Kansas City Zoo. Sample size, N, is listed next to the total number of individuals in the herd during the sampling time.

Sample	N	Range	Median	25% Quartile	75% Quartile
FRWC – Oct (EPG + OPG)	19 (25)	2600	575	412.5	700
KCZ – Oct (EPG + OPG)	16 (24)	2300	1600	1100	2000
FRWC – Feb (EPG)	17 (25)	850	150	50	325
FRWC – Feb (OPG)	17 (25)	1800	450	300	750
KCZ – Feb (EPG)	16 (24)	100	0	0	25
KCZ – Feb (OPG)	16 (24)	450	150	62.5	237.5
FRWC – Apr (EPG)	17 (28)	550	150	50	250
FRWC – Apr (OPG)	17 (28)	1300	300	200	775
KCZ – Apr (EPG)	16 (22)	450	0	0	1375
KCZ – Apr (OPG)	16 (22)	350	200	100	250
FRWC – July (EPG)	21 (28)	4100	350	175	600
FRWC – July (OPG)	21 (28)	9550	450	275	2000
KCZ – July (EPG)	16 (22)	350	0	0	875
KCZ – July (OPG)	16 (22)	450	100	50	150

Table 5

Scimitar-horned oryx with high levels of eggs per gram (>500) of gastrointestinal parasites in feces or oocysts per gram (>1000) of feces sampled from Fossil Rim Wildlife Center and Kansas City Zoo in October 2013, February 2014, April 2014, and July 2014 with coordinating medians and ranges of the herd from the same sampling.

Sample	EPG/OPG	Median	Range
Subordinate Male (KCZ) – Oct	2650 (EPG + OPG)	1600	2300
Pregnant Female (FRWC) – Oct	3250 (EPG + OPG)	1600	2600
Pregnant Female (FRWC) – Oct	2200 (EPG + OPG)	1600	2600
Pregnant Female (FRWC) – Feb	2000 (OPG)	450	1800
Pregnant Female (FRWC) – April	1300 (OPG)	300	1300
6 week calf (FRWC) – July	9700 (OPG)	450	9600
8 week calf (FRWC) – July	6050 (OPG)	450	9600
12 week calf (FRWC) – July	1500 (OPG)	450	9600
12 week calf (FRWC) – July	1650 (OPG)	450	9600
12 week calf ^b (FRWC) – July	7350 (OPG)	450	9600
12 week calf ^b (FRWC) – July	4100 (EPG)	350	4100
13 week calf (FRWC) – July	2850 (OPG)	450	9600
16 week calf (FRWC) – July	2350 (OPG)	450	9600

^bSame calf.

slides from KCZ during February, April, and July, 66.7% did not have ova present on McMaster slides due to close proximity of deworming, therefore, 25% were ≤ 200 , and 8.3% were between 201 and 500. The majority of the OPG counts from FRWC were ≤ 500 (43.6%), with 18.2% ≤ 200 , 14.6% ≤ 900 , and 23.6% were > 1000 . For the KCZ, 77.1% of the OPG were ≤ 200 , and 22.9% were ≤ 500 .

3.2. Identified genera via microscopy

Parasite genera found at each location were identified using wet mounts, and densities of eggs or oocysts present for each genera

Table 6

Genera of parasites found in the feces of scimitar-horned oryx from Fossil Rim Wildlife Center and Kansas City Zoo in February 2014, April 2014, and July 2014, reported as range (median).

Genus	Location	Feb 2014	April 2014	July 2014
<i>Strongylus</i>	FRWC	850 (150)	500 (0)	650 (200)
<i>Strongylus</i>	KCZ	100 (0)	450 (0)	350 (0)
<i>Nematodirus</i>	FRWC	0	450 (0)	50 (0)
<i>Trichuris</i>	FRWC	300 (0)	50 (0)	500 (0)
<i>Eimeria</i> (sp. 1)	FRWC	650 (100)	150 (50)	250 (0)
<i>Eimeria</i> (sp. 1)	KCZ	250 (100)	100 (25)	100 (0)
<i>Eimeria</i> (sp. 2)	FRWC	0	0	3900 (0)
<i>Isospora</i>	FRWC	1700 (300)	1250 (250)	9600 (450)
<i>Isospora</i>	KCZ	150 (50)	350 (150)	450 (50)

were determined for samplings occurring in February, April, and July (Table 6). Microscopy (Fig. 1) revealed the presence of several genera of nematodes including *Strongylus*, *Trichuris*, and possibly *Nematodirus*. Several protozoa were also found on wet mounts that came from the class Coccidia, in particular two from the genus *Eimeria*, and one from the genus *Isospora*. The genera of parasites found varied between locations. *Strongylus*, one species from *Eimeria*, and *Isospora*, were found at KCZ. *Strongylus*, *Trichuris*, *Nematodirus*, two species from the genus *Eimeria*, and *Isospora*, were all found at FRWC. Images of parasites seen via microscopy are shown in Fig. 1A–E. Fig. 2 shows the OPG differences between the two sites.

3.3. Comparison of parasite genera and analysis of FRWC

All genera of parasites were compared between sites for each season except for one species of *Eimeria*, *Trichuris*, and *Nematodirus*

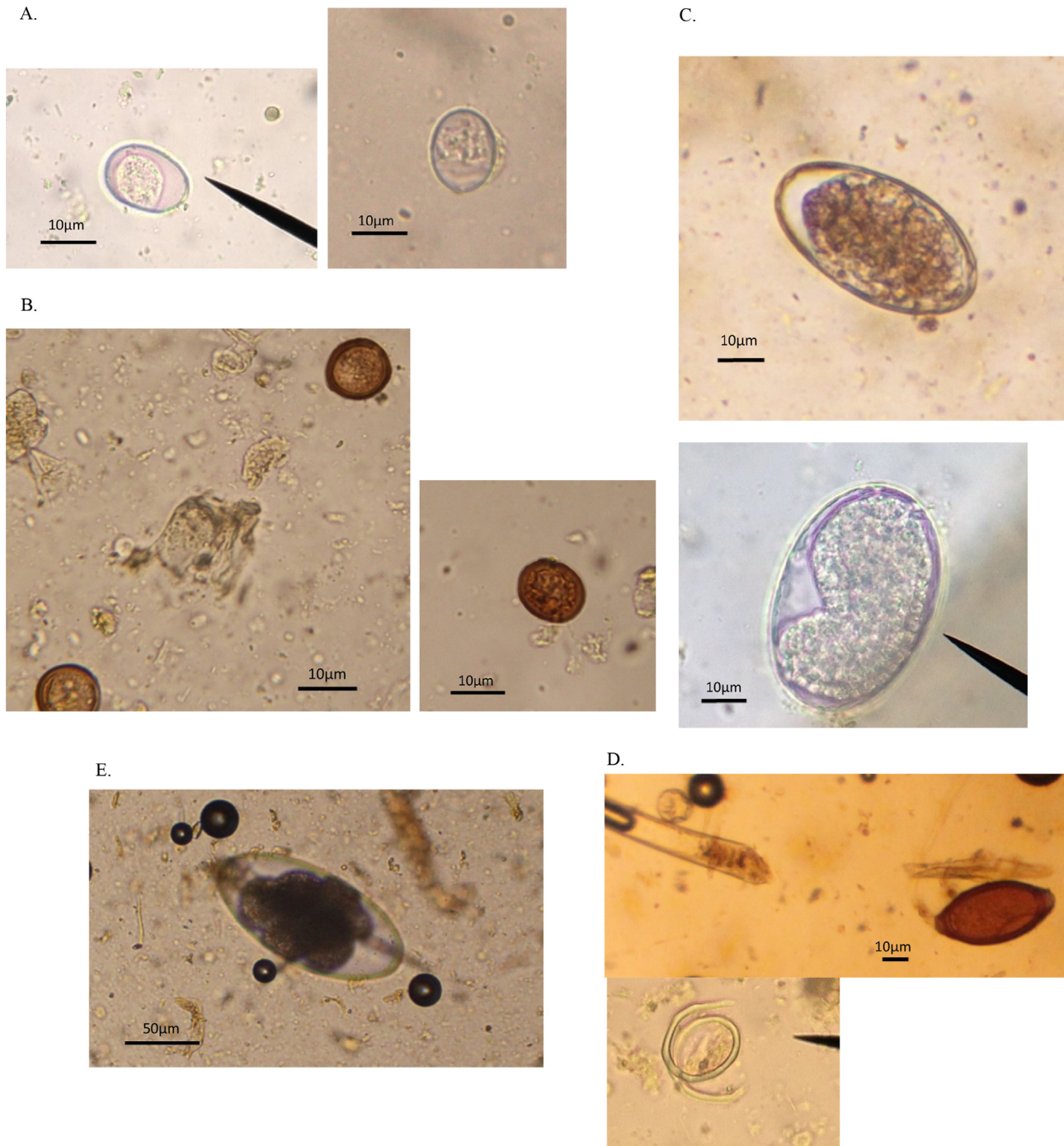


Fig. 1. Pictures of the parasites found in *O. dammah* from Fossil Rim Wildlife Center located in Glen Rose, Texas and Kansas City Zoo located in Kansas City, Missouri during the following time frames, October 2013, February 2014, April 2014, and July 2014. A. *Isospora* spp. in *O. dammah* feces from the Kansas City Zoo in October 2013. (40 × magnification). B. *Eimeria* spp. in *O. dammah* feces from Fossil Rim Wildlife Center in October 2013. (40 × magnification). C. *Strongylus* spp. in *O. dammah* feces from Kansas City Zoo in February 2014. (10 × magnification). D. *Trichuris* spp. in *O. dammah* feces from Fossil Rim Wildlife Center in February 2014. (10 × magnification). E. Possible *Nematodirus* spp., in *O. dammah* feces from Fossil Rim Wildlife Center in April 2014. (10 × magnification).

which all appeared only in the FRWC herd. For the species of *Eimeria* found at both locations, overall there was not a statistically significant difference between the herds. There was an overall significant difference between the herds for *Isospora* ($P = 0.003$, $t = 3.083$) and *Strongylus* ($P < 0.001$, $t = 4.413$). There were significant differences in concentrations of parasites from specific genera between herds within certain months, as well as significant differences in the amount of parasites from specific genera between

months within each herd (Table 7).

Using a general linear model with a Poisson distribution to analyze the data from FRWC, several significant interactions were found in the FRWC herd between genera of parasites, age, sex, pregnancy status, and month. Significance was indicated with a p -value < 0.05 . *Eimeria* (sp. 1) had a significant interaction with season ($P < 0.0005$) with the highest amount occurring in February. *Eimeria* (sp. 2) had a significant interaction with season ($P < 0.0005$)

Mean Oocysts Per Gram (OPG)

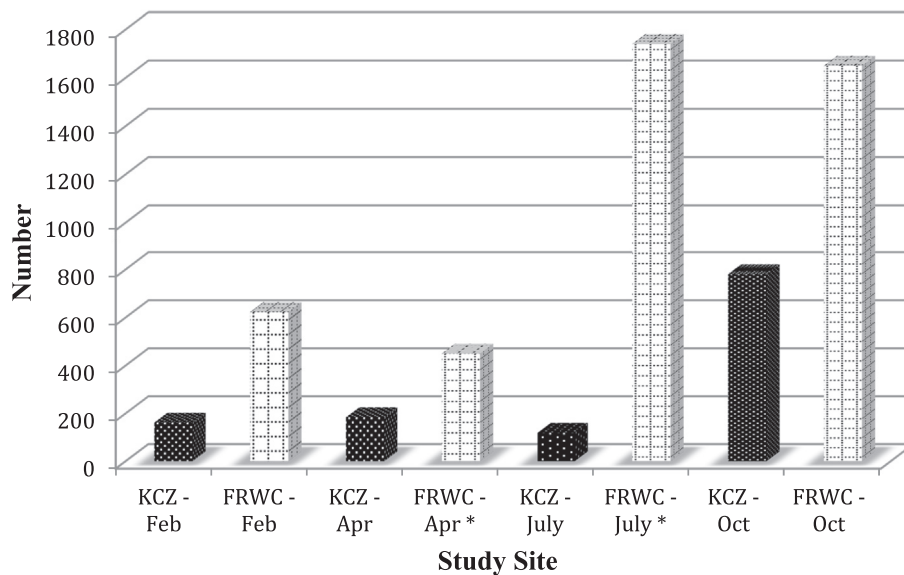


Fig. 2. Mean oocysts per gram of parasites seen.

Table 7

Significant differences in number of parasites from specific genera between months for certain herd. Significant differences in number of parasites from specific genera between herds for certain months. The alpha level was 0.05 for all.

Genus	Location(s)	Month(s)	P-value	t
<i>Eimeria</i> (sp. 1)	FRWC	Feb vs. April	<0.001	4.263
<i>Eimeria</i> (sp. 1)	FRWC	Feb vs. July	<0.001	3.804
<i>Eimeria</i> (sp. 1)	KCZ	Feb vs. April	0.010	2.856
<i>Eimeria</i> (sp. 1)	KCZ	Feb vs. July	0.016	2.856
<i>Isospora</i>	FRWC	July vs. Feb	0.006	3.025
<i>Isospora</i>	FRWC	July vs. April	0.006	3.164
<i>Isospora</i>	FRWC vs. KCZ	July	<0.001	3.890
<i>Strongylus</i>	FRWC vs. KCZ	February	<0.001	3.398
<i>Strongylus</i>	FRWC vs. KCZ	July	0.002	3.218

as it only appeared in July, sex ($P = 0.001$), and age, only appearing in calves ($P < 0.0005$). *Strongylus* had a significant interaction with season ($P = 0.036$) with the highest amount occurring in July and age with the highest amounts in young calves ($P = 0.001$). *Isospora* had significant interactions with all factors ($P < 0.0005$) and the highest amounts occurred in July.

3.4. Genera confirmed by sequencing

October samples were excluded due to the combined OPG and EPG counts. Out of the remaining 55 samples from FRWC, DNA was isolated from 9 samples. These samples were tested for all parasites observed through microscopy and 21 successful amplifications were achieved. Out of 48 samples from KCZ, DNA was isolated from 4 samples with 10 successful amplifications. Several bright electrophoresed PCR bands were seen in the 1% TAE agarose gels. In order to determine which band identified the parasite the primers were designed for, several bands were cut out using a scalpel and sent in for DNA sequencing. The results suggested the other bands as *Camelostromylus mentulatus* and *Haemonchus contortus*.

Sequencing suggested the presence of *Strongylus vulgaris* (FRWC, KCZ; 96% homology, E-value 0.46), *Angiostrongylus cantonensis* (FRWC; 88%, E-value 0.33), *Camelostromylus mentulatus*

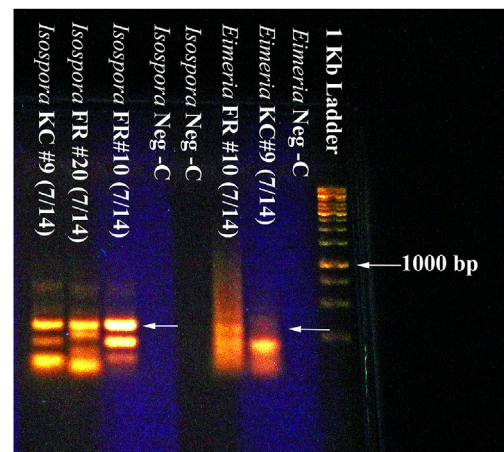


Fig. 3. A 1% agarose gel showing the presence of *Isospora* and *Eimeria* from feces of scimitar-horned oryx.

(KCZ; 99%, E-value $9e-111$), *Haemonchus contortus* (KCZ; 98%, E-value $4e-159$), *Trichuris* (*T. leporis*, *T. ovis*, and *T. discolor*) (FRWC; 100%, E-value 0), *Isospora gryphoni* (FRWC, KCZ; 98%, E-value $2e-137$), and *Eimeria zuernii* (FRWC and KCZ; 99%, E-value $4e-129$), and *Eimeria bovis* (FRWC; 97%, E-value $1e-129$). Attempts to isolate and amplify DNA from samples that contained *Nematodirus* spp. were unsuccessful. Representative agarose gel results are shown in Figs. 3 and 4. It should be noted that a BLAST detects similarities and is therefore not a definitive match.

4. Discussion

Parasite loads and genera did differ between the management styles and although some species appeared in both herds, three species were specific to FRWC. This could be indicative of specific inter-species interactions, type of grass or food supplied, how long animals were allowed to graze and how short the grass was (Jones, 1993), removal of feces from the grazing area and enclosures, a

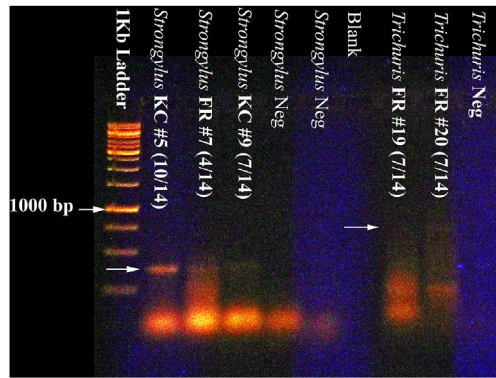


Fig. 4. A 1% agarose gel showing the presence of *Strongylus* and *Trichuris* from feces of scimitar-horned oryx.

drier versus humid climate, a longer parasite season due to milder winters in Texas, and even physiological differences between the herds, such as the number of pregnant females (11 at FRWC and 3 at KCZ). FRWC manages *O. dammah* in as natural of a setting as possible. In order to accomplish this, animals are able to forage free-range within a 100-acre pasture, which reduces the high possibilities of re-infestation of intestinal parasites typically associated with close confinement in grazing areas. Animals are monitored and administered a dewormer only if monitoring indicates high levels of gastrointestinal parasites. The FRWC herd had higher EPG and OPG levels in comparison to KCZ; however, in comparing the FEC/OPG counts to wild ruminants in North America, both EPC and OPG counts were much lower than those found by Hoberg et al. (2001). The highest OPG levels occurred in July and were associated with calves; the youngest calf sampled had the highest concentration of oocysts present in the feces. Kahn and Line (2010) determined that coccidiosis is common in calves born in early April through June, and this study utilized several calves born from early April through June. The highest OPG levels contained high levels of *Eimeria* spp., which is also typically the most prevalent endoparasite in domestic calves (Jäger et al., 2005). High numbers of oocysts are expected in the feces of young calves, with a decrease typically beginning around 7 weeks of age (Jäger et al., 2005). This pattern was observed at FRWC during our study. The reduction of protozoa as calves mature indicates developing immunity (Craig, 2003; Jäger et al., 2005).

The management style at KCZ requires periodic use of dewormers because animals are housed indoors during evenings and during cold weather, resulting in a higher likelihood an animal could be either infected or re-infected. The importance of utilizing appropriate dewormers to the situation was observed in three intact males housed together in an indoor enclosure. Dewormer was placed in food for all three animals to share, but was primarily consumed by the dominant male. Not surprising, the dominant male had very low EPG and OPG levels of 400 combined, but the most subordinate male demonstrated a combined total EPG and OPG count of 2500, made up predominantly of strongyles. This indicates the importance of monitoring and dewormers. These findings support the hypothesis that the concentration of parasitic eggs and oocysts found in *O. dammah* feces would vary between study sites due to different management styles. Different genera of parasites were found between study sites; in addition, several species of parasites within the same genera were seen between sites as well supporting our second hypothesis.

Although parasite loads were substantially different between herds, both locations managed herds within or below EPG levels

seen in domestic animals, or animals managed in zoological parks. Only a few individuals showed EPG levels above published guidelines at different times throughout the sampling period, which has been previously seen by Jäger et al. (2005), Fagiolini et al. (2010), and Lester and Matthews (2014). The majority of the EPG counts, including ova from *Strongylus* spp., *Trichuris* spp., and *Nematodirus* spp., from FRWC were ≤ 500 (77%), and only 16.5% were above 500. For KCZ, 67% of samples were devoid of ova on McMaster slides due to close proximity of deworming, and 33% of the samples were ≤ 500 . The majority of OPG counts from FRWC samples were ≤ 500 (62%) and 23.5% were > 1000 . Counts included both species of *Eimeria* spp. and *Isospora* spp. For the KCZ samples, all OPG levels were ≤ 500 . Individuals demonstrating high EPG or OPG counts were a subordinate male, pregnant females, or young calves.

The most commonly found helminth was the genus *Strongylus*. This supports Goossens et al. (2005) previous study of 100% of *O. dammah* samples showed strongyle type parasites. Analysis indicated a significant difference in *Strongylus* levels between herds, with FRWC having higher levels. Ezenwa (2003) found that within an individual habitat, an increase in bovid species increased the individual mean strongyle numbers significantly. This could explain one reason why increased numbers at FRWC were observed, as *O. dammah* are involved in higher numbers of inter-species interactions than those at the KCZ. That study also found strongyle abundance in captive managed African bovids exceeded 500 EPG, with some individuals exceeding 1000 EPG (Ezenwa, 2003). Ezenwa (2003) also found *Coccidia* ranges from 7 to 7846 EPG, possibly due to competition with other parasites. Our study found similar results in regards to higher numbers of both strongyles and coccidia.

Isospora as a genus is commonly found in *O. dammah* and domestic bovines. The match to *Isospora gyrphoni* was unexpected as it is commonly recovered from goldfinches (Olson et al., 1998). Several possibilities exist for this match, including a species of *Isospora* that has not been previously identified or accidental ingestion of ova. Hay is often utilized as a perch or for nests of various birds. The possibility of the animals acquiring the parasite from the food source should be investigated further, as the likelihood of acquiring the parasite from foraging on grass alone seems unlikely. Significant interactions existed for season, sex, pregnancy status, age, and *Isospora* in the FRWC herd. *Isospora* was detected in all but one sample throughout the study at FRWC, and counts varied tremendously so these significant interactions were expected. Although we were not able to analyze the herd at KCZ, raw data shows only 11 samples that did not identify *Isospora* through microscopy. *Isospora* spp. as with all species of *Coccidia* are treated with sulfas, not anthelmintics. The increased treatments at KCZ did not eliminate *Coccidia*; however, counts were much lower in comparison to FRWC which was indicated in the analysis with a significant difference between the herds.

Upon initial trials of identifying DNA bands amplified via PCR, one occurrence each of *Haemonchus contortus* and *Camelostrongylus mentulatus* were identified at KCZ. We did not test any further bands for ID but the presence of these two species is interesting. For example, *Camelostrongylus mentulatus* is typically seen in sheep and goat, but has been documented in giraffes (Fukumoto et al., 1996; Garijo et al., 2004), and roe deer (Rossi and Ferroglio, 2001). For the KCZ herd, the *O. dammah* are allowed to interact with giraffe, but without a parasite profile database of all animals, it would be difficult to make a positive correlation to the said interaction. *Haemonchus contortus* is widely known for causing economic losses in sheep and goats. With widespread anthelmintic resistance, monitoring levels of this parasite could become more important to ensure *O. dammah* survival and fecundity.

5. Conclusion

With the current progress towards reintroducing the *O. dammah* to the wild, this study is important for the captive management programs that currently house these oryx to ensure healthy maintenance of the species. Understanding the variability in parasites within the species can help increase the success of anthelmintic programs, as well as reduce the introduction of non-native parasites in other regions. Our study emphasizes the need for facilities to ensure proper anthelmintic treatment for their herd due to their unique management style. Currently *O. dammah* are managed very similar to cattle and are typically treated for parasites typical of cattle. Typical anthelmintics used in zoological parks are approved for livestock (Hosey et al., 2009). Although this is an efficient management style, it is important to know that parasites outside of the range of the typical ones found in cattle can exist in *O. dammah*. A specialized anthelmintic treatment can be established once the generalist, and specialist, parasites found within a species have been determined. This study also showed the variance in genera of parasites present in *O. dammah* in different regions, which is necessary to understand for animal transportation to different regions and facilities in order to prevent the introduction of non-native parasites. These parasites are commonly transmitted to other animal species in captivity, and thus the scimitar-horned oryx can serve as a reservoir of these parasites.

Interspecies interactions for potential parasite accrual were different at each facility. The additional parasites found incidentally confirm parasites are shared between giraffe or camel and *O. dammah*, but we do not know if the *O. dammah* had acquired the parasite before being housed at KCZ, just as the *Isoospora* species discovered could have been an interaction at a different park or zoo. The findings support different parasite management systems for *O. dammah* depending on the type of facility they are housed in. Over time, a parasite database would provide valuable parasite and host relationship information. Future studies should include a more sensitive PCR method, such as qPCR, in order to detect species of low prevalence and discovering co-infections that may exist (Mejia et al., 2013). Utilizing a more sensitive method can also detect species that may not be identified in GenBank, and determine a more precise concentration of parasites.

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Conflict of interest

The authors declare no conflict of interest.

There is no financial or personal interest that could affect the outcome of this manuscript.

This manuscript has been read and approved by all authors listed. Individuals who contributed to the work are listed as authors and there were no other individuals who satisfied the criteria for authorship. We have followed all ethical protocols for our institution to carry out this research.

It is understood that the corresponding author listed is the sole contact for the editorial process. The corresponding author will keep open and direct communication with all authors in regards to any aspect of the editorial process. All information given for the corresponding author is up to date and current.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ijppaw.2016.11.001>.

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