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

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A B S T R A C T

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 fecal 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, Camelostongylus mentulatus), Tri-churis (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, and pregnancy status in the FRWC herd. Sampling limitations prevented further analysis of the KCZ herd. Understanding interactions between parasite load 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-
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’ productivity 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 Trichos strongestus 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 vari

### 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 genera 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/ml 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 30 μl contained 0.125 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 Shapiro-Wilk test. The genera were then separated out, tested for normality using the Shapiro-Wilk, and pairwise comparisons utilizing the Holm-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 15.5% 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

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**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 |
|--------|---------------------------------|------------------------------|------|
| Strongylus | GGTGTAGCCGATAACGGAGAA               | TGCCACCAACCACTTAA             | 897 bp |
| Trichuris | GATGCCCTTACGGTCTATTT               | GCCAGAGTCTCCTGTTATC            | 525 bp |
| Eimeria  | ATTGCCTGCTGCTCTCA                  | CCTGCAACTCCTGAGAATC            | 307 bp |
| Isospora | TCTGCACCTCCTGAGAATC                | GACACCGAAACTCCTCTAA            | 330 bp |

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

**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 | Eimeria<sup>a</sup> | Trichuris<sup>ab</sup> | Isospora<sup>c</sup> | Strongylus<sup>d</sup> |
|----------------|---------------------|-----------------------|---------------------|----------------------|
| Mastermix      | 10                  | 12.5                  | 10                  | 12.5                 |
| DNA            | 1                   | 2                     | 1                   | 2                    |
| Primer (each F & R) | 0.5              | 0.125                 | 0.5                 | 1.2                  |
| MgCl2          | –                   | –                     | –                   | –                    |
| Sterile ddH2O  | 8                   | 10.25                 | 8                   | 8.1                  |

<sup>a</sup> Demeler et al. (2013).

<sup>b</sup> Gerhold et al. (2011).

<sup>c</sup> Rutkowski et al. (2001).

<sup>d</sup> Bisset et al. (2014).
 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 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 Isospora, and one from the genus Eimeria. 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.

Table 3

| Thermocycler parameter | Eimeria<sup>a</sup> | Trichuris<sup>b</sup> | Isospora<sup>c</sup> | Strongylus<sup>d</sup> |
|------------------------|-------------------|---------------------|---------------------|---------------------|
| 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 45 s      |
| 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       | 72 °C for 2 m       | 72 °C for 45 s      |
| # cycles (steps 2–4)   | 40                | 40                  | 35                  | 40                  |

<sup>a</sup>Kawahara et al., 2010.
<sup>b</sup>Demeler et al., 2013.
<sup>c</sup>Ruttkowski et al., 2001.
<sup>d</sup>McLean et al., 2012.

Table 4

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

Table 5

| 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 (FRWC) – July | 7350 (OPG) | 450 | 9600 |
| 12 week calf (FRWC) – July | 4100 (OPG) | 450 | 9600 |
| 13 week calf (FRWC) – July | 2850 (OPG) | 450 | 9600 |
| 16 week calf (FRWC) – July | 2350 (OPG) | 450 | 9600 |

<sup>b</sup>Same 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% >1000. For the KCZ, 77.1% of the OPG were ≤200, and 22.9% were ≥500.
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 (\(P < 0.0005\))

![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).]
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. 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 Camelostryngylus 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), Camelostryngylus mentulatus (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

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

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

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 comparisons 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 (Fukimoto et al., 1996; Garrio et al., 2004), and roe deer (Rossi and Ferroglia, 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.

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), Fagioliini 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 species interactions than those at the KCZ. That study also found strongyle abundance in captively 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 gyrophoni* 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.

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

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 OPG levels occurred in July and were 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).

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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 (Fukimoto et al., 1996; Garrio et al., 2004), and roe deer (Rossi and Ferroglia, 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.

Although parasite loads were substantially different between herds, both locations managed herds within or below EPG levels
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 *Isospora* species discovered could have had 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 correct.

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### Appendix A. Supplementary data

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