Detection of *Trichuris* eggs in feces and soil from giraffe (*Giraffa camelopardalis*) and other hoofstock enclosures under human care in the USA

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A B S T R A C T

Trichuris spp. are nematode parasites infecting wild ruminants in zoological institutions worldwide. These helminths cause significant morbidity in giraffe (*Giraffa camelopardalis*) and other hoofstock located in zoological institutions throughout the United States. Historically, studies and institutions have used a variety of nematode detection methods with various flotation solutions. Optimization of *Trichuris* egg detection is necessary for monitoring collections. Fecal and soil optimized protocols were generated in this study using samples containing *Trichuris* eggs from multiple semi free-ranging zoological institutions. First, Sheather's sugar (specific gravity (SG) 1.27), sucrose (SG 1.40), magnesium sulfate (SG 1.26), and zinc sulfate (SG 1.18) were compared as flotation solutions by quantitative eggs per gram using a modified Stoll method. Then a soil recovery method was optimized comparing Tween 20, sodium hydroxide, Dawn™ (Procter and Gamble) detergent, and sodium chloride as liberating solutions to free eggs from the soil. We found that Sheather's sugar and sucrose solutions were the most effective for *Trichuris* egg detection, and either sodium hydroxide or sodium chloride liberated eggs from soil.

1. Introduction

Trichuris spp. are common nematode parasites found in a variety of host species. In zoos and conservation reserves, *Trichuris* eggs are commonly found on routine hoofstock fecal detection (Mir et al., 2016; Pauling et al., 2016; da Silva Barbosa et al., 2020), including in giraffe (*Giraffa camelopardalis*) (VanderWaal et al., 2014; Kyriánová et al., 2017). *Trichuris* in giraffe is a consistent clinical finding for wildlife institutions worldwide (Bertelsen, 2015). The life cycle of *Trichuris* makes it difficult to eradicate. *Trichuris* eggs are shed in feces unembryonated, and when outdoors, these feces become mixed with the soil in each enclosure. The egg then larvates in the environment to an infective stage in about three weeks. The eggs are very hardy, resistant to desiccation, and can survive for months or years in the environment. Ingestion of a larvated egg by a susceptible host allows the larvae to migrate through the gastrointestinal tract, mature into an adult, reproduce, and then female adults deposit eggs in the host's feces (Miller et al., 2012). An infected host can experience a range of clinical signs, including diarrhea, weight loss, decreased appetite, hypoproteinemia, anemia and possible mortality (Van Metre et al., 2008).

Collection giraffes are susceptible to many of the same types of parasites as confined domestic ruminants, including *Trichuris* (Bertelsen, 2015). *Trichuris* infection treatment protocol is different for each institution. Some facilities treat on a timeline schedule, performing routine deworming on a monthly basis (Pauling et al., 2016). Other facilities deworm when animals are identified as infected (Kyriánová et al., 2017). According to Fowler's Zoo and Wild Animal Medicine Volume 8, treatment for *Trichuris* should be instituted even if only a few eggs are found on a fecal examination, due to *Trichuris*' low fecundity and inconsistent shedding (Bertelsen, 2015). Thus, a single egg found on a fecal examination can still indicate that the host is infected with many adult nematodes.

Another variation among facilities is the method and the flotation solutions used to recover *Trichuris* eggs in detection protocols. For example, zinc sulfate (ZnSO$_4$) with a specific gravity (SG) of 1.18 has been used for *T. vulpis* detection in canines (Becker et al., 2016), *T. felis* in felines (Geng et al., 2018), *T. trichiura* in gorillas (Sleeman et al., 2000) and *T. ovis* in soil recovery (Golek and Al-Saeed, 2019). Saturated

sodium chloride (NaCl) with a SG of 1.18 has been used for *T. suis* detection in pigs (Tan et al., 2018). Magnesium sulfate (MgSO$_4$) with a SG of 1.26 has been used for *Trichuris* spp. detection in roe deer (Body et al., 2011). Finally, sucrose solution with SG between 1.27 and 1.33 is a common flotation solution (Dryden et al., 2005) and is used for detection of *Trichuris* spp. at many zoo institutions.

Each species of *Trichuris* egg has a unique SG, for example 1.1299 for *T. suis* and 1.1453 for *T. vulpis* (David and Lindquist, 1982). Therefore, a flotation solution which is denser than the *Trichuris* eggs is necessary to float and detect the eggs in solution. Detection of eggs using the modified Stoll method permits quantitative fecal egg count (Williamson et al., 2014; Zajac and Conboy, 2012) and allows for comparison of different flotation methods to measure relative recovery rates. Additionally, modified Stoll is a sensitive fecal flotation method, allowing detection of 10 EPG (eggs per gram) of fecal material (Zajac and Conboy, 2012) and is well suited for low fecundity nematodes like *Trichuris*.

Trichuris is a soil-transmitted helminth and eggs require time to moult to an L3 infective stage in the environment, therefore soil egg recovery could serve as an additional means of routine *Trichuris* detection and subsequent treatment. If *Trichuris* eggs are detected in soil, facilities could implement measures to better prevent infection, such as quicker removal of feces or temporary movement of highly susceptible hosts from infected enclosures. Before eggs can be detected in soil samples by flotation, they must first be liberated from the soil and concentrated. Ionic solutions can be used to dissociate parasite eggs from soil through displacement of anions found on the parasite egg wall from cations found in soil (Collender et al., 2015); otherwise, if only water is used the eggs may remain adherent or associated with soil particles and not rise through the flotation solution. Different ionic, egg liberating solutions that have been used in previous studies are Tween (Steinbaum et al., 2017) and NaCl (Tun et al., 2015).

In this study, individual animal and environmental surveys of *Trichuris* detection in different giraffe collections was conducted and evaluation of methods occurred. Fecal and soil protocols for *Trichuris* detection were optimized to compare the multiple flotation and liberating solutions used in previous studies. The first aim of this study was to use modified Stoll's method to optimize a fecal flotation protocol and determine which flotation solution recovered the most *Trichuris* eggs. The second aim of this study was to optimize a soil egg recovery protocol to evaluate four liberating solutions to dissociate *Trichuris* eggs from soil and allow egg recovery. We hypothesized that sucrose solution with a SG of 1.40 would yield the most eggs, as this was the highest SG flotation solution tested. We also hypothesized that there would be no significant difference between the liberating solutions tested in their ability to dissociate for the detection of *Trichuris* eggs from soil.

2. Materials and methods

2.1. Study site locations

The facilities chosen in this study were White Oak Conservation (WO) in Yulee, Florida; Fossil Rim Wildlife Center (FR) in Glen Rose, Texas; The Wilds (TW) in Cumberland, Ohio; and Binder Park Zoo (BP) in Battle Creek, Michigan.

2.2. Helminth management survey of the facilities

Each study site was surveyed, and information was gathered regarding fecal flotation procedure and protocol for *Trichuris*, as well as feeding location and feces removal protocols. These locations were chosen in an attempt to gather samples from a range of temperate regions with differing environmental and soil conditions, and institutional management styles. Weather data over the past 30 years was reviewed and Koeppen-Geiger Climate Classifications were summarized from the Midwestern Regional Climate Center (Wilson, A.). WO and FR reside in humid, subtropical climates, however WO has an annual average total

precipitation of 1.4 m while FR is more drought prone and has an annual average total precipitation of 1.0 m. Neither has dry summer months or a record minimum temperature below −11.7 °C. TW and BP reside in humid, continental climates; however, TW has slightly hotter summers compared to BP's warm summers. Both sites receive around 0.5–1.0 m of snow per year, with BP receiving a bit more than TW.

2.3. Study sites and sample collection

Giraffe *Trichuris* positive fecal and adjacent soil samples were obtained from each zoological facility. At the time of this study the giraffe populations sampled included 13 to 15 giraffes at WO, ten giraffes at FR, eight giraffes at TW, and seven giraffes at BP. These zoological institutions manage giraffes on large pastures when temperatures allow; and smaller yards with heated barns when temperatures or weather met specific criteria. For example, at TW, giraffes are locked in their barn when temperatures fall below 7 °C, and they are given barn access when temperatures are below 10 °C with situational adjustments based on other factors, such as wind and rain. While on open pasture there may be various interspecies interactions. For example, at FR, the giraffes share a pasture with red deer (*Cervus elaphus*), Arabian oryx (*Oryx leucoryx*), Hartmann's mountain zebra (*Equus zebra hartmannae*), Nile lechwe (*Kobus megaceros*), and fallow deer (*Dama dama*). Whereas BP manages its giraffe herd in the spring through fall in a 19 acre mixed species exhibit with addax (*Addax nasomaculatus*), bontebok (*Damaliscus pygargus pygarus*), Grant's zebra (*Equus burchelli*), addra gazelle (*Nanger dama*), and common waterbuck (*Kobus ellipsiprymnus*).

Samples, fecal and soil, were collected in Ziploc® bags and stored at either −15.6 °C or room temperature until shipped to The Ohio State University (OSU), College of Veterinary Medicine, Diagnostic Parasitology Laboratory no later than 2 weeks after collection. Fecal samples were collected after witnessing the individual animal defecate and when personel could safely collect the feces. Soil samples were also sent from sites where the giraffe are often housed and defecate and followed the same collection and shipping protocols as feces. An average of 100–400 g of soil was collected from each giraffe site. Coordinates from each soil sample site were run through a soil classification program (Wilson et al., 2007) at https://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx and soil types were recorded. The fecal and soil samples were all individually labelled, stored at room temperature, periodically moistened with water, and antibiotic and antimycotoxin solution (100 units/ml of penicillin, 100 μg/ml of streptomycin, and 0.25 μg/ml of Amphotericin B) in water were added to fecal samples as needed at OSU until processed. Once received at OSU, fecal and soil processing occurred anywhere from 7 days to about 3 months after receipt. Prior to processing for egg recovery, samples were well mixed to obtain a homogeneous mixture to ensure even distribution of the eggs through the representative sample.

2.4. Determination of the most effective flotation solution for fecal samples

All of the fecal samples were subjected to modified Stoll method (Zajac and Conboy, 2012) with different flotation media for an egg recovery quantitative analysis. Each sample was tested using four flotation solutions: Sheather's sugar (SG 1.27), sucrose (SG 1.40), ZnSO$_4$ (SG 1.18), and MgSO$_4$ (SG 1.15). These solutions were chosen based on previous study utility and the density of *Trichuris* eggs. Briefly, 2 g of feces were well mixed with 98 ml of water in a flask. From this flask, a quadruplicate set of 10 ml aliquots were placed into 15 ml centrifuge tubes. The tubes were spun at ~1800 g for 5 min. The water was removed, and the resulting pellet was resuspended in the designated flotation solution to be evaluated. The nearly full tubes underwent another centrifugation at ~1800 g for 5 min. After centrifugation, more flotation solution was added to make a bulging meniscus on each tube. Coverslips were placed on the meniscus and allowed to sit for 10 min

before eggs were counted by microscopy. After the first coverslip was removed, more flotation solution was added to each tube to make a second bulging meniscus and another coverslip was allowed to sit for 10 min and read by microscopy to capture any additional eggs not recovered on the first coverslip. The EPG for each tube was recorded by multiplying the total number of *Trichuris* eggs found under both coverslips by five.

Based on preliminary results demonstrating disparate recovery of *Trichuris* eggs when comparing the sugar-based flotation solutions (greater quantity of eggs recovered) to the $ZnSO_4$ and $MgSO_4$ flotation solutions, an additional experiment was conducted. This involved using the fecal sediment material remaining at the bottom of the tube after the completion of the $ZnSO_4$ and $MgSO_4$ processed sample coverslips were read. This residual sediment is generally considered waste and discarded. However, fecal residual sediment samples from WO and FR sites post -$ZnSO_4$ and post -$MgSO_4$ were selected to undergo additional modified Stoll method using Sheather's sugar flotation to determine if the eggs remained in the residual sediment.

2.5. Determination of most efficient soil egg liberating solution

A soil recovery protocol was initially optimized testing TW-derived soil with *Trichuris* egg recovery and evaluated using four different detergents: 0.05% v/v Tween 20 in water, 0.1 N NaOH in water, 0.05% v/v Dawn™ (Procter and Gamble) in water, and 0.9% w/v NaCl in water on each individual soil sample. These ionic detergents were chosen based on their accessibility in a clinic setting and their non-toxic nature relative to waste generation and disposal. For BP, FR and WO, only NaCl and NaOH were used as the dispersion and first wash solution based on preliminary results of unreadable microscope slides using v/v Tween 20 and Dawn™ in water. This was followed by the use of Sheather's sugar (SG 1.27) as a flotation solution. Sheather's sugar was chosen as the flotation medium based on the results from the egg recovery from the fecal samples.

The details of the soil egg recovery involved using 50 g of soil in a glass beaker with 450 ml of the detergent or ionic solution. This was mixed by hand with a large, flat hand-held blade for 1 min, allowing the ionic solution to liberate the eggs from the soil. This slurry was then poured through a two to three mm wire weave strainer into a pilsner glass, allowed to sit for 30 s and then the supernatant containing the eggs was decanted into a 500 ml beaker. The eggs were allowed to settle in the beaker for 2 h. The supernatant was then carefully decanted, and the remaining sediment was rinsed into 15 ml centrifuge tubes. We aimed to have less than 5 ml of sediment in each centrifuge tube, so three to four centrifuge tubes were used per beaker of sample. The sediment was centrifuged (~1800 g) for 5 min and the supernatant was decanted. Approximately 3–5 ml of water was added to the centrifuge tubes and centrifuged (~1800 g) for 5 min again to wash the pellet and the supernatant was decanted. Sheather's sugar solution was added to the pellet and mixed well using mixing applicant sticks. The sediment-flotation media was centrifuged (~1800 g) for 5 min. After centrifugation, more flotation solution was added to make a bulging meniscus on each tube. Coverslips were placed on the meniscus and allowed to sit for 10 min. To obtain a number of eggs per 50 g of soil from each site, the coverslips were read by microscopy for *Trichuris* eggs and counts from the same site and liberating solution were summed.

2.6. Statistical analysis

To test the hypothesis that the solutions were different from one another in their ability to recover *Trichuris* eggs, pairwise comparisons of each solution were made using a two-sided Wilcoxon signed rank test using the EPG data on fecal samples and the egg count data on soil samples. This test was chosen due to the nonparametric data and the multiple observations for each sample. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Survey results

Survey results for each study site are summarized (Table 1). Each facility differed on their detection protocols for *Trichuris* in giraffe. At WO, giraffe have access to elevated grain and hay rack feeding areas and hanging browse, and it is estimated that the heaviest defecation is between 100 and 300 feet from these feeding areas. At FR, giraffe are provided elevated feed troughs. At TW, in the case of new arrivals, weekly fecal exams are conducted while the giraffe is in quarantine and held in the same facility as the current collection. Clinical signs warranting fecal testing include abnormal stool production or decreasing weight or body condition. Giraffe are provided hanging browse and elevated feeders. At BP, clinical signs warranting fecal testing include soft stool or decreasing weight or body condition. Giraffe are also provided hanging browse and elevated feeders. Information collected from the study site surveys was used as background descriptive information, with no further comparative analysis relative to the soil and fecal data collected and analyzed.

3.2. Sample collection

Two individual fecal samples were collected from WO, three individual fecal samples were collected from FR, five individual fecal samples were collected from TW, and three individual fecal samples were collected from BP. Additionally, soil samples were collected from two sites at WO, two sites from FR, four sites from TW, and three sites from BP. The collection sites, coordinates, and soil types are presented (Table 2).

3.3. Egg recoveries from feces and soil

The *Trichuris* EPG of the modified Stoll's fecals are presented (Table 3a). EPGs of fecal flotation where $ZnSO_4$ and $MgSO_4$ flotation were initially used followed by immediate re-processing of sediment using Sheather's sugar flotation is presented (Table 3b). The *Trichuris*

Table 1
Site location survey results.

Location	Flotation procedure	Scheduled protocols	Feces removal and feeding methods
WO	Modified McMaster	Monthly fecal exam of all individuals and fecal examination based on clinical signs	Feces removed from giraffe barn daily, corral monthly, and never from pasture. Elevated feeding areas provided.
FR	Modified McMaster	Twice per year fecal examination of all individuals and fecal examination based on clinical signs	Feces removed from inhabited giraffe barns and yards weekly and never from pasture. Elevated feeding areas provided.
TW	Modified Stoll	Monthly fecal examination of all individuals and fecal examination based on clinical signs. Weekly fecal examination while in quarantine	Feces removed from inhabited giraffe barns daily and never from pastures. Elevated feeding areas provided.
BP	Passive flotation	Quarterly fecal examination on all individuals and fecal examination based on clinical signs. Monthly fecal examination on giraffe <1 yr old and new arrivals during their first year after exiting quarantine period. Weekly fecal examinations while in quarantine	Feces removed from giraffe indoor stalls and outdoor holding yards daily and never from pasture. Elevated feeding areas provided.

Table 2
Description, coordinates, and soil types from study sites.

Location	Enclosure	Coordinates	Soil Type
WO	Swale (in pasture) and behind corral	30.742643, −81.730845	Chaires fine sand and Goldhead fine sand
FR	Main pasture	32.169325, −97.805368	Sunev clay loam and Maloterre gravelly clay loam
FR	Giraffe pasture	32.160912, −97.798373	Brackett soils, Venus loam, and Frio silty clay
TW	Feeder in pasture, water trough in pasture, and giraffe barn fence line	39.8367, −81.7189	Morristown silty clay loam, reclaimed
BP	G1, G2, and G4[a]	42.241512, −85.167761	Oshtemo sandy loam

[a] Facility outdoor holding yard designation.

egg counts recorded from soil recovery are presented (Table 4).

3.4. Statistical analysis and egg recovery comparison

The results of the fecal EPG statistical analysis are recorded (Table 5) and support the hypothesis that the solutions are different regarding egg recovery. Sheather's sugar and sucrose solutions consistently recovered significantly more eggs compared to $MgSO_4$ and $ZnSO_4$. Often, a solution recovered no eggs using $MgSO_4$ or $ZnSO_4$, whereas eggs were recovered using Sheather's sugar or sucrose. The Sheather's sugar and sucrose solutions were not statistically different, meaning one solution did not detect more eggs than the other. However, sucrose was more technically difficult to obtain a good mixture of the sediment due to its intense viscosity as compared to Sheather's sugar solution. Additionally, $MgSO_4$ and $ZnSO_4$ were not significantly different in their egg recoveries compared to one another.

For soil recovery of *Trichuris* eggs, NaCl and NaOH dispersion solutions were not significantly different (p = 0.25). This may be due to the low number of *Trichuris* positive soil samples. Initially after processing soil samples from TW, it was determined that Tween 20 and Dawn™ (Procter and Gamble) detergent generated too many bubbles under the coverslip for readable slides, thus their use was discontinued.

It was determined that the number of eggs recovered from individual giraffe feces did not parallel the number of eggs recovered from the corresponding soil location. Individual giraffes with the highest EPG came from FR and WO; however, the most soil eggs were recovered from BP. The Wilds had the highest number of positive individuals (n = 5), but neither the highest EPG or soil egg recovery.

4. Discussion

The survey results of this study found that many sites were currently utilizing passive flotation techniques to analyze their fecal samples. The use of a modified Stoll technique, which utilizes centrifugation, rather than a passive flotation protocol for *Trichuris* egg detection is further supported by other fecal detection protocol comparison studies. In a study comparing passive fecal flotation results to $ZnSO_4$ (SG 1.2) centrifugation, passive fecal flotation identified *Trichuris* positive results in only 54.2% of *T. vulpis* positive canine feces, thereby missing 45.8% of *Trichuris* positive individuals (Gates and Nolan, 2009). In another study which compared camelid fecal examination using a modified McMasters technique to a centrifugation-flotation procedure, the centrifugation-flotation procedure yielded more positive *Trichuris* results, as well as more positive results for *Eimeria macusaniensis* oocysts, *Nematodirus,* and capillarids-type eggs (Cebra and Stang, 2008). The mean egg recovery rate for the modified McMasters procedure was determined to be 46.4% with reliable positive results as of 500 EPG, whereas centrifugation-flotation technique with $ZnSO_4$ flotation (SG

Table 3a
Individual modified Stoll procedures results.

Location	Giraffe Identifier	Flotation Solution	First Coverslip	Second Coverslip	EPG in Feces
WO	1	Sheather's	29	1	150
		Sucrose	23	2	125
		$ZnSO_4$	0	6	30
		$MgSO_4$	2	1	15
WO	2	Sheather's	0	0	0
		Sucrose	0	0	0
		$ZnSO_4$	0	0	0
		$MgSO_4$	0	1	5
FR	3	Sheather's	28	3	155
		Sucrose	58	5	315
		$ZnSO_4$	0	0	0
		$MgSO_4$	0	0	0
FR	4	Sheather's	9	2	55
		Sucrose	14	7	105
		$ZnSO_4$	0	0	0
		$MgSO_4$	0	0	0
FR	5	Sheather's	3	0	15
		Sucrose	1	3	20
		$ZnSO_4$	1	3	20
		$MgSO_4$	0	1	5
TW	6	Sheather's	0	0	0
		Sucrose	1	1	10
		$ZnSO_4$	0	0	0
		$MgSO_4$	0	0	0
TW	7	Sheather's	0	0	0
		Sucrose	1	1	10
		$ZnSO_4$	0	0	0
		$MgSO_4$	0	0	0
TW	8	Sheather's	0	1	5
		Sucrose	0	0	0
		$ZnSO_4$	0	0	0
		$MgSO_4$	0	0	0
TW	9	Sheather's	2	0	10
		Sucrose	8	0	40
		$ZnSO_4$	2	0	10
		$MgSO_4$	0	0	0
TW	10	Sheather's	1	0	5
		Sucrose	0	0	0
		$ZnSO_4$	0	0	0
		$MgSO_4$	0	0	0
BP	11A[a]	Sheather's	1	0	5
		Sucrose	0	0	0
		$ZnSO_4$	1	0	5
		$MgSO_4$	0	0	0
BP	11B[a]	Sheather's	3	1	20
		Sucrose	5	1	30
		$ZnSO_4$	0	0	0
		$MgSO_4$	1	0	5
BP	12	Sheather's	0	0	0
		Sucrose	0	0	0
		$ZnSO_4$	0	0	0
		$MgSO_4$	0	0	0
BP	13A[b]	Sheather's	1	0	5
		Sucrose	2	0	10
		$ZnSO_4$	0	0	0
		$MgSO_4$	0	0	0
BP	13B[b]	Sheather's	7	0	35
		Sucrose	5	0	25
		$ZnSO_4$	0	2	10
		$MgSO_4$	0	0	0

[a] 11A and 11B were taken 1 week apart from the same individual.

[b] 13A and 13B were taken 1 week apart from the same individual.

1.3) had reliable positive results as of 80 EPG (Becker et al., 2016). These studies all support that the use of a centrifugation-flotation procedure is more effective at identifying positive *Trichuris* egg shedding individuals than a passive fecal flotation, and that eggs from low fecundity parasites like *Trichuris* are less likely to be detected using a modified McMaster technique.

Though some samples were kept at room temperature for 2 weeks

Table 3b
Results of initial and reprocessing of selected samples.

Location	Giraffe	Initial[a]	EPG[b]	EPG[c]
WO	1	$ZnSO_4$	0	205
WO	1	$MgSO_4$	10	215
FR	3	$ZnSO_4$	0	350
FR	3	$MgSO_4$	0	450

[a] Flotation solution used for initial processing.

[b] Eggs per gram recovered after initial processing.

[c] Eggs per gram results from residual sediment reprocessed using Sheather's sugar (SG 1.27).

Table 4
Results of soil recovery protocol.

Location	Enclosure	Suspensory fluid	Egg count	Observations
TW	Feeder	Tween	0	
		Dawn	0	Too many bubbles
		NaOH	1	57.5 μm x 32.5 μm[a]
		NaCl	1	
TW	Water trough	Tween	0	
		Dawn	0	
		NaOH	0	
		NaCl	0	
TW	Barn fence line	Tween	0	
		Dawn	0	Too many bubbles
		NaOH	0	Too many bubbles
		NaCl	0	
FR	Main pasture	NaOH	0	Too many bubbles
		NaCl	0	
FR	Pasture	NaOH	0	Many mites and strongyles
		NaCl	0	Many mites and strongyles
BP	G1[b]	NaOH	7	
		NaCl	87	67.5 μm × 35.0 μm
BP	G2[b]	NaOH	5	
		NaCl	34	72.5 μm × 35.0 μm
BP	G4[b]	NaCl	10	
WO	Swale	NaOH	1	55.0 μm × 37.5 μm
		NaCl	0	
WO	Behind corral	NaOH	0	
		NaCl	2	70.0 μm × 37.5 μm

[a] Recovered egg length by width measurements in microns.

[b] Facility outdoor holding yard designation.

Table 5
Two sided Wilcoxon signed rank test p-values for pairwise comparisons.

	Sheather's	Sucrose	$ZnSO_4$
Sucrose	0.1953		
$ZnSO_4$	0.0195[a]	0.0039[a]	
$MgSO_4$	0.0029[a]	0.002[a]	0.1094

[a] Values less than 0.05 were significantly different.

without any preserving solution before processing, *Trichuris* eggs were still recovered from held soil and fecal samples. This egg recovery ability is consistent with other studies which found that *Trichuris* has the ability to remain in the environment for years (Burden and Hammet, 1979; Burden et al., 1987). This finding also supports that enclosure and soil contamination with *Trichuris* eggs poses a risk to susceptible host species within that enclosure for potentially years.

In a study comparing flotation-centrifugation with zinc chloride (SG 1.45) to flotation-centrifugation with sucrose (SG 1.2), zinc chloride was able to identify more *Trichuris* positive results and recover more *Trichuris* eggs (Taglioretti et al., 2014). This differs from our finding that sucrose SG 1.40 flotation recovered statistically similar numbers of eggs to Sheather's sugar SG 1.27. Additionally, a soil egg recovery method has been previously described by Horiuchi and Uga (2016) which found that using 0.05% Tween as a detergent and sucrose (SG 1.2) was effective at recovering *Toxocara* eggs in soil, and also detected *Trichuris* eggs in soil. However, we found that 0.05% v/v Tween produced unreadable slides. The 0.1N NaOH or 0.9% w/v NaCl produced readable slides as compared to the detergent based dispersion solution which did not.

Possible variables which could alter the number of *Trichuris* eggs found in fecal and soil samples include host treatment protocol for *Trichuris* being utilized at each site, other direct hosts or aberrant hosts for *Trichuris* that may be sharing enclosures with the giraffe affected, and population density of the giraffe relative to enclosure size and soil substrate matrix of the environment. In a study where soil samples were gathered from Kenya and Bangladesh, it was found that texture of the soil effected soil transmitted helminths (STH) egg recovery, where sandy samples had higher recovery than loamy samples (Steinbaum et al., 2016). According to Collender et al. (2015), the recovery efficiency from highest to lowest would be sand, loam, and then clay. In this study, *Trichuris* eggs were found in clay, loam soil at TW and sandy, loam soil at BP. Eggs were not found in fine sand soil at WO or in clay, loam at FR.

Individual susceptibility parameters for parasitism may also contribute to increased parasite loads, such as age, body mass, and pregnancy status (Pauling et al., 2016). Additionally, due to the fecal-oral spread of *Trichuris*, other factors which can impact exposure include type and quality of forage supplied, how long and on what substrate animals are allowed to graze, and time at which feces are removed from the grazing area and enclosures. Feed or feces which is left near egg contaminated soil for longer than three weeks is at greater risk of containing infective *Trichuris* eggs. At all sites, giraffe are provided elevated feeding areas and feces are removed from indoor areas daily or weekly. However, routine feces removal on pasture is not conducted. Therefore, giraffe on pasture are at greater risk of ingesting infective eggs, especially those which graze. In a wild environment, giraffe are known to be browsers which may reduce their exposure to STH, such as *Trichuris* (VanderWaal et al., 2014). However, at all sites sampled in this study the giraffe are allowed grazing on pasture thereby increasing their exposure to STH, though some individuals graze more than others. For example, at TW giraffe are often seen grazing on pasture, whereas at FR and WO grazing is not witnessed often.

The small number of animals sampled with respect to total herd sizes is a limitation of this study. However, this study determined that a modified Stoll method utilizing higher specific gravity Sheather's sugar or sucrose flotation solution was significantly more effective than $ZnSO_4$ or $MgSO_4$ for detection of *Trichuris* eggs from the sampled giraffe feces. Both Sheather's sugar SG 1.27 and sucrose SG 1.40 identified positive individuals, recovering statistically similar EPGs. As soil dispersion solutions, the NaOH and NaCl were not statistically different and trended towards being more effective at liberating the giraffe *Trichuris* eggs from soil compared to Tween 20 or Dawn™ (Procter and Gamble) due to their ability to produce readable slides. Another limitation of this study was the low number of positive *Trichuris* soil samples. Statistical significance may have been determined between the NaOH and NaCl had more positive soil samples been analyzed. Additionally, the different sized *Trichuris* eggs recovered from each site suggest the presence of different *Trichuris* spp. contaminating the enclosures or among giraffe populations.

The fecal and soil protocols optimized in this study can be utilized by institutions for routine detection of *Trichuris* and subsequent treatment. In the cases where *Trichuris* infection is a chronic herd problem, routine fecal and soil monitoring can support decisions which limit exposure of susceptible individuals. For example, *Trichuris* positive individuals should have their feces removed from enclosures as soon as possible.

Routine monitoring of *Trichuris* is important for host health, as well as judicious treatment protocols.

In conclusion, this study demonstrates that the use of ZnSO4 SG 1.18 or MgSO4 SG 1.15 for centrifugation fecal flotation will underestimate or miss animals shedding *Trichuris* eggs. The study results support the use of Sheather's sugar or sucrose flotation solution with at least SG 1.27 along with centrifugation for fecal egg detection. In addition, NaOH and NaCl are successful ionic solutions for liberating eggs from the soil types analyzed in this study.

Declaration of competing interest

To the best of the authors' knowledge regarding conflict of interest, declarations of interest: none.

References

Becker, A.C., Kraemer, A., Epe, C., Strube, C., 2016. Sensitivity and efficiency of selected coproscopical methods—sedimentation, combined zinc sulfate sedimentation-flotation, and McMaster method. Parasitol. Res. 115, 2581–2587.

Bertelsen, M.F., 2015. Giraffidae. Fowler's Zoo and Wild Animal Medicine, vol. 8. W. B. Saunders, Philadelphia, pp. 602–610.

Body, G., Ferté, H., Gaillard, J., Delorme, D., Klein, F., Gilot-Fromont, E., 2011. Population density and phenotypic attributes influence the level of nematode parasitism in roe deer. Oecologia 167 (3), 635–646.

Burden, D.J., Hammet, N.C., 1979. The development and survival of *Trichuris suis* ova on pasture plots in the south of England. Res. Vet. Sci. 26, 66–70.

Burden, D.J., Hammet, N.C., Brooks, P.A., 1987. Field Observations on the longevity of *Trichuris suis* ova. Vet. Rec. 121, 43.

Cebra, C.K., Stang, B.V., 2008. Comparison of methods to detect gastrointestinal parasites in llamas and alpacas. J. Am. Vet. Med. Assoc. 232 (5), 733–741.

Collender, P.A., Kirby, A.E., Addiss, D.G., Freeman, M.C., Remais, J.V., 2015. Methods for quantification of soil-transmitted helminths in environmental media: current techniques and recent advances. Trends Parasitol. 31 (12), 625–639.

da Silva Barbosa, A., Pinheiro, J.L., Dos Santos, C.R., de Lima, C.S.C.C., Dib, L.V., Echarte, G.V., Augusto, A.M., Bastos, A.C.M.P., Uchôa, C.M.A., Bastos, O.M.P., Santos, F.N., Fonseca, A.B.M., Amendoeira, M.R.R., 2020. Gastrointestinal parasites in captive animals at the rio de Janeiro zoo. Acta Parasitol. 65, 237–249.

David, E., Lindquist, W., 1982. Determination of the specific gravity of certain helminth eggs using sucrose density gradient centrifugation. J. Parasitol. 68 (5), 916–919.

Dryden, M.W., Payne, P.A., Ridley, R., Smith, V., 2005. Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. Vet. Therapeut. 6, 15–28.

Gates, M.C., Nolan, T.J., 2009. Comparison of passive fecal flotation run by veterinary students to zinc-sulfate centrifugation flotation run in a diagnostic Parasitology laboratory. J. Parasitol. 95 (5), 1213–1214.

Geng, J., Elsemore, D.A., Oudin, N., Ketzis, J.K., 2018. Diagnosis of feline whipworm infection using a coproantigen ELISA and the prevalence in feral cats in southern Florida. Vet Parasitol Reg Stud Rep 14, 181–186.

Golek, H.I., Al-Saeed, A.T., 2019. Contamination of soil with Toxocara and other helminths in soils of amadyia district, duhok governorate, Kurdistan region - Iraq. Appl. Ecol. Environ. Res. 17 (6), 14883–14891.

Horiuchi, S., Uga, S., 2016. Modified flotation method, an effective technique for recovering helminth eggs in soil. Parasitol. Int. 65 (5), 576–579.

Kyriánová, I., Drnek, J., Langrová, I., Peřinková, P., Nechybová, S., 2017. Gastrointestinal parasites in giraffes kept in zoological gardens of the Czech republic. Sci. Agric. Bohem. 48 (3), 122–126.

Miller, J.E., Kaplan, R.M., Pugh, D.G., 2012. Internal Parasites. Sheep and Goat Medicine, second ed. W. B. Saunders, Philadelphia, pp. 106–125.

Mir, A.Q., Dua, K., Singla, L.D., Sharma, S., Singh, M.P., 2016. Prevalence of parasitic infection in captive wild animals in Bir Moti Bagh mini zoo (Deer Park), Patiala, Punjab. Vet. World 9 (6), 540–543.

Pauling, C.D., Oller, A.R., Jackson, V., 2016. Fecal parasite identification by microscopy and PCR in scimitar-horned oryx, Oryx dammah, managed at two sites. Int J Parasitol Parasites Wildl 5 (3), 312–320.

Sleeman, J., Meader, L., Mudakikwa, A., Foster, J., Patton, S., 2000. Gastrointestinal Parasites of Mountain Gorillas (*Gorilla gorilla beringei*) in the Parc National des Volcans, Rwanda. J. Zoo Wildl. Med. 31 (3), 322–328.

Steinbaum, L., Kwong, L.H., Ercumen, A., Negash, M.S., Lovely, A.J., Njenga, S.M., Nelson, K.L., 2017. Detecting and enumerating soil-transmitted helminth eggs in soil: new method development and results from field testing in Kenya and Bangladesh. PLoS Neglected Trop. Dis. 11 (4), e0005522.

Taglioretti, V., Sardella, N., Fugassa, M., 2014. Effectiveness of coproscopic concentration techniques. Helminthologia 51 (3), 210–214.

Tan, L., Wang, A., Yi, J., Liu, Y., Li, J., Liu, W., 2018. Prevalence and phylogenetic analyses of *Trichuris suis* in pigs in hunan province, subtropical China. Kor. J. Parasitol. 56 (5), 495–500.

Tun, S., Ithoi, I., Mahmud, R., Samsudin, N.I., Kek Heng, C., Yee Ling, L., 2015. Detection of helminth eggs and identification of hookworm species in stray cats, dogs and soil from Klang valley, Malaysia. PloS One 10 (12), e0142231.

Van Metre, D.C., Tennant, B.C., Whitlock, R.H., Divers, Thomas J., Peek, Simon F., 2008. Chapter 6 - infectious diseases of the gastrointestinal tract. In: Rebhun's Diseases of Dairy Cattle, second ed. W.B. Saunders, pp. 200–294. ISBN 9781416031376.

VanderWaal, K., Omondi, G.P., Obanda, V., 2014. Mixed-host aggregations and helminth parasite sharing in an East African wildlife–livestock system. Vet. Parasitol. 205 (1–2), 224–232.

Williamson, Lisa H., Van Saun, Robert J., Johnson, LaRue W., 2014. Chapter 6 - anthelmintic resistance in camelid parasites. In: Cebra, Christopher, Anderson, David E., Tibary, Ahmed, Llama, Care, Alpaca, Saunders, W.B. (Eds.), pp. 16–22, 9781437723526.

Wilson, A. State Climate Office of Ohio. The Ohio State University. NOAA Cooperative Network Data Obtained from the Midwestern Regional Climate Center, Cli-MATE (MRCC Application Tools Environment, on/CLIMATE/"). Accessed online on April 12, 2021.

Wilson, M.A., Burt, R., Indorante, S.J., Jenkins, A.B., Chiaretti, J.V., Ulmer, M.G., Scheyer, J.M., 2007. Geochemistry in the modern soil survey program. Environ. Monit. Assess. 139 (1–3), 151–171.

Zajac, A.M., Conboy, G.A., 2012. Veterinary Clinical Parasitology. John Wiley & Sons, Incorporated, Ames, IA. ISBN 9781118292037.