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Not playing by the rules: Unusual patterns in the epidemiology of parasites in a natural population of feral horses (*Equus caballus*) on Sable Island, Canada

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ABSTRACT

Sable Island, Nova Scotia, Canada hosts one of few natural populations of feral horses (*Equus caballus*) never exposed to anthelmintics. Coproculture revealed cyathostomes, *Strongylus equinus*, *S. edentatus*, and *S. vulgaris*, with *S. equinus* (unusually) dominating in adult horses and cyathostomes dominating in young horses (< 3 years of age). We examined 35 horses found dead in the springs of 2017 and 2018, as well as fecal samples from live horses in spring (n = 45) and summer 2018 (n = 236) using McMaster fecal flotation and Baermann larval sedimentation on fresh samples, and modified Wisconsin flotation and sucrose gradient immunofluorescent assay for *Giardia* and *Cryptosporidium* on frozen samples. Mean strongyle fecal egg counts were 666 eggs per gram (EPG) in dead horses, 689 EPG in live horses in spring, and 1105 EPG in summer; domestic horses are usually treated at counts exceeding 200 EPG. Adult horses (unusually) had patent infections with the lungworm *Dictyocaulus arnfieldi* and ascarids (*Parascaris* spp.), and in spring, dead horses had 5 times higher odds of having patent ascarid infections than live horses, likely due to malnutrition and corresponding immunodeficiency. Fecal prevalence and intensity of *D. arnfieldi* and *Parascaris* spp. were significantly higher in young horses, and in spring versus summer. A higher proportion of fecal samples were positive for strongyle and ascarid eggs using a centrifugal flotation technique on previously frozen feces, as compared to a passive flotation method on fresh feces. Eggs of the tapeworm *Paranoplocephala mamillana* were present in fecal samples from 28% of live, and 42% of dead, horses in spring. This research represents several new geographic records (*S. edentatus*, *D. arnfieldi*, and *Eimeria leuckarti*), provides insight into unusual patterns of parasite epidemiology in a nutrition-limited environment, and has conservation and biosecurity implications for this unique equine population, as well as for parasite management in domestic horses.

1. Introduction

The study of parasites in unmanaged feral host populations on islands provides a unique opportunity to explore natural transmission in a closed population, and can provide insight into the ecology, epidemiology, and genetics of hosts and parasites alike (Gulland, 1992;

Debeffe et al., 2016; Gold et al., 2019). Sable Island National Park Reserve is a 34 km² (49 km long, maximum 1.3 km wide), vegetated sand bar located approximately 275 km east of Halifax, Nova Scotia, Canada (43° 55' N, 60° 00' W) (Fig. 1). Feral horses (*Equus caballus*) are the only terrestrial mammals on the island, founded in the mid-1700s with repeated introductions until 1935 (Welsh, 1975). The horses have

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Fig. 1. Map of Sable Island, Canada, which is about 50 km long, 1 km wide at its widest point, and in total, 34 km² (from Gold et al., 2019).

been legally protected from human interference since 1961, and Sable Island became a National Park Reserve in 2013, with the horses now classified as ‘naturalized wildlife’. The population is free-ranging, and the horses survive despite high pasture densities, a high component of sand in their diet, and absence of human interventions such as supplementary feeding, anthelmintic treatment, dentistry, and farriery. To our knowledge, Sable Island supports one of few feral horse populations where no members have received anthelmintics, the population is closed, and individual animals are identifiable from a photographic database dating back to 2007. Genetic analyses of the Sable horses suggests that they are unique, highly inbred, and share a common ancestor with Newfoundland, Icelandic and Fjord breeds (Plante et al., 2007; Tollett, 2018). As such, they represent a study system of great scientific and veterinary interest, and are equally important from a conservation perspective.

The last and only other post-mortem gastrointestinal helminth parasite survey in the Sable horse population examined 32 adult horses and 15 foals in 1971–72. Findings included ascarids (*Parascaris* spp.), a cestode (*Paranoplocephala mamillana*), 2 large strongyles (*Strongylus vulgaris* and *S. equinus*), and at least 9 cyathostomes, or small strongyles (Welsh, 1975). More recent work, based on non-invasive fecal sampling every summer since 2014, demonstrated that Sable Island horses have high strongyle fecal egg counts (FEC) (mean of ~1500 eggs per gram in summer), and that high individual FEC correlated with poor body condition in lactating mares (Debeffe et al., 2016; Gold et al., 2019).

Since strongyle species cannot be inferred from egg morphology, the first objective of the current study was to determine the strongyle species currently present in Sable horses, especially the large strongyles, which are highly pathogenic and increasingly rare in domestic horse populations (McCraw and Slocombe, 1985; Young et al., 1999). The second objective was to update the post-mortem parasite study from 1971–72, bringing new understanding of parasite taxonomy and molecular tools to bear on parasite identification. For this, we examined natural mortalities on the island in April 2017, following an unusually large die-off of approximately 10% of the population, and a smaller number of horses (a more typical 1% of the population) in April 2018; the causes of these mortality events (the primary proximate cause was emaciation) will be reported in another manuscript. Finally, the third aim of the study was to explore the effects of season, age, and sex on parasite shedding in free-ranging horses under completely natural transmission dynamics, difficult to replicate even in anthelmintic-free research herds (Lyons et al., 2006; Nielsen et al., 2010).

Our results are of general interest because both host and parasite populations are of conservation significance. The Sable Island horses have a unique, archaic, bottlenecked parasite fauna that has never been subjected to anthelmintic treatment, where parasite transmission occurs naturally and is thus of interest epidemiologically, and ecologically with respect to the study of density-dependent population regulation (Gulland, 1992).

2. Materials and methods

Collection of samples from live and dead horses were approved by the University of Saskatchewan Animal Research Ethics Board (Protocol 20090032), and Parks Canada Agency Research and Collections (Permits SINP 2013-14314 and SINP 2017-20436).

2.1. Strongyle species identification on coproculture

To identify strongyle species in Sable horses, we morphologically identified third stage larvae (L3) cultured from feces. This was necessary because sampling from live horses is limited to ground-collected feces, and many different nematode species produce morphologically indistinguishable strongyle-type eggs (including the three migratory large strongyle species, *S. vulgaris*, *S. edentatus*, and *S. equinus*, over 50 small strongyle species, and a few other trichostrongyles and non-migratory large strongyles). 89 fecal samples were opportunistically collected in the summer of 2014, as part of systematic census of the island, in which fecal samples were collected within minutes of defecation from individual horses. From 8 horses, two fecal samples were collected within a 2 week period, and for these, counts were averaged; therefore, the total number of individual horses examined was 81. Fresh feces were stored in nitrile gloves in the field, and kept at ambient temperature for a maximum of 7 h. Briefly, 30 g feces from each sample were mixed with three heaped teaspoons of vermiculite in a glass tumbler and moistened with tap water. Tumblers were then covered with a medium plastic Petri dish and kept moist at 26 °C for 6 days. Larvae were extracted overnight using the glass-over-petri-dish method (Dunn, 1978), and aliquots were fixed in 10% formalin for morphological identification. From each sample, up to 200 L3 were morphologically identified (Dunn, 1978; Madeira de Carvalho et al., 2007) (Table 1). The proportion of larvae of each species/genus in each sample was calculated, and the average proportion for each equine age group (foals, young horses < 3 years of age, and adult horses ≥ 3 years) reported for the 5 most common parasites.

2.2. Parasite examination of dead horses

We opportunistically examined fresh carcasses of horses found dead of natural causes during systematic survey of the island in April 2017 (n = 30) and April 2018 (n = 5). There were 19 females and 16 males, 15 young and 20 adult horses. Horses were aged based on size and dentition (and in some cases, known date of birth from photographic observations), and ranged from 4 months to late teens. Horses between 4 months and 2.5 years of age (inclusive) were considered young, and horses ≥ 3 years old were considered adults. The airways were cut open from the epiglottis to the end of the cartilaginous bronchi in the caudal lung lobes and examined for lungworms. Adult lungworms were identified using morphological (Cameron, 1926; Lichtenfels, 1975) and

Table 1

Morphological characters and measurements used to identify third-stage larvae cultured from strongyle-type eggs present in horse feces (Dunn, 1978; Madeira de Carvalho et al., 2007).

Species	# of gut cells	Length (µm) (mean and range)	Mean width (µm)	Special Features
Small strongyles	8	850 (590–975)	25	Distinct gut cells
<i>Strongylus edentatus</i>	18–20	790 (660–870)	23	Indistinct, thin gut cells
<i>S. equinus</i>	16	901 (890–1000)	18	Indistinct, thin, elongated gut cells; trilobed tail process
<i>S. vulgaris</i>	28–32	936 (795–1295)	32	Distinct gut cells
<i>Poteriostomum</i> sp.	16	786 (675–850)	28	Broad
<i>Triodontophorus</i> sp.	18–20	850 (750–950)	28	Distinct gut cells
<i>Trichostrongylus axei</i>	16	738 (682–780)	24	None
<i>Gyalocephalus capitatus</i>	12	731 (634–880)	27	None

molecular methods (Epe et al., 1997). PCR product of the second internal transcribed spacer (ITS2) of ribosomal DNA was sequenced by Macrogen, Korea. Sequences were trimmed, and aligned using CLUSTAL Omega (1.2.4; <https://www.ebi.ac.uk/Tools/msa/clustalo/>). The entire small intestine was opened, stripped, and the presence of ascarids (*Parascaris* spp.) and cestodes (*Paranoplocephala mamillana*) noted and recorded. Rectal fecal samples were collected for a quantitative McMaster passive flotation on fresh feces in the field (Debeffe et al., 2016). Briefly, 4 g of feces were homogenized in 26 ml of Sheather's sugar solution (S.G. 1.27), filtered, mixed, and aliquoted into 2 0.15 ml chambers of a McMaster slide (Chalex Corp. USA). The number of strongyle and ascarid eggs were counted, and eggs per gram of feces (EPG) for each egg type calculated by taking the sum of the two chambers and multiplying by 25. A second fecal sample from each horse was frozen at -20°C until examination at the Zoonotic Parasite Research Unit (ZPRU) in Saskatoon using a modified, quantitative Wisconsin (centrifugal flotation in Sheather's solution) (Cox and Todd, 1962) for helminth eggs and protozoan oocysts, and sucrose gradient concentration (Olson et al., 1997) followed by immunofluorescent assay for *Giardia/Cryptosporidium* spp. (Waterborne Inc., New Orleans).

2.3. Parasite examination of live horses

In April 2018, during systematic survey of the entire island, freshly deposited fecal samples were opportunistically collected from 45 individual live horses (8 young, 36 adults, and 1 of unknown age; 24 males, 17 females, and 4 of indeterminate sex); we are confident that no horses were sampled twice since the island was surveyed only once in this period, and defecation is a relatively rare event at this time of year with little vegetation available for grazing. Samples were processed in the field for McMaster fecal egg counts (as above) and a modified beaker Baermann sedimentation for larvae (Forrester and Lankester, 1997; Jenkins et al., 2005). Briefly, 5 g of feces from each sample were placed into an envelope made of window screen and a single layer of laboratory grade tissue (Kimwipes™, Kimberly Clark, Mississauga, Ontario) immersed in tap water in a glass tumbler with straight sides, incubated at room temperature with the lights on for 8–12 h, and the sediment examined in a Petri dish under a dissecting scope. Larvae were transferred to a slide under a compound scope for definitive identification; larvae of *D. arnfieldi* were differentiated from free-living and hatched strongyle larvae on the basis of their size (300–430 µm long, 15–20 µm wide), granular appearance, pharyngeal shape, and stylet (or spear-shaped) tail (Fig. 2). Modified Wisconsin fecal egg counts (helminths and protozoans) and *Giardia/Cryptosporidium* sucrose gradient isolation and immunofluorescent assay on previously frozen feces were performed in the laboratory in Saskatoon as described above. In summer 2018 (Jul 21–Aug 6, inclusive), during systematic survey of the island, we collected freshly deposited fecal samples from 236 live horses (53 young and 183 adult horses; 137 males, 98 females, and 1 of indeterminate sex); 32 horses were repeat sampled since the island was surveyed twice in this period. Fresh feces were examined in the field by McMaster and Baermann examination as described above, with the

(a)



(b)



Fig. 2. a) *Dictyocaulus arnfieldi* first-stage larvae showing typical granular appearance and beginning of cuticular separation b) closer view of tail showing stylet, or spear.

exception that larvae in Baermann sediments were heat-killed, fixed in 10% neutral-buffered formalin, and counted at ZPRU.

2.4. Statistical analyses

Data were entered into Excel for Mac 16.16.11 and imported into Stata MP 16 for analyses. There were 3 main groups compared

statistically: dead horses in spring, live horses in spring, and live horses in summer. Age and sex were also considered group defining variables; however, as sex was not significant for any of the calculations, only stratification by age was performed for statistical analyses and reporting. Among the three groups, we compared prevalence (proportion of samples positive) for larvae of *Dictyocaulus arnfieldi*, and ascarid eggs on McMaster, using chi-square or Fishers exact if group size was 4 or less. Two-way comparisons (e.g. dead vs live spring, live spring vs live summer) were held to a significance level of $p < 0.025$ using the Bonferroni correction; otherwise, p was < 0.05 . To follow up on the univariate analyses, multivariate logistic regression was used to explore effects of age and season on ascarid status. Continuous variables with non-normal distributions (strongyle and ascarid FEC, *D. arnfieldi* LPG) were compared among the three groups using Kruskal Wallis, then Wilcoxon Rank Sum (Mann Whitney) for two-way comparisons using the Bonferroni correction. Finally, for ascarids, we compared prevalence (proportion positive) for dead horses on necropsy, McMaster, and Wisconsin using the McNemar chi-square; if not significantly different, kappa was calculated for test agreement. Strongyle egg shedding classifications (low < 200 EPG, medium 200–500 EPG, and high > 500 EPG) developed for domestic horses were taken from the American Association of Equine Practitioners Parasite Control Guidelines (Nielsen et al., 2013).

3. Results

3.1. Strongyle identification

Fecal samples were cultured for 81 individual horses: 40 males and 41 females; 5 foals, 20 young horses, and 56 adult horses. While ideally 200 larvae/sample were examined, for 13 horses, less than 200 larvae were available (range 16–180). Overall, of the 17,381 larvae characterized, 39% were cyathostomes, 33% were *S. equinus*, 16% were *S. edentatus*, and 2% were *S. vulgaris* (together, large migratory strongyles comprised 51% of the larval population). In the 5 samples from foals, larvae with rhabditiform pharynxes predominated, which could be free-living nematodes and/or *Strongyloides westeri*, followed by cyathostomes; one foal had a larva of the large migratory strongyle *S. vulgaris*. For young horses, cyathostomes pre-dominated, followed by the large migratory strongyles (*S. equinus*, *S. edentatus*, and *S. vulgaris*, in that order). In adult horses, *S. equinus* pre-dominated, followed by cyathostomes, *S. edentatus*, and *S. vulgaris* (Fig. 3). Other species that were identified in horses greater than 1 year of age included larvae of the non-migratory large strongyles *Poterostomum* sp. and *Triodontophorus* sp., the generalist stomach nematode *Trichostrongylus axei*, and the

cyathostome *Gyalocephalus capitus*.

3.2. Adult lungworm identification

Consensus sequence from the entire ITS2 nuclear locus (404 bp) was obtained from adult nematodes from the airways of 4 dead horses in spring 2017 (B41, B141, B180, and B150). Four sequences (2 from B41, 1 each from B141 and B180) had almost complete coverage of the 403 bp reference sequence, and on BLAST, were more than 98% identical with the reference sequence for *D. arnfieldi* (Genbank U37715.1, from a donkey in Germany). All 4 complete Sable sequences had 5 single nucleotide polymorphisms (SNPs) from the reference sequence at positions 45 (Sable A, reference C), 53 (Sable T, reference C), 249 (Sable A, reference G), insertion of G between positions 319 and 320, and 355 (Sable A, reference G). A representative sequence was deposited in Genbank (Accession number MN496151). Sequence from B150 was missing the first 120 bp but was otherwise identical to the 4 complete Sable sequences.

3.3. Parasite examination of dead horses (n = 35)

Adult nematodes of *D. arnfieldi* were present in the airways of 66% of all horses examined at necropsy, with higher prevalence in young (80%) versus adult horses (55%) (Table 2). All horses were shedding strongyle eggs in feces on Wisconsin examination, with lower proportion positive (91%) on McMaster examination; median and mean strongyle fecal egg counts on McMaster examination are reported in Table 3. Using shedding classifications developed for domestic horses, 25% were low (< 200 EPG), 25% were moderate (200–500), and 50% were high (> 500 EPG). Adult *Parascaris* spp. were present in the small intestines of 82% of horses, including 74% of adult horses; some were single worm infections, but at least half of adult horses had patent infections (i.e. were shedding *Parascaris* spp. eggs in feces) (Table 4). Prevalences of *Parascaris* spp. based on necropsy, Wisconsin, and McMaster fecal examination (Table 4) were not significantly different, with an overall kappa value for test agreement of 0.511 ($p = 0.000$); on pairwise comparison, the two fecal based tests had the highest agreement (kappa 0.778, $p = 0.000$). On univariate analysis, dead horses were significantly more likely to shed ascarid eggs on McMaster fecal examination, and had significantly higher ascarid fecal egg counts, than live horses in spring (Table 5). On multivariate logistic regression, dead horses had 4.6 (1.6–13) times higher odds of having ascarid eggs present on McMaster (after accounting for age) ($p = 0.005$), and young horses had 4.9 (1.5–14.9) higher odds of being positive than adult horses ($p = 0.008$). At necropsy, the cestode *Paranoplocephala mamillana* was observed in the small intestine of 32% (95% CI: 17–51) of horses, with higher prevalence based on detection of eggs in feces on Wisconsin examination (42%, 95% CI: 25–61). Three young horses, but no adults, were shedding coccidia (*Eimeria leuckarti*) in feces. *Giardia* and *Cryptosporidium* spp. were not detected in feces of any dead horses.

3.4. Parasite examination of live horses in spring 2018 (n = 45)

Larvae of the lungworm, *D. arnfieldi*, were detected in fecal samples of 47% of live horses, including adult horses (36% had patent infections); young horses had higher prevalence (100%) and median intensity (12 vs 0.8 LPG) (Table 2). All horses were shedding strongyle eggs in feces on Wisconsin examination, with slightly lower proportion positive (98%) on McMaster examination. Median and mean strongyle fecal egg counts on McMaster are reported in Table 3. Of 45 live horses, 18% were low shedders (< 200 EPG), 27% were moderate (200–500 EPG), and 55% were high (> 500 EPG). On McMaster fecal examination, young horses were more likely to shed ascarid eggs in feces, and ascarid fecal egg counts were higher in young horses than adult horses; however, 19% of adult horses also had patent infections (Table 5). The proportion of samples positive for ascarid eggs was higher on Wisconsin

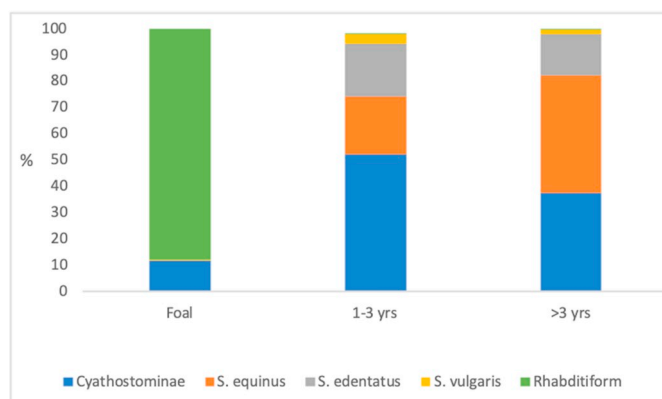


Fig. 3. Proportions of third-stage larvae of large and small strongyle species cultured from feces of 81 Sable Island horses in summer 2014, showing an unusual dominance of *S. equinus* in adult horses. Larvae with a rhabditiform pharynx were rare in young (1–3 years) and adult horses (≥ 3 years), but common in foals, which could represent larvae of *Strongyloides westeri*.

Table 2

Dicytocaclus arnfieldi (lungworm) prevalence (% positive with 95% confidence interval, CI) and median larvae per gram of feces, LPG (25th–75th percentile) in 35 dead horses in spring 2017 and 2018 (based on detection of adult nematodes in airways), on Baermann examination of feces from 45 live horses in spring 2018 (age was not recorded for one animal), and on Baermann examination of feces from 222 live horses (175 adult and 47 young) in summer 2018 on Sable Island.

Status	Adult ^a % (CI)	Young ^b % (CI)	Overall % (CI)	Adult ^b LPG (25–75th)	Young ^b LPG (25–75th)	Overall LPG (25–75th)
Dead spring	55 (32–77)	80 (52–96)	66 (48–81)	NA	NA	NA
Live spring	36 ^c (21–54)	100 ^c (63–100)	47 ^c (32–62)	0.8 (0.4–5)	12 (4–16)	3 ^d (0.8–11)
Live summer	10 ^c (6–16)	13 ^c (5–26)	11 ^c (7–16)	0.9 (0.4–3)	0.9 (0.8–2)	0.9 ^d (0.4–2)

^a Overall (n = 302), young horses were significantly more likely to be positive for lungworm than adult horses (p = 0.001).

^b Overall (n = 302), young horses had significantly higher larval counts than adult horses (p = 0.0139).

^c Horses were significantly more likely to shed larvae in spring than summer (p = 0.000) and this effect remained significant when stratified by age.

^d For live horses, larval shedding was significantly higher in spring than summer (p = 0.03).

on frozen feces (33%, 95% CI: 18–90) than McMaster on fresh feces (24%, 95% CI: 13–40). On Wisconsin, eggs of the tapeworm *Paranoplocephala mamillana* were observed in feces of 28% (95% CI: 15–44) of horses, and 3 young horses and 1 adult were shedding coccidia (*Eimeria leuckarti*) in feces. *Giardia* and *Cryptosporidium* spp. were not detected in feces of any live horses sampled in spring.

3.5. Parasite examination of live horses in summer 2018 (n = 236)

Larvae of *D. arnfieldi* were detected in fecal samples of 11% of live horses in summer, with no difference in prevalence and median intensity between young (13%, 0.9 LPG) versus adult horses (10%, 0.9 LPG) (Table 2). The proportions of adult and young horses shedding larvae, and the median larvae per gram of feces, were significantly lower in summer than in spring (Table 2). Almost all (98%) of horses were shedding strongyle eggs in feces on McMaster examination. Strongyle fecal egg counts overall and in adult horses were significantly higher in summer than in spring (Table 3). Of 236 live horses, 9% were low shedders (< 200 EPG), 25% were moderate (200–500 EPG), and 66% were high (> 500 EPG). The proportion of horses shedding ascarid eggs was significantly lower in summer than in spring (Table 5).

4. Discussion

We describe a unique, archaic parasite fauna in feral horses of Sable Island, including large migratory strongyles and a lungworm that are now considered highly unusual in domestic horses in North America. We report new geographic records for the large migratory strongyle *S. edentatus*, a non-migratory large strongyle *Poteriostomum* sp., the lungworm *D. arnfieldi*, and a protozoan, *E. leuckarti*. It is not surprising that the last two parasites had not been documented previously, as previous necropsies did not examine the lungs (Welsh, 1975), and fecal surveys were primarily focused on quantifying strongyle eggs (Debeffe et al., 2016; Gold et al., 2019). However, it was unexpected that *S. edentatus*, which is the largest of the large migratory strongyles and represented almost 20% of larvae in samples from horses over a year old in our study (Fig. 3), was not described previously. This may reflect the

Table 3

Median strongyle eggs per gram of feces (EPG, 25th–75th percentile) and mean EPG (standard deviation, stdev) on McMaster examination of fresh feces in 32 dead horses in spring 2017 and 2018, 45 live horses in spring 2018, and 236 live horses in summer 2018 on Sable Island. Almost all horses were positive (91% of dead horses and 98% of live horses). Zero values were included for calculation of medians and statistical comparisons, but excluded for calculation of mean intensity.

Status	Adult ^c EPG (25–75th)	Young ^c EPG (25–75th)	Overall median EPG (25–75th)	Overall mean EPG (stdev)
Dead spring ^a	325 (50–700)	825 (450–975)	513 (163–950)	666 (474)
Live spring ^b	525 ^d (225–938)	938 (688–1575)	650 ^d (225–1000)	689 (523)
Live summer	775 ^d (325–1500)	1075 (375–1625)	800 ^d (325–1525)	1105 (943)

^a Of the 35 carcasses, 2 adult horses had no fecal material due to intestinal accident, and one yearling was necropsied on the day of departure from the island.

^b age not reported for one horse.

^c Overall (n = 313), young horses had higher strongyle fecal egg counts than adult horses, which approached significance (p = 0.0578).

^d Strongyle FEC were significantly higher in summer than in spring (p = 0.0104); when stratified by age, this effect remained significant for adult horses (p = 0.0073).

understanding of taxonomy at the time (1975), confusion with *S. equinus*, or genuine absence from the horses examined at the time (32 horses and 15 foals) (Welsh, 1975). It is unlikely that the parasite was introduced since the 1970s, since the population is closed, and strongyle eggs are not particularly robust and therefore not likely to survive on boots or fomites contaminated off the island.

4.1. Strongyles – unusual dominance of large strongyles and tolerance of “high” FEC

Compared to domestic horse populations in North America, feral horse populations elsewhere in North America (Young et al., 1999), and Przewalski horses (Kuzmina et al., 2009), we found an unusual predominance of large (vs small) strongyles in coproculture from horses of Sable Island. While cyathostomes (small strongyles) dominated in young horses (< 3 years of age) on Sable Island, the large strongyle *S. equinus* dominated in larval cultures from adult horses, and all three large strongyles (*S. equinus*, *S. edentatus*, and *S. vulgaris*) were present in horses over a year of age. The most comparable horse population to Sable Island is likely the Assateague population, which is another closed, island population with no record of anthelmintic treatment since 1972 (Young et al., 1999). In this horse population, cyathostomes represented 96%, *S. equinus* 2%, and *S. vulgaris* 1%, of the total cultured larval population. In contrast, cyathostomes represented only 39% of the total larval population from the Sable horses, with large strongyles (primarily *S. equinus*) representing 51% of the total larval population. This may represent a founder effect in these two island feral horse populations, differing bioclimatic conditions that differentially favor large strongyle transmission on Sable Island, or the possibility that historical anthelmintic treatment records are incomplete. Large migratory strongyles in general, and *S. equinus* in particular, are almost extinct in managed domestic horse populations (McCraw and Slocombe, 1985; Young et al., 1999; Nielsen et al., 2010). This is in part due to their susceptibility to anthelmintics, largely used to prevent verminous arteritis and colic caused by migration of larvae of *S. vulgaris* through the cranial mesenteric artery (Slocombe, 1985). Therefore, the dominance of large strongyles in Sable horses may well reflect the

Table 4

Proportion positive (%) (95% confidence interval, CI) and median fecal egg counts (eggs per gram of feces, EPG) (25th to 75th percentile) of ascarids (*Parascaris* spp.) in dead horses using three different methods: necropsy (n = 34), Wisconsin on previously frozen feces (n = 33), and McMaster on fresh feces (n = 32).

Method	Adult % (CI)	Young % (CI)	Overall % (CI)	Adult EPG (25–75th)	Young EPG (25–75th)
Necropsy	74 (49–91)	93 (68–100)	82 (65–93)	NA	NA
Wisconsin	56 (31–78)	100 (78–100)	76 (58–89)	32 (14–61)	98 (36–165)
McMaster	50 (26–74)	86 (57–98)	66 (47–81)	175 (100–300)	500 (425–750)

“natural” situation, prior to anthelmintic interventions which have virtually eliminated large strongyles in domestic horse populations (Kaplan and Nielsen, 2010).

Overall, mean strongyle FEC in Sable horses (approximately 650 EPG in spring, 1100 EPG in summer) were considered high by domestic horse standards, but were not unusual compared to mean FEC in other feral horse populations using the same method (McMaster): e.g. 559 EPG in July on Assateague Island, USA (Young et al., 1999); 824 EPG in August on Delft Island, Sri Lanka (Dissanayake et al., 2017); 1017 EPG and 1163 EPG in Przewalski's horses in the Ukraine and Russia (Kuzmina et al., 2009, 2017); and 1443 EPG in feral horses in Australia (Harvey et al., 2019). In individual feral horses, high FEC have been linked to lower body condition and decreased reproductive success (Rubenstein and Hohmann, 1989; Debeffe et al., 2016; Gold et al., 2019). However, our results do not suggest that higher strongyle FEC are correlated with mortality; i.e. dead horses did not have higher strongyle FEC than live horses sampled in the same season (spring) (Table 3). As well, despite these high FEC, the Sable population is stable around 550 animals, and in summer, most horses appear to be in moderate-excellent body condition.

Using FEC classifications of low, medium and high developed for domestic horses, 50–66% of Sable horses were in the “high” category (> 500 EPG). This is comparable to 89% in feral horses in Australia and 64% in feral horses in the USA (Cain et al., 2018; Harvey et al., 2019), and 60% of domestic horses on one relatively unmanaged farm in Saskatchewan, Canada (Misuno et al., 2018). These classifications are used to guide targeted treatment decisions in domestic horses, with the current “best practice” recommendation to treat only those animals with medium to high FEC (> 200 or 500 EPG), in order to decrease pasture contamination while maintaining a refugium of unexposed parasites to preserve anthelmintic efficacy (Nielsen et al., 2013). Using the spring collection from live horses on Sable Island as an example, if following the guideline to treat all horses with FEC > 200 EPG, we would have recommended treatment of 37 horses (82% of 45). In comparison, if we treated only the top 20% of horses (which harbour approximately 80% of the total parasite burden - Kaplan and Nielsen, 2010), we would have treated only 10 horses, those with egg counts higher than 1050 EPG. Therefore, this suggests that targeted selective treatment recommendations should be based on the proportion of high

shedders calculated for a specific population or herd, instead of absolute FEC thresholds that may not be applicable across all equine populations, seasons, and methods.

4.2. Lungworm – unusual in the absence of donkeys, and unusual in adult horses

Detection of the lungworm *D. arnfieldi* in necropsied horses in spring of 2017 was unexpected, as this parasite is generally thought to be reservoired in donkeys and “spills over” into co-pastured horses, although horse-horse transmission has been documented (Clayton and Duncan, 1981; Slocombe, 1985; Boyle and Houston, 2006). Donkeys have not been present on Sable Island for at least 60 years, making this finding enigmatic. Transmission of lungworm occurs on pasture and is thought to be maintained by foals, the only age class of horses to develop patent infections; larvae are not supposed to develop into mature nematodes in adult horses (Clayton and Duncan, 1981; Boyle and Houston, 2006). Although prevalence and intensity of patent infections with *D. arnfieldi* were higher in young horses in our study, it was unexpected to observe that a high proportion of live adult horses (36%) were shedding larvae in spring 2018, and adult nematodes were detected in the lungs of an even higher proportion of adult horses at necropsy (55%) (Table 2).

This suggests an unusual role for adult horses in the epidemiology for lungworm on Sable Island, which might be attributable to malnutrition, with correspondingly decreased energy available to allocate to immune response. There may also be high levels of infection pressure due to high pasture densities (~500 horses on 35 km²), and year-round transmission due to a natural breeding system, with foals born almost year-round, and a temperate, wet climate, which favors larval survival on pasture (Boyle and Houston, 2006). Lungworm is thought to be particularly pathogenic in adult horses, where it can cause a mucopurulent bronchitis manifested as a chronic cough and nasal discharge (Slocombe, 1985; Boyle and Houston, 2006), and higher prevalence has been observed in equids in poor body condition (Solomon et al., 2012). Future work will investigate the role of lungworm and other respiratory pathogens in the health of horses on Sable Island. As well, further work is needed to determine the phylogenetic relationship of *D. arnfieldi* on Sable Island with that from donkeys and domestic horses elsewhere; we

Table 5

Ascarid (*Parascaris* spp.) prevalence (% positive with 95% confidence interval, CI) and median fecal egg counts (FEC) (in eggs per gram of feces, EPG) on McMaster examination of fresh feces from 32 dead horses in spring 2017 and 2018, 45 live horses in spring 2018, and 236 live horses in summer 2018 on Sable Island.

Status	Adult ^c % (CI)	Young ^c % (CI)	Overall % (CI)	Adult ^d median EPG (25–75th)	Young ^d median EPG (25–75th)	Overall median EPG (25–75th)
Dead spring ^a	50 ^e (26–74)	86 (57–98)	66 ^e (47–81)	175 ^g (100–300)	500 (425–750)	425 ^g (175–600)
Live spring ^b	19 ^{e,f} (8–36)	50 ^f (16–84)	24 ^{e,f} (13–40)	50 ^g (25–100)	800 (263–1163)	75 ^g (50–450)
Live summer	1.6 ^f (0.3–5)	1.9 ^f (0.1–10)	1.7 ^f (0.4–4)	75 (25–100)	50 (NA)	63 (38–88)

^a Of the 35 carcasses, 2 adult horses had no fecal material due to intestinal accident, and one yearling was necropsied on the day of departure from the island.

^b age not reported for one horse.

^c Overall (n = 313), young horses were significantly more likely to shed ascarid eggs than adult horses (p = 0.001).

^d Overall (n = 313), young horses had significantly higher ascarid FEC than adult horses (p = 0.000).

^e Dead horses were significantly more likely to have patent infections with *Parascaris* spp. than live horses in spring (p = 0.000), and this remained significant for adult horses when stratified by age (p = 0.02).

^f Live horses were significantly more likely to have patent ascarid infections in spring than in summer, overall and when stratified by age (p = 0.000).

^g Dead horses had significantly higher ascarid FEC than live horses in spring (p = 0.0188), and this remained significant for adult horses when stratified by age (p = 0.0054).

described 5 mutations (4 SNPs and an insertion) present in the ITS2 region of *D. arnfieldi* from Sable horses compared to reference sequence from a donkey in Europe. While genetic differences are expected after isolation from other equids for at least 60 years, the ITS2 region is generally well conserved and often used to differentiate species of parasites; therefore, this level of difference suggests that the Sable Island lungworm is beginning to diverge and may represent a unique strain or sub-species.

4.3. *Ascarids – higher prevalence and intensity in dead horses, and unusual in adult horses*

Similar to lungworm, it was unexpected to observe that a number of adult horses (≥ 3 years of age) from Sable Island had patent ascarid infections. *Parascaris* spp. are rare in domestic horses older than a year, and patent infections are generally only observed in foals less than 7 months of age (Slocombe, 1985; Boyle and Houston, 2006; Bellaw et al., 2016); but see (Lyons et al., 2006). Therefore, it was unexpected to routinely observe patent infections in both live and dead adult horses on Sable Island, as well as non-patent intestinal infections (i.e. adult ascarids, often single worm infections, in the absence of eggs in feces) in 5 of 20 dead adult horses. Dead horses were significantly more likely to have patent ascarid infections, and higher ascarid eggs per gram of feces, than live horses. It is not possible to determine if this is cause or effect, but it is likely that ascarid parasites in adult horses on Sable Island are a symptom of other problems, such as malnutrition and corresponding immunodeficiency. In addition, we observed much higher ascarid prevalence at necropsy (82%) than previously reported on necropsy of a comparable number of horses in 1970–72 (16%) (Welsh, 1975), which could represent differences in methods, season, or a genuine increase in prevalence; the density of horses currently on Sable Island is more than double that in the 1970s (Welsh, 1975; van Beest et al., 2014).

In young horses, ascarid infections are associated with respiratory disease (due to migration of larvae through the lungs) and decreased weight gain, and high intensities can cause impaction and intestinal perforation (Slocombe, 1985; Boyle and Houston, 2006). It is not known if ascarids are contributing to foal and yearling mortality on Sable Island, although some of the intestinal infections observed in yearlings at necropsy were of very high intensity. Future work is needed to determine the impact and identity (*P. equorum* vs *P. univalens*) of ascarids in Sable Island horses (Nielsen et al., 2014).

4.4. *Effects of season, age, and method on parasite epidemiology*

Season and age, but not sex, appear to be important drivers of prevalence and intensity of helminth parasites in Sable horses, with prevalence and fecal intensity of *Parascaris* spp. and *D. arnfieldi* significantly higher in young animals, and in spring vs summer. For strongyle FEC, young horses generally had higher egg counts than adult horses, and counts were significantly higher in summer versus spring. Season and age are known to be important drivers of parasite epidemiology; however, some studies report no significant differences between adult and yearling feral horses (Rubenstein and Hohmann, 1989). The lack of gender effect was somewhat unexpected (although consistent with Debeffe et al., 2016), as male biases in parasitism are more the norm in host-parasite relationships (Schalk and Forbes, 1997). However, female biases in mortality have been observed on Sable Island and other island populations of feral horses (Welsh, 1975; Rubenstein and Hohmann, 1989), suggesting an unusual situation in these populations.

We also had the opportunity to compare two fecal-based methodologies. The standard practice for both Sable Island and domestic horse populations is to use a simple passive flotation method on fresh feces (the McMaster technique) for fecal egg counts, an important practice for guiding anthelmintic treatment decisions in domestic

horses. The McMaster technique generally has a detection limit (25 EPG in our case) (Boyle and Houston, 2006) but is useful at high prevalence and intensity of strongyle eggs. Centrifugation based methods, such as the Wisconsin technique, are known to have higher sensitivity, and can also be quantitative. Freezing decreases both strongyle and ascarid egg counts in horse feces (Schurer et al., 2014); however, it was not possible to perform Wisconsin examinations on fresh samples from horses on Sable Island. Therefore, duplicate samples were frozen at -20°C and analyzed in the laboratory. Despite the decrease in FEC, Wisconsin examination on frozen feces consistently detected a higher proportion of ascarid and strongyle egg positive samples than McMaster examination on fresh feces, had excellent agreement with McMaster findings for ascarid eggs (kappa of 0.778), and also allowed detection of rare parasites, including the coccidian *Eimeria leuckarti* and the cestode *Paranoplocephala mamillana*. Of note, we observed higher cestode prevalence at necropsy (32%) than previously reported on necropsy of a comparable number of horses in 1970–72 (13%) (Welsh, 1975). Therefore, these findings are reassuring for fecal parasite investigations in wildlife and remote regions, where samples must be frozen prior to transport and analyses.

4.5. *Management significance and conclusions*

Our findings will help guide design of future parasite studies, targeting appropriate age groups and seasons of collection to unravel the unusual epidemiology of parasites in Sable Island horses (for example, sampling in summer is not ideal for studies on ascarids or lungworm). As well, our findings emphasize the need to develop population-specific treatment guidelines for domestic horses based on proportions of high shedders rather than absolute FEC, and that feral horses can tolerate higher levels of parasitism (based on FEC) than generally accepted in managed horses. Sable Island horses harbor parasites no longer commonly found in domestic horses in Europe and North America, such as *S. equinus* and *D. arnfieldi*, and their cyathostome and ascarid populations (which show multi-drug resistance in most domestic horse populations - Matthews, 2014) represent a critical refugium that have never experienced selection pressure from anthelmintic use. Conversely, it is important to note the apparent absence of important equine and zoonotic parasites, including the protozoans *Giardia* and *Cryptosporidium*, cestodes in the genus *Anoplocephala*, and the stomach nematodes *Habronema* and *Drascheia*, on Sable Island. At moment, migratory birds, grey seals (*Halichoerus grypus*), and people are the major potential routes of introduction of parasites (and other pathogens) to the island. Our findings support biosecurity measures currently in place to prevent introduction of parasites and pathogens onto the island, including rules for all visitors (government personnel, researchers, contractors, and tourists from cruise ships) such as boot and hand-washing, toileting, and quarantine of people experiencing gastrointestinal illness; consideration should be given to implementing exit biosecurity measures as well, to prevent transmission from horses on Sable Island to companion horses. Both the horse and parasite populations on Sable Island are of conservation and scientific significance, in which future work will explore genetic markers of parasite resistance and the effects of parasitism and nutrition interactions on host survival and fitness.

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