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Research paper

Wild ruminants as reservoirs of domestic livestock gastrointestinal nematodes

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ABSTRACT

Gastrointestinal nematode (GIN) infections in cattle cause appetite suppression which leads to poor feed conversion, reduced weight gain and reduced milk production. Overuse and exclusive reliance on anthelmintic drugs has resulted in widespread resistance in many parasitic nematode species infecting livestock making control increasingly difficult. Wild ruminants are competent hosts of a number of nematode species that typically infect and are best adapted for cattle, sheep, and goats. Thus, the potential exists for wild ruminants to act as reservoirs in the translocation of domestic GIN, including those carrying anthelmintic resistance mutations as well as susceptible genotypes. The potential for parasite exchange is heightened by interfaces or ecotones between managed and wild rangelands, and by perturbations linked to climate warming that can increasingly alter the distributions of wild ungulates and their interactions with domestic and free-ranging ruminants. To investigate the extent to which wild ruminants harbour parasites capable of infecting domestic ruminants we first performed an epidemiological study of feces from wildlife hosts that spanned 16 states and included white-tailed deer (85 % of the samples), pronghorn, elk, mule deer, bighorn sheep, moose, cattle, and caribou across the United States. All samples were cultured to third stage larvae and nematode DNA was isolated and PCR amplified. Among the 548 wild ruminant samples received, 33 % (181 samples) were positive for nematode DNA, among which half (84 samples) contained DNA from GIN species commonly found in cattle. DNA from cattle GIN species was detected in 46 % of samples from the Northeast, 42 % from the Southeast, 10 % from the Midwest, 0 % from the Southwest and 11 % from the West. Deep amplicon sequencing of the ITS-2 rDNA indicated that *Ostertagia* and *Trichostrongylus* were present in 90 % and 69 % of the nematode DNA positive samples, respectively, whereas *Haemonchus, Cooperia* and *Oesophagostomum* were present in 26 %, 2 % and 10 % of the samples, respectively. These data clearly show that wild ruminants commonly harbour multiple parasite species whose primary hosts are domestic cattle, and suggest that further work is warranted to investigate their specific roles in the management of anthelmintic resistance.

1. Introduction

Gastrointestinal nematode (GIN) infections have a major impact on the production characteristics of grazing ruminant species. Anthelmintics initially provided high efficacy management of these parasitic infections; however, misuse and overuse have resulted in the widespread development of anthelmintic resistance which has severely compromised control measures. The most prevalent GIN fauna in ruminant livestock includes species of *Haemonchus*, *Cooperia*, *Trichostrongylus*, *Ostertagia*, *Nematodirus*, and *Oesophagostomum.* Wild cervids such as deer, caribou, elk, and pronghorn are also ruminants that can acquire GIN infections by grazing on pastures contaminated with infective larval stages (e.g., Hoberg et al., 2001). Thus, transmission of GIN among domestic and wild ruminant host species has the potential to interfere with efforts to manage GIN infections in domestic livestock.

GIN species of domestic livestock demonstrate variation in host range related to environmental opportunity and the distribution of host-based resources that are available and can be exploited by parasites in space and time (e.g., Araujo et al., 2015; Hoberg and Zarlenga, 2016). Consequently, wild ruminants may become infected with species common to grazing cattle, sheep, and goats (McGhee et al., 1981), consistent with processes defined by ecological fitting (e.g., Agosta et al., 2010; Brooks et al., 2019). Thus, understanding the potential and

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realized host range(s) involved in persistence of GIN is essential. Reflected is the competence of free-ranging artiodactyls, which may be involved in maintenance of viable populations of parasites, in the absence of sheep and cattle, and which may serve as significant reservoirs for infection of domestic stock.

The hypothesis that pasture-borne parasites are transferred between domestic and free-ranging ruminants has a deep history, and especially with reference to shared pastures and the interface between managed and wild-land habitats (e.g., Hoberg et al., 2001, 2008; Hrabok et al., 2006; McGhee et al., 1981; Chintoan-Uta et al., 2014). For example, a relationship between parasite faunas in domestic stock and reindeer had been previously advanced by Hrabok et al. (2006), showing that reindeer are susceptible hosts to important GIN of sheep (*Teladorsagia circumcincta* and *Haemonchus contortus*) and cattle (*Ostertagia ostertagi* and *Trichostrongylus axei*). Using experimental animals, McGhee et al. (1981) showed that cross-transmission of *H. contortus* can occur between deer and domestic livestock where white-tailed deer are significant hosts for these haemonchines across the southeastern region of North America (Hoberg et al., 2001). Chintoan-Uta et al. (2014) reported the first account of *H. contortus* with benzimidazole resistant genotypes in wild roe deer, but concluded that in-field studies were needed to assess the levels of cross-transmission between deer and livestock and how this may contribute to the occurrence of anthelmintic resistance on livestock farms. Recent studies have further shown that there is overlap between host-parasite communities, but concluded that the consequences of these parasite communities on each host species are not well understood (Zaffaroni et al., 2000; Matthee et al., 2004).

Despite recent efforts to better manage GIN infections in livestock, there have been no large-scale surveys to our knowledge that assess the extent to which co-grazing wildlife are infected with the major species of cattle gastrointestinal nematodes. A central question relates to understanding whether free-range ungulates are sources which amplify, or sinks which diminish, the potential exchange of parasites and genes for anthelmintic resistance. Although wild ungulates may pick up and circulate anthelmintic-resistant parasites, they may also harbour an untreated refugia population of parasites which would benefit the control of resistance (Chintoan-Uta et al., 2014; Walker and Morgan, 2014). Further, it has been suggested that ecological disturbance emerging from climate warming, and changing patterns of distribution and diversity for GIN are synergistic with dissemination of anthelmintic resistance (Hoberg and Zarlenga, 2016).

In this paper, we describe the use of an ITS-2 rDNA PCR assay, followed by deep amplicon nemabiome sequencing, to investigate the presence of the major cattle gastrointestinal trichostronglyid nematode species in a large number of fecal samples collected from wild ruminant species across the USA. Developing consistent baselines to demonstrate the distribution and dynamics of parasite diversity is increasingly important in the context of accelerating climate warming, ecological disruption and rapidly changing geographic and host ranges for parasites and diseases on local to regional scales (Hoberg and Zarlenga, 2016; Brooks et al., 2014, 2019).

2. Materials and methods

2.1. Sample collection

Fecal samples from wild ruminants were collected between July 2017 and March 2018 directly from the animal's rectum or from the ground. Collections were made in geographical regions where temperatures averaged 19.3°C (-2.2°C to 32.8°C) and included the following 16 states; Massachusetts, Rhode Island, Vermont, New York, Maryland, Kentucky, North Carolina, South Carolina, Arkansas, Louisiana, Iowa, Kansas, Nebraska, New Mexico, Wyoming, and Alaska (Fig. 1). Sample collectors encompassed local, natural resource agencies, hunters, state wildlife departments, state veterinarians, and biologists. Protocols and materials for both sampling and shipping were provided to all sample collectors (see **Supplementary Document 1**). Individual samples (10 − 20 g of feces per animal) (n = 548) were obtained from wild ruminants of varying ages and species including white-tailed deer (*Odocoileus virginianus*, n = 489), mule deer (*Odocoileus hemionus*, n = 5), elk (*Cervus canadensis*, n = 2), moose (*Alces alces*, n = 3), pronghorn (*Antilocapra americana*, n = 10), bighorn sheep (*Ovis canadensis*, n = 19), and caribou (*Rangifer tarandus*, n = 20). Additionally, 28 samples were collected from domestic cattle (*Bos taurus*, n = 28). Fecal samples were kept in collection gloves that were tied closed and shipped in cardboard boxes at ambient temperature (~22°C) to the Animal Parasitic Diseases Laboratory in Beltsville, MD.

2.2. Sample preparation

Upon arrival, fecal cultures were immediately prepared without refrigeration using a modified coproculture technique as described by Roberts and O'Sullivan (1949). For each sample, whole fecal pellets were placed into a small moisture chamber for 14 days at room temperature (~22°C), after which larvae were harvested using a modified Baermann apparatus. All samples were flooded with water for at least 6 h, or overnight. After allowing any L3 to migrate out of the sample, the feces were removed and L3 were allowed another hour to settle. Samples were concentrated by sedimentation down to ~0.5 mL. Each sample was split into two equal aliquots. One aliquot was used for DNA isolation and the other was frozen in aqueous buffer at −80 °C for potential morphological identification.

2.3. DNA purification from L3

To prepare DNA from isolated L3 (suspended in water), Qiagen DNeasy® Blood & Tissue Kit (Cat. # 69504 and 69506) was used and the corresponding Quick-Start Protocol was followed with slight modifications. Excess water was evaporated by incubating the sample at 95°C. Sheathed L3s were transferred to 0.5 mL thin wall PCR tubes containing 180 μL buffer ATL, 20 μL proteinase K, and 1.4 μL beta-mercaptoethanol (2-mercaptoethanol, BioRad, Cat # 1610710, Hercules, CA, USA). Mixtures were incubated for 1 h at 56°C after which they were transferred to the supplied spin columns and the DNA was eluted in 100 μL of elution buffer. Positive and negative controls were DNA extracted from *O. ostertagi* and nuclease-free H_2O, respectively. All DNAs were stored at -80°C in fresh microcentrifuge tubes until analysis.

2.4. PCR and deep amplicon sequencing of the ITS-2 rDNA region

Isolated DNA was first analyzed using Clade V, nematode specific, ITS-2 r DNA primers NC1 and NC2 (Avramenko et al., 2015). The Clade designations were derived from Blaxter et al. (1998) where Clade V encompasses gastrointestinal strongylid parasitic nematodes of cattle, chosen as the target parasites. PCR reaction conditions were 0.25 μL TaKaRa Ex Taq (5 U/μL), 2.5 μL 10X Ex Taq buffer (Mg^{2+}), 0.5 μL dNTP mixture (2.5 mM each dNTP), 0.125 μL of each primer (50 μM stock), and 5 μL L3 DNA in a 25 μL reaction. The thermocycling parameters were 94°C for 4 min, then 40 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 2 min, followed by a final extension of 72°C for 5 min. Amplified DNA was run on a 2 % NuSieve™ 3:1 agarose gel stained with Gel Red. All positive samples were repeated to validate results and further analyzed by deep amplicon sequencing of the ITS-2 rDNA locus as described by Avramenko et al. (2015, 2017). Sequencing was performed on an Illumina MiSeq platform with the 2 × 300 v3 Reagent Kit (Illumina Inc., San Diego, CA, USA).

2.5. Bioinformatic analysis

Samples were analyzed to determine the relative proportions of reads mapping to one of 44 different ruminant GIN species present in a trichostrongylid database as described by Avramenko et al. (2017);

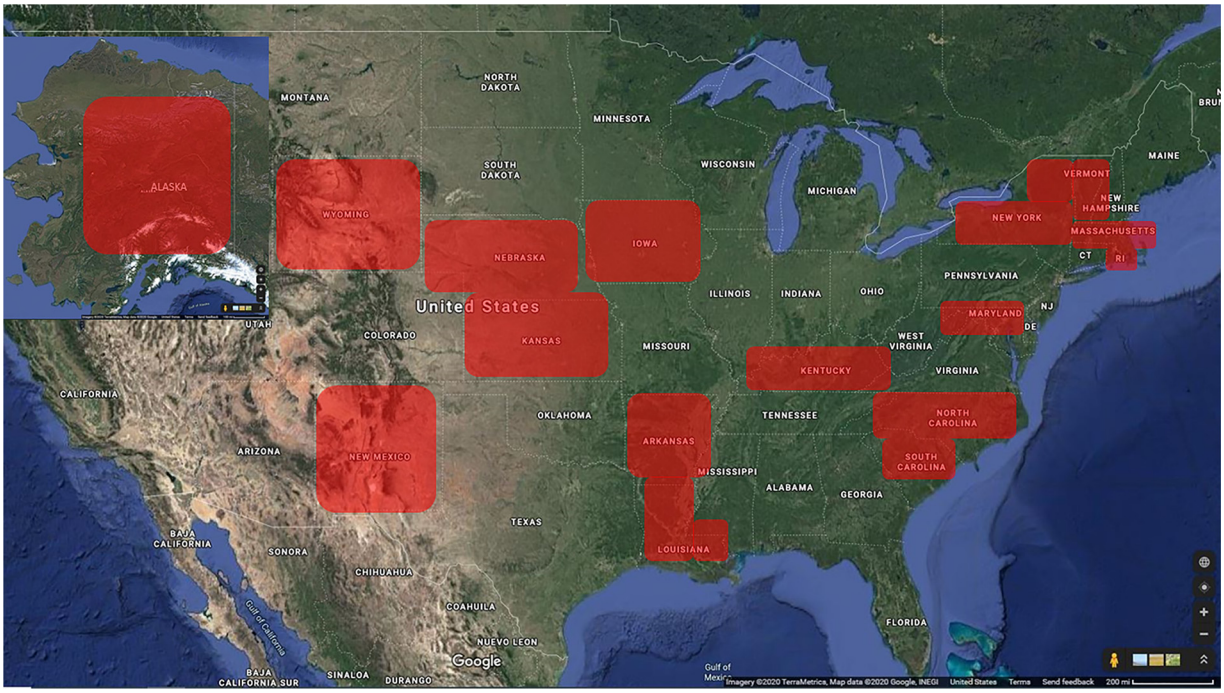

Fig. 1. Sample Distribution (Google Earth). Map of the United States showing the locations of the 16 states from where wild ruminant samples were collected.

further details are available at https://www.nemabiome.ca. Briefly, using the Mothur pipeline (version 1.36.1, Schloss et al., 2009), forward and reverse reads were merged to form one contig per read pair, which were then compared to a previously built reference database consisting only of domestic ruminant trichostronglyid nematode species (**Supplementary Data S1**). Samples were not considered further if they did not contain at least 2000 reads; the lower threshold for assessing parasite ratios in samples harboring more than one species (Avramenko et al., 2015). Samples which had less than 20 % of the reads classified as one of the GIN species in the database (available at Nematode ITS2 database version 1.3) using the Mothur pipeline were discarded from further analysis. These samples likely contained a large percentage of other nematode species that would not map to the cattle nematode species pipeline and database (see discussion). A species was only deemed present if at least 1 % of the reads were assigned to that species (Avramenko et al., 2015).

3. Results

3.1. PCR analysis of L3 DNA

Using PCR and gel electrophoresis, one-third of the samples were positive for Clade V nematode DNA based on the successful amplification of an ITS-2 rDNA product using the NC1 and NC2 primers (Table 1). Among the wild ruminant samples, 181 of 548 samples (33 %) were positive for Clade V nematode DNA and of the domestic cattle samples, 7 of 28 samples (25 %) were positive for Clade V nematode DNA. When grouped by geographical region, wild ruminant samples were positive for Clade V nematode DNA in 18 of 39 samples (46 %) from the Northeast, 152 of the 361 samples (42 %) from the Southeast, 9 of the 93 samples (10 %) from the Midwest, and 4 of the 35 samples (11 %) from the West; none of the 20 samples received from the Southwest were positive (Table 1). When grouped by host species, Clade V nematode DNA was detected in 176 of 489 (36 %) white-tailed deer samples, 1 of 2 (50 %) elk samples, 7 of 28 (25 %) cattle samples, 4 of 20 (20 %) caribou samples and 1 of 5 (20 %) mule deer samples. Clade V nematode DNA was not found in samples from bighorn sheep (19 samples), moose (3 samples) or pronghorn (10 samples) (Table 2).

Table 1
Regional distribution of wild cervid samples.

Region	Number of samples tested	Collection location (state abbreviation)	Positive for Clade V nematode DNA
Northeast	39	MA, RI, VT, NY, MD	46 %
Southeast	361	KY, NC, SC, AR, LA	42 %
Midwest	93	IA, KS, NE	10 %
Southwest	20	NM	0 %
West	35	WY, AK	11 %
Total	**548**	**All 16 States**	33 %

3.2. Speciation by ITS-2 rDNA deep amplicon sequencing

Among the 188 ITS-2 rDNA PCR positive samples, 153 generated > 2000 illumina reads (mean = 32.300 reads per sample), 89 of which presented with at least 20 % of the reads that mapped to a nematode species in the bespoke domestic ruminant ITS-2rDNA reference database. The mean number of mapped reads for the 89 samples used for further analysis was 21.141 (Fig. 2; Table 3).

At the genus level, *Ostertagia, Trichostrongylus* and *Haemonchus* were present in 76 (90 %), 58 (69 %), and 22 (26 %) of the 84 wild cervid samples, respectively, whereas *Cooperia* (2 of 84 samples; 2 %) and *Oesophagostomum* (8 of 84 samples; 10 %) were in significantly lower numbers. At the species level, *O. ostertagi* (46 of 84 samples; 55 %), *Trichostrongylus colubriformis* (29 of 84 samples; 35 %), and *H. contortus* (20 of 84 samples; 24 %), all common livestock parasites, were well represented in the wildlife samples, specifically the samples collected from white-tailed deer (Table 4). *Ostertagia leptospicularis* (23 of 84 samples; 27 %), a common wildlife parasite, was also well represented (Table 4). Of the 5 sequenced domestic cattle samples, *O. ostertagi* was well represented in 4 of the 5 samples (80 %), while *Oesophagostomum radiatum, Haemonchus placei, and T. axei* were each present in 1 of the 5 samples (20 %) (Table 5).

4. Discussion

The aim of this study was to improve our understanding of the

Table 2
Diversity of host species.

Host species	Number of samples collected	Collection location (state abbreviation)	Positive for Clade V nematode DNA
White-tailed deer	489	KS, MD, NY, RI, NE, NC, IA, AR, LA, SC, KY, VT, MA	36 %
Elk	2	WY, KS	50 %
Moose	3	AK	0 %
Caribou	20	AK	20 %
Cattle	28	KS	25 %
Bighorn sheep	19	NM	0 %
Mule deer	5	NM, WY, NE	20 %
Pronghorn	10	WY	0 %

prevalence of domestic ruminant GIN among wild and domestic cervids in the United States because of their potential to act as reservoirs for propagating GIN infections among domestic livestock. Specifically, we investigated the extent to which wild ruminants in the USA are infected with GIN species that commonly infect domestic ruminants such as cattle, sheep and goats. We applied a novel genome-based approach to assess faunal diversity for GIN, and methods that should be broadly applicable for developing large scale baselines against which to identify connectivity and change over time.

Our study found that at least one-third (181 of 548) of the wild ruminants sampled across the U.S. harbored Clade V nematodes. Geographically, the highest infection rates were among samples from Eastern US, but this is anecdotal at best given the disproportionally lower number of samples obtained from the Midwest and Western US. In like manner, 85 % of the samples originated from white-tailed deer, making it hard to accurately compare infection rates across different wildlife host species. Given that white-tailed deer tend to have a home range of less than one square mile (Saunders, 1988), it may be the best indicator host species for transmission at local and regional scales, especially across landscapes among neighboring farms and therefore domestic livestock. Sequencing results showed that 90 % of samples which generated amplifiable DNA contained parasites belonging to the genus *Ostertagia*; one of the most prevalent cattle GIN that significantly and detrimentally affects production traits.

It is generally assumed that pathogen transmission at the livestock-wildlife interface is bi-directional (Hoberg et al., 2001; Bengis et al., 2002; Miller et al., 2013), circulating between wildlife and livestock. In North America, it is estimated that more than 79 % of emerging infectious diseases are supported by a wildlife-livestock transmission cycle (Miller et al., 2013). One example is the correlation observed between the high levels of antibodies to paratuberculosis (*Mycobacterium avium paratuberculosis*) in cattle, and direct or indirect contact with red deer (*Cervus elaphus*) which gain access to Norwegian dairy pastures (Fredriksen et al., 2004). A similar finding was observed in

Table 3
Origins of the 89 sequenced samples.

Sample numbers	Host species	Collection location (state abbreviation)
1-2	Domestic Cattle	KS
3-5	White-tailed Deer	KS
6-8	Domestic Cattle	KS
9	White-tailed Deer	MD
10	White-tailed Deer	NY
11-27	White-tailed Deer	NC
28-40	White-tailed Deer	AR
41-57	White-tailed Deer	LA
58-73	White-tailed Deer	SC
74-78	White-tailed Deer	KY
79-85	White-tailed Deer	VT
86-89	Caribou	AK

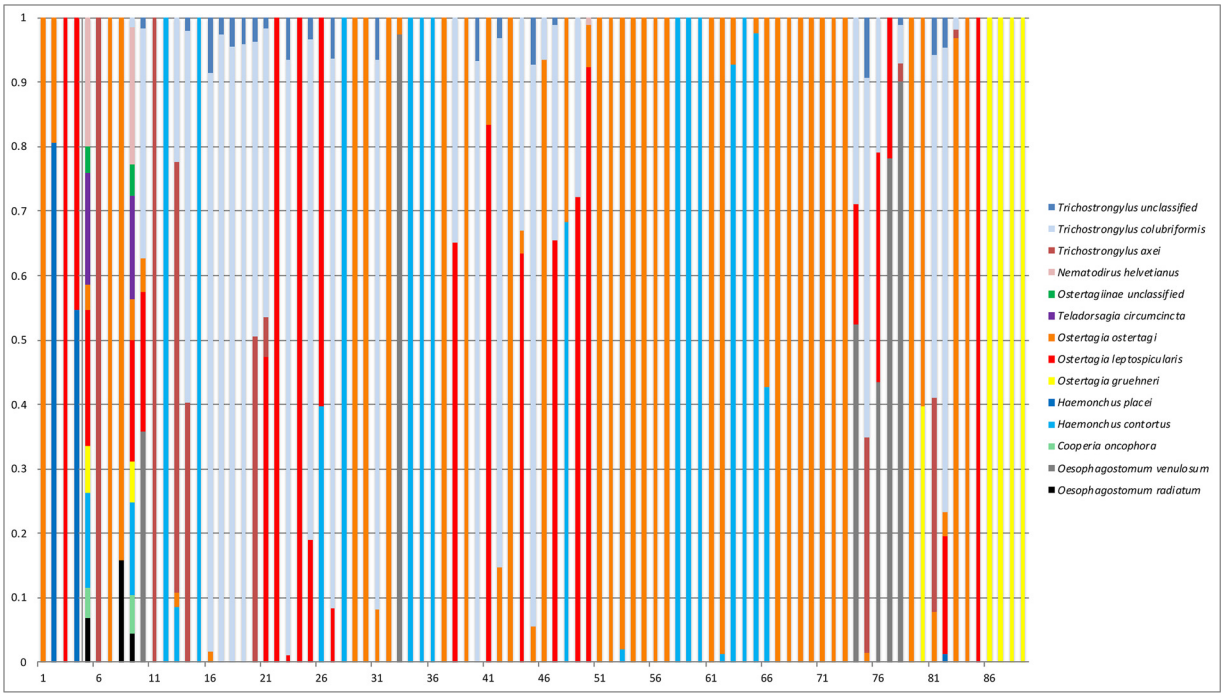

Fig. 2. Relative proportions of ITS-2 rDNA sequence reads mapping to domestic ruminant trichostrongylid gastrointestinal nematode in 89 fecal samples obtained from wild ruminants and domestic cattle. Each bar represents one sample.

Table 4
Parasite species found in 84 wild cervid samples identified by next generation sequencing.

Species	# of samples	% of samples
Oesophagostomum radiatum	2	2.4 %
Oesophagostomum venulosum	6	7.1 %
Cooperia oncophora	2	2.4 %
Haemonchus contortus	20	23.8 %
Haemonchus placei	2	2.4 %
Ostertagia gruehneri	7	8.3 %
Ostertagia leptospicularis	23	27.4 %
Ostertagia ostertagi	46	54.8 %
Teladorsagia circumcincta	2	2.4 %
Unclassified Ostertagiinae	2	2.4 %
Nematodirus helvetianus	3	3.6 %
Trichostrongylus axei	9	10.7 %
Trichostrongylus colubriformis	29	34.5 %
Trichostrongylus unclassified	20	23.8 %

Table 5
Parasite species found in 5 domestic cattle samples identified by next generation sequencing.

Species	# of samples	% of samples
Oesophagostomum radiatum	1	20 %
Haemonchus placei	1	20 %
Ostertagia ostertagi	4	80 %
Trichostrongylus axei	1	20 %

Minnesota dairy herds where 62 % of 114 dairy herds examined exhibited at least 1 positive fecal pool or environmental sample containing *M.a. paratuberculosis* from free-range deer and rabbits (Raizman et al., 2005). The correlation between the spread of disease and contact between domestic animals and wildlife has been well documented for cattle as well as other species.

Synanthropic transmission cannot occur without a common environment where wild ruminants and domestic livestock share resources (Winter et al., 2018). Previous investigations have shown that factors including the number of water sources on a cattle farm (Kaneene et al., 2002; Berentsen et al., 2014) and feed sources such as undergrazed pastures were positively correlated with the number of visits deer would make to farms (Berentsen et al., 2014). Interestingly, a minority of deer (19–43 %) were responsible for the majority (80–88 %) of these visits (Berentsen et al., 2014). The minority percentage (19–43 %) of deer that revisit farms coincides with our findings that at least 33 % of the wild ruminants were infected with Clade V nematodes. This suggests that only a subset of deer make recurring visits to farms and may help explain the results of our study. We speculate that the farm-visiting deer ingest infective larvae by grazing these pastures but also deposit eggs via defecation thereby circulating the infection among both domestic and wildlife hosts. Furthermore, Berentsen et al. (2014) reported that the visits by the deer were seasonal and concentrated in the spring during peak parasite transmission season. Our hypotheses do not rule out transmission among sylvatic hosts.

The overlap in faunas among wild free ranging ungulates and domestic ungulates in North America has been extensively reviewed (Hoberg et al., 2001) and provides an essential window into the structure and distribution of nematode faunas. Hoberg and Zarlenga (2016) discussed the potential for interaction between expanding anthelmintic resistance and in synergy, the impact of climate on ecological structure and parasite distribution (mosaics and faunal mixing). A range of biotic responses to climate change (increase in global temperature) have been previously discussed (e.g., Hoberg et al., 2008; Hoberg, 2010; Peters, 1992; Dobson and Carper, 1992). From this, it has been proposed that changes in host and parasite distribution can lead to overlapping ranges and increased host-colonizations (Hoberg et al., 2008; Dobson and Carper, 1992). In addition, with climate change, a broader distribution of pathogens can be expected as shifts in the geographic ranges are driven by habitat alteration and the persistence or shift in environmental permissiveness for free-living stages of GIN (Hoberg et al., 2008; Hoberg and Brooks, 2015; Dobson and Carper, 1992). Alternatively, in regions of higher latitude, climate change may have different impacts leading to increased rates of transmission, larval survivability, and increased prevalence and intensity for parasites (Hoberg, 2010; Altizer et al., 2013). Climate is critically important, and the expanding links for ecological disruption, pathogens and climate change are crucial for management of wild, free-ranging ungulates and domestic ungulates globally. Thus, this study begins to provide another avenue for assessing the structure of diversity, assembling baselines and developing an understanding of how faunal assemblages may change in space and time.

Walker and Morgan (2014) found that of 30 white-tailed deer referenced, they had 26 parasite species, 9 unique to them, 10 shared with domestic ungulates, and 7 shared with other wild ungulates. Like Walker and Morgan (2014), the current study found parasite species unique to wild ruminants as well as those present in domestic livestock. These data are consistent with a deeper historical picture for the structure of GIN communities in North American ungulates and globally (e.g., Hoberg et al., 2001). Stromberg et al. (2015), analyzed 99 cattle samples via PCR and found *Cooperia spp*. (91 %), *Ostertagia spp*. (79 %), *Haemonchus spp*. (53 %), *Oesophagostomum spp*. (38 %), and *Trichostrongylus spp*. (3 %) present in the samples. While the same list of species was present in the current study, *Ostertagia* spp. were found in a similar percentage; 79 % of cattle samples (Stromberg et al., 2015) compared with 90 % of wild ruminant samples in the current study. A notable difference between the two studies was the percentage of samples with *Cooperia spp*.; 91 % of cattle samples (Stromberg et al., 2015) compared with 2 % of wild ruminant samples in the current study. *Cooperia spp*. can have significant negative impacts on cattle productivity (Gasbarre et al., 2015; Stromberg et al., 2012) and are highly resistant to many current anthelmintics (Gasbarre et al., 2015). While wild ruminants are certainly acting as reservoir hosts of common cattle parasite species, *Cooperia*, a highly dominant parasite species in cattle (both pre-treatment (Stromberg et al., 2015; Gasbarre et al., 2015) and post-treatment (Gasbarre et al., 2015)), was found in only 2 % of the wild ruminant samples. These results support and reflect previous findings that intensity and prevalence of *Cooperia* species are minimal in wild hosts and are known not to infect cervids as well as *Ostertagia* and *Haemonchus* (Hoberg et al., 2001).

The ITS-2 rDNA deep amplicon sequencing assay, the analysis pipeline and the ITS-2 rDNA database used in this study are specifically designed to detect those trichostrongylid nematode species that commonly infect domestic ruminants such as cattle, sheep, and goats. This allowed us to address the prevalence of these particular species in wild ruminants in the USA. The NC1 and NC2 primers used in the PCR are complementary to the 5.8S and 28S rDNA coding regions, respectively, that flank the ITS-2; these primer sites are conserved among Clade V nematodes (Gasser et al., 1993; Avramenko et al., 2015, 2017). However, this approach does not rule out amplification of members of other clades. To consistently detect members of other clades, more comprehensive primers spanning the other nematode clades would be required as well as a modification of the bioinformatic pipeline to encompass a greater ITS-2 rDNA size range, and a more comprehensive nematode ITS-2 rDNA database.

Our data indicate that a large proportion of the samples contained GIN parasite species other than those commonly found in domestic ruminants; ITS-2 amplicons > 500bp were commonly detected which is outside the size range of the amplicons typically generated from domestic ruminant trichostrongylid parasite species. In some samples, up to 80 % of the reads did not map to the trichostrongylid parasite species in our databases; preliminary BLAST analysis suggests many of these mapped to either wild ruminant species or to free-living nematodes.

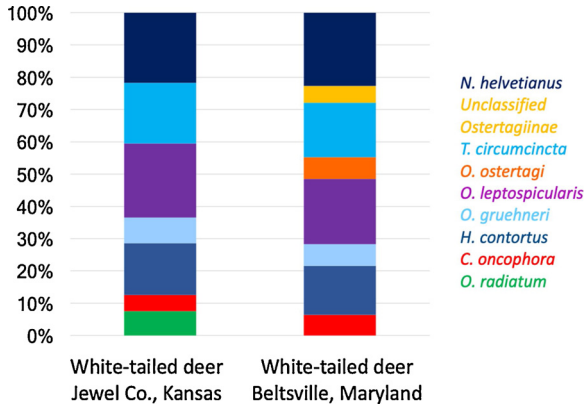

Fig. 3. Domestic ruminant GIN trichostrongylid species diversity found in two white-tailed deer samples.

Although egg counts would have been beneficial to this study, the accuracy of this data would have been questionable given the significant time delay between sample collection and receipt, and the shipment of samples under ambient temperature conditions. Another limitation was the ability to accurately assess true species diversity in each sample. In some samples that were collected from the ground rather than collected from the rumen, the numbers of free-living nematodes in some of the samples were sufficiently high to render larval counts difficult and inaccurate. Nevertheless, some interesting trends were observed that might inform a future, more quantitative study. Furthermore, in an effort to minimize sample-to-sample variation, a standard protocol of 14 day incubation (~22°C) was applied to all larval cultures. This allowed for all strongylid parasites present in the fecal samples (with the exception of *N. helvetianus*) to reach their arrested L3 stages by day 10 of culture (Avramenko et al., 2015; Roeber and Kahn, 2014; Dobson et al., 1992).

There are many confounding factors associated with epidemiological studies that make it difficult to identify cause and effect, and that support the findings related to geographical regions or hosts. Temperature, precipitation and humidity, broader weather patterns, season, terrain, host population density, farming practices and anthropogenics (urban vs rural areas) can all play a role in the results. For example, none of the 20 samples from the Southwest region of the U.S. were positive for Clade V nematode DNA, which could be due to any of the previously mentioned factors. However, these 20 samples were the only ones to arrive covered in what looked like a mold and/or fungus, thus introducing an additional factor that could have affected the hatchability of the eggs and the viability of the worms.

Considering geographical location, we observed differences in species percentages. For example, when the samples are pooled together by state, only *H. contortus* and *O. ostertagi* were present in 16 deer samples from South Carolina whereas 3 deer samples from Kansas collectively harbored 8 different species (Fig. 3). Kansas has 46 million acres of agricultural land and about 5.9 million cattle while South Carolina has only 4.9 million acres and cattle are not considered a major livestock commodity (0.3 million cattle) (USDA, 2012a,b). It is plausible that the parasite diversity seen in the samples from Kansas is related to animal numbers and/or density. Similarly, in the 7 deer samples from Vermont, there were 6 different parasite species present. Vermont has 1.3 million acres of agricultural land with 0.3 million cattle (USDA, 2012a, b). With a similar number of cattle as South Carolina, but on a quarter of the acreage, there was more parasite diversity with more cattle per acre. A similar relationship was observed in samples from a National Wildlife Preserve in Alaska which contained only one species (*Ostertagia gruehneri*) which is commonly found in wild ruminants and not in domestic livestock. Although our sampling is relatively small from each of these regions, the data is consistent with turnover, animal density and the potential for interaction between the domestic and sylvatic transmission cycles, all contributing to parasite diversity. Understanding the infection patterns, management practices and parasite diversity and not just the infection rates, may provide better insight into the circulation and transmission patterns of these GINs.

5. Conclusion

Overall, our hypothesis that wild ruminants harbor GIN common to domestic livestock that can act as reservoirs for maintaining infections in productions herds was supported by this study. *Ostertagia* was found in 90 % of the 84 sequenced wildlife samples which also contained *Haemonchus*, *Cooperia*, *Trichostrongylus*, and *Oesophagostomum* species to varying degrees. These results are consistent with the hypothesis that wild ruminants can play a significant role in the transmission of domestic ruminant nematodes and calls for more thorough investigations in particular those that involve the ecosystems that are proximal to production facilities and in ascertaining the level of anthelmintic resistant and susceptible species in the wildlife hosts.

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Ethics approval

This epidemiological study adhered to all applicable international, national, and/or institutional guidelines for the care and use of animals was approved as defined in Objective 3 of the Agricultural Research Service Research Project 8042-32000-105-00D.

Author contributions

Conceptualization & methodology: CDB EPH DSZ JSG JW. Investigation & analysis - sample collection organization, sample prep, DNA isolation and PCR: CDB. Investigation & Analysis - deep amplicon sequencing and bioinformatics: JW JSG. Writing – Original Draft: CDB DSZ JSG JW. Writing – Review and Editing: CDB EPH DSZ JSG JW.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetpar.2020.109041.

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