THE UNIVERSITY of EDINBURGH

Edinburgh Research Explorer

Nemabiome metabarcoding shows a high prevalence of Haemonchus contortus and predominance of Camelostrongylus mentulatus in alpaca herds in the northern UK

Citation for published version:
Zahid, O, Butler, M, Hopker, A, Freeman, E, Martins Costa Junior, L, Chaudhry, UN & Sargison, N 2024, 'Nemabiome metabarcoding shows a high prevalence of Haemonchus contortus and predominance of Camelostrongylus mentulatus in alpaca herds in the northern UK', *Parasitology Research*, vol. 123, no. 5, 201, pp. 1-10. https://doi.org/10.1007/s00436-024-08226-w

Digital Object Identifier (DOI):
10.1007/s00436-024-08226-w

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Parasitology Research

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 04. Jul. 2024

1 **Nemabiome metabarcoding shows a high prevalence of *Haemonchus contortus* and**
2 **predominance of *Camelostrongylus mentulatus* in alpaca herds in the northern UK**
3

4 Osama Zahid[a*], Meghan Butler[a], Andy Hopker[a], Emily Freeman[a], Livio M. Costa Júnior[b], Umer Chaudhry[c],
5 Neil Sargison[a]
6

7 a University of Edinburgh, Royal (Dick) School of Veterinary Studies and Roslin Institute, Easter Bush
8 Veterinary Centre, Midlothian, EH25 9RG, UK

9 b Federal University of Maranhão, Pathology Department, São Luís, Maranhão, Brazil.

10 c St. George's University, School of Veterinary Medicine, St. George's Grenada, West Indies.

11 Current address for Meghan Butler: Galedin Veterinary, Kelso TD5 7BH, UK

12 *Corresponding author: Osama Zahid (osamazahid308@gmail.com, s1885932@ed.ac.uk)
13

14 **Abstract**

15 Gastrointestinal nematodes (GINs) are a common threat faced by pastoral livestock. Since
16 their major introduction to the UK in the early 1990s, South American camelids have been co-
17 grazed with sheep, horses, and other livestock, allowing exposure to a range of GIN species.
18 However, there have been no molecular-based studies to investigate the GIN populations present
19 in these camelids. In the current study, we sampled nine alpaca herds from northern England and
20 southern Scotland and used high-throughput metabarcoded sequencing to describe their GIN
21 species composition. A total of 71 amplicon sequence variants (ASVs) were identified representing
22 eight known GIN species. *Haemonchus contortus* was the most prevalent species found in almost
23 all herds in significant proportions. The identification of *H. contortus* in other livestock species is
24 unusual in the northern UK, implying that alpacas may be suitable hosts and potential reservoirs
25 for infection in other hosts. In addition, the camelid-adapted GIN species *Camelostrongylus*
26 *mentulatus* was identified predominantly in herds with higher faecal egg counts. These findings
27 highlight the value of applying advanced molecular methods, such as nemabiome metabarcoding
28 to describe the dynamics of gastrointestinal nematode infections in novel situations. The results
29 provide a strong base for further studies involving co-grazing animals to confirm the potential role
30 of alpacas in transmitting GIN species between hosts.

31

32 Keywords: alpaca gastrointestinal nematodes, nemabiome metabarcoding sequencing,
33 *Haemonchus contortus*, *Camelostrongylus mentulatus*, internal transcribed spacer 2

34 ## 1 Introduction

35 Gastrointestinal nematodes (GINs) impact animal health and welfare through both direct
36 pathological and indirect immune-mediated effects (Stromberg and Gasbarre, 2006); leading to a
37 reduction in productivity and significant economic losses to the livestock industry worldwide
38 (Charlier et al., 2009; Mavrot et al., 2015). In addition, they substantially increase the industry's
39 carbon footprint due to consequential higher maintenance requirements for natural resources
40 relative to production output. Mathematical modelling suggests controlling the spread and severity
41 of nematode infections can help to reduce these effects (Kenyon et al., 2013; Nieuwhof and Bishop,
42 2005).

43 GINs live in complex communities of multiple co-infecting species (Agneessens et al.,
44 1997; Burgess et al., 2012; Giudici et al., 1999; Stromberg et al., 2015; Vlassoff, 1976); with each
45 having potentially different epidemiology, pathogenicity, clinical presentation, and drug resistance
46 status (Besier et al., 2016; Whitlock et al., 1980). The composition of these communities is affected
47 by various factors such as temperature and humidity (O'Connor et al., 2006), different farming
48 practices and the age and immune status of the host animals (Redman et al., 2019). GIN species
49 are adapted to characteristics of different hosts, and many are considered host-specific (Van Wyk
50 et al., 2004); albeit cross-infections of these species can occur. For example, *Haemonchus*
51 *contortus* is generally considered a small ruminant-adapted nematode with a host preference for
52 sheep; but has also been reported in cattle, buffalo, and bison (Avramenko et al., 2018; Ali et al.,
53 2019). Thus, coinfections arising from co-grazing domestic and wild animal populations must be
54 considered when exploring GIN infections within specific host populations.

55 Traditionally, the morphological identification of coprocultured third-stage larvae (L_3) has
56 been the primary method employed to determine the species composition of nematode populations.

This relies on the microscopic examination of the shape, size, and arrangement of various anatomical features in L_3s (Saidi et al. 2020; Van Wyk et al. 2004; Van Wyk and Mayhew 2013). This classical approach has served as the basis for nematode identification for decades, providing valuable insights into the taxonomy and epidemiology of parasites of farmed small and large ruminants for which keys have been developed. However, this approach is time-consuming and highly dependent on the expertise of skilled taxonomists. In addition, distinguishing between L_3 of closely related species with overlapping morphological traits poses significant challenges in discerning between species accurately; thus necessitating the exploration of advanced molecular techniques (Roeber and Kahn 2014).

High-throughput metabarcoding of ribosomal nematode DNA provides an example of how next-generation sequencing technology has revolutionised the field of nematode identification. This technique referred to as 'nemabiome metabarcoding', involves extracting nematode DNA from nematode eggs, first (L_1), or third stage larvae (L_3) and targeting conserved rDNA ITS-2 primer binding regions to amplify and sequence nematode clade V-specific DNA (Avramenko et al. 2017). The resulting amplicon sequence variants are then filtered and compared to reference databases to identify the nematode species present.

In recent years, nemabiome sequencing has been successfully employed in various livestock hosts including small ruminants (Redman et al. 2019), large ruminants (Avramenko et al. 2017) and horses (Sargison et al. 2022) to determine the composition and diversity of nematode communities. These studies have demonstrated the utility of nemabiome metabarcoding sequencing in accurately identifying gastrointestinal nematode species and providing a comprehensive understanding of the parasitic landscape within these hosts.

79 Compared to traditional methods, nemabiome metabarcoding sequencing offers several
80 distinct advantages. Firstly, it provides a rapid and high-throughput approach for nematode
81 identification, allowing for the simultaneous detection and characterisation of multiple nematode
82 species within a single sample. This capability is particularly valuable when dealing with mixed
83 infections, or when studying the dynamics of nematode populations over time. Secondly,
84 nemabiome sequencing enhances the accuracy and objectivity of nematode species identification.
85 Unlike traditional morphological methods, which are prone to subjectivity and intra- and inter-
86 observer variability, nemabiome sequencing relies on DNA sequence data, which provides a robust
87 and reproducible basis for species determination. This objectivity minimises biases and increases
88 the consistency of results, enabling comparisons and interpretations across studies. Furthermore,
89 even when compared to species-specific molecular methods, nemabiome metabarcoding has the
90 potential advantage of uncovering amplicon sequence variants representing previously unknown
91 or cryptic species and hybrids. Phylogenetic analysis of amplicon sequence variants can detect
92 subtle genetic variations that may indicate the presence of new, or closely related species (Sargison
93 et al. 2022). Such discoveries contribute to the broader understanding of nematode biodiversity
94 and aid in refining taxonomic classifications.

95 The use of nemabiome metabarcoding outside of traditional farm animal species is
96 comparatively uncommon; partly because of the difficulty in creating reference databases
97 containing sequences for GINs that might be present in these wildlife, or other unusual hosts.
98 Recently, we developed a sequence library for horses that included the sequences for many wildlife
99 species (Sargison et al. 2022). In the current study, we applied this library to study the presence
100 and proportional abundance of nemabiome amplicon sequence variants of GINs present in alpacas

101 in the northern UK. The work aimed to improve understanding of the GIN communities infecting
102 UK camelid herds, as a basis for improved control strategies.

103 **2 Methods**

104 *2.1 Sample collection and processing*

105 Faecal samples were collected from nine alpaca farms in the north of England and south of
106 Scotland between July and November 2018. The freshly voided samples were obtained from the
107 ground of communal defecation sites. Precautions were taken to avoid any cross-contamination
108 for the faeces of any co-crazing animals. Available information was gathered on co-grazing and
109 GIN management, as shown in **table 1**.

110 One gram of faeces from each sample was used to perform a faecal egg count (FEC) using
111 a saturated salt flotation and the cuvette method with a detection threshold of 1 egg per gram (epg)
112 (Christie and Jackson, 1982). Equal amounts of the remaining faecal material (>10 grams) were
113 pooled for each farm and incubated at about 20ºC for 14 days for L_3 coproculture. L_3 were isolated
114 by Baermannisation (Großbritannien, 1986) and fixed in 70% ethanol. All of the L_3 obtained from
115 the coprocultures were used to produce DNA lysates.

116 For DNA extraction, 1000 µl Direct PCR Lysis Reagent (Viagen), 50 µl of proteinase K
117 (Quiagen) solution, and 50 µl of 1M dithiothreitol (DDT) were added to create a worm lysis
118 solution. 20 µl of this worm lysis solution was added to each sample and incubated at 60°C for
119 two hours, followed by 15 minutes at 85°C to inactivate the proteinase K (Evans et al., 2021). The
120 lysates were stored at -20°C until further use.

121 *2.2 Adapter and barcoded PCRs*

122 Previously published primers and conditions (Avramenko et al., 2015) were used to
123 amplify the rDNA ITS-2 region. PCR products were purified with AMPure XP magnetic beads
124 according to the manufacturer's guidelines, followed by the second round of PCR amplification to
125 add unique barcode combinations to each sample using the previously described method (Rehman
126 et al., 2020). Finally, the samples were pooled (10 ul PCR product from each sample) and purified
127 using a Qiagen gel extraction and purification kit, followed by further purification through
128 AMPure XP magnetic beads. 20 µl of the pooled sample was submitted to Edinburgh Genomics
129 for Illumina MiSeq, using a 500-cycle paired-end reagent kit (MiSeq Reagent Kits v2, MS-103-
130 2003) at a concentration of 15 nM with the addition of 15% PhiX Control v3 (Illumina, FC-11-
131 2003). Each resequencing step followed Illumina's standard protocol.

132 The numbers of L_3 recovered varied greatly between farms, and the DNA amount could
133 not be equalised between samples; hence the results are focused on describing the GIN species
134 present on individual farms, rather than direct proportional comparisons.

135 *2.3 GIN Species analysis*

136 The FASTQ files obtained from the post-run Illumina MiSeq processing, representing
137 sequences present in each index-recognised sample, were analysed following the adapted Illumina
138 MiSeq protocols for nemabiome in Mothur v1.39.5 (Schloss et al., 2009). The steps involved
139 joining paired forward and reverse reads and screening sequences shorter than 200 bp, longer than
140 450 bp, or with any ambiguous bases before they were aligned to a bespoke reference sequence
141 library (https://github.com/drosamazahid/uk_alpaca) containing ruminant, horse, camelid, and
142 wild animal nematode species using a Needleman-Wunsch pairwise alignment method, following
143 the described workflow (https://www.nemabiome.ca/mothur_workflow.html). The sequence

144 library had previously been developed and used to study equine GIN populations, for which there
145 was limited *a priori* knowledge of what species might be present (Sargison et al., 2022). The
146 sequences were then classified into different species/groups according to the taxonomy file of the
147 reference library using the k-Nearest Neighbor algorithm (knn) method. Finally, a summary file
148 showing a total of about 342,000 (average 38,000 per sample; range 6,176 - 74,168) aligned
149 sequences belonging to different species in each sample was created and inputted to R (R Core
150 Team, 2021) for further analysis. Before calculating the relative abundance of different species,
151 any species which had less than 1% of the total reads (3,420) was removed to avoid stochastic
152 effects arising from low egg counts and to negate the effects of bleeding/index hoping during the
153 sequencing process, leaving 337,000 total reads (average 37,444 per sample; range 6,176 - 70,951).
154 Correction factors (available at https://www.nemabiome.ca/mothur_workflow.html) were not
155 used, as none are available for *Camelostrongylus mentulatus*. Bar charts were produced to show
156 the proportion reads and intensity of infection (obtained by multiplying the proportion reads with
157 FEC) of GIN species on different farms (**Figs. 1 and 2**).

158 *2.4 Confirmation of species identity*

159 Sequences of each species were separated, and the identical sequences present at least twice
160 were collapsed using FaBox DNA collapser
161 (https://birc.au.dk/~palle/php/fabox/dnacollapser.php) to obtain amplicon sequence variants
162 (ASVs). A total of 61 ASVs were obtained which were then blasted on NCBI to confirm their
163 identities. These matched 100% with the results obtained from our library. The top five blast results
164 for each ASV, along with their percentage identity match with the previous sequences in NCBI
165 Genbank are available at https://github.com/drosamazahid/uk_alpaca.

166 ### 2.5 *Phylogenetic analysis*

167 Phylogenetic trees of both the field sequences and the NCBI Genbank sequences of all the
168 species found in the field samples were constructed in MEGA X to show where the field sequences
169 aligned with previously reported NCBI Genbank sequences. The Genbank sequences were
170 obtained by manually searching for each species. Partial sequences were removed and duplicates
171 were merged before constructing the tree using the Tamura 3-parameter model (Tamura, 1992)
172 The tree with the highest log likelihood (-25627.67) is shown in **Fig 3.** Initial tree(s) for the
173 heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to
174 a matrix of pairwise distances estimated using the Tamura 3 parameter model and then selecting
175 the topology with superior log-likelihood value. A discrete gamma distribution was used to model
176 evolutionary rate differences among sites [5 categories (+G, parameter = 0.9576)]. This analysis
177 involved 207 nucleotide sequences, and there was a total of 362 positions in the final dataset.

178 ## 3 Results

179 ### 3.1 *Faecal egg count and management data*

180 The mean FEC results are shown in **table 1**. Overall, the counts were low and varied
181 between 1 and 24 eggs per gram (epg) for trichostrongyle eggs and 1 and 8 epg for *Nematodirus*
182 spp. eggs. Co-grazing animals and anthelmintic treatments of the alpacas during the previous 12
183 months are also shown in **table 1**. Most of the herds had been treated with one of moxidectin,
184 doramectin, ivermectin and fenbendazole during the previous six months, except farm 6 which had
185 no anthelmintic treatment history. Similarly, all but farm 3 had a history of co-grazing with
186 different animals including sheep, horses, donkeys, cattle, pigs and wild deer. It is worth noting
187 that the study adopted a convenience-based sampling approach; hence it is possible that the
188 selected farms might not be wholly representative of alpaca herds in the northern UK.

189 *3.2 The abundance of different GIN species*

190 The species analysis confirmed six known GIN species in the nine herds studied (**Fig. 1)**.
191 *Haemonchus contortus* was the most common species, present in all except farm 4, where a very
192 low FEC and consequent L_3 yield might have accounted for its detection failure. *Trichostrongylus*
193 *axei* was found in all herds, except those on farms 1 and 4. *Trichostrongylus colubriformis* and *C.*
194 *mentulatus,* which were present in six (farms 1, 3, 4, 6, 7 and 9) and four (farms 2, 5, 7 and 9)
195 herds, respectively. *Cooperia oncophora* was identified on farms 3 and 5, and *Trichostrongylus*
196 *vitrinus* on farms 5 and 7.

197 The GIN populations identified in the herds on farms 5 and 7 showed the greatest diversity
198 with five different GIN species present. Those on farms 3 and 9 were ranked second with four GIN
199 species each, followed by three species each on farms 2 and 6. Only two species each were
200 identified in the herds on farms 1 and 8. Farm 4 showed the least diversity with just one GIN
201 species; albeit the FEC was very low (1.2 epg), and consequently, a minimal number of larvae may
202 have been amplified.

203 The proportional FEC chart (**Fig 2**) shows most species to have similar abundances in
204 different herds, regardless of the total egg count. The most abundant species are *H. contortus* and
205 *T. axei*; while *C. mentulatus* seems to be predominant on farms with relatively higher egg counts.

206 *3.3 Phylogenetic analysis*

207 The ML tree of the field and NCBI Genbank ASVs (**Fig 3**) shows the GIN species separated
208 into different clades. The *Trichostrongylus* spp. sit very close to each other in a single large clade.
209 *C. oncophora, C. mentulatus*, and *H. contortus* are separated into their own clades. The tree also
210 shows all field samples sitting with their respective clades of Genbank sequences; supporting the
211 species identity of those ASVs

4 Discussion

Camelids have been kept in increasing numbers in the UK since major imports during the 1990s, especially alpacas which are mostly kept for fibre production, recreational enterprises, and as therapy animals and pets. Nevertheless, except for a few faecal egg counts, copro-cultured larval morphology studies and post-mortem reports (de B Welchman et al., 2008; Mitchell et al., 2016; Tait et al., 2002), there is little published information regarding their GIN infections; and none involving confirmation through molecular techniques.

The current study, based on high throughput sequencing (Avramenko et al., 2015), provides a robust insight into the GIN species present in UK alpacas. The FECs were low in all the samples, in agreement with the previous studies (Kultscher et al., 2019). This might be partially attributed to the distinct alpaca behaviour of establishing communal defecation and urination sites referred to as latrines (McGregor, 2002; McGregor and Brown, 2010). Since alpacas do not normally graze around these latrines when sufficient grazing is available, they may avoid acquiring a high level of infection. It is important to note that this is not always the case and that alpacas have been found to have higher FECs (>300 epg) in some studies (Bedenice et al, 2022); which might be attributed to the differences in the location of these latrines. It is not the scope of this study, but it will be interesting to investigate how this behaviour changes the overall composition of GINs in alpacas as it might favour certain GIN species over others. It might also be important to study any co-grazing animals, as they may maintain and spread most of the GIN species found, and could affect the challenge and burdens in the camelids.

The low FECs highlight a limitation of the study. Nevertheless, they do not greatly impact our results, because adequate numbers of sequence reads from multiple GIN species were generated from each sample (except for those from farm 4). DNA was extracted from copro-

235 cultured L_3 to allow easy processing of samples with low FECs. Other methods to isolate DNA
236 directly from faeces (Pafčo et al., 2018), from eggs (Redman et al., 2019), or from L_1 (Queiroz et
237 al., 2020) have been described along with arguments concerning their ability to represent GIN
238 diversity that is present. However, the choice of method ought not to affect the presence/absence
239 of GIN species, which was the focus of this study. There are correction factors available for some
240 of the GIN species to account for slight differences in the efficiency of DNA amplification
241 (https://www.nemabiome.ca/mothur_workflow.html). However, we did not apply these, primarily
242 because they are unavailable for camelid-adapted GIN species, *C. mentulatus* in particular.

243 Another potential limitation arises from the methodology of pooling samples for each farm
244 regardless of individual FEC, and the lack of DNA quantification before sequencing. While this
245 approach reflects the real-world conditions within farms, it complicates comparisons across
246 different farms. Additionally, the variability in anthelmintic treatment schedules among the farms
247 adds another layer of complexity. For instance, moxidectin was potentially administered within 12
248 weeks of sampling at farms 1 to 3, which could influence parasite loads and species dynamics.
249 Given these challenges, the study's results and discussions have been primarily focused on
250 analyzing the dynamics of individual GIN species, with particular focus on *H. contortus* and *C.*
251 *mentulatus*, rather than making broad comparisons between farms.

252 Most of the GIN species identified in the current study were of presumed small-ruminant
253 origin (*H. contortus* and *Trichostrongylus* sp.), while *C. oncophora* is considered a cattle species.
254 No apparent correlation was seen between the GIN species found and the co-grazing or
255 anthelmintic treatment history, albeit the small sample size prevented any kind of statistical
256 analysis.

257 *Nematodirus lamae* was previously identified in UK Alpacas (Becklund, 1963; Mitchell et
258 al., 2016). While *Nematodirus* sp. eggs were identified in the current study, these would not have
259 consistently hatched in the coprocultures (Zajac, 2006). Hence, it was not possible to confirm their
260 species identity using molecular methods.

261 The predominance of *H. contortus* is noteworthy because it is relatively very uncommon
262 in its preferred sheep hosts in the study region in the northern UK (Sargison et al., 2007). Similar
263 results have also been reported in German alpaca herds (Kultscher et al., 2019). The distinct
264 digestive physiology or grazing behaviour of camelids might make them a well-adapted host for
265 *H. contortus*. The investigation of the potential for alpacas to act as a reservoir for *H. contortus*
266 affecting small ruminants could be worthwhile.

267 *Camelostrongylus mentulatus* was the only camelid-specific GIN species found.
268 Interestingly, it was present on the 3 farms with the highest FECs. This species has previously
269 been reported in UK alpacas (de B Welchman et al., 2008), but its presence has not been confirmed
270 with molecular biology. *Camelostrongylus mentulatus* has also been reported to infect small
271 ruminants in different countries (Beveridge and Ford, 1982; de Ybáñez et al., 2003; Hilton et al.,
272 1978; Mayo et al., 2013); hence it will be interesting to investigate the GINs in sheep and goats
273 co-grazing with alpacas in future studies.

274 In summary, the study demonstrates the value of next-generation resequencing methods to
275 study the composition and diversity of GIN communities in a novel host species. The results
276 confirm the presence of at least six different GIN species in alpacas in the northern UK. The
277 unexpectedly high prevalence of *H. contortus* and predominance of *C. mentulatus* in herds with
278 the highest FECs prompt the need to revise the sustainable GIN control practices for alpacas and
279 co-grazing livestock species. The study also shows the importance of molecular techniques such

280 as nemabiome sequencing to describe changing GIN coinfections in different hosts and inform
281 effective and sustainable parasite control.

282 **Declarations**

283 **Animal Ethics and Consent to Participate**

284 Ethical approval for the utilization of animal data was obtained from the R(D)SVS Veterinary
285 Ethical Review Committee (VERC) under reference number 122.17. No human data were used in
286 the study and the exact locations of alpaca farms are not disclosed.

287 **Competing interests**

288 We have no conflicts of interest to disclose.

289 **Authors' contributions**

290 Meghan Butler collected the samples, and Emily Freeman processed them under the supervision
291 of Neil Sargison, Andy Hopker and Umer Chaudhry. Osama Zahid prepared the library, analysed
292 the results and wrote the manuscript, with help from Livio M. Costa Júnior, Umer Chaudhry and Neil
293 Sargison. All authors reviewed the manuscript.

294 **Funding**

295 All the work was done at the Roslin Institute, UK, using facilities funded by the Biotechnology
296 and Biological Sciences Research Council (BBSRC), UK.

297 **Availability of data and materials**

298 The sequence library used, along with the resultant sequences obtained can be accessed on GitHub:
299 https://github.com/drosamazahid/uk_alpaca

300 **References**

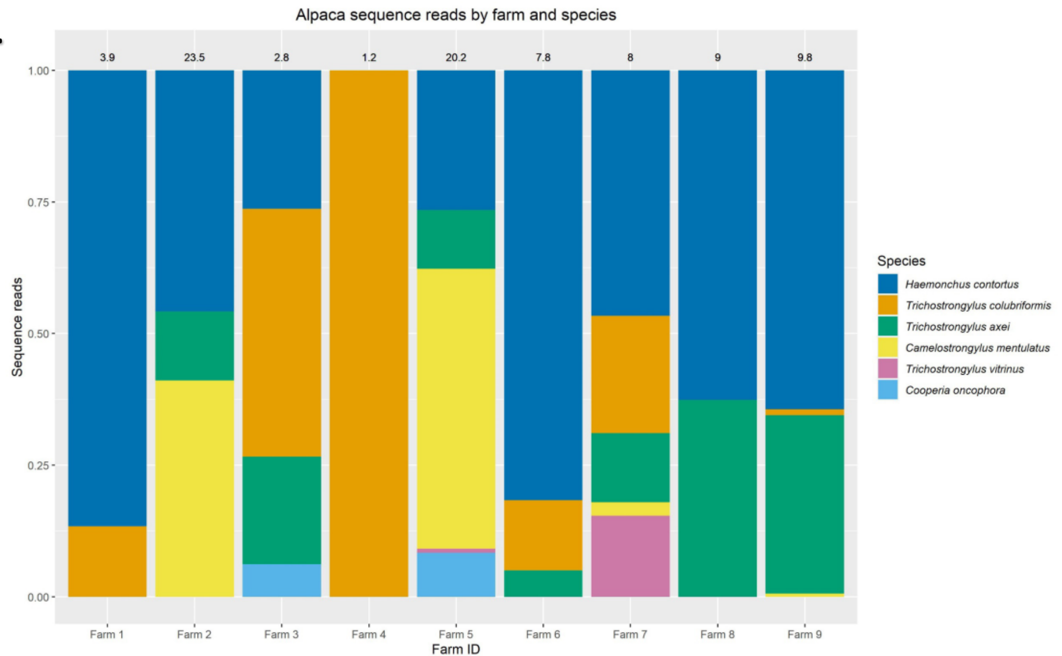
301 Agneessens, J., Dorny, P., Hollanders, W., Claerebout, E., Vercruysse, J., 1997. Epidemiological
302 observations on gastrointestinal nematode infections in grazing cow-calf pairs in Belgium.
303 Veterinary Parasitology 69, 65-75.
304 Ali, Q., Rashid, I., Shabbir, M.Z., Shahzad, K., Ashraf, K., Sargison, N.D., Chaudhry, U., 2019. Emergence and
305 the spread of the F200Y benzimidazole resistance mutation in *Haemonchus contortus* and
306 *Haemonchus placei* from buffalo and cattle. Veterinary Parasitology 265, 48-54.
307 Avramenko, R.W., Bras, A., Redman, E.M., Woodbury, M.R., Wagner, B., Shury, T., Liccioli, S., Windeyer,
308 M.C., Gilleard, J.S., 2018. High species diversity of trichostrongyle parasite communities within
309 and between Western Canadian commercial and conservation bison herds revealed by
310 nemabiome metabarcoding. Parasites & Vectors 11, 1-13.
311 Avramenko, R.W., Redman, E.M., Lewis, R., Bichuette, M.A., Palmeira, B.M., Yazwinski, T.A., Gilleard, J.S.,
312 2017. The use of nemabiome metabarcoding to explore gastro-intestinal nematode species
313 diversity and anthelmintic treatment effectiveness in beef calves. International Journal For
314 Parasitology 47, 893-902.
315 Avramenko, R.W., Redman, E.M., Lewis, R., Yazwinski, T.A., Wasmuth, J.D., Gilleard, J.S., 2015. Exploring
316 the gastrointestinal "nemabiome": deep amplicon sequencing to quantify the species
317 composition of parasitic nematode communities. PLoS One 10, e0143559.
318 Becklund, W.W., 1963. *Lamanema chavezi* gen. n., sp. n. and *Nematodirus lamae* sp. n.(Nematoda:
319 Trichostrongylidae) from the Alpaca, Lama pacos, and the Vicuña, Vicugna vicugna, in Perú. The
320 Journal of Parasitology, 1023-1027.
321 Bedenice, D., Resnick-Sousa, J., Bookbinder, L., Trautwein, V., Creasey, H. N., & Widmer, G. (2022). The
322 association between fecal microbiota, age and endoparasitism in adult alpacas. *Plos one*, *17*(8),
323 e0272556.
324 Besier, R., Kahn, L., Sargison, N., Van Wyk, J., 2016. Diagnosis, treatment and management of *Haemonchus*
325 *contortus* in small ruminants. Advances In Parasitology 93, 181-238.
326 Beveridge, I., Ford, G., 1982. The trichostrongyloid parasites of sheep in South Australia and their regional
327 distribution. Australian Veterinary Journal 59, 177-179.
328 Burgess, C.G., Bartley, Y., Redman, E., Skuce, P.J., Nath, M., Whitelaw, F., Tait, A., Gilleard, J.S., Jackson,
329 F., 2012. A survey of the trichostrongylid nematode species present on UK sheep farms and
330 associated anthelmintic control practices. Veterinary Parasitology 189, 299-307.
331 Charlier, J., Höglund, J., von Samson-Himmelstjerna, G., Dorny, P., Vercruysse, J., 2009. Gastrointestinal
332 nematode infections in adult dairy cattle: impact on production, diagnosis and control. Veterinary
333 Parasitology 164, 70-79.
334 Christie, M., Jackson, F., 1982. Specific identification of strongyle eggs in small samples of sheep faeces.
335 Research in Veterinary Science 32, 113-117.
336 de B Welchman, D., Parr, J., Wood, R., Mead, A., Starnes, A., 2008. Alpaca and llama nematodes in Britain.
337 The Veterinary Record 162, 832.
338 de Ybáñez, M.R., Garijo, M., Carpintero, M., Martínez-Carrasco, C., Ortiz, J., 2003. *Camelostrongylus*
339 *mentulatus* in domestic goats from the Iberian Peninsula. Journal Of Helminthology 77, 371-372.
340 Evans, M., Chaudhry, U.N., Costa-Júnior, L., Hamer, K., Leeson, S.R., Sargison, N., 2021. A 4 year
341 observation of gastrointestinal nematode egg counts, nemabiomes and the benzimidazole
342 resistance genotypes of Teladorsagia circumcincta on a Scottish sheep farm. International Journal
343 for Parasitology 51, 393-403.
344 Giudici, C., Aumont, G., Mahieu, M., Saulai, M., Cabaret, J., 1999. Changes in gastro-intestinal helminth
345 species diversity in lambs under mixed grazing on irrigated pastures in the tropics (French West
346 Indies). Veterinary Research 30, 573-581.

347 Großbritannien, M.o.A., 1986. Manual of veterinary parasitological laboratory techniques: 160 S.: Ill. HM
348 Stationery Office.
349 Hilton, R., Barker, I., Rickard, M., 1978. Distribution and pathogenicity during development of
350 *Camelostrongylus mentulatus* in the abomasum of sheep. Veterinary Parasitology 4, 231-242.
351 Kenyon, F., Dick, J.M., Smith, R.I., Coulter, D.G., McBean, D., Skuce, P.J., 2013. Reduction in greenhouse
352 gas emissions associated with worm control in lambs. Agriculture 3, 271-284.
353 Kultscher, L., Hinney, B., Schmäschke, R., Joachim, A., Wittek, T., 2019. Current anthelmintic treatment is
354 not always effective at controlling strongylid infections in German alpaca herds. Parasites &
355 Vectors 12, 1-10.
356 Mavrot, F., Hertzberg, H., Torgerson, P., 2015. Effect of gastro-intestinal nematode infection on sheep
357 performance: a systematic review and meta-analysis. Parasites & Vectors 8, 1-11.
358 Mayo, E., Ortiz, J., Martínez-Carrasco, C., Garijo, M., Espeso, G., Hervías, S., Ruiz de Ybáñez, M., 2013. First
359 description of gastrointestinal nematodes of Barbary sheep (*Ammotragus lervia*): the case of
360 *Camelostrongylus mentulatus* as a paradigm of phylogenic and specific relationship between the
361 parasite and its ancient host. Veterinary Research Communications 37, 209-215.
362 McGregor, B., 2002. Comparative productivity and grazing behaviour of Huacaya alpacas and Peppin
363 Merino sheep grazed on annual pastures. Small Ruminant Research 44, 219-232.
364 McGregor, B., Brown, A., 2010. Soil nutrient accumulation in alpaca latrine sites. Small Ruminant Research
365 94, 17-24.
366 Mitchell, S., Hopkins, B., Corfield, C., 2016. *Nematodirus lamae* identified in an alpaca in the UK. The
367 Veterinary Record 178, 271.
368 Nieuwhof, G.J., Bishop, S., 2005. Costs of the major endemic diseases of sheep in Great Britain and the
369 potential benefits of reduction in disease impact. Animal Science 81, 23-29.
370 O'Connor, L.J., Walkden-Brown, S.W., Kahn, L.P., 2006. Ecology of the free-living stages of major
371 trichostrongylid parasites of sheep. Veterinary Parasitology 142, 1-15.
372 Pafčo, B., Čížková, D., Kreisinger, J., Hasegawa, H., Vallo, P., Shutt, K., Todd, A., Petrželková, K.J., Modrý,
373 D., 2018. Metabarcoding analysis of strongylid nematode diversity in two sympatric primate
374 species. Scientific Reports 8, 1-11.
375 Queiroz, C., Levy, M., Avramenko, R., Redman, E., Kearns, K., Swain, L., Silas, H., Uehlinger, F., Gilleard,
376 J.S., 2020. The use of ITS-2 rDNA nemabiome metabarcoding to enhance anthelmintic resistance
377 diagnosis and surveillance of ovine gastrointestinal nematodes. International Journal for
378 Parasitology: Drugs and Drug Resistance 14, 105-117.
379 Redman, E., Queiroz, C., Bartley, D.J., Levy, M., Avramenko, R.W., Gilleard, J.S., 2019. Validation of ITS-2
380 rDNA nemabiome sequencing for ovine gastrointestinal nematodes and its application to a large
381 scale survey of UK sheep farms. Veterinary Parasitology 275, 108933.
382 Roeber F, Kahn LJVP (2014) The specific diagnosis of gastrointestinal nematode infections in livestock:
383 larval culture technique, its limitations and alternative DNA-based approaches. 205(3-4):619-628
384 Rehman, Z.U., Zahid, O., Rashid, I., Ali, Q., Akbar, M.H., Oneeb, M., Shehzad, W., Ashraf, K., Sargison, N.D.,
385 Chaudhry, U., 2020. Genetic diversity and multiplicity of infection in Fasciola gigantica isolates of
386 Pakistani livestock. Parasitology International 76, 102071.
387 Sargison, N., Wilson, D., Bartley, D., Penny, C., Jackson, F., 2007. Haemonchosis and teladorsagiosis in a
388 Scottish sheep flock putatively associated with the overwintering of hypobiotic fourth stage
389 larvae. Veterinary Parasitology 147, 326-331.
390 Sargison, N., Chambers, A., Chaudhry, U., Júnior, L.C., Doyle, S.R., Ehimiyein, A., Evans, M., Jennings, A.,
391 Kelly, R., Sargison, F., 2022. Faecal egg counts and nemabiome metabarcoding highlight the
392 genomic complexity of equine cyathostomin communities and provide insight into their dynamics
393 in a Scottish native pony herd. International Journal for Parasitology 52, 763-774.

394 Saidi A, Mimouni R, Hamadi F, Oubrou WJUjov, sciences a (2020) Some larval morphological
395 characteristics of Camelostrongylus mentulatus and Nematodirus spathiger. 3(2):7-11
396 Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley,
397 B.B., Parks, D.H., Robinson, C.J., 2009. Introducing mothur: open-source, platform-independent,
398 community-supported software for describing and comparing microbial communities. Applied
399 and Environmental Microbiology 75, 7537-7541.
400 Stromberg, B.E., Gasbarre, L.C., 2006. Gastrointestinal nematode control programs with an emphasis on
401 cattle. Veterinary Clinics: Food Animal Practice 22, 543-565.
402 Stromberg, B.E., Gasbarre, L.C., Ballweber, L.R., Dargatz, D.A., Rodriguez, J.M., Kopral, C.A., Zarlenga, D.S.,
403 2015. Prevalence of internal parasites in beef cows in the United States: Results of the National
404 Animal Health Monitoring System's (NAHMS) beef study, 2007-2008. Canadian Journal of
405 Veterinary Research 79, 290-295.
406 Tait, S., Kirwan, J., Fair, C., Coles, G., Stafford, K., 2002. Parasites and their control in South American
407 camelids in the United Kingdom. The Veterinary Record 150, 637.
408 Tamura, K., 1992. Estimation of the number of nucleotide substitutions when there are strong transition-
409 transversion and G+ C-content biases. Mol Biol Evol 9, 678-687.
410 Van Wyk, J., Cabaret, J., Michael, L., 2004. Morphological identification of nematode larvae of small
411 ruminants and cattle simplified. Veterinary Parasitology 119, 277-306.
412 Van Wyk JA, Mayhew EJOJoVR (2013) Morphological identification of parasitic nematode infective larvae
413 of small ruminants and cattle: A practical lab guide. 80(1):1-14
414 Vlassoff, A., 1976. Seasonal incidence of infective trichostrongyle larvae on pasture: the contribution of
415 the ewe and the role of the residual pasture infestation as sources of infection to the lamb. New
416 Zealand Journal of Experimental Agriculture 4, 281-284.
417 Whitlock, H., Sangster, N., Gunawan, M., Porter, C., Kelly, J., 1980. *Trichostrongylus colubriformis* and
418 *Ostertagia sp* resistant to levamisole, morantel tartrate and thiabendazole: isolation into pure
419 strain and anthelmintic titration. Research in Veterinary Science 29, 31-35.
420 Zajac, A.M., 2006. Gastrointestinal nematodes of small ruminants: life cycle, anthelmintics, and diagnosis.
421 Veterinary Clinics: Food Animal Practice 22, 529-541.

422 <u>**Figure Legends**</u>

Fig. 1

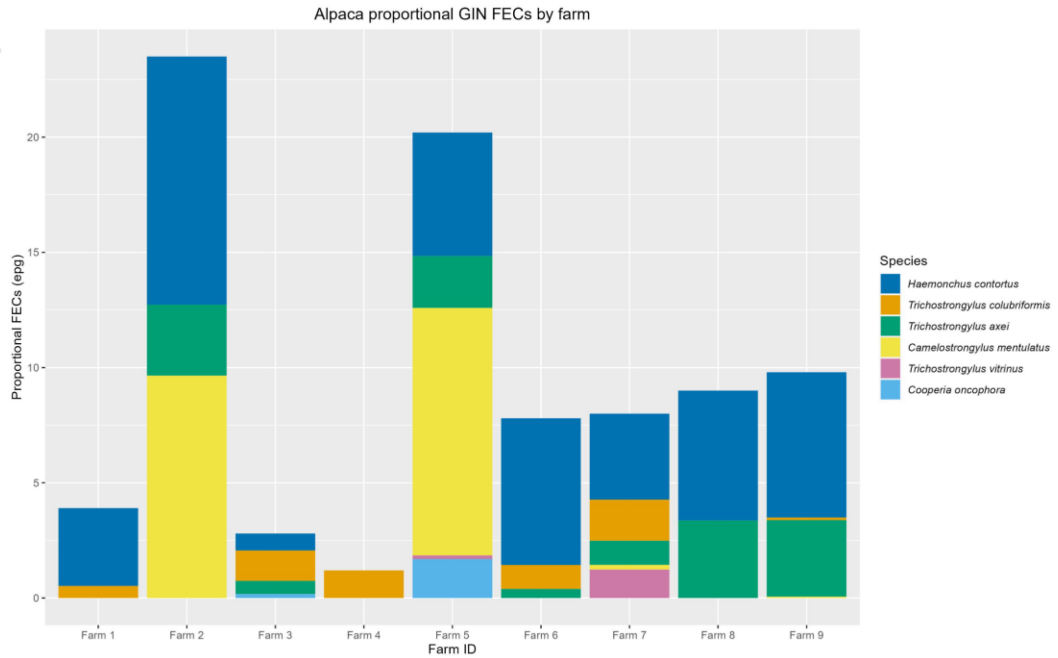


423

424 **Fig. 1.** The relative abundance of nematode species on different farms. Each color represents a
425 separate species, and each bar is a different farm. The x-axis shows the sequence reads proportion
426 for each GIN species, with different farms on the y-axis. Each farm's mean faecal worm egg count
427 is shown on the top of each bar. The legend shows the colour of each GIN species as well as their
428 arrangement within the bar chart.

429

Fig. 2

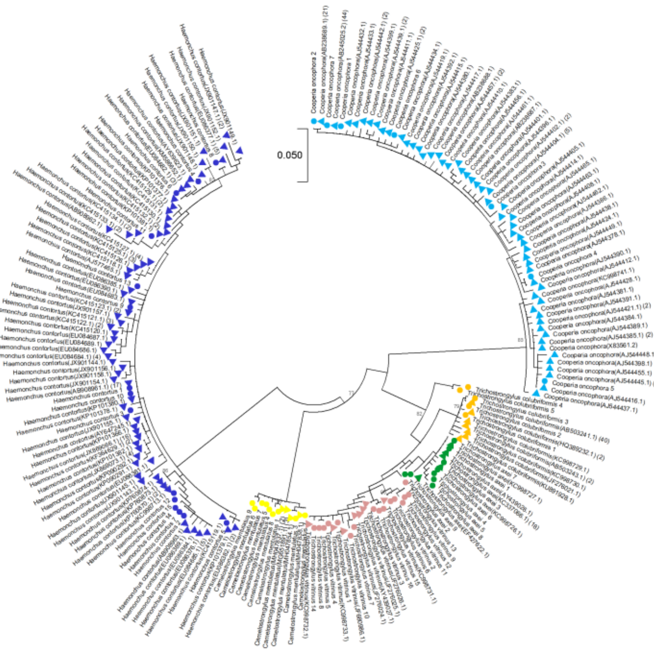


430

431 **Fig. 2.** The proportional feacal egg count (FEC) of each nematode species in different farms. Each
432 color represents a separate species, and each bar is a different farm. The x-axis shows the
433 proportional FEC for each GIN species, obtained by multiplying the FEC with proportional reads.
434 Different farms are on the y-axis. The legend shows the colour of each GIN species as well as their
435 arrangement within the bar chart.

436

Fig. 3



437

438 **Fig. 3.** Phylogenetic tree of GIN species found in alpaca herds in the northern UK and the Genbank
439 sequences**.** The phylogenetic tree of field ASVs and Genbank sequences was constructed using the
440 Maximum Likelihood method and the Tamura 3-parameter model. The tree with the highest log
441 likelihood (-25627.67) is shown. Initial tree(s) for the heuristic search were obtained automatically
442 by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using
443 the Tamura 3 parameter model and then selecting the topology with the superior log-likelihood
444 value. A discrete Gamma distribution was used to model evolutionary rate differences among sites
445 (5 categories (+G, parameter = 0.9576)). This analysis involved 207 nucleotide sequences, and
446 there was a total of 362 positions in the final dataset. Evolutionary analyses were conducted in
447 MEGA X. The numbers on branches show bootstrap values. Triangles show Genbank sequences
448 with their accession number identity, while circles represent field ASVs. Each colour represents a
449 different GIN species. The Genbank accession numbers are shown in brackets after the name. The
450 second bracket shows the number of identical Genbank sequences merged to simplify the analysis.

451

Farm ID	*n*	Trichostrongyle counts (epg)		*Nemotodirus* spp. counts (epg)		Other animals co-grazed with the alpacas	Typical anthelmintic treatment history
		Range	Mean	Range	Mean		
1	16	0-12	3.9	0-6	0.8	wild deer	October (Moxidectin); May (Moxidectin)
2	11	0-155	23.5	0-14	2.5	sheep; horse	April (Moxidectin)
3	12	0-12	2.8	0-3	1.8	none	November (Moxidectin); March (Doramectin)
4	15	0-9	1.2	0-12	2.8	horses	May (Febendazole)
5	22	0-22	20.2	0-7	4.2	donkeys	May (Doramectin)
6	7	0-30	9.0	0-3	0.9	wild deer; sheep; cattle; pigs	None
7	4	3-12	9.8	0-30	8.3	sheep	March (Ivermectin)
8	5	0-18	7.8	0-12	3.0	sheep; horses; pigs	June (Moxidectin)
9	12	0-69	8.0	0-6	0.8	sheep; cattle	June (Febendazole); October (Febendazole)

452

453 **Table 1: Average Trichostrongyle and *Nematodirus* faecal egg count at the**
454 **beginning and end of a grazing season, with co-grazing and GIN management data.** The
455 number of alpacas tested (n) and the ranges and means of trichostrongyle and *Nematodirus* FECs
456 (eggs per gram) on each farm are given in this table. Animals reported to co-graze with the
457 alpacas and anthelmintic treatments for GINs of the alpacas during the previous 12 months are
458 also shown.

459

460