Using deep-amplicon sequencing to understand the dynamics of gastrointestinal nematodes present in co-grazed host species kept in a Scottish zoological collection.

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Introduction

Significance of gastrointestinal nematodes in farmed ruminant populations in the UK:

- Helminths are **ubiquitous** in grazing host populations worldwide. **Industry**
- Estimated costs of helminths, including gastrointestinal nematodes (GIN), to the UK livestock industry estimated in 2020:
 - Treatment costs: ~£80 million per annum.

Charlier J, et al. Initial assessment of the economic burden of major parasitic helminth infections to the ruminant livestock industry in Europe. Prev. Vet. Med. 182, 105103, 2020

Gastrointestinal nematodes in mixed host-species populations:

- Mixed species grazing is a more common form of livestock production in pastoral and smallholder systems in low-middle income countries.
- Grazing animal species kept in mixed grazing systems can be at risk of significant GIN infections, due to life-cycle maintenance in confined grazing areas and proximity to other



- Production costs: ~£223 million per annum. Herd and flock level







susceptible hosts.

- Yet little work has been undertaken to understand the GIN species dynamic within cograzed host-species with likely complex GIN transmission networks related to host susceptibility.
 - For example, host-specific parasites account for <50% of the parasite species infecting a host.
- Pasture-based zoological collections in the UK provide an opportunity to explore GIN dynamics of nematode species in a mixed species grazing system.
- Deep-amplicon sequencing of ITS-2 shows potential for describing nematode dynamics between hosts.

Walker, J. G., & Morgan, E. R. (2014). Generalists at the interface: Nematode transmission between wild and domestic ungulates. IJP: Parasites and Wildlife, 3(3), 242–250. 2014

Aim: This pilot study aimed to describe the species diversity of nematode species found in faeces of grazing animals in a Scottish zoological collection.

Materials and Methods



- **Population:** The study was conducted in a zoological collection on the west coast of Scotland. Host species included goats, sheep, alpacas, horses, deer, camels and tapir.
- All animal, except the deer, were routinely given anthelmintic treatments 2-4 times per annum. Benzimidazoles, levamisole and macrocyclic lactone and praziquantel are used.
- Sampling (Summer 2021): single time point sampled a range of grazing host species at a zoological collection:
 - Host species, grazing management and treatments.
 - 2. Fresh free catch faecal sample for up to 10 animals per enclosure and pooled for:
 - A. Faecal worm egg count (FEC) using the cuvette method.
 - B. Larval culture with subsequent deep-amplicon sequencing of ITS-2.



- Figure 1. highlights where animals were grazed at time of sampling. Although no official records, species had been rotated on multiple pastures previously. Therefore, it is assumed that host species co-graze multiple pastures.
- **Analysis:**
 - Description of sample.
 - Comparison of FEC between species.
 - Graphical comparison of GIN species distribution between hosts.



ITS-2 ASVs across all hosts

Figure 1: 10x pooled faecal b. samples per enclosure. C. • Limitation: not all

Coronocyclus_coronatus

Haemonchus_contortus

Cyathostominae_unclassified

Cylicostephanus_longibursatus

Oesophagostomum_asperum

Oesophagostomum_venulosur

Spiculopteragia_unclassified

Strongyloidea_unclassified

Teladorsagia_circumcincta

Trichostrongylus_colubriformis

Trichostrongylus_axei

Trichostrongylus_vitrinus

animals were sampled in

multi-species enclosures.



Results



Figure 2: Bar plots by pooled faecal samples per host species A. proportional to FEC & **B.** proportional reads to GIN species. • Goats and sheep contributed to the ylicostephanus_longibursatus largest FECs and may act as a Spiculopteragia_unclassified significant Trichostrongylus_colubriformis multiplier host. • Goats and deer had a relatively high GIN species diversity.



Figure 3: Heat map by pooled faecal samples per host species by GIN species and ITS-2 allele strain variant (ASV). • For *H. contortus*, certain strains are shared between alpacas, deer, goats and sheep. A proportion of strains are restricted to alpacas, highlighting bottlenecks in transmission. • T. circumcinta, T.

columbriformis and T. vitrinus also highlight single or multiple host



alpacas, sheep and goats. • Horse GIN species appear to be the most restricted to this host species.

• *H. contortus* is the

across all species,

being prevalent in

Figure 4: Phylogenic tree by GIN species and ITS-2 allele strain variant (ASV).

• The tree highlights potential routes to GIN strain bottlenecks in certain host species such as demonstrated in *H. contortus*.



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Conclusion

- GIN can be ubiquitous in mixed grazed host species in zoological collections.
- Small ruminant GIN species predominated (H. contortus, T. circumcinta and T. colubriformis) across all grazed species and transmission facilitated by communal pasture.
 - Deep amplicon sequencing to generate a nemabiome is useful to explore variation in GIN species in co-grazed host species.
- Goats are a possible multiplier host for certain GIN species, e.g. *H. contortus,* and their transmission to other co-grazed hosts.
 - Strains of *H. contortus* may differ in host preference, highlighting different risk profiles for transmission and potential associated disease.
 - **Opportunities:** using the ITS-2 allele strain variant platform, along with additional mitochondrial markers, to understand the drivers for GIN species diversity and impact on anthelmintic resistance profiles overtime.

preference across strains.