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Research paper

Gastrointestinal nematode species diversity in Soay sheep kept in a natural environment without active parasite control

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ABSTRACT

Molecular methods based on ITS2 sequence analysis were used to identify strongylid parasites and describe their diversity in a management intervention and anthelmintic drug treatment-free sheep flock. Fourteen different nematode parasite species were identified in the flock and the results showed a greater level of nematode species diversity than is normally reported in managed farmed flocks, with the presence of parasites such as *Bunostomum trigonocephalum*, *Ostertagia leptospicularis*, *Spiculopteragia houdemeri* and *Trichostrongylus retortaeformis* that are considered to be absent or rare in sheep kept in comparable localities. The implied prevalences of *Haemonchus contortus* in lambs, and of *Trichostrongylus axei* in lambs, ewes and rams, were higher than those in farmed sheep kept in similar regions, while those of *Teladorsagia circumcincta* and *Trichostrongylus vitrinus* were lower. Comparison of the patterns of nematode parasite infection between the summer and autumn sampling periods showed differences from the scenarios that are commonplace in comparable managed flocks; with *T. vitrinus* burdens of the lambs being higher in the summer than in the winter, and *Oesophagostomum venulosum* being the predominant nematode species in the adult sheep during the summer, while more-or-less absent from these groups during the winter. Rams played an important role in the epidemiology of certain parasitic nematode species. The relatively non-pathogenic *O. venulosum* was the only parasitic nematode species to predominate in any group during the study. This preliminary characterisation of the nematode parasite burdens of sheep extensively grazed on diverse unimproved pastures will aid in the understanding of the parasitological consequences of intensive grazing management and of the manner in which modern agriculture upsets the equilibrium between parasites and their hosts. These factors must be accounted for when defining the concept of sustainable parasite control and informing sustainability with reference to commercially efficient sheep farming.

1. Introduction

Sheep are potential hosts to numerous genera and species of gastrointestinal nematode parasites. The relationship between nematode parasites and their hosts has evolved over millions of years, but has been upset in relatively recent times by domestication and farm management practices that either inadvertently select for more susceptible hosts, or create environments that enable the differential establishment of larger numbers of free-living stages of the parasites (Sutherland and Scott, 2010). Consequently, *Teladorsagia circumcincta*, *Haemonchus contortus*, *Trichostrongylus vitrinus* (or *colubriformis* in warmer regions) and *Nematodirus battus* have become the major production limiting species affecting managed, improved sheep in temperate climates.

Most studies of sheep parasitic nematodes have been conducted in managed flocks, which are grazed on improved grass pastures that are conducive to differentially high rates of free-living stage larval development and survival and are treated with anthelmintic drugs with differential efficacy or persistence. In these situations, most hosts are infected with just a few nematode species, often showing sequential variation in the predominance of just one or two (Boag and Thomas, 1977; Paton et al., 1984). A seasonal trend in the predominance of individual major species is driven by temper-

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ature, moisture and physical characteristics of the biomes occupied by the parasites' free-living stages (Stromberg, 1997; Wang et al., 2014), immunologically mediated host responses (Stewart, 1955) and regulatory influences of one nematode species upon another (Coop and Field, 1983). The manner whereby factors might act independently and, or, interactively to determine the impact of the burden of particular parasitic nematode species on their sheep hosts is poorly defined. Better understanding of these trends and interactions is required to inform sustainable control strategies, and depends upon accurate characterisation of the hosts' nematode parasite burdens.

Extrinsic factors such as climate, management systems and the influence of geography and flora on the ecological niche occupied by free-living stages of the gastrointestinal parasitic nematodes, clearly exert a large influence on the size of the infective larval challenge (Jackson et al., 1992) and its potential to limit animal productivity (Morgan, 2013). The manner in which these factors might also influence the seasonal prevalence of different species, contributing to the temporal development of heavy mono-specific burdens in sheep grazed on managed grass and clover pastures is poorly understood. Conversely, it is unclear if these factors might in part allow for the development of lower burdens of a larger number of gastrointestinal parasitic nematode species, causing less severe production loss, in sheep grazed in more diverse natural environments. Preliminary characterisation of the nematode parasite burdens of sheep extensively grazed on diverse unimproved pastures will therefore aid in the understanding of the parasitological consequences of intensive grazing management.

The manner in which modern agriculture upsets the equilibrium between parasites and their hosts must be accounted for when defining the concept of sustainable parasite control (Morgan et al., 2012). Neither naturally unmanaged grazing, nor planned evasive nematode management strategies are sustainable, and the prerequisite understanding of conditions influencing the biotopes of different parasite populations is inadequate with regards to the development of sustainable systems (Morgan et al., 2013). Dependence on pharmaceutical control of nematode parasites inevitably selects for anthelmintic resistance, hence is also unsustainable (Kaplan and Vidyashankar, 2012).

This study provided an insight to the parasitic nematode species diversity in unimproved Soay sheep kept in a natural environment without active management or pharmaceutical nematode control measures. The aim was to improve general understanding of the impact of management on nematode parasite diversity and interactions, as a prerequisite for the development of sustainable control (Lello et al., 2004) of gastrointestinal nematode parasites in commercially-managed sheep.

2. Materials and methods

2.1. Study group and farm

The closed Soay sheep population of 11 lambs, 23 ewes and 10 rams (during the study period) was co-grazed with six native ponies on 30 ha of natural rough grazing, historically improved pastures and woodland, between about 25 and 100 m above sea level in coastal Argyll, Scotland. There had been neither animal or grazing management intervention, nor modern broad-spectrum anthelmintic drug treatments given since the establishment of the flock, about 12 years previously. Individual animals were uniquely identified using pictures and a panel of phenotypic descriptive indices. Thus, the flock afforded a unique opportunity to observe domesticated livestock under conditions that reflected those applying prior to the implementation of modern management systems.

2.2. Parasitological methods

Faecal samples voided by every animal in the Soay sheep flock were collected immediately off the ground during the summer (August 2013) and winter (January 2014), without handling the animals. This was done by watching each animal in turn. Samples were collected within one minute of being voided to minimise potential contamination by free living nematodes or environmental stages of unrepresentative parasitic nematodes. Faecal nematode egg counts (FECs) were performed using a modified McMaster method (MAFF 1986) where one egg counted represented 50 eggs per gram (epg) (conducted on farm in summer), and a salt floatation and cuvette method (Christie and Jackson, 1982) with a potential sensitivity of 3 epg (conducted in the laboratory in winter). The different methods were used for practical reasons, and while the results cannot be compared at less than 100 epg due to the differences in sensitivities, we have shown the two methods have been shown to give practically comparable results at higher egg counts (data on file). *Nematodirus* spp. eggs were enumerated and recorded separately from those of other genera. Approximately 5 g of faeces from each animal was pooled for lambs, rams and ewes and incubated for 72 h at 20 °C (±2 °C) to promote egg hatching. Hatched L_1 was recovered by Baermannisation and fixed in ethanol (final concentration ~70% ethanol). Hatching was monitored in subsamples of extracted eggs to ensure that more than 95% of eggs, excluding those of *Nematodirus* spp. developed to the larval stage. This was done to account for potential species-proportional differences in egg hatching rates.

Ethanol-fixed samples were bathed in 1x phosphate buffered solution (PBS) for 30 min to rehydrate the larvae (1/100 v/v). 88 individual L_1 were transferred into individual wells of 96 well plates (Axygen), containing 50 μl of lysis buffer (50 mM KCl; 10 mM Tris [pH8.3]; 2.5 mM $MgCl_2$; 0.45% Nonidet P-40; 0.45% Tween-20; 0.01% gelatine and 0.1 mg/ml proteinase K (Bioline)). Plates were placed at −20 °C overnight and incubated at 56 °C for a minimum of 4 h. The lysates were then heated to 95 °C for 15 min to deactivate proteinase K. DNA was precipitated by adding 100 μl ethanol to each well; plates were kept at −20 °C overnight and centrifuged (4000G, 40 min at 4 °C). The supernatant was removed and plates were air-dried briefly. Extracted DNA was then re-suspended in 50 μl of DNA-free water.

2.3. Species identification by PCR

Species identification was carried out following the method developed by the Moredun Research Institute (Melville et al., 2016). Briefly, a published multiplex PCR assay was used to identify five nematode species most commonly found in the UK; *T. circumcincta*, *H. contortus* and the *Trichostrongylus axei*, *T. colubriformis* and *T. vitrinus* in a single reaction (Bisset et al., 2014). Amplification of each species results in a unique product length for each gastrointestinal nematode species. Positive controls were included in each PCR reaction using genomic DNA extracted from single adult nematodes of each the five species tested following morphological species identification (MAFF, 1986) to verify results and ensure that band size were accurately and specifically analysed. Multiplex PCR products were analysed using QIAxcel advanced capillary electrophoresis using the QIAxcel DNA High resolution kit (Qiagen) following the manufacturers' protocol. The Qiaxcel advanced capillary system used provides a much more accurate measure of PCR product size compared to conventional agarose gel electrophoresis. Analysis was completed using QIAxcel ScreenGel software (Qiagen).

Generic pan-nematode primers (ITS2GF and ITS2GR) (Bisset et al., 2014) were also included in the multiplex PCR. Detection of a PCR product from the pan-nematode primer set but not from species specific primers indicated the presence of nematode larvae DNA of a species not included in the test. As all of PCR speciation