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Short communication

Ovine nematodes in wild lagomorphs in Australia and first record of *Trichostrongylus rugatus* in free living lagomorphs

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ABSTRACT

Gastrointestinal parasites are a major problem for sheep graziers and anthelmintic resistance has been reported worldwide. Given that in experimental circumstances, sheep helminths are able to develop in rabbits and hares, and that hares are very mobile and sympatric with sheep in many regions of Australia, free-living lagomorphs were investigated regarding carriage of ovine nematode parasites under field conditions. We performed specific worm counts in the gastrointestinal tracts of 88 rabbits and 110 hares. We revealed that ruminant worms are common in hares (prevalence 79%) and that they are occasionally found in rabbits (9%). Statistical analyses showed that the ruminant worm *Trichostrongylus colubriformis* occurred frequently in hares whilst rabbits were commonly infected with lagomorph-specific *Trichostrongylus retortaeformis*. Detection of the ovine worm *Trichostrongylus rugatus* is reported for the first time in wild lagomorphs. The potential for cross-transmission between hares and sheep in the natural environment is much more prevalent than previously believed.

1. Introduction

Areas of common grazing between sheep (*Ovis aries*), hares (*Lepus europaeus*) and rabbits (*Oryctolagus cuniculus*), are widespread in south eastern Australia. Pastures on farms are infested for much of the year with large numbers of infective larvae of livestock to which grazing lagomorphs are inevitably exposed. A number of parasitological surveys have shown that hares and rabbits sympatric with sheep are occasionally infected with ovine nematodes (Alzaga et al., 2009; Hesterman and Kogon, 1963; Mackerras, 1958; Saulai and Cabaret, 1998). However, few hares were examined in these studies and/or very few animals were infected with ruminant nematodes. There have been successful attempts to establish parasitism with ovine nematodes in laboratory rabbits (Musongong et al., 2004) and hares (Stott et al., 2009). The major problem affecting sheep graziers worldwide is the spread of resistant strains of ruminant gastrointestinal parasites (Coles et al., 2006). There are two factors directly correlated with this problem: leporids are sympatric with sheep; and hares are highly mobile mammals with home ranges ~200 ha in area (Stott, 2003). Lagomorphs can easily cross property boundaries, keep worms *in refugia* from anthelmintics, and contribute to the spread of resistant strains of ruminant gastrointestinal parasites.

The aim of the present investigation was to assess infestation levels with ovine internal parasites in larger samples of free-living hares and rabbits sympatric with sheep in different regions of south-eastern Australia.

2. Materials and methods

2.1. Parasites and morphology

The project was approved by the University of Adelaide Animal Ethics Commitee (S-2010-096).

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Sympatric hares and rabbits were shot in paddocks previously grazed by sheep or in vineyards adjacent to sheep pastures in the Adelaide region of South Australia, the western district of Victoria (hares only) and central western New South Wales. The gastrointestinal tract was removed from each carcass and contents were washed extensively with tap water, poured through wire mesh screens to remove debris and then, poured into Petri dishes for observation. Total helminth counts were performed, and individual specimens were identified to genus after Mönnig and Lapage (1962). Because the genus *Graphidium* is monotypic, individual specimens were assumed to be *G. strigosum*, and because the source populations for the hares and rabbits came from Britain, individuals of the genus *Passalurus* were assumed to be *P. ambiguus*. Adult male *Trichostrongylus* spp. were cut in half, and the posterior half was cleared with lactophenol. Spicules were identified to species using identification keys based on spicule morphology (Mönnig and Lapage, 1962).

2.2. Extraction of genomic DNA and PCR

DNA was isolated by QIAamp DNA Mini Kit by Qiagen. Generic *Trichostrongylus* spp. primers were designed around the second internal transcribed spacer (ITS-2) based on existing data from Genbank; Accession Nos: HQ844229; AB503252; AB503251; HQ389232; EF427624; EF427622; X78066; Y14818; AY439027; X78064 5′-TCGAATGGTCATTGTCAA-3′ (forward); 5′-TAAGTTTCTTTTCCTCCGCT-3′ (reverse). The final concentrations of $MgCl_2$ and dNTP, in each 25 μL tube were 1.5 mM and 0.2 mM, respectively. Forward and reverse primers had a final concentration of 0.4 μM. 1 Unit of Taq DNA polymerase, 5 μL of genomic template and negative controls were included in the runs. PCR was conducted in a Kyratec SC 200 thermal cycler at 94 °C (denaturation) for 45 s (38 cycles), 56 °C for 45 s (annealing) and 72 °C for 1 min (extension). The PCR run was finalised with another final extension at 72 °C for 5 min and one cooling cycle at 20 °C. Amplicons were subjected to electrophoresis in 2% agarose gel. Samples were sequenced in the Australian Genome Research Facility Ltd and results were analysed with Nucleotide BLAST®.

Fifty-two nucleotide sequences of the *Trichostrongylus* species found in this study were deposited in Genbank® under the accession numbers **JX04,6418.1** and **KC52,1364–KC52,1414**.

2.3. Statistical analysis

One way ANOVA on ranks (Kruskal–Wallis) was performed to detect any effect of region on intensity of infection. The non-parametric sign test was utilised to verify difference in prevalence of a specific nematode species between the populations of hares and rabbits. Any value of $P < 0.05$ was considered significant.

3. Results

Of the total 110 hares examined, 63 (57.3%) were infected with one or more species of nematode parasites. Of the total of 88 rabbits examined, 81 (92%) were infected. Five species of nematodes were recovered and amongst them, two were ovine: *Trichostrongylus colubriformis* (Giles, 1892) – found in the small intestine of 37 hares and two rabbits; and *Trichostrongylus rugatus* (Mönnig, 1925) – small intestine of four hares and one rabbit and is recorded for the first time in free-living lagomorphs. The other species found were lagomorph-specific: *Graphidium strigosum* (Dujardin, 1845) – stomach of 44 rabbits and one hare; *Passalurus ambiguus* (Rudolphi, 1819) – caecum and colon of five rabbits and one hare; and *Trichostrongylus retortaeformis* (Zeder, 1800) – small intestine of 15 hares and 54 rabbits. Some *Trichostrongylus* could not be identified to species level.

Tables 1 and 2 show the number of lagomorphs affected by different species of nematode parasites in different regions of south-eastern Australia.

Kruskal–Wallis test showed that, in general, nematode parasite infestations in hares or rabbits were not significantly different amongst the different regions examined ($P > 0.05$). However, the sign test showed that the prevalence of *T. colubriformis* was distinctly higher in hares, whereas *T. retortaeformis* and *G. strigosum* occurrences were more pronounced in rabbits ($P < 0.05$). Macroscopic disruptions at the form of thickening of portions of the duodenal mucosa were observed in a few hares affected only by *T. retortaeformis*.

PCR was entirely consistent with morphological identification and it was used as a diagnostic tool when only female worms were retrieved.

4. Discussion

Nematode species normally regarded as ovine parasites occurred in all free-living lagomorph populations examined. *T. colubriformis* appeared to have had a high prevalence in hares but not in rabbits (Tables 1 and 2). In fact, hares were more commonly infected by ovine than by lagomorph-specific nematodes (Fig. 1A). Their high mobility could be an explanation.

In the sheep regions of south eastern Australia, hares and rabbits show different levels of infestation with ovine and lagomorph larvae. By examining the grazing and defecation pattern of sheep (White and Hall, 1998); along with the overlapping home range (Stott, 2003), density, and digesta throughput of hares (Stott, 2008), ingestion of infective ovine strongyle larvae by hares would occur often. Conversely, the focused grazing patterns of rabbits close to cover (Stott, 2003) would result in the ingestion of few ovine but many lagomorph larvae.

In 1982, Beveridge and Ford indicated that in South Australia, *T. vitrinus* was the most prevalent *Trichostrongylus* in sheep and it seemed to be prominent in more humid areas of the state. Conversely, *T. rugatus* was abundant in the drier and hotter northern areas of the state. According to the authors, *T. colubriformis* had a relatively constant prevalence in sheep in South Australia. However, in our study, *T. colubriformis* was the most prevalent species in hares for all regions examined. *T. rugatus* had a very modest contribution, not precisely occurring in the drier areas of South Australia. Despite conjecture based