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Short communication

Influence of earthworms on development of the free-living stages of gastrointestinal nematodes in goat faeces

S. d'Alexis [a], G. Loranger-Merciris [b], M. Mahieu [a], M. Boval [a,*]

[a] INRA, UR143, Unité de Recherches Zootechniques, Domaine Duclos, 97170 Petit Bourg, Guadeloupe (F.W.I.)
[b] EA 926 DYNECAR, Université des Antilles et de la Guyane, Laboratoire de Biologie et de Physiologie Végétales, BP 592, 97159 Pointe à Pitre Cedex, Guadeloupe (F.W.I.)

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ABSTRACT

With the important infection of small ruminants by gastrointestinal nematodes, and in the face of reduced efficacy of anthelminthic treatments, a search for other biological options is necessary. The effect of earthworms on the free-living stages of *Haemonchus contortus* and *Trichostrongylus colubriformis* in faeces from goats naturally infected in tropical pastures was evaluated. Two levels (0 vs 14 individuals per container) of indigenous earthworms (50% *Pontoscolex corethrus* and 50% *Perionyx excavatus*) were added to containers filled with soil and faeces collected from 20 grazing goats. After 1 week, the numbers of free-living stages of each infective larvae species was measured. The addition of earthworms reduced by 34% ($P < 0.006$) the number of infective larvae recovered in the faeces of goats. The reduction was significant for both larvae species, 29% and 33% for *H. contortus* and *T. colubriformis*, respectively. The ratio of the two species of larvae, without or with earthworms, did not vary significantly ($P > 0.21$). These results must be confirmed in experiments on pasture, with other ratios and combinations of earthworms.

1. Introduction

Gastrointestinal nematodes (GIN) have a major effect on the productivity of grazing ruminants. In tropical pastures, 80% of pre-weaned kid mortality is due to gastrointestinal nematodes (Aumont et al., 1997b). Control of GIN relies mainly on anthelmintic drugs, but the worldwide development of anthelmintic resistance has engendered a search for alternative strategies for controlling GIN infections in livestock.

Integrated approaches such as selective breeding of resistant animals (Stear and Murray, 1994), vaccination of young animals (Jackson and Miller, 2006; Vercruysse et al., 2004), pasture management (Aumont and Gruner, 1989), dietary supplementation with protein (Coop and Holmes, 1996), use of tanniferous forages (Niezen et al., 1993; Paolini et al., 2003), or targeted drenching (Bath et al., 1996; Mahieu et al., 2007), are being investigated as sustainable alternatives. Biological agents, such as nematophagous fungi (Larsen, 2000) and earthworms, can also directly affect the development of free larval stages in faeces, thus reducing the population of infective GIN larvae in pasture.

There have been few reports concerning the impact of earthworms on the development of infective larvae (Hyvonen et al., 1994; Ilieva-Makulec and Makulec, 2002). However, biological control by earthworms seems to be promising, since the infective larvae of gastrointestinal nematodes are located in dung and faecal organic matter, the main food resource for several earthworm species (Scown and Baker, 2006). Earthworms may ingest considerable numbers of nematodes (Dash et al., 1980; Yeates, 1981), and in some studies, mostly with cattle and sheep (Hyvonen et al., 1994; Ilieva-Makulec and Makulec,

* Corresponding author. Tel.: +590 590 255989; fax: +590 590 255936.
E-mail address: maryline.boval@antilles.inra.fr (M. Boval).

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2002), the presence of earthworms was related to a reduction in nematode populations.

The aim of this study was to measure the effect of a mixed population of indigenous earthworms (*Pontoscolex corethrus* and *Perionyx excavatus*) on the count and proportion of GIN larvae of *Haemonchus contortus* and *Trichostrongylus colubriformis* recovered in faeces of naturally infected Creole goats.

2. Materials and methods

The study was conducted at the experimental station of the National Agronomic Research Institute (INRA) in the French West Indies, Guadeloupe, (16°16′N, 61°30′W) in 2007.

2.1. Container preparation and collection of earthworms

Forty aluminium containers (910 cm^3) were filled with 400 g of ground ferralitic soil, dried and sieved (4 mm mesh) to eliminate plant remains and large invertebrates. The containers were pierced at the base for aeration and drainage, and were covered with nylon fabric and held at −20 °C for 48 h in order to kill all soil nematodes. Subsequently, the containers were held at ambient temperature (25–30 °C). They were watered to field capacity and kept 1 week before the addition of earthworms.

P. corethrurus Müller (Glossoscolecidae) was collected from ferralitic soil at a depth of 0–25 cm in the forest contiguous with the experimental station. *P. corethrurus* is indigenous and widespread in tropical zones (Lavelle, 1983), although invasive elsewhere in tropical areas outside America. This endogeic geophagous species was commonly found in tropical lands cleared for cultivation. A second species, *P. excavatus* Perr. (Megascolecidae) identified by P. Lavelle (University of Paris 6/IRD, France) was collected from the manure of stall fed goats. *P. excavatus* is an epigeic earthworm found commonly over a large area of tropical Asia (Stephenson, 1930; Gates, 1972), and has been transported to Europe and North America. This species inhabits organic waste, and its potential for breaking down and processing organic wastes is well known (Kale et al., 1982; Reinecke et al., 1992). Seven *P. corethrurus* and seven *P. excavatus* were chosen for their vigour and their large size. They were then added into each of 20 of the containers filled with soil as described above. The 20 remaining containers without earthworms were used as a control.

2.2. Collection of nematodes and experimental design

Twenty Creole goats naturally infected by *H. contortus* and *T. colubriformis*, grazing paddocks based on *Digitaria decumbens*, were used. The goats' faeces were collected in faecal bags 8 days after the addition of the earthworms to the containers. Two 20 g samples per doe were randomly assigned to 1 of the 20 containers with earthworm or 1 of the 20 containers without earthworms. The sub-samples were deposited on the surface of the soil, as they would be on pasture and were incubated for 7 days (i.e. the duration of the egg to infective larva development). Then the containers were covered with aluminium foil to prevent the escape of the earthworms.

At the same time, 20 sub-samples of faeces (5 g per goat) were kept for the determination of the faecal egg count (FEC). Faecal eggs counts were estimated using a modified Mc Master method (Aumont et al., 1997a) providing a lower limit of detection of about 30 eggs per gram (epg). Coprocultures (about 10 g per goat) were also prepared to assess the species composition of GIN burden.

2.3. Recovery of GIN larvae

One week after addition of faeces to the containers, the faeces not ingested were recovered and the infective larvae (L3) present therein were extracted by 24 h Baermann extraction.

Cleaned samples were held at 5 °C, and concentrated to 10 ml by sedimentation and siphoning off the supernatant water. The larvae recovered were stained with Lugol's iodine, identified to genus and counted at 40× magnification.

The initial nematode species composition was determined after recovering L3 from coprocultures using the same Baermann extraction.

2.4. Statistical analysis

Data were normalized by a logarithmic transformation and then subjected to ANOVA with 20 replications using the GLM procedure of SAS (SAS software, V8.01, SAS Institute Inc., Cary, NC, USA). The model included the effects of treatment and does. The LSMEANS option was used to calculate treatment means reported as geometric values, calculated as the exponential of the mean of the logged data. Significant effects were considered at $P < 0.05$ and trends at $P < 0.10$.

3. Results

3.1. Level of infestation of faeces collected at pasture

The number of GIN eggs in the faeces of the goats was 451–2967 epg. After 1 week of culture, the number of larvae was 6–555 g^{-1} of faeces, and two species of nematodes were identified: *Haemonchus* sp. and *Trichostrongylus* sp. (Fig. 1). The proportion of each species was similar, 51% *Haemonchus* sp. and 49% *Trichostrongylus* sp. ($P > 0.05$). The rate of development from egg to infective larva was estimated to be 7% on average.

3.2. Effect of addition of earthworms on the number of infective larvae recovered

The number of nematode larvae recovered after 1 week in the containers with earthworms was significantly lower ($P = 0.006$), compared with the controls. The average reduction of larvae in the presence of earthworms was about 34%, compared with the controls, irrespective of the species of the nematode, *Haemonchus* sp. or *Trichostrongylus* sp. The proportions by which the two nematode species lower were 29% for *Haemonchus* ($P = 0.02$) and 33%